M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: Aquatic Invasive Species Research Center
SUBPROJECT MANAGER: Nicholas Phelps
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $8,700,000
AMOUNT SPENT: $8,383,770
AMOUNT REMAINING: $316,230

Sound bite of Project Outcomes and Results

This project established the Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota. Through this appropriation, MAISRC has supported 32 subprojects on many of Minnesota's most important aquatic invasive species, significantly advancing our scientific understanding and ability to manage AIS, and engaging thousands of stakeholders and partners.

Overall Project Outcome and Results

This project successfully established the Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota, a vibrant and durable research program that develops research-based solutions to Minnesota's aquatic invasive species (AIS) problems. MAISRC has quickly become a global leader in the field and a go-to resource for managers, the public and researchers. In total, 32 subprojects were supported from this project – significantly advancing our scientific understanding and ability to manage AIS. New tools have been developed and knowledge gaps filled on many of Minnesota's most important AIS, including: zebra mussels, bigheaded and common carps, starry stonewort, non-native *Phragmites*, Eurasian watermilfoil, curlyleaf pondweed, Heterosporosis, and spiny waterflea. The results of this work have been broadly disseminated to end-users via research reports, peer-reviewed manuscripts, fact sheets, white papers, news media, newsletters and presentations (on the MAISRC website). An annual Research and Management Showcase has been held since 2014, with 700+ unique attendees in total. MAISRC has also created an award-winning and sustainable citizen science program ("AIS Detectors") that has trained hundreds of people from across the state. This project supported efforts to ensure effectiveness and efficiency of a Center-based research model, including a 10-year strategic plan, a comprehensive process for prioritizing research needs, increased collaboration and coordination between researchers and managers, an annual competitive and peer-reviewed request for proposals, the formation of external and internal advisory boards, research dissemination and outreach, support of a world class research facility, and creation of communication and development plans. Minnesota is much better equipped to address our AIS problems than we were prior to this project – MAISRC has significantly advanced the science of AIS management and engaged thousands of stakeholders and partners from across the state and world. This project will continue with Phase II and III appropriations awarded in 2017 and 2019.

Project Results Use and Dissemination

MAISRC currently has a social media following of just under 2,300 and an e-newsletter list with just under 3,500 recipients. Social media posts about research findings, events, AIS Detector workshops, and general invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects. In addition, MAISRC has recorded consistent growth in the number of unique visitors and total website views since the website launch in February 2016. This increase shows that MAISRC is growing in name recognition and being seen as an important resource for different stakeholders around the state. Over the course of the last six years, MAISRC has been in approximately 350 news stories in roughly 117 different outlets. The most common outlets have been the *Star Tribune*¸ Minnesota Public Radio, and KSTP-TV. Other notable outlets include *The New York Times, The Washington Post,* and Minnesota Bound. Nine videos were created highlighting MAISRC subproject research. Six AIS Research and Management Showcases were held with 700+ unique attendees. The AIS Detectors program was formally launched in March 2017 and we now have 299 certified Detectors around the state.

- The nine videos highlighting MAISRC subproject research included:
  - AIS Detectors
  - Starry stonewort research
  - Spiny waterflea research
  - Impacts of AIS on walleye
  - Using pathogens to control invasive carp
  - Novel methods for controlling common carp
  - Valuing AIS management
  - Genetic control of invasive carp
  - Using the Whooshh fish transport system (not released yet)

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2013 Work Plan Final Report

Date of Status Update Report: November 11, 2019

FINAL REPORT

Date of Work Plan Approval: June 25, 2013

Project Completion Date: June 30, 2019

Project Title: Aquatic Invasive Species Research Center

Project Manager: Nicholas Phelps

Affiliation: University of Minnesota

Address: 135 Skok Hall, 2003 Upper Buford Circle

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Location:

Counties Impacted: Statewide

Ecological Section Impacted: Lake Agassiz Aspen Parklands (223N), Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B), Northern Minnesota and Ontario Peatlands (212M), Northern Minnesota Drift and lake Plains (212N), Northern Superior Uplands (212L), Paleozoic Plateau (222L), Red River Valley (251A), Southern Superior Uplands (212J), Western Superior Uplands (212K)

Total ENRTF Project Budget:	**ENRTF Appropriation $:**	8,700,000
	Amount Spent $:	8,383,770
	Balance $:	316,230

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

Appropriation Language:
$4,350,000 the first year and $4,350,000 the second year are from the trust fund to the Board of Regents of the University of Minnesota to develop and support an aquatic invasive species (AIS) research center at the University of Minnesota that will develop new techniques to control aquatic invasive species including Asian carp, zebra mussels, and plant species. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Aquatic Invasive Species Research Center

II. PROJECT SUMMARY:

Aquatic invasive species (AIS) are causing irreparable damage to Minnesota's fisheries and wildlife and their habitats, as well as to our outdoor heritage. This threat is expanding as new exotic species arrive, most of which are poorly understood. New ideas and approaches are needed to develop real solutions. The Minnesota state legislature awarded the University of Minnesota $3,800,000 in 2012 to create an Aquatic Invasive Species (AIS) Research Center. The goal of the Research Center (Laws of 2012, Chapter 264, article 2, section 4 and article 4, section 3) is to develop and implement solutions to control aquatic invasive species. It will do this by developing scientific expertise in variety of disciplines so that new solutions can be devised and extant ones improved while educating management agencies and the public. The Center will function in collaboration with the Minnesota Department of Natural Resources as well as other federal and state governmental agencies and private citizens groups. Initial funding was allocated to establish the administrative structure for this center, renovate University facilities, and start studies of zebra mussels and Asian carp. The present project will provide operating funds so that the scope of research can be extended to include common carp, pathogens designed to control invasive fishes, risk analysis of AIS, as well as establish as an extension and education component. This new funding will also establish an administrative structure for the Center which will both administer funds and reporting and coordinate collaborations with the DNR and other groups with an advisory board as well was as a board of technical experts. The Center will coordinate anonymous peer-reviews of center projects to insure high quality research. The new funding will give the center a life through 2019 and the opportunity to create to raise supplemental funding from other sources.

The work supported by this new proposal will initially include 11 sub-projects:

1. Coordinating, synergizing and promoting expertise: Establishing the administrative structure;
2. Delaying the spread of AIS: Monitoring the abundance and distribution of AIS using new molecular tools so techniques to delay their spread can be implemented;
3. Reducing and controlling AIS: Developing effective tools to attract and locate aggregations of invasive carp;
4. Reducing and controlling AIS: Developing effective bio-control techniques to control common and/or Asian carp;
5. Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants;
6. Reducing and controlling AIS: Simulation modeling to identify and evaluate AIS control methods;
7. Developing eradication tools: Exploring whether native pathogens can be used to control AIS;
8. Implementing findings: An applied ecologist - extension specialist position and program;
9. Implementing Findings: Implementing new tools for zebra mussel control;
10. Implementing findings: An extension educator or outreach position; and
11. Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods**.**

These sub-projects will all be evaluated at 2 -3 year intervals through a peer-review process at which time detailed budgets will be assigned. Sub-projects may be added or eliminated depending upon progress and needs for AIS control in the state. Evaluation of results and implementation of changes (if necessary) will be evaluated by a Center Advisory Board (CAB) which will provide recommendations to the Director who would then suggest project amendments. Final approval of plans and changes to them must come from an internal Center Administrative Review Board and then ultimately from the LCCMR as an amendment to the work plan. This first work plan has been written following advice provided by the DNR and LCCMR staff using knowledge available as of June 2013.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of August 30, 2013*:*
Revisions and corrections have been made to the budget to resolve issues such as formula errors, updating fringe rates to reflect current university policy, and rebalancing travel and supplies allocations for consistency among similar projects. This has resulted in a change in each subproject budget and a shift in the reserve amounts accordingly:

Subproject 1: $2,083,419 to $2,034,394; reserve from $1,668,657 to $1,445,927
Subproject 2: $953,014 to $978,220; reserve from $953,014 to $978,220
Subproject 3: $674,917 to $666,335; reserve from $674,917 to $666,335
Subproject 5: $630,776 to $650,280; reserve from $470,758 to $426,998
Subproject 6: $331,628 to $352,790; reserve from $246,917 to $230,116
Subproject 7: $864,888 to $806,535; reserve from $569,401 to $471,308
Subproject 8: $1,056,222 to $1,037,134; reserve from $785,223 to $758,341
Subproject 10: $395,416 to $390,196; reserve from $319,711 to $283,694
Subproject 11: In addition to the corrections mentioned above, an error was fixed so that this project has a duration of two years (the original intent) rather than of 3.5 years. Budget shifted from $282,988 to $171,932; reserve from $168,797 to $0

Additionally, Attachment A now shows allocations for the entire 2-year duration of the first round of subprojects (#s 1,5,6,7,and 11), which will extend over three fiscal years. This also explains the change in the reserve amounts listed above for those subprojects.

Amendment Request as of August 30, 2013:
In addition to the type of corrections mentioned above, programmatic changes were made to three subprojects. We hereby request an amendment for the following changes:

Subproject 4: We have increased the fish ecologist time from 50% to 75% in the first year to allow for a possible earlier start. Together with the corrections mentioned above, this results in the budget for this subproject changing from $943,058 to $990,584; the reserve from $849,072 to $842,358.

Subproject 8: Change in job title. Conversations with the Extension service (Dr. M . Schmitt) have revealed that we cannot presently ask for formal status within Extension Service for this position (they lack space and funding, and have their own hiring procedures) so we have dropped this term from the position description. Nevertheless, there is a good possibility that this individual may work with an extension specialist (which we will pursue) and language to that effects is now in the subproject description.

Subproject 9: We have increased the zebra mussel program by half a year and included some expenses to reflect a more updated understanding of the needs of this program. Together with the corrections mentioned above, this results in the budget for this project changing from $483,674 to $621,600; the reserve from $483,674 to $621,600.

Subproject 10: We slightly increased the salary based on updated information on this type of position. We also delayed the start and reduced it to a 75% position because of inadequate funds. We are seeking non ENRTF matching funds to make this a full time position. The job title of this position has also changed because conversations with the Extension service (Dr. M . Schmitt) have revealed that we cannot ask for formal status within extension service for this position (they lack space and funding, and have their own hiring procedures) so we have dropped the 'extension' designation. Nevertheless, there is a good possibility that this individual may work with extension educators (which we will pursue) and language to that effect is now in the subproject description as well as the fact this individual will assist with communications. Together with the corrections mentioned above, this results in the budget for this project changing from $395,416 to $390,196; reserve from $319,711 to $283,694.

Further adjustments to these projects will be needed as project proposals are received. We will submit to LCCMR updates and/or further amendment requests as needed at those times.
Amendment Request approved contingent on revision of Attachment A format: September 23, 2013

Project Status as of *February 10, 2014*
As planned, the Center's administration and care of shared resources, as well as the Center's initial research, continues to be funded through its 2012 ENRTF appropriation. Please see the 2012 workplan and budget for progress reports on these activities.

No funds have been drawn down from the 2013 ENRTF award as SUB-PROJECT 1 continues to be paid from 2012 ENRTF Funds and SUB-PROJECTS 2, 3, 4, 6, 8, and 10 are not slated to begin yet. SUB-PROJECT 9 is initially being paid for with other funds, as described below.

Three research subprojects proposed with 2013 funds (SUB-PROJECTS 5, 7, and 11) have now completed the proposal and peer review process for their first phase of work, have been recommended for funding by the Scientific Director, and have now been approved by the Center Administrative Review committee. Detailed work plans and budgets for these subprojects will soon be submitted by these researchers to LCCMR.

These subprojects are:

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Phase 1 Budget: $214,995
Estimated Start Date: June 2014
This work will be guided by Professor Ray Newman over the next two and a half years and will have a phase 1 budget of $214,995.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: $335,225
Estimated Start Date: May 2014
This work will be conducted under the guidance of Professor Nick Phelps over the next two years and will have a phase 1 budget of $335,225.

SUB-PROJECT 11: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods. Phase 1: Problem formulation for invasive Asian carp.
Project Manager: David Andow
Phase 1 Budget: $110,185
Estimated Start Date: May 2014
This first phase in a two- phase Ecological Risk Assessment effort will be guided by Professor David Andow and will have a phase 1 budget of $110,185.

The first phase of SUB-PROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure has also now been approved by the Center Administrative Review Committee. This work will be completed over two and a half years and will have a phase 1 budget of $913,893**.** A detailed subproject 1 budget is attached.

The Center has hired its first new Research Assistant Professor, Dr. Michael McCartney, who will be committed to studying zebra and quagga mussels. The first phase of this work will be funded through the Clean Water Fund. Subsequent work is anticipated to be funded as part of SUB-PROJECT 9. Implementing Findings: Applying new methods to control zebra mussels under this 2013 work plan.

Changes to the projected budgets on several of the subprojects have been made since the August 30, 2013 update. Explanations for these changes follow:

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure.
Project Manager: Susan Galatowitsch
Phase 1 Budget: $913,893
Estimated Start Date: April/May 2014
An administrative and communications assistant has been added, and a technician has been converted to a lab manager for the Engineering and Fisheries Laboratory, which was recently designated for the Minnesota Aquatic Invasive Species Research Center's use as a central holding and research facility. Additional funds were also included in supplies, capital equipment, and repairs in anticipation of MAISRC's increased responsibility for upkeep of this facility.

SUBPROJECT 2: Delaying the spread of AIS: Monitoring the abundance and distribution of AIS using new molecular tools and metagenomics to delay their spread.
Project Manager: Michael Sadowsky
Phase 1 Budget: $365,756.00
Estimated Start Date: December 2014
University of Minnesota Professor and Director of the Biotechnology Institute, Mike Sadowsky, will now alone guide this subproject rather than the Center hiring a new research assistant professor to do so. This will allow the MAISRC to collaborate with a renowned expert in the field of metagenomics and also to get this research started sooner than previously planned.

SUBPROJECT 3: Reducing and controlling AIS: Developing effective tools to attract and locate aggregations of invasive carp.
Project Manager: Peter Sorensen
Phase 1 Budget: TBD
Estimated Start Date: July 2015
Additional funds for supplies, travel, and services were added to the budget.

SUBPROJECT 4: Reducing and controlling AIS: Developing effective bio-control techniques to control common and/or Asian carp.
Project Manager: TBD
Phase 1 Budget: TBD
Estimated Start Date: October 2014
No progress to report at this time as the project is not anticipated to start until early 2015

SUB-PROJECT 6: Reducing and controlling AIS: Simulation modeling to identify and evaluate AIS control methods.
Project Manager: Paul Venturelli

Phase 1 Budget: TBD
Estimated Start Date: July 2015
This sub-project has been delayed to more appropriately sequence it after additional empirical data has been gathered by the Center. It is anticipated that this project will move ahead with a project proposal and start sometime after July 1, 2015. The budget has been reduced accordingly.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.
Project Manager: TBD
Phase 1 Budget: TBD
Estimated Start Date: Workplan date July 2014; realistic date January 2015
Funds have been added to this project in anticipated need of additional boat(s) and or a vehicle (the specifics would be proposed to LCCMR as part of the subproject workplan and budget)

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
Phase 1 Budget: TBD
Estimated Start Date: July 2016 (currently being funded through Clean Water Funds)
The budget for half a year of this project has been added to this workplan.

SUBPROJECT 10: Implementing Findings: An educator-outreach position.
Project Manager: TBD
Phase 1 Budget: TBD
Estimated Start Date: Workplan date July 2014; realistic date March 2015
The educator-outreach position has been made full time for the first two years (years 3-6 continue to be 75%) and additional funds have been provided for field supplies (nets and boat gas) and printing services in anticipation of this person generating informational brochures and other educational materials.

SUBPROJECT 11: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Phase 1 Budget: $110,185
Estimated Start Date: May 2014
Following the project proposal process, this project has been extended and the budget has been adjusted accordingly. Additionally, based on peer review of this project, it will now be two phases, with the design and implementation of the second phase being conditioned on the results of phase 1.

Modifications to the total project budgets and reserves on the remaining projects (SUB-PROJECTS 4, 5, and 7), which have not yet begun, were made to accommodate the above changes. The net result of these budget changes are as follows:

Subproject 1: from $2,034,394 to $2,307,760; reserve from $1,445,927 to $1,393,867
Subproject 2: from $978,220 to $729,512; reserve from $978,220 to $729,512
Subproject 3: from $666,335 to $702,736; reserve from $666,335 to $702,736
Subproject 4: from $990,585 to $920,521; reserve from $842,358 to $920,521
Subproject 5: from $650,280 to $643,394; reserve from $426,998 to $428,399
Subproject 6: from $352,790 to $248,261; reserve from $230,116 to $248,261
Subproject 7: from $806,535 to $780,434; reserve from $471,308 to $445,210
Subproject 8: from $1,037,134 to $987,253; reserve from $758,341 to $987,253
Subproject 9: from $621,600 to $712,438; reserve from $621,600 to $712,438
Subproject 10: from $390,196 to $434,378; reserve from $283,694 to $434,378
Subproject 11: from $171,932 to $233,313; reserve from $0 to $123,128

These new budgets are reported on a new Overall Budget spreadsheet agreed to by LCCMR and MAISRC. The Subproject 1 revised budget is reported on a similarly approved new Subproject Budget spreadsheet. These changes have all been approved by the Center Administrative Review committee as the final initial budget of the 2013 appropriation. Any future budget changes will follow the processes set forth in the Center's MOU and the "Summary of LCCMR reporting and process 120213 final with attachment" document that are both on hand with LCCMR staff.

Project Status as of *August 31, 2014*

The Center's administration and care of shared resources, as well as the Center's initial research, continues to be funded through its 2012 ENRTF appropriation. Please see the 2012 workplan and budget for progress reports on these activities. No funds have therefore been drawn down on SUBPROJECT 1 as these activities continue to be paid from 2012 ENRTF Funds. SUBPROJECT 5 was approved on July 31, 2014; work has begun, however no funds have been drawn down as of the date of this report. SUBPROJECTS 7 and 11 were approved in May and April 2014 respectively. Progress from their July 31, 2014 workplan updates are provided in the IV Activity sections below. SUBPROJECTS 2, 3, 4, and 6 are all beginning the project proposal process now for estimated project start times in Spring and Summer 2015. SUBPROJECT 9 has been approved and is underway with funding from the Clean Water Fund. SUBPROJECTS 8 and 10 involve hiring additional faculty and staff. Progress has been made with both of these positions and MAISRC is proceeding with these hires in reliance on the previous budget and workplan approvals provided by LCCCMR for these subprojects. Before these hires begin, a request for approval of initial budgets for these subprojects will be requested to LCCMR. Additional updates on these subprojects are provided in the IV Activity sections below.

Please note, all reserve balances except for $822,000 for SUBPROJECT 8 and $220,000 for SUBPROJECT 10 have been moved to a central reserve holding place under the SUBPROJECT 1 BUDGET. The attached Overall budget and the following status updates reflect this change.

Project Status as of *February 28, 2015*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Sue Galatowitsch
Phase I Budget: $913,893
The Center has made significant strides since the last update. The workplan is continuing to be implemented as originally laid out by the founder of the Center, with attention to expediting the initiation of sub-projects that had been delayed in the first year and a half of the Center's existence and to launching all of the remaining sub-projects within the estimated timeframe laid out in the February and August 2014 updates. Included in these efforts is hiring new staff to complete the work described in these sub-projects. The new extension educator (subproject #10) has been hired and an initial coordination meeting was held with Minnesota DNR, Minnesota Sea Grant, MAISRC, and Minnesota Extension to insure maximum value added by this new position. The Extension Specialist position (subproject #8) hiring process has progressed and is on target for filling this now-permanent position by Fall to focus on aquatic plant management and restoration.

In anticipation of all of the Center's ENRTF funded sub-projects soon being underway, the Center has also begun its first systematic research needs assessment to identify top priorities for its next "phase" of research to be undertaken. Additionally, the Center has engaged its board and faculty in a 10-year strategic planning process to identify key issues and strategies for moving the Center forward in its critical work of finding solutions to Minnesota's AIS problems.

The Center's first research and management showcase, during which all Center faculty, staff, and students shared updates, information, and findings affecting AIS Management in Minnesota, was held in November, 2014, and was attended by over 200 people. Staff and faculty continue to give talks and serve in advisory and other roles outside the University, contributing to sound planning and coordination around Minnesota's collective AIS efforts.

The research and holding facility renovation is now nearing completion of the detailed design phase and construction is still on target to begin in May, 2015.

The Center's core operations are now being funded through this, ENRTF 2013, appropriation as the operations portion of the ENRTF 2012 appropriation has been fully spent down.

SUBPROJECT 2: Delaying the spread of AIS: Monitoring the abundance and distribution of AIS using new molecular tools and metagenomics to delay their spread.
Project Manager: Michael Sadowsky
Phase 1 Budget: $365,756.00
Estimated Start Date: July 2015
It was hoped that this sub project could be accelerated to start in December 2014, however this was not possible due to health issues of the PI. The project proposal has now been received and is currently undergoing peer review. Anticipated start time is July, 2015 with a focus on using metagenomics to develop biocontrol strategies for AIS.

SUBPROJECT 3: Reducing and controlling AIS: Developing effective tools to attract and locate aggregations of invasive carp.
Project Manager: Peter Sorensen
Phase 1 Budget: TBD
Estimated Start Date: July 2015
This sub project proposal has been received and is currently undergoing peer review. This sub-project was envisioned to build upon and continue research being conducted as part of the ENRTF 2012 work plan, once those prior phases were complete. Work on subproject 3 will therefore begin July 2015 or as soon as work is completed and ENRTF 2012 funds for activities 3, 4, 5 and 6 are spent down.

SUBPROJECT 4: Reducing and controlling AIS: Developing effective bio-control techniques to control common and/or Asian carp.
Project Manager: Przemek Bajer
Phase 1 Budget: TBD
Estimated Start Date: July 2015
Dr. Przemek Bajer has been identified as the project manager to lead this subproject. Due to existing common carp control research commitments, the PI elected to submit his proposal in January, 2015. The proposal has now been received, is currently undergoing peer review, and is anticipated to start in July 2015. The topic of the proposal is developing control approaches for common carp in shallow lakes, including use of a species-specific toxin for common carp in hypoxia- prone lakes. Previous work by the PI and other team members has focused on control approaches for larger lakes.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Phase 1 Budget: $214,995
Start Date: July 2014
This subproject was approved on July 31, 2014 and is currently underway. Finding a postdoctoral associate has been harder than anticipated. A candidate has just accepted the position and started work on March 9, 2015.

Data collection for the curlyleaf pondweed project will then accelerate and field work will begin on Eurasian watermilfoil this summer.

SUB-PROJECT 6: Reducing and controlling AIS: Simulation modeling to identify and evaluate AIS control methods.
Project Manager: Paul Venturelli
Phase 1 Budget: TBD
Estimated Start Date: July 2015
The project proposal has been received and is currently undergoing peer review, with an aim to start research July 2015. The proposal aims to address key knowledge gaps by providing, through modeling, an initial estimate of the threat caused by the parasite *Heterosporis* to populations of common game species, such as yellow perch, in Minnesota lake systems.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: $335,225
Start Date: May 2014
This subproject was approved in May 2014 and is progressing as planned with the first six months focused on hiring a post doc, purchasing laboratory equipment, collecting samples, and building networks to meet additional sample collection needs.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.
Project Manager: TBD
Phase 1 Budget: TBD
Estimated Start Date: Fall 2015
As previously reported, Dr. Galatowitsch was able to leverage this position from a term-limited position to a more competitive and permanent tenure- track position within the Department of Fisheries, Wildlife, and Conservation Biology. Per University procedures, a search committee was created, the position was posted, and candidates were interviewed. An offer was made recently; we hope the position will be filled this spring and the new hire will begin in August 2015.

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
Phase 1 Budget: TBD
Estimated Start Date: July 2016 (currently being funded through Clean Water Funds)
The preliminary phases of this project continue to advance with funding from the Clean Water Fund.

SUBPROJECT 10: Implementing Findings: An educator-outreach position.
Project Manager: Susan Galatowitsch
Phase 1 Budget: TBD
Start Date: February 2015
Danielle Quist started work February 26, 2015 as the new Extension Educator for the Center. Ms. Quist is meeting with key partners and stakeholders while she works with Extension and MAISRC to develop a detailed program plan. This program plan will be focused on outreach and programming related to AIS control, which is consistent with the programming gaps identified by DNR, Minnesota Sea Grant, MAISRC, and Extension in preliminary outreach coordination meetings. Dr. Galatowitsch will continue to serve as project manager of this Subproject, with Ms. Quist as the key implementing staff.

SUBPROJECT 11: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow

Phase 1 Budget: $110,185
Start Date: April 2014
The first phase (“problem definition”) of this two-phase Ecological Risk Assessment was approved in April, 2014 and is currently underway. The researchers have engaged in informational interviews and have conducted four focus groups to obtain input on priority potential adverse effects of and management options for Asian carp in Minnesota. The final focus group is scheduled. In-depth interviews and a survey will be conducted next. Analysis of this data collected is anticipated to be complete by September 30, 2015. All of this information will shape the analysis stage of a risk assessment to be conducted in Phase 2 of this project.

Project Status as of *September 24, 2015*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Sue Galatowitsch
Phase I Budget: $913,893

The Center continues to make significant strides forward. The proposal, peer review and workplan development process is now complete for four new research projects (Subprojects 2, 3, 4 and 6) and the extension and outreach project (Subproject 10). Dr. Larkin (Subproject 8) has officially started work under an initial approved workplan to develop his project proposal. Dr. Andow (Subproject 11) has requested continuation with a Phase 2 of this project, which has now been approved by MAISRC and LCCMR. Please see below for an amendment request to transfer funds from the reserve budget into all of these subprojects.

In continuation of our strategic planning efforts, we are currently developing a request for proposals for new research projects that support collaborative teams to address MAISRC’s strategic research priorities as defined through its first systematic research needs assessment. Funding to support this research will be made available through cost savings primarily in Subproject 1 as well as from funds on hand from the Clean Water Fund. We will request LCCMR review of the RFP before releasing it. Additionally, a draft 10 year strategic plan is now being routed for comment. A final version will be presented to the CAB at its fall meeting.

Demolition is complete and construction is underway at the research and holding facility, washdown facility, and new storage facility.

The MAISRC’s second annual research and management showcase was held September 16, 2015 with approximately 175 attendees. Staff and faculty continue to give talks and serve in advisory and other roles outside the University, contributing to sound planning and coordination around Minnesota’s collective AIS efforts.

SUBPROJECT 2: This subproject proposal has now completed peer review and the workplan has been approved by MAISRC and LCCMR. Please see below for amendment request.

SUBPROJECT 3: This subproject proposal has now completed peer review and the workplan has been approved by MAISRC and LCCMR. Please see below for amendment request.

SUBPROJECT 4: This subproject proposal has now completed peer review and the workplan has been approved by MAISRC and LCCMR. Please see below for amendment request.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Phase 1 Budget: $214,995

Start Date: July 2014
A postdoc was hired and started work in March. Queries for curlyleaf pondweed data sets were sent out and suitable lakes have been identified for analysis this winter. Undergraduate assistants were hired in May and field equipment and supplies were acquired and assembled. Weevil/herbivore surveys have been conducted, enclosures have been deployed, and sampling for sunfish diet assessments has begun.

SUB-PROJECT 6: This subproject proposal has now completed peer review and the workplan has been approved by MAISRC and LCCMR. Please see below for amendment request.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: $335,225
Start Date: May 2014
Significant progress has been made to perform diagnostic tests on the previously collected common carp with hundreds of carp testing negative for a variety of potential pathogens and with one still unknown virus identified. Two novel viruses have been identified from common carp and grass carp mortality events with one of them being the first report associated with fish mortality in the United States. Efforts are underway with new partners to collect silver carp this summer/fall. An update on this project was invited to be presented at the Great Lakes Fisheries Commission – Great Lakes Fish Health Committee meeting held in July 2015.

SUBPROJECT 8: An initial subproject workplan has been approved by MAISRC and LCCMR. Please see below for amendment request.

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
Phase 1 Budget: TBD
Estimated Start Date: July 2016 (currently being funded through Clean Water Funds)
The preliminary phases of this project continue to advance with funding from the Clean Water Fund.

SUBPROJECT 10: This project has now completed external review and the workplan has been approved by MAISRC. Please see below for amendment request.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Phase 1 Budget: $110,185
Start Date: April 2014
The subproject team has finished the research for both parts of Phase 1, has finished the report on the potential adverse effects, and is in the process of analyzing and writing the report on the management interviews. A proposal for Phase 2 has been made, reviewed, approved, and a workplan has been approved by LCCMR. Please see amendment request below to add and fund this Phase 2 project.

Amendment request as of *September 24, 2015***:**

We seek an amendment to begin four subprojects that have been reviewed and approved by the MAISRC Director and Center Administrative Review Board (CAR) and another three subprojects that have been approved by MAISRC but that don't require CAR approval. All of these have been approved by LCCMR. A total of $1,666,717 will be moved from the reserve line item in Subproject 1 (Coordinating, synergizing and promoting expertise: Establishing an administrative structure Project Manager: Sue Galatowitsch) to each of the subprojects in the amounts shown below. Additionally, $130,000 from the reserve budget line in Subproject 8 and $220,000 of the reserve budget line in Subproject 10 have been allocated within that subproject. The subprojects are as follows:

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $303,217
LCCMR approval and start date: June 20, 2015 (2 years)
This sub- project was specified in original 2013 work plan, however it has been modified from *detection* of various AIS to *control* of water milfoil, zebra mussels. The budget has also shifted downward based on need. The subproject title, description, budget and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen
Phase 2 Budget: $500,000
LCCMR approval and start date: July 9, 2015 (2.5 years)
This sub-project was specified in the original 2013 workplan and has been revised to reflect findings from (and in some cases is intended to extend) work funded through 2012 ENRTF Activities 3,4,5,6, and 8. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 4: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer
Phase 1 budget: $413,247
LCCMR approval and start date: July 7, 2015 (2 years)
This sub-project was specified in the original 2013 work plan, however it has been modified to include carp-specific toxins, a priority identified in MAISRC's 2015 research needs assessment. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $127,650
LCCMR approval and start date: June 15, 2015 (2 years)
The original 2013 work plan scoped this sub-project to model common carp populations; the project has been redirected to investigate the risk of an invasive pathogen identified as a priority in MAISRC's 2015 research needs assessment. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.
Project Manager: Dan Larkin
Phase 1 Budget: $130,000 (initial)
LCCMR approval and start date: August 31, 2015 (~4 years)
An initial workplan has been created by MAISRC and Dr. Larkin to cover program development, peer review, and workplan development and review. An updated workplan is expected to be submitted to LCCMR by March 15, 2016 at which time the subproject title, description, and outcomes below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: will be revised to reflect changes.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Sue Galatowitch
Phase 1 Budget: $419,475

LCCMR approval and start date: October 1, 2015 (~4 years)
This sub-project was identified in the original 2013 workplan, however it has been further refined into three components: 1) development and implementation of a program to train 400 citizen scientists and professionals to rapidly identify and report AIS. This increases capacity and allows DNR resources to focus where they need to be: on rapid response to new findings 2) development and implementation of a program to train 100 citizen scientists and professionals to survey and monitor populations of AIS using standardized protocols in order to guide and evaluate effectiveness of AIS management 3) development of an interactive, web based data repository that can be used in association with existing formats (e.g. EDDMapS) to allow for entry and sharing of data generated from the above activities as well as from other treatment efforts around the state. Standardized data collection protocols and data sharing through this database will allow AIS managers to benefit from lessons learned by others and will allow researchers to evaluate effectiveness of different management treatments.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis
Project Manager: David Andow
Phase 2 Budget: $123,128
LCCMR approval and start date: September 23, 2015
This project will conduct a risk assessment with a variety of experts and stakeholders by evaluating which adverse effects identified in Phase I are most salient and by determining the likelihood of impact and consequence in various watersheds. Through risk communication, the results and implications of these findings will be shared with a broader set of stakeholders, researchers, managers, and decision makers from relevant state and federal agencies. Areas of disagreement, remaining uncertainties, and additional research needs will be identified. By fostering conversation among researchers, managers, stakeholders, and decision makers, this project will promote needed dialogue and communication to support decision making in the face of complexity and uncertainty.

Amendment approved: October 14, 2015

Amendment request: October 29, 2015:

MAISRC seeks approval to issue a request for proposals (RFP) to fund additional research on topics and species as determined through MAISRC's research needs assessment process. We anticipate the amount of ENRTF funds used in this process will range from $250,000- 400,000. Projects awarded ENRTF funding would be added as additional subprojects to this award and reflected in additional workplans and amendment requests. An outcome regarding this RFP has been added to Subproject 1 below.

Amendment approved: October 30, 2015

Project Status as of *February 29, 2016*

SUBPROJECT 1: All initial subprojects for the Center are now either approved or in the peer review and workplan development stage. The exception is Subproject 9, which was envisioned to be the 2nd phase of zebra mussel work that is currently being funded through the Clean Water Fund through December 2016. Individual project updates are provided below.

In order to address additional unmet statewide research needs and as identified in MAISRC's strategic plan, a request for proposals was announced in November 2015 to seek collaborations on top priority research needs that had been identified in the 2015-2106 MAISRC Research Needs Assessment process. We received seventeen proposals, totaling $3.2 million, which were then vetted by a committee made up of MAISRC scientists and advisory board members. The top three proposals have been advanced to the full proposal stage and are currently undergoing scientific peer review.

The Center's 10 year strategic plan was endorsed by the Center Advisory Board at its Fall 2015 meeting and is now considered final. A new advisory board chair has been elected and the board is now looking at implementation of other key aspects of the plan, including long term funding for the Center's operations. A new funding proposal is also being developed for submission to LCCMR for its 2017 call.

Construction on the research and holding facility, washdown facility, and new storage facility is complete. Commissioning is now underway at the research and holding facility and researchers will be able to begin populating it once all systems are shown to be in working condition. A ribbon cutting event is scheduled for March 2. In order to help support future operations of the facility, MAISRC staff has developed draft cost share policies and procedures consistent with University of Minnesota policies on Internal Service Organizations and similar to the UMN greenhouses and BSL 2 and 3 Quarantine facilities. This has also been discussed with LCCMR staff.

Director Sue Galatowitch continues to be involved in managing the content and direction of Subproject 10. We have also hired a new Extension Educator who will begin early April to lead the AIS Trackers program.

MAISRC has identified the date for its 2016 Showcase on the St. Paul campus (September 22) and continues to broadcast updates on MAISRC progress and findings via talks, social media, and newsletters, and now also via a revamped website launched earlier this month.

MAISRC participated in developing the agenda for the Governor's Clean Water Summit on 2/28/16, attended the summit, and will be involved in helping to organize input received by attendees for delivery to the Governor.

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $303,217
LCCMR approval and start date: June 20, 2015 (2 years)
A postdoctoral associate was hired to start August 31 and an undergrad has been assisting him. Sampling for Eurasian watermilfoil was conducted at three different sites in Cedar Lake and DNA extracts were submitted for sequencing. A milfoil decay experiment was also performed. Zebra and quagga mussels were collected from six lakes, were dissected, and DNA samples submitted for sequencing. Analysis for all will be conducted this spring.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen.
Phase 2 Budget: $500,000
LCCMR approval and start date: July 9, 2015 (2.5 years)
Experiments were conducted in late summer of 2015 to test food and pheromones as attractants to drive common carp aggregation. While data is still being analyzed, it is clear that food was able to drive aggregations, especially at night. Novel techniques for both eDNA and pheromone levels were able to measure the aggregations with more sensitivity. Plans for this coming summer will be formulated once we have analyzed all the data.

SUBPROJECT 4: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer.
Phase 1 budget: $413,247
LCCMR approval and start date: July 7, 2015 (2 years)
Outcome goals have been achieved—experimental lakes have been selected for whole lake biocontrol experiments; monitoring is continuing over the winter; and next steps for stocking will be identified in the Spring. Winter aeration data were compiled and paired with DNR fish assessments, however the resulting

sample size was too small to analyze, so a higher resolution case-study is being pursued. Experimental design for the selective control by antimycin A tests has been finalized and ponds have been selected at the USGS facility.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed. Project Manager: Ray Newman.
The Newman lab has received and collated curlyleaf pondweed datasets for 57 lakes from state and county agencies, watershed districts, and consultants. Several discussions regarding analytical approaches have taken place. Eight of 14 lakes surveyed for weevils/herbivores were resurveyed in August and September. Lower than average weevil densities were found in 5 of the 8 resurveyed lakes; only three showed an increase in weevil density. Enclosures were surveyed for weevils and plants and diets were collected from sunfish at six lakes.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $127,650
LCCMR approval and start date: June 15, 2015 (2 years)
Model development is well under way. We have collected a quarter to a third of necessary parameter values, and beginning to code the subroutines that simulate disease and energy dynamics. In collaboration with the MN DNR, we collected 1,221 yellow perch and other fishes from three lakes in September. Preliminary results from the lab suggest that ~8% of fish are infected and that most of these fish were yellow perch. Winter gill netting is now under way so that we can determine if the frequency and intensity of heterosporosis infection is seasonal or temperature-dependent. To determine if infected fish are more or less susceptible to angling, we have also distributed to log books to resorts on all three lakes. Finally, we have obtained ~1100 yellow perch for laboratory experiments, which will begin once the new research facility is operational.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: $335,225
Start Date: May 2014
Significant progress has been made to collect new common carp samples from different sites. Total of 94 common carp were collected from three different sites in Minnesota. In addition, 120 silver carp from the Fox and Illinois rivers were collected. Significant progress had been made to perform diagnostic tests on the previously and recently collected common carp as well as silver carp. Bighead carp samples were also collected from mortality even from US Geological Survey, Columbia Environmental Research Center, Columbia, MO. Samples have been processes for virus isolation and molecular diagnostic. Multiple novel viruses have been isolated and are currently being characterized by next generation sequencing from common carp collected this last fall. Due to delays in the construction of the MAISRC biocontainment facility, Activity 3 will no longer be completed during this project period and, due to the unavailability of the commercial ELISA kit for testing prior exposure to KHV, we have had to rely on PCR testing, which does not give us as much information as planned. It is still a useful, however, in this first-ever attempt to survey common carp in Minnesota for this important virus.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.
Project Manager: Dan Larkin
Phase 1 Budget: $130,000 (initial)
LCCMR approval and start date: August 31, 2015 (~4 years)
This project is currently undergoing peer review. Additionally, an ecological niche model has been developed to determine the threat of starry stonewort spread in Minnesota. The model indicated that this species is persisting in novel habitats – meaning that it is occurring in areas here that are climatically distinct from its native range, and that conditions in portions of the upper Midwest and other regions in the U.S. are ideal for its growth and

spread. Additionally, a convening in the next months of researchers and managers with starry stonewort experience is being led by Dr. Larkin to determine current research and management knowledge and gaps.

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
This project is not anticipated to start until after December, 2016.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Sue Galatowitch
Phase 1 Budget: $566,550
LCCMR approval and start date: October 1, 2015 (~4 years)
A template for the online portion of the AIS Detectors course has been designed and is organized in six modules with specific learning outcomes. The course will initially focus on ten AIS species, which were chosen in consultation with the MAISRC technical committee. An educator for the AIS Trackers program has been hired and is expected to begin early April. We decided not to pursue additional funding for this program from the Initiative Foundation, so have had to rebudget this Subproject to make it whole. An amendment to this effect follows.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Revised Phase 1 Budget: $93,343
Start Date: April 2014
This phase of the project, which identified potential adverse effects from Asian carp to inform a subsequent risk assessment and characterized the tensions and conflicts that are hampering Asian carp management, completed in November. Two reports were released. An amendment was approved in November by LCCMR to move the remaining balance to Phase 2, which continues the work with a full risk assessment of Asian carp impacts and a risk communication session.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis
Project Manager: David Andow
Phase 2 Budget: $139,970
LCCMR approval and start date: September 23, 2015
The risk assessment meeting, which will convene Asian carp experts from around the country, has been scheduled for March 8 and 9, 2016. An online survey to help guide the assessment meeting has been designed. Remaining funds from 11-1 (Phase 1) were transferred here, resulting in the budget to change from 123,128 to 139,970.

New LCCMR approved language was added to section VI A below.

Amendment request as of *February 29, 2016*

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Sue Galatowitch
Phase 1 Budget: $566,550
LCCMR approval and start date: October 1, 2015 (~4 years)
MAISRC decided to withdraw its application to the Initiative Foundation for approximately 75% of the costs of Activities 1-2 due to difficulty meeting the prescribed match requirement for that program, which would have resulted in a need for us to secure additional private funds within a timeframe that was unfeasible. Additionally, it has been determined that additional staff assistance is needed for Activities 3-5.

Therefore, we request that $147,075 funds are transferred from the reserve to fully fund this Subproject 10. This would result in the overall reserve changing from $4,416,986 to $4,269,911. Subproject 10 budget would change from $419,475 to $566,550.

Specifically, the $147,075 funds would be transferred to Subproject 10 and would result in the following budget changes:

Activity 1:
Services from $2,300 to $5,200
Professional services from $5,000 to $20,000
Supplies from $500 to $1,200
Supplies and Equipment from $1,875 to $7,500
Travel from $1,900 to $5,900
Room rental from $625 to $2,000

Activity 2:
Services from $425 to $3,700
Professional services from $750 to $3,000
Supplies and Equipment from $8,000 to $32,500
Travel from $21,950 to $34,800

Activity 3: Personnel from $77,300 to $97,000
Activity 4: Personnel from $45,900 to $73,000
Activity 5: Personnel from $134,200 to $162,000

Amendment Approved March 3, 2016

Amendment request as of *May 5, 2016*

We seek an amendment to fully fund Subproject 8, which has a peer reviewed proposal and a workplan and budget that has been approved by MAISRC. We also seek an amendment to add Subproject 12 and Subproject 13 to fund two proposals received in response to the MAISRC RFP issued this past fall. Seventeen proposals were received and the top three were invited to submit full proposals. All three have undergone peer review and are in different stages of revision and workplan development. We anticipate funding all three; however only two will be funded through this 2013 ENRTF appropriation (the other will be funded with Clean Water Funds).

SUBPROJECT 8: Risk assessment, control, and restoration research on aquatic invasive plant species
Project Manager: Dan Larkin
Phase 1 Budget: $822,000
LCCMR approval and start date: August 31, 2015 (~4 years)

This project has completed peer review, revision, and its workplan and budget have now been approved by MAISRC. This amendment would result in $692,000 from the Budget Reserve of Subproject 8 being allocated within the project so that the full project budget is $822,000

The project description has been updated in the IV Subprojects and Outcomes section, below.

SUBPROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology
Project Manager: Donn Branstrator (UMD)
Phase 1 Budget: $207,766
Estimated Start Date: August 2016 (~2.5 years)

This project has completed peer review and is in process of revision and workplan development for approval by MAISRC and LCCMR. The work will be guided by Professor Donn Branstrator from University of Minnesota Duluth over the next two and a half years. We seek an amendment to move $207,766 from the Subproject 1 Reserve budget into Subproject 12.

The project description has been added to IV Subprojects and Outcomes section, below, and will be updated as needed following approval of a Sub project workplan and budget by MAISRC and LCCMR.

SUBPROJECT 13: Eco-epidemiological model to assess AIS management
Project Manager: Dr. Nicholas Phelps
Phase 1 Budget: $215,000
Estimated Start Date: June 2016 (~2 years)

This project has completed peer review and is in process of revision and workplan development for approval by MAISRC and LCCMR. This work will be guided by Professor Nick Phelps over the next two years. We seek an amendment to move $215,000 from the Subproject 1 Reserve budget into Subproject 13.

The project description has been added to IV Subprojects and Outcomes section, below, and will be updated as needed following approval of a Sub project workplan and budget by MAISRC and LCCMR.

In summary, $207,766 will be moved from the Subproject #1 Reserve to Subproject #12 reserve. $215,000 will be moved from Suproject #1 Reserve to Subproject #13 Reserve. Therefore Subproject #1 reserve will decrease $422,766 total, from $4,269,911 to $3,847,145.

Amendment Approved by LCCMR 5-11-2016

Project Status as of *August 31, 2016*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Sue Galatowitsch
Phase I Budget: $913,893

Three subprojects were reviewed for continuation per the Center's project continuation policy. As a result, two of the three projects will be considered for funding after receipt and peer review of full research proposals. Significant effort was put into getting the Extension programs (Subproject 10) launched, including writing and reviewing science-based online training materials and classroom curriculum so that the AIS Detectors program may pilot this Fall.

Staff continued to work closely with the design and construction teams to properly commission the newly renovated research and holding facilities. Issues discovered during this process have resulted in the need for ongoing attention by MAISRC staff beyond the original timeframe envisioned. Construction of the new storage facility is finished and MAISRC staff have outfitted the space and coordinated the move of all MAISRC faculty gear.

Transition planning and a search were conducted by MAISRC leadership and staff, which led to the hiring of Nick Phelps as the new Director of the MAISRC starting July 1. He and Sue Galatowitsch will serve as co-directors for the first year to ensure a smooth transition.

Planning was conducted and arrangements were made for the 2016 Showcase, which will be held September 12 on the St. Paul campus. Dissemination of research progress continues through talks, papers, newsletter, website and other social media formats. An amendment is being sought to take Phase 2 of Subproject 1 out of reserve and into Subproject 1 budget to sustain this subproject to the end of the project period (June 30, 2019).

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $303,217
LCCMR approval and start date: June 20, 2015 (2 years)
Sequence analysis has been completed for samples collected last year, and a broader sampling regime for both Eurasian watermilfoil (EWM) and zebra mussels (ZM) has been implemented this year. All samples were processed for nutrient and microbiological features and DNA extracts sent to UMGC for bacterial and fungal sequencing. A new tech was also hired.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen.
Phase 2 Budget: $500,000
LCCMR approval and start date: July 9, 2015 (2.5 years)
Water samples collected for eDNA and pheromone evaluation were analyzed and a baiting scheme perfected. Experiments from last summer showed that a third of the population of mature common carp could be attracted and then measured with eDNA and pheromones with a level of sensitivity, precision and accuracy previously unseen. Pheromone-releasing Judas carp were also attractive. A third study successfully measured common carp mating pheromones in waters near mating carp. Finally, a pilot study using food to attract Bigheaded carp was completed in Illinois with the University of South Illinois as collaborators. Whether this behavior enhanced our ability to measure them using eDNA or pheromones (as shown with carp) is presently being evaluated.

SUBPROJECT 4: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer.
Phase 1 budget: $413,247
LCCMR approval and start date: July 7, 2015 (2 years)
Research continues to advance and outcome goals have been achieved. Experiments are underway for activity 1a: carp and bluegills have been stocked in ponds, egg and larval densities assessed, and water quality assessments taken to document productivity and zooplankton abundance. Activity 1b has been adapted to allow analysis of a higher quality dataset provided by DNR to determine which lakes are capable of supporting bluegill populations to control common carp. Corn-based bait containing antimycin has been formulated for Activity 2, and has been shown to be lethal to common carp through preliminary gavage studies, however leaching is occurring and rates higher than expected. The bait is currently being re-formulated.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Phase 1 Budget: $214,995
Start Date: July 2014
Compilation of the curlyleaf pondweed data sets and ancillary data was completed and analyses conducted and a talk was given on the analysis and results at the Aquatic Plant Management Society meeting in Grand Rapids, MI in July. Enclosures (at Cedar and Peltier Lakes) have been installed, stocked with fish and pre- and mid-experiment samples have been collected. Fish diets were obtained and are now being collected from other lakes as well. Herbivore surveys have been conducted in 14 lakes and additional lakes are being selected for surveys in August. The milfoil weevil portion of the project will conclude December, while a possible extension will be requested in January to conduct additional curlyleaf pondweed analysis.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control

Project Manager: Paul Venturelli
Phase 1 budget: $127,650
LCCMR approval and start date: June 15, 2015 (2 years)

We have a working model that combines bioenergetics and population dynamics to model perch in the absence of heterosporosis, and are beginning to couple this model with the disease sub-model (Activity 1). We have completed one cycle of field work (Activity 2) to determine if heterosporosis varies seasonally or with size, sex, or species. Preliminary results suggest that ~3% of fish are infected with heterosporosis, which is consistent with the 2% reported by the two resorts with which we are working. We are on pace with model development and field work, but not lab experiments. Unfortunately, lab experiments (Activity 2) will be delayed at least 9 months because the MAISRC laboratory is not yet operational due to unforeseen construction delays. As a result of these delays, we i) will have to purchase new experimental fish (the batch that we obtained in fall have grown too large), ii) have cancelled the experiment to determine if perch can recover from heterosporosis, and iii) have adjusted the timelines and sample sizes of the remaining experiments.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: $335,225
Start Date: May 2014

Samples from apparently healthy invasive carp and those from mortality events were screened by virus isolation, targeted PCR and next generation sequencing (NGS) Illumina MiSeq for molecular identification of viruses. Novel RNA viruses belonging to six different families were identified since the previous update, including three picornaviruses, two reoviruses, hepatovirus, astrovirus, hepatitis E virus, and betanodavirus. The analysis of DNA Miseq sequences from all samples and both RNA and DNA sequences from a recent mortality event will be complete in the coming weeks. Analysis of complete NGS work will fulfill the aim of Activity 2 in Phase I, which is to generate baseline data of local invasive carp pathogens. The manuscript on RNA viruses of invasive carp populations in Minnesota is in preparation.

Activities 1, 2, 4, and 5 are complete and all outstanding balances will be reconciled with unused funds being returned to MAISRC at the January 31, 2017 update and a final report summary for all activities will be provided shortly thereafter. Activity 3 is still in progress pending amendment approval.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.
Project Manager: Dan Larkin
Phase 1 Budget: $130,000 (initial)
LCCMR approval and start date: August 31, 2015 (~4 years)
This Sub-project was approved in May with an understanding that its next status update would be provided January 31, 2017

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
This project is not anticipated to start until after December, 2016.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Sue Galatowitsch
Phase 1 Budget: $-566,550
LCCMR approval and start date: October 1, 2015 (~4 years)
The two part curriculum for the AIS Detectors program has been developed and will be pilot-tested in the September and early October. Part 1 is an online course and Part 2 is an all-day classroom session that will be

pilot tested in Brainerd. Based on feedback received, we will revise the online and classroom sessions, so the program is ready for a statewide launch in Spring 2017. For AIS Trackers program, various assessments and reviews have been completed to help build the A-DRUM database, develop curriculum and training materials, and select methods needed to monitor AIS population changes and identify trends from AIS treatments.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Revised Phase 1 Budget: $93,343
Start Date: April 2014
Final report submitted September 2015.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis
Project Manager: David Andow
Phase 2 Budget: $139,970
Start Date: September, 2015 (~1.2 years)
The two day risk assessment workshop was held with twenty-three experts on bigheaded carps and Minnesota's waterways. The risk assessment focused on the impacts to game fish, non-game fish, species diversity/ecosystem resilience, and recreation (from the silver carp jumping hazard). Four watersheds were chosen to be studied and participants characterized the likelihood that bigheaded carps would establish in each watershed, the resulting abundance of bigheaded carp in each watershed, and the severity of each potential adverse effect in each watershed. The risk assessment report is being written by project researchers and a subset of the workshop participants.

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology
Project Manager: Donn Branstrator
Budget: $207,766
This project was approved by LCCMR June 20, 2016 with a start date of August 1, 2016 (~3 years). A number was transposed in the IV budget below and on the overall budget in the last update and has now been corrected.

SUB-PROJECT 13: Eco-epidemiological model to assess AIS management
Project Manager: Nicholas Phelps
Budget: $215,000
This project was approved by LCCMR on September 2, 2016 (~3 years)

Amendment Request as of *August 31, 2016 and received October 4, 2016*
Phase 1 funds for Subproject 1 were comprised of the 2012 ENRTF appropriation (Activity #1) as well as some funding from the Clean Water Fund. The Center will rely primarily on this 2013 ENRTF appropriation (Subproject 1) for continuation of its core operations now until the end of the appropriation and so proposes an amendment at this time. We seek an amendment to move $891,966 of the Phase 2 budget for Subproject 1 out of reserve and into the subproject budget. This should be enough funding to cover the Center's core operations through the end of the appropriation (June 30, 2019). Any remaining funds in the Center's overall reserve will then be dedicated to research and outreach.

The amendment would result in the following:

- Personnel budget increase from $809,588 to $1,564,487 to support current core personnel through June 30, 2019
- Services increase from $9,000 to $16,221 to allow for continued services at approximately the same rate as required to-date.

- Lab and Medical services will increase from $1,000 to $72,049 to account for anticipated costs of the newly remodeled Engineering and Fisheries Lab. Since it is a new lab, the exact annual costs for operating this facility are currently unknown. Costs included here are those not covered through University cost pools such as preventative maintenance for equipment and for laboratory cleaning services. It is also anticipated that a portion of the costs will be paid by individual users. This is similar to other facilities of this type on campus.
- Rental budget increase from $0 to $13,500 to account for facility rental and accommodations for our annual Showcase event cost to be covered entirely through ENRTF 2013 (and partially offset by registration fees) rather than through Clean Water Fund.
- Supplies budget increase from $10,525 to $14,108 to cover costs at approximately the same rate as required to-date.
- Non capital equipment budget increase from $4,000 to $12,421to support at approximately the same rate as required to date, including hoses, pumps, and other items required for the shared laboratory and wash-down space.
- Travel increase from $9,540 to $20,669, which is a slight increase in prior spending in order to allow us to develop a speaker series to bring out of town experts to campus for public events to increase state knowledge and capacity.
- Telecommunications decrease from $1000 to $582 to account for lower than anticipated costs.

With the above amendment, the remaining balance for Subproject 1 for 9/1/16- 6/30/16 would be $1,265,477. The total reserve would change by $891,966 from $3,847,145 to $2,955,179.

Amendment approved 10-10-16

Project Status as of *February 28, 2017*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Sue Galatowitsch
Phase I Budget: $1,805,859
Start Date: February 2015 (5.3 years)

The Center is continuing to progress in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota. We evaluated two current sub-projects for continuation and advanced both to the full proposal and workplan development stage. An amendment is being sought to fund both of these subprojects from the overall reserve budget. We have begun the continuation evaluation process for two additional sub-projects that we hope to launch this Spring.

We completed our 2016 biennial Research Needs Assessment, which included soliciting input from a broad range of experts and stakeholders including AIS managers, researchers, and resource- users. We received 383 submissions that were vetted by our 20-member Research Needs Assessment Team and ultimately resulted in a list of 26 priorities that were supported by the Center Advisory Board.

We also announced our 2016- 2017 RFP in order to find and fund scientists to conduct research on these priorities. We sent the RFP directly to ~300 people, including to high potential researchers at 4 campuses of the UMN, 8 other Minnesota colleges and universities, and 10 regional universities as well as state and federal agencies. We received and convened a committee to review 15 pre-proposals and have invited three to submit full proposals. Two Director projects were reviewed and considered with a separate pot of funds under a new conflict of interest policy. Both were invited to full proposal and peer review.

The AIS Detectors program piloted to a small cohort of DNR staff and citizens in Fall of 2016 and substantial effort by MAISRC communications staff has continued in order to prepare for seven statewide sessions to be held in 2017. Part of this effort includes creating a professional, scientifically vetted AIS (and look-alikes) identification book that will set a new standard in the state for this kind of publication.

We held our 2016 Showcase in September, which attracted 171 non-MAISRC attendees and provided 16 presentations spread out among 21 speakers. 90% of attendees rated the event as excellent or very good. MAISRC core staff also attended conferences and presented on MAISRC's Research Needs Assessment process, which has gained attention as an efficient, inclusive solutions-oriented model. Efforts to broadcast research progress continue through talks, meetings, papers, newsletters, website and other social media formats.

We have brought the MAISRC Containment Lab ("MCL") online through a difficult commissioning process and began paperwork to create the MCL as an Internal Service Organization and to develop sustainable pricing. Usage policies are currently being developed. MAISRC technicians continue to manage the facility and trouble-shoot when issues arise. Also as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

We have continued transitioning to a new Director. In order to better align with strategic aims, we have expanded and diversified CAB membership, and revised our external MOU with DNR accordingly. With assistance from our newly expanded board, we have also developed an annual MAISRC budget and have begun pursuing funding to sustain the center after 2019.

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $303,217
LCCMR approval and start date: June 20, 2015 (2 years)
The project has made significant progress since the last project update and is on schedule for completion in July. Field sampling in 2016 including collecting EWM, native macrophytes, zebra mussels, sediment, and water sampled from 25 lakes. Samples were processed, DNA extracted, and high-throughput DNA sequencing of bacteria and fungi was performed. Sequencing results showed a distinct clustering of microbes by each sample type with the greatest number of operational taxonomic units (OTUs) observed in sediment samples, and the lowest in EWM and ZM samples. Several OTUs were identified that were present in higher relative abundance in EWM and ZMs. Additionally, it was determined that EWM harbored elevated levels of fecal indicator bacteria, such as *E.coli* and *Enterococcus*.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen.
Phase 2 Budget: $500,000
LCCMR approval and start date: July 9, 2015 (2.5 years)
Work is on schedule. An experiment was conducted to determine whether adult male common carp can be attracted to pheromones in small ponds. Pilot data suggest that they can so a final experiment is now planned for spring 2017. Analyses of common carp induced to aggregate around pheromone-implanted Judas fish are also nearly complete. Another experiment was conducted to determine whether adult silver carp can be attracted to food in small ponds. Once again the results were positive so this experiment will be repeated as well next spring. As eluded to in the previous report, a re-budgeting and amendment is proposed and is pending. A meeting to discuss the update with LCCMR has been set.

SUBPROJECT 4: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer.
Phase 1 budget: $413,247
LCCMR approval and start date: July 7, 2015 (2 years)
The 2016 field season ended and data are currently being analyzed. Outcome goals have been achieved, or exceeded. Activity 1a has concluded and mark-recapture estimates were made for young-of-year (YOY) carp in

each of the four ponds. We found that the two ponds without bluegill sunfish had approximately 6.5 times more YOY carp than ponds with bluegill. For activity 1b, the analysis of bluegill sunfish abundance (carp biocontrol) in lakes of southern Minnesota is currently underway. Modeling and analyses have been conducted to determine which lake types have strong carp biocontrol in Minnesota. All for experiments in Activity 2, control of common carp using antimycin-laden bait, have been conducted and data has been analyzed. A manuscript that we anticipate submitting in February is in preparation. Our results suggest that ANT-impregnated bait has potential to target carp without harming most native species.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Start Date: July 2014 (2.5 years)
Field work was completed in fall and all data were entered and analyzed. Eighteen lakes were assessed for milfoil weevil densities, which ranged from none found to 0.27/stem, lower than for most lakes in 2015. Densities were lower in 2016 compared to 2015, and 2015 generally had lower densities than in previous years. Sunfish stomach contents were analyzed. Benthic and macrophyte associated invertebrates were common in the diets but only one milfoil weevil was found. Enclosure experiments were completed in August. Despite methodological improvements and an earlier start in June we were unable to get definitive results from the enclosure experiments. Curlyleaf analysis was continued and the data sets were organized and systematized to allow an analysis of the effects of curlyleaf and curlyleaf control on the associated native plant communities. The final report and abstract for this project will be submitted by 2/28/17. The remaining funds are being moved to the overall project reserve. Please see amendment request below.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $127,650
LCCMR approval and start date: June 15, 2015 (2 years)
We are on pace with model development, but not lab experiments. We have a working aggregate model to predict perch dynamics in a system with varying degrees of disease prevalence and virulence (Activity 1). We are now parameterizing this model so that it can generate predictions and perform a sensitivity analysis (Activity 3). We have finished microscope analysis on field samples from the fall and winter, resulting in a 6% and 1% prevalence of heterosporosis in Leech Lake, respectively. We are still processing samples from the spring and summer. We are behind on lab experiment due to delays in facility construction and difficulties in finding and culturing *Heterosporis*. We were able to run a small experiment and only one fish tested positive for the disease. Given our remaining timeline and the challenges associated with infecting perch in the lab, we are cancelling experiments to determine heterosporosis effects on consumption, activity or recovery, and will instead focus on lab experiments to determine heterosporosis transmission rates via direct contact among fathead minnows. We have initiated work on Activity 3, and have started planning and structuring the model to best implement the sensitivity analysis.

SUB-PROJECT 7-1. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Start Date: May 2014 (2.2 years)

This Subproject has completed and the final report and abstract have been approved by LCCMR Please see the amendment below to move remaining funds to the Overall budget reserve.

SUBPROJECT 8: Risk assessment, control, and restoration research on aquatic invasive plant species
Project Manager: Dan Larkin

Phase 1 Budget: $822,000
LCCMR approval and start date: August 31, 2015 (~4 years)
This funding has enabled an active research program addressing applied issues in aquatic invasive plant management in Minnesota lakes. Research on starry stonewort has addressed spread risk using ecological niche modeling and environmental characteristics. Culturing of starry stonewort is being refined to enable laboratory experiments addressing starry stonewort climate and desiccation tolerance and chemical control. Field sampling and experimental germination of starry stonewort bulbils from areas treated with algaecides and/or mechanical harvesting revealed high capacity for reinvasion of treated areas. In-lake outcomes of starry stonewort management efforts are being monitored in collaboration with DNR and other external partners. Research on Eurasian watermilfoil and curly-leaf pondweed has shown that shallow lakes with higher native plant diversity are more vulnerable to invasion, and that these invasive plants are associated with rapid biotic homogenization of vegetation in these lakes. We are compiling monitoring data from past treatments of Eurasian watermilfoil and curly-leaf pondweed in Minnesota lakes to investigate how management decisions and environmental conditions influence effectiveness of control and capacity for recovery of native plant communities. Finally, our research is being integrated with joint MAISRC-Extension efforts to develop the Trackers citizen science program (Subproject 10).

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
This project has undergone continuation review and its workplan has been approved by MAISRC and LCCMR. Please see amendment request below.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Dan Larkin
Phase 1 Budget: $566,550
Start Date: November 2015 (3.5 years)
Progress was made in several key areas of Detectors and Trackers. The full web-based Detectors course was pilot-tested this past fall and the participants provided feedback that is being used to revise the curriculum. Groundwork has been laid for full implementation of the AIS Detectors program in spring of 2017. Advanced training opportunities are being developed. Development of the Trackers program is in progress, with a detailed plan for program roll-out. In addition, progress has been made in refining the scope of the Trackers database and we have met with a vendor and agreed on a timeline for development of the data management system.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Revised Phase 1 Budget: $93,343
Start Date: April 2014

Final report submitted September 2015.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis
Project Manager: David Andow
Phase 2 Budget: $139,970
Start Date: September, 2015 (~1.75 years)

Since the last project update, the report for the Minnesota Bigheaded Carps Risk Assessment has been drafted and reviewed by risk assessment workshop participants. Project researchers: 1) transcribed key documents from the risk assessment workshop for volunteer authors from each watershed to use in drafting their section of the report 2) calculated the overall risk for each watershed 3) drafted the introduction, methodology, overall risk characterization, and discussion sections of the overall report, and 4) sent the full draft report to all risk assessment workshop participants for their review. Comments have been received and the risk assessment report is in the process of being revised. Planning for the risk communication meeting has also begun. The project's completion date has now been extended from December 30st, 2017 to May 31st, 2017, however still within the appropriation timeframe.

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology
Project Manager: Donn Branstrator
Budget: $207,766
LCCMR approval Date: June 2016

We have been preparing for the field season (February and March, 2017) when we will collect sediment cores from the 4 study lakes (Kabetogama, Leech, Mille Lacs, and Winnibigoshish) on this project. This preparation has included the hiring of an undergraduate research assistant (Mr. Ben Block), application for a permit to remove lake bottom sediment from Lake Kabetogama in Voyageurs National Park (a federally protected area), ordering of additional supplies for the field work, and the collection and interpretation of information from the MNDNR and Voyageurs National Park on suitable coring locations (latitude, longitude. During an upcoming meeting of the research team), final coring locations will be chosen.

SUB-PROJECT 13: Eco-epidemiological model to assess AIS management
Project Manager: Nicholas Phelps
Budget: $215,000
Start Date: September, 2016 (~3 years)

The ecological niche model for Heterosporosis was developed to achieve outcome 1 from Activity 1. Thus, we were able to identify the geographic areas in Minnesota with suitable conditions for the establishment or presence of this fish disease and produce risk maps for use by managers and researchers. These findings will be submitted for peer-review in late January to the open access journal *Frontiers in Veterinary Science*. A second manuscript is currently under review in the scientific journal *Reviews in Fisheries Science and Aquaculture,* with a broad overview of MAISRC studies, including this project, ("Aquatic invasive species in the Great Lakes region: An overview."). Data for the zebra mussels risk maps were collected and cleaned and models are under development by Dr. Huijie Qiao, the visiting researcher involved with the project.

Amendment request as of February 28, 2017:

Two subprojects have completed, requiring MAISRC to move all remaining unspent funds from the subproject budget into the overall budget reserve so that they can be redistributed to other priority efforts. In addition, two new subprojects have begun, which require moving funds from the overall budget reserve into the new subproject budgets. The specific requests follow:

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Phase 1 Budget: $194,415
Start Date: July 2014 (2.5 years)

This subproject completed in December. MAISRC wishes to move the remaining balance of $20,581 to the overall reserve. Once the final project abstract has been approved by LCCMR, it will be incorporated into IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUB-PROJECT 7-1. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 1 Budget: 206,754
Start Date: May 2014 (2.2 years)

This subproject completed in July 2016 and the final report and abstract have been approved by LCCMR. MAISRC wishes to move the remaining balance of $128,470 to the overall reserve. The final budget, outcomes and project summary are provided in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUB-PROJECT 7-2: Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents
Project Manager: Nick Phelps
Phase 2 Budget: $445,210
Start Date: February 2017 (2.33 years)
This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR this month. We wish to move $445,210 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 9: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol.
Project Manager: Michael McCartney
Phase 2 Budget: $427,950
Start date: February 2017 (2.33 years)

This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR this month. We wish to move $427,950 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

As a result of this amendment, the overall reserve balance would change from $2,955,179 to $2,231,070, a reduction in $724,109.

Project	Starting Budget	Ending Budget	Impact on Reserve
SUB-PROJECT 5:	$214,995	$194,415	+$20,581
SUB-PROJECT 7-1:	$335,225	$206,754	+128,470
SUB-PROJECT 7-2:		$445,210	-$445,210
SUBPROJECT 9:		$427,950	-$427,950
Total			-$724,109

Amendment approved for overall report by LCCMR 3/29/2017

Project Status as of *August 31, 2017*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Nicholas Phelps
Phase I Budget: $1,805,859
Start Date: February 2015 (5.3 years)

The Center is continuing to make significant advances in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota.

We have completed the continuation evaluation process for two projects (SUBPROJECT 2 and SUBPROJECT 4) and are in the process of closing out these Phase I grants and starting work on Phase 2 as approved by LCCMR. Details on these awards is provided below in their respective project update.

As a result of our 2016 Research Needs Assessment process and RFP, we reviewed, evaluated, and peer reviewed four new projects (SUBPROJECTS 14, 15, 16, and 18) that were subsequently approved by LCCMR. Two additional projects (SUBPROJECTS 17 and 19) were reviewed through a separate process as guided by our conflict of interest policy. Details on these awards is provided below in their respective project update.

We have also been working on two additional needs identified in the RNA: a conference on the ethics and regulations of genetic biocontrol as well as generating white papers that summarize the best known science on the prevention, detection, control of four priority species.

We have begun implementing additional goals from our strategic plan, including formalizing what it means to be a MAISRC researcher, reorganizing the internal coordination structure in part to reduce administrative burden, and updating the Center's MOU. With support from the College and advisory board, efforts were made this past legislative session to secure additional funding. A renewed commitment was also made to the strategic plan goals of updating the MAISRC communications plan and creating a development plan. Both efforts are underway.

We have also been preparing for our 2017 update to MAISRC's species and research priorities lists, which is a less involved process than the biennial comprehensive effort completed last year. This update will take place in September with help from the Center's Technical Committee in anticipation of another RFP being announced in late October. We anticipate using remaining funds from 2013 combined with new funds from ML 2017 in these awards.

MAISRC staff continued to be involved in implementation of the AIS Detectors program, which rolled out statewide over the summer, with 121 new detectors having passed their tests after taking online and in-person training. Over 200 volunteers participated in our first ever Starry Trek August 5 to search at 211 public accesses on 178 lakes for Minnesota's most recent invader. MAISRC staff played essential role in the planning, promotion, and reporting related to this event.

The identification book MAISRC staff created is also now available online as a free download or for purchase, which includes spiral bound copy printed on waterproof paper that is expandable as needed. We have been in conversation with DNR to incorporate additional pages and to adapt the book for multiple uses.

We have been putting in a considerable effort to prepare for our 2017 Showcase in September, which will have the largest number of speakers to date and will also include a poster session at the end of the day.

Efforts to broadcast research progress continue through talks, meetings, papers, newsletters, website and other social media formats. MAISRC post docs and staff collaborated on a review paper of AIS in the Great Lakes that has been accepted in *Reviews in Fisheries Science & Aquaculture.* We also worked on several media stories, including the Star Tribune on an in-depth two-issue article on zebra mussels in Minnesota and how "science is fighting back."

We continue efforts to get the MAISRC Containment Lab ("MCL") online and have worked to overcome obstacles related to proper functioning of the water decontamination system. We are also continuing to work with the college and the Agricultural Experiment Station to create user policies, reservation and pricing systems, and maintenance procedures for its operation.

Also as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

The transition to a new Director completed when Nicholas Phelps became the sole Director as of July 1, 2017. We are seeking an amendment below to change the Project Manager from Dr. Galatowitsch to Dr. Phelps. In the meantime, Dr. Galatowitsch has continued to assist with workplan review and approval.

SUBPROJECT 2-1: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $303,217
LCCMR approval and start date: June 20, 2015 (2 years)

This project has finished and a final report will be submitted by 9/30/17. We are seeking an amendment to move remaining funds to the MAISRC reserve and to fund a second phase. Please see below.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen.
Phase 2 Budget: $500,000
LCCMR approval and start date: July 9, 2015 (2.5 years)

A new activity was added to this project. Please see amendment request below to move funds from the reserve to enable this additional work.

SUBPROJECT 4-1: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer.
Phase 1 budget: $413,247
LCCMR approval and start date: July 7, 2015 (2 years)

This project has finished and a final report submitted 8/31/17. We are seeking an amendment to move remaining funds to the MAISRC reserve and to fund a second phase. Please see below.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Start Date: July 2014 (2.5 years)

Final report was approved by LCCMR 6/30/17.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $127,650
LCCMR approval and start date: June 15, 2015 (2 years)

This project has completed. A final report will be submitted by 9/30/17. We are seeking an amendment to move remaining funds to the MAISRC reserve. Please see below.

SUB-PROJECT 7-1. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Start Date: May 2014 (2.2 years)

Final report was approved by LCCMR 2/21/17.

SUB-PROJECT 7-2. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 2 budget: $445,210
Start Date: February 1, 2017

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 8: Risk assessment, control, and restoration research on aquatic invasive plant species
Project Manager: Dan Larkin
Phase 1 Budget: $822,000
LCCMR approval and start date: August 31, 2015 (~4 years)

This project is making progress. The title of this project has been updated here and on the budget. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
Budget: $427,950
LCCMR approval and start date: February 22, 2017

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Dan Larkin
Phase 1 Budget: $566,550
Start Date: November 2015 (3.5 years)

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Revised Phase 1 Budget: $93,343
Start Date: April 2014

Final report submitted September 2015.

SUBPROJECT 11-2: Reducing and controlling AIS Phase 2: Risk assessment
Project Manager: David Andow
Phase 2 Budget: $139,970
Start Date: September 2015 (~1.75 years)

This project is complete. A final report is being finalized. We are requesting an amendment to transfer the remaining balance to the MAISRC reserve. Please see below.

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology
Project Manager: Donn Branstrator
Budget: $207,766
LCCMR approval Date: June 2016

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUB-PROJECT 13: Eco-epidemiological model to assess AIS management
Project Manager: Nicholas Phelps
Budget: $215,000
Start Date: September, 2016 (~3 years)
This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

Amendment request as of August 31, 2017

The transition to a new MAISRC Director is complete. We therefore wish to change the project manager for this 2013 ENRTF appropriation and for SUBPROJECT 1 from Dr. Sue Galatowitsch to Dr. Nicholas Phelps. SUBPROJECT 2 and SUBPROJECT 4 have completed phase 1 and have had Phase 2 approved through the Center's continuation review process. We request to return remaining balances from these Phase I projects to the MAISRC reserve and move funds from the reserve to fund the Phase 2 projects. SUBPROJECT 6 and SUBPROJECT 11-2 are finished and we request remaining funds to be returned to the MAISRC reserve to be reallocated to other priorities. A new activity was added to SUBPROJECT 3, requiring funds to be transferred to the project from the MAISRC reserve. Additionally, six news projects (SUBPROJECTS 14-19) have been initiated requiring funds to be transferred from the reserve. Following are the specifics for each requested action:

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Nicholas Phelps
Phase I Budget: $1,805,859
Start Date: February 2015 (5.3 years)
The transition to a new MAISRC Director is complete. We therefore wish to change the project manager for this 2013 ENRTF appropriation and for SUBPROJECT 1 from Dr. Sue Galatowitsch to Dr. Nicholas Phelps

SUBPROJECT 2-1 Metagenomic Approaches to Develop Biological Strategies to Control AIS
Project Manager: Mike Sadowsky
Phase 1 Budget: $299,849
Phase 1 has now completed and we request that the remaining balance of $3368 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by September 30.

SUBPROJECT 2- Phase 2: Development of potential microbiological control agents for AIS
Project Manager: Mike Sadowsky
Phase 2 Budget: $303,217
This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR 5/22/17. We wish to move $303,217 from the overall reserve to fund this project. The project

description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed; Determining if and how a sound-bubble system can be combined with light in the laboratory to deter carp while examining potential impacts to native fishes.
Project Manager: Sorensen
Budget: $682,969
Dr. Sorensen created a new activity (approved by LCCMR 4/18/17), funded through rebudgeting existing activities plus transferring $182,968 from the overall reserve. We wish to move $182,968 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below. The title on the budget spreadsheet has been updated.

SUBPROJECT 4- Phase 1: Developing realistic management solutions for common carp: testing the potential for biocontrol and assessing the possibility for developing carp-specific toxins
Project Manager: Przemek Bajer
Phase 1 budget: $384,231
Phase 1 is now completed and we request that the remaining balance of $29,016 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by August 31.

SUBPROJECT 4- Phase 2: Developing realistic management solutions for common carp: testing the potential for biocontrol and assessing the possibility for developing carp-specific toxins
Project Manager: Przemek Bajer
Phase 2 budget: $406,000
This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR on 6/27/17. We wish to move $406,000 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $111,889
Phase 1 has now completed and we request that the remaining balance of $15,761 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by September 30.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods
Project Manager: David Andow
Phase 2 Budget: $126,677
Phase 2 has now completed and we request that the remaining balance of $13,294 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by September 30.

The following new projects have been approved. Project funds will be moved from the budget reserve to fully fund these projects accordingly:

SUBPROJECT 14: Cost- effective monitoring of lakes newly infested with zebra mussels
Project Manager: John Fieberg
Budget: $266,500

LCCMR approval date: 6/27/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 15: Determining Highest-Risk Vectors of Spiny Waterflea
Project Manager: Valerie Brady
Budget: $122,640
LCCMR approval date: 6/27/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 16: Sustaining walleye populations: assessing impacts of AIS (DNR)
Project Manager: Gretchen Hansen (DNR) Valerie Brady
DNR Budget: $117,584
NRRI Budget: $81,116
Total budget: $198,700
LCCMR approval date: 7/6/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 17: Building scientific and management capacity to respond to invasive Phragmites (common reed) in Minnesota
Project Manager: Daniel Larkin
Budget: $246,800
LCCMR approval date: 6/27/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota
Project Manager: Ray Newman
Budget: $221,375
LCCMR approval date: 7/7/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 19: Decision-making tool for optimal management of AIS
Project Manager: Nick Phelps
Budget: $172,465
LCCMR approval date: 7/6/17
The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

As a result of these amendment requests, the total budget reserve will be reduced from $2,231,070 to $171,843. $19,767 of this reserve is available to Dr. Sorensen upon his request. It is our intent that the reserve remaining after that ($152,076) will be awarded along with M.L. 2017 funds to research priorities identified through our research needs assessment and made available through an RFP to be announced late October 2017.

Project Status as of *February 28, 2018*

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure
Project Manager: Nicholas Phelps

Phase I Budget: $1,805,859
Start Date: February 2015 (5.3 years)

MAISRC is continuing to make significant advances towards implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota. MAISRC is currently supporting 15 subprojects from ML 2013 – all workplans have been approved by LCCMR and summarized within this overall report.

The MAISRC Technical Committee recommended priority species and research needs that were then vetted by MAISRC's Faculty Group, MN DNR, and the MAISRC Advisory Board. Ultimately, the MAISRC Director finalized a list of up-to 40 high priority species and a list of 20-25 high priority research needs based on their recommendations and offered a competitive request for proposals (RFP). The full list of high priority species and research needs are available on the MAISRC website or upon request. We encourage other funding agencies to review this list when setting their own AIS research priorities.

We opened our most recent RFP in November 2017 with the intention to fund $1.0 million worth of projects from the reserves of ML 2013 and new funds from ML 2017. In total, 20 pre-proposals were submitted in January 2018 requesting a total of $4.2 million, with one more submitted through a separate process as guided by our conflict of interest policy. All projects were reviewed by a committee and recommendations were made to the Director. A meeting was also held with LCCMR staff to discuss the preproposal selections prior to investigator notification. Full proposals have now been requested from six selected preproposals.

In an effort to promote a culture of collaboration and inclusion, on and off campus, MAISRC created an administrative structure for the affiliations of MAISRC Research Fellow (PhD level scientists) and MAISRC Graduate Research Fellow (Students). This was formally launched in December and we now have 29 Research Fellows and 13 Graduate Research Fellows.

We continue efforts to offer the MAISRC Containment Lab as a unique and fully functional AIS research facility and have worked to overcome obstacles related to proper functioning of the water decontamination system, water heating and alarm systems. We have also worked with the college and the Agricultural Experiment Station to finalize user policies, reservation and pricing systems, and maintenance procedures for its operation. We went "live" with accounting for the new Internal Service Organization starting January 1, 2018. MAISRC technicians continue to manage the facility and trouble-shoot when issues arise.

To support the hard work of MAISRC researchers and staff, we have given out awards at our biannual All-MAISRC meetings. The most recent award was given to Dr. Dan Larkin and his team for leading the highly successful AIS Detectors program.

As part of our strategic plan we created a development plan for the Center. A finalized plan was reviewed and supported by the College and the Center Advisory Board. We are now working on revising our communications plan to incorporate strategies from the development plan.

Each year we host an annual Research and Management Showcase and the event continues to grow. In 2017 we had more 260 attendees – than ever before. Importantly, nearly half of the attendees attended for the first time, an indication of MAISRC's expanding reach and credibility.

We have also spent considerable effort with communicating the outcomes of our research. This is discussed in more detail in the dissemination section. We also presented at the International Conference on Aquatic Invasive Species on MAISRC's process for research prioritization, which is quickly becoming a model for other research organizations.

Also as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

In October 2017, Becca Nash left MAISRC. We hired Cori Mattke as the new Associate Director in January 2018.

SUBPROJECT 2-1: Metagenomic approaches to develop biological control strategies for aquatic invasive species.
Project Manager: Michael Sadowsky.
Phase I budget: $299,849
LCCMR approval and start date: June 20, 2015 (2 years)

Phase 1 of project is complete. Final report submitted 8/30/2017.

SUBPROJECT 2-2: Development of potential microbiological control agents for AIS
Project Manager: Mike Sadowsky
Phase 2 Budget: $303,217
LCCMR approval and start date: May 22, 2017

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed
Project Manager: Peter Sorensen.
Phase 2 Budget: $682,969
LCCMR approval and start date: July 9, 2015 (2.5 years)

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 4-1: Common carp management using biocontrol and toxins
Project Manager: Przemek Bajer.
Phase 1 budget: $384,231
LCCMR approval and start date: July 7, 2015 (2 years)

Phase 1 of project is complete. Final report submitted 8/31/2017.

SUBPROJECT 4-2: Developing realistic management solutions for common carp: testing the potential for biocontrol and assessing the possibility for developing carp-specific toxins
Project Manager: Przemek Bajer
Phase 2 budget: $406,000
LCCMR approval and start date: 6/27/2017 (2 years)

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.
Project Manager: Ray Newman
Start Date: July 2014 (2.5 years)

Final report was approved by LCCMR 6/30/17.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control
Project Manager: Paul Venturelli
Phase 1 budget: $111,889
LCCMR approval and start date: June 15, 2015 (2 years)

Final report was approved by LCCMR 10/25/2017.

SUB-PROJECT 7-1. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Start Date: May 2014 (2.2 years)

Final report was approved by LCCMR 2/21/17.

SUB-PROJECT 7-2. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.
Project Manager: Nick Phelps
Phase 2 budget: $445,210
Start Date: February 1, 2017

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 8: Risk assessment, control, and restoration research on aquatic invasive plant species
Project Manager: Dan Larkin
Phase 1 Budget: $822,000
LCCMR approval and start date: August 31, 2015 (~4 years)

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.
Project Manager: Michael McCartney
Budget: $427,950
LCCMR approval and start date: February 22, 2017

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response
Project Manager: Dan Larkin
Phase 1 Budget: $566,550
Start Date: November 2015 (3.5 years)

This project is making progress. Please see update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods:
Project Manager: David Andow
Revised Phase 1 Budget: $93,343

Start Date: April 2014

This project has completed. Final report submitted September 2015.

SUBPROJECT 11-2: Reducing and controlling AIS Phase 2: Risk assessment
Project Manager: David Andow
Phase 2 Budget: $126,677
Start Date: September 2015 (~1.75 years)

This project is complete. Final report submitted 7/31/2017

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology
Project Manager: Donn Branstrator
Budget: $207,766
LCCMR approval Date: June 2016

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUB-PROJECT 13: Eco-epidemiological model to assess AIS management
Project Manager: Nicholas Phelps
Budget: $215,000
Start Date: September, 2016 (~3 years)

This project is making progress. Please see the update in section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 14: Cost- effective monitoring of lakes newly infested with zebra mussels
Project Manager: John Fieberg
Budget: $266,500
LCCMR approval date: 6/27/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 15: Determining Highest-Risk Vectors of Spiny Waterflea
Project Manager: Valerie Brady
Budget: $122,640
LCCMR approval date: 6/27/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 16: Sustaining walleye populations: assessing impacts of AIS (DNR)
Project Manager: Gretchen Hansen (DNR) Valerie Brady
DNR Budget: $117,584
NRRI Budget: $81,116
Total budget: $198,700
LCCMR approval date: 7/6/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 17: Building scientific and management capacity to respond to invasive Phragmites (common reed) in Minnesota
Project Manager: Daniel Larkin
Budget: $246,800
LCCMR approval date: 6/27/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota
Project Manager: Ray Newman
Budget: $221,375
LCCMR approval date: 7/7/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 19: Decision-making tool for optimal management of AIS
Project Manager: Nick Phelps
Budget: $172,465
LCCMR approval date: 7/6/17

The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

Project status updates as of June 30, 2019:

Subproject	Subproject Title	Project Manager	Total Budget	LCCMR Approval Date	Subproject Completion Date	Project Status*
1	Coordinating, synergizing and promoting expertise: Establishing an administrative structure.	Nicholas Phelps	Budget: $1,372,730 Final: $1,351,424	June 25, 2013	June 30, 2019	Complete
2.1	Phase 1: Metagenomic approaches to develop biological control strategies for aquatic invasive species	Michael Sadowsky	Budget: $303,217 Final: $299,363	June 20, 2015	July 31, 2017	Complete
2.2	Phase II: Development of Potential Microbiological Control Agents for Aquatic Invasive Species	Michael Sadowsky	Budget: $303,217 Final: $286,610	May 23, 2017	June 30, 2019	Complete

3	Attracting carp so their presence can be accurately assessed	Peter Sorensen	Budget: $682,969 Final: $663,719	July 9, 2015	June 30, 2019	Complete
4.1	Phase I: Common carp management using biocontrol and toxins	Przemyslaw Bajer	Budget: $413,247 Final: $384,231	July 7, 2015	July 31, 2017	Complete
4.2	Phase II: Common carp management using biocontrol and toxins	Przemyslaw Bajer	Budget: $406,000 Final: $348,913	June 27, 2017	June 30, 2019	Complete
5	Developing and evaluating new techniques to selectively control invasive plants	Raymond Newman	Budget: $214,996 Final: $194,415	July 31, 2014	December 31, 2016	Complete
6	Determining Heterosporosis Threats to Inform Prevention, Management, and Control	Paul Venturelli	Budget: $127,650 Final: $111,889	July 31, 2016	August 31, 2017	Complete
7.1	Developing eradication tools for invasive carp species – Phase I: Understanding the virome of carp species in the Upper Midwest	Nicholas Phelps	Budget: $335,224 Final: $206,754	April 24, 2014	June 30, 2016	Complete
7.2	Developing eradication tools for invasive species – Phase II: Virus Discovery and evaluation for use as potential biocontrol agents	Nicholas Phelps	Budget: $445,210 Final: $422,667	February 1, 2017	June 30, 2019	Complete
8	Risk assessment, control, and restoration research on aquatic invasive plant species	Daniel Larkin	Budget: $822,000 Final: $820,251	August 13, 2015	June 30, 2019	Complete
9	Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol	Michael McCartney	Budget: $427,950 Final: $380,318	February 22, 2017	December 31, 2018	Complete

10	Citizen Science and Professional Training Programs to Support AIS Response	Daniel Larkin	Budget: $525,389 Final: $520,850	November 16, 2015	June 30, 2019	Complete
11.1	Phase I: Reducing and controlling AIS, Risk analysis to identify AIS control priorities and methods	David Andow	Budget: $110,185 Final: $93,343	May 16, 2014	September 30, 2015	Complete
11.2	Phase II: Reducing and controlling AIS, Risk analysis to identify AIS control priorities and methods	David Andow	Budget: $139,970 Final: $126,676	September 23, 2015	May 31, 2017	Complete
12	Characterizing spiny water flea impacts using sediment records	Donn Branstrator	Budget: $212,266 Final: $211,708	June 20, 2016	June 30, 2019	Complete
13	Eco-epidemiological Model to Assess Aquatic Invasive Species Management	Nicholas Phelps	Budget: $215,000 Final: $195,249	September 2, 2017	June 30, 2018	Complete
14	Cost-effective monitoring of lakes newly infested with zebra mussels	John Fieberg	Budget: $266,500 Final: $225,533	June 27, 2017	June 30, 2019	Complete
15	Determining Highest Risk Vectors of Spiny WaterFlea Spread	Valerie Brady	M.L.2013: $92,932 Final: $92,756 M.L.2017: $26,581	June 27, 2017	June 30, 2019	M.L. 2013: Complete M.L. 2017: In Progress
16	Sustaining walleye populations: assessing impacts of AIS	Gretchen Hansen	Budget: $198,700 Final: $197,568	July 6, 2017	June 30, 2019	Complete
17	Building scientific and management capacity to respond to invasive Phragmites (common reed) in Minnesota	Daniel Larkin	Budget: $283,568 Final: $269,773	June 27, 2017	June 30, 2019	Complete
18	Eurasian and hybrid watermilfoil genotype	Raymond Newman	Budget: $221,375 Final:	July 7, 2017	June 30, 2019	Complete

	distribution in Minnesota		$220,412			
19	Decision-making tool for optimal management of AIS	Nicholas Phelps	Budget: $172,465 Final: $80,469	July 6, 2017	June 30, 2019	Complete
20	A Novel Technology for eDNA Collection and Concentration (Year 1)	Abdennour Abbas	M.L.2013: $94,599 Final: $90,263 M.L.2017: $96,264	July 31, 2018	June 30, 2020	M.L. 2013: Complete M.L. 2017: In Progress
21	Early detection of zebra mussels using multibeam sonar	Jessica Kozarek	Budget: $96,550 Final: $96,175	July 31, 2018	June 30, 2019	Complete
22	Copper-based control: zebra mussel settlement and non-target impacts (Year 1)	James Luoma	M.L.2013: $66,866 Final: $62,436 M.L.2017: $148,460	November 15, 2018	June 30, 2020	M.L. 2013: Complete M.L. 2017: In Progress
23	AIS Management: An Eco-economic Analysis of Ecosystem Services (Year 1)	Amit Pradhananga	M.L.2013: $131,845 Final: $131,149 M.L.2017: $110,245	July 31, 2018	June 30, 2020	M.L. 2013: Complete M.L. 2017: In Progress
24	Genetic method for control of invasive fish species (Year 1)	Michael Smanski	M.L.2013: $110,112 Final: $109,000 M.L.2017: $140,004	July 31, 2018	June 30, 2020	M.L. 2013: Complete M.L. 2017: In Progress
25	What's In Your Bucket? Quantifying AIS Introduction Risk (Year 1)	Nicholas Phelps	M.L.2013: $111,642 Final: $101,540 M.L.2017: $88,142	July 31, 2018	June 30, 2020	M.L. 2013: Complete M.L. 2017: In Progress

26	Updating an invasive and native fish passage model for locks and dams	Anvar Gilmanov	Budget: $90,826 Final: $88,296	July 31, 2018	June 30, 2019	Complete

*Pending Approval; In Progress; Complete; Not Completed

Amendment Request August 31, 2018:
As we prepare to launch our final subprojects on 2013 ENRTF funds, we request a series of budget amendments to move a total of $672,342 to fund an increase in budget for Subproject 17 and new Subprojects 20-21, 23-26. Additionally, we request a transfer of balance funds from Subproject 13.

Amendment Request 1
Subproject 1 - move $504,077, decreasing budget from $1,805,859 to $1,301,782 to fund new Subprojects 20-21, 23-26. This decrease will impact the Subproject 1 budget as follows:

- Personnel – $1,564,487 to $1,199,487
- Professional/Technical Services and Contracts – $127,010 to $41,510
    - *Services - Lab and Medical* – decrease to $49 to zero-out budget. Generally, lab Services are now covered under the MAISRC Containment Lab's ISO.
    - *Rentals* – decrease to $0. No planned rentals before the end of M.L. 2013 in June 2019.
- Equipment/Tools/Supplies - $69,111 to $31,534
    - *Supplies - Lab and Field* – decrease to $5,005 to zero-out budget. Generally, lab supplies are now covered under the MAISRC Containment Lab's ISO.
- Capital Expenditures - $16,000 to $0
    - No planned capital purchases planned before the end of M.L. 2013 in June 2019.

Amendment Request 2
Subproject 26 – move $41,161 from Subproject 10, decreasing budget from $566,550 to $525,389, to partially fund new Subproject 26 (total budget $90,827). Decrease in Subproject 10 budget was approved by LCCMR 07/31/2018.

Amendment Request 3
Subproject 26 – move $26,581 from Subproject 15, decreasing budget from $122,640 to $96,059, to partially fund new Subproject 26 (total budget $90,827). Decrease in Subproject 15 budget was approved by LCCMR 07/31/2018.

Amendment Request 4
Subproject 26 – move $23,085 from Subproject 1 to partially fund new Subproject 26 (total budget $90,827).

Amendment Request 5
Subproject 21 – move $96,550 from Subproject 1 to fund new Subproject 21 at a total amount of $96,550.

Amendment Request 6
Subproject 20 – move $94,599 from Subproject 1 to fund new Subproject 20 at a total amount of $94,599 for year one. Year two will be funded by M.L. 2017 reserves.

Amendment Request 7
Subproject 23 – move $131,845 from Subproject 1 to fund new Subproject 23 at a total amount of $131,845 for year one. Year two will be funded by M.L. 2017 reserves.

Amendment Request 8
Subproject 24 – move $110,112 from Subproject 1 to fund new Subproject 24 at a total amount of $110,112 for year one. Year two will be funded by M.L. 2017 reserves.

Amendment Request 9
Subproject 25 – move $47,886 from Subproject 1 and $63,756 from reserves to fund new Subproject 24 at a total amount of $111,642 for year one. Year two will be funded by M.L. 2017 reserves.

Amendment Request 10
Subproject 17 – move $36,768 from reserves to fund an increase in budget and scope for Subproject 17, increasing overall budget to $244,663. This increase will be allocated to a 0.5 FTE research fellow (full-time, 6 months). Additional staffing will enable subproject to a landscape-scale assessment of the potential for *Phragmites* control. Addition to project scope and budget approved by MAISRC 07/19/2018 and by LCCMR on 07/31/2018.

Amendment Request 11
Subproject 13 – move $19,751 from Subproject 13 into reserves. Subproject 13 ended on June 30, 2018 with a budget balance of $19,751. Funds moved from Subproject 13 into reserves will be used to fund additional MAISRC subprojects/activities.

Amendment Approved: **[09/19/2018]**

Amendment Request February 28, 2019:
Amendment 1
Subproject 22 – we request a budget amendment to move $66,866 from reserves to fund new Subproject 22 at a total amount of $66,866 for one year. Year two will be funded by M.L. 2017 reserves.

Amendment 2
Subproject 12 – move $4,500 from reserves to fund an increase in budget and scope for Subproject 12, increasing overall budget to $212,266. This increase will be allocated to hire two undergraduate researchers (40 hrs/week at $10.26/hr). Additional staffing will enable the subproject to extend the search for subfossil evidence of spiny water flea to earlier time periods, with the objective of finding the transition between presence and absence. Addition to project scope and budget approved by MAISRC 01/30/2019 and is pending approval from LCCMR.

Amendment 3
Subproject 1 – we request approval to increase Capital Expenditures in Subproject 1 from $0 to $65,000 in order to purchase a new electrofishing boat for use by current and future MAISRC research projects, increasing the Subproject 1 budget to $1,366,782.

A new electrofishing boat is a critical need for the upcoming 2019 field season and beyond. MAISRC's current electrofishing boat is a shared resource with the Fisheries, Wildlife, and Conservation Biology (FWCB) Department at the University of Minnesota and has degraded to the point of being no longer viable for safe and effective use in the field. We anticipated the need to upgrade the electrofishing boat and budgeted for a new backpack electrofishing unit in our M.L. 2017 budget, however simply updating the current boat or relying on a backpack unit is no longer a fiscally responsible and mechanically feasible solution. Knowing this, we included funds for a new boat in our pending M.L. 2019 budget.

As we begin to wrap up work on M.L. 2013, we have identified sufficient budget savings among our projects to purchase a new electrofishing boat on M.L. 2013, in partnership with the FWCB Department. Purchasing a new boat will allow MAISRC to support multiple current projects through the coming field season and completion of their research in June 2019. This will also provide much needed capacity as we launch new projects on M.L. 2017 and M.L. 2019 this summer, with several expected to need an electrofishing boat.

In order to leverage our resources, MAISRC is working with the FWCB Department to share the cost of a new electrofishing boat and are developing a shared use policy for availability, maintenance, and repairs. Once purchased, the boat will get extensive use as a shared resource with MAISRC and FWCB. MAISRC's portion of this expenditure will be up to $65,000 and will be compiled from the following sources:

- Subproject 15 budget savings (Amendment 4, below) – $3,127
- Subproject 9 budget savings (Amendment 5, below) – $47,632
- Subproject 1 reserve balance (Amendment 6, below) – $14,241

Purchasing a new electrofishing boat this spring on M.L. 2013 will allow MAISRC to continue our lines of research efficiently and effectively, and will free-up additional funds for new research on M.L. 2017 and M.L. 2019. Funds budgeted for the electrofishing boat backpack on M.L. 2017 and the new electrofishing boat on M.L. 2019 will be moved into reserves and will be made available in future MAISRC RFPs.

In alignment with LCCMR's *Policy on Eligible and Ineligible Expenses,* the electrofishing boat will be available for use by any MAISRC funded or MAISRC partnership project. MAISRC use of the boat will be in proportion to the percent investment by MAISRC/LCCMR in its purchase. MAISRC staff will also provide oversight of the management of the boat, to ensure that it is being used proportionally for the purpose of advancing AIS research in Minnesota. This oversight will continue throughout the useful life of the boat.

Amendment 4
Subproject 15 – move $3,127 from Subproject 15, decreasing budget from $96,059 to $92,932, to Subproject 1, increasing overall budget to $1,304,909. This increase will be allocated to capital expenditures in Subproject 1 for the purchase of an electrofishing boat (see Amendment 3). Decrease in Subproject 15 budget was approved by LCCMR 02/26/2019.

Amendment 5
Subproject 9 – move $47,632 balance from completed Subproject 9 to Subproject 1, increasing overall budget to $1,352,541. This increase will be allocated to capital expenditures in Subproject 1 for the purchase of an electrofishing boat (see Amendment 3). Subproject 9 ended on 12/31/2018 and all expenses have cleared.

Amendment 6
Subproject 1 – move $14,241 from reserves to Subproject 1, increasing overall Subproject 1 budget to $1,366,782 and decreasing the reserves balance to $5,948. This increase in the Subproject 1 budget will be allocated to capital expenditures for the purchase of an electrofishing boat (see Amendment 3).

Amendments Approved: **03/18/2019**

Amendment Request May 28, 2019
As we wind down ML 2013 funding for the establishment of MAISRC, we request the following budget amendments:

Amendment 1
We request an amendment to move $5,000 from *Travel-MN,* reducing the budget from $20,669 to $15,669, in order to increase the *Supplies-office & gen oper* budget from $17,108 to $22,108. This increase will allow for the purchase of additional operating supplies such as ink/toner, printer paper, mailing envelopes, meeting provisions for our spring All MAISRC meeting, and materials for our upcoming Research and Management Showcase. While our Research and Management Showcase is scheduled for September 2019 (after the end of ML 2013 funding) we plan to do a significant amount of prep prior to June 30. Purchasing these supplies will

allow MAISRC to wrap-up first generation subprojects that end on June 30 and plan ahead for the dissemination of research findings.

Amendment 2
We request a second budget amendment to increase the budget for *Equipment-non capital lab and field* from $9,421 to $21,204. This $11,783 increase will impact the Subproject 1 budget as follows:

- Professional/Technical Services and Contracts – $41,510 to $35,675
    - *Professional Services and Contracts* – decrease to $165 to zero-out the budget. No planned guest lecturers or speakers in the remaining weeks of the project.
    - *Repairs – Lab and Field* – decrease to $19,240. While we plan to do some repairs on shared equipment in the coming weeks, we do not anticipate spending down all remaining funds.
- Budget Reserve – $5,948 to $0
  Following the final RFP issued on ML 2013 funds (2017/2018), the total *Budget Reserve* was not sufficient to allocate toward an additional subproject.

Funds allocated to *Equipment-non capital lab and field* with this amendment will be used to transition MAISRC into permanent office space, which will allow MAISRC to support research teams and AIS projects well into the future. At the beginning of Subproject 1, leased office space was acquired in a US Forest Service research building. While this space has served us well as we have been getting MAISRC up and running, we were informed at the beginning of 2019 that our lease would not be renewed. Since then, we have worked with the College of Food, Agricultural, and Natural Resource Sciences (CFANS) at the University of Minnesota to secure long-term, stable office space in a university-owned building. Our new location was confirmed in May 2019.

The majority of office equipment (chairs, tables, etc.) that are currently being used by MAISRC staff and researchers are included in our lease with the US Forest Service and will not be able be to transferred with MAISRC to our new space. We request this budget amendment so that we can purchase refurbished, modular conference tables and chairs for our new office space, allowing MAISRC to continue to grow our capacity to build interdisciplinary teams and focus on collaboration. While these equipment purchases come at the end of the ML 2013 grant period, allocating existing funds to secure MAISRC in functional, designated space will provide the last puzzle piece in establishing an AIS research center.

Amendments Approved by LCCMR: **06/12/2019**

IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES:

SUB-PROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure.

Project Manager: Nicholas Phelps

Description: The promise of the center lies in its ability to promote synergies, share facilities, and disseminate information. These activities require scientific and administrative leadership that can organize meetings of center participants in the form of an advisory group as well as a technical group and faculty, while running peer-review, sponsoring symposia, raising funds, and both creating and disseminating reports to the legislature. Sub-Project 1 consolidates the framework for this leadership. As it becomes fully operational (an outcome of this work plan), the Center will be called 'The Minnesota Aquatic Invasive Species Research Center '(MAISRC) and it will be based in the College of Food, Agricultural and Natural Resource Sciences (CFANS) at the University of Minnesota. The MAISRC's Director is Dr. Susan Galatowitsch and she will devote approximately 30% of her time to administering the Center and providing overall leadership and direction. Dr. Galatowitsch will be assisted by a fulltime Associate Director (1.0 FTE for 5 years) who will be fully funded by this activity after startup funding ends in 2014. The Associate Director will continue to work with the Director to run an advisory board (Center Advisory Board [CAB], that includes the DNR (see below), establish and coordinate a technical board (MTC), organize peer-reviews, organize working groups, compile and produce reports and budgets, track spending, produce media releases, and organize peer reviews. Working with the Director and Extension specialist, the Associate Director will also organize regular meetings of Center faculty and staff and a symposium on campus each year, and keep a website up to date. An annual report for the Center will also be produced and biannual reports to the LCCMR.

A Memorandum of Understanding (effective 12/2/2013) between the MAISRC, the College of Food, Agricultural, and Natural Resource Sciences, and the Department of Fisheries, Wildlife, and Conservation Biology memorializes the policies guiding MAISRC. A document entitled “Summary of LCCMR reporting and process 120213 final with attachments” guides the procedures for seeking approvals from and reporting to LCCMR. These documents are on hand with LCCMR staff. Key policies and procedures from those documents are highlighted here.

The Scientific Director will be advised by CAB. This board will meet at least twice per year to review and provide feedback on center activities, new developments on AIS in the state, provide advice to the Director on overall research directions, new funding sources, and new collaborations. This board will also review any proposed changes in research (sub-project) direction or scope (i.e. identified outcomes) and provide recommendations to the Director for implementation according to the parameters of funding agencies (LCCMR and potential future funding contributors). The Director may add or eliminate sub-projects depending on progress and needs according to the processes set out in the Center’s MOU with the department and college. All proposed changes to the Center’s work plan must ultimately be approved by the LCCMR which would have to approve an amendment to the work plan. The Commissioner of the DNR (or designee) will initially lead CAB. In addition, the Board will include the Dean of CFANS (or designee; ex officio), two federal representatives; 2 representatives from state government; 2 representatives from local government; and 2 representatives that do not represent any particular entity. The Director (*ex officio* and non-voting) and Commissioner of the DNR may appoint work groups to address special issues of mutual concern such as how the Center can address key AIS challenges facing the DNR. Work groups would report to CAB and have a limited life.

A Center Administrative Review Board (“CAR”) will provide administrative oversight for MAISRC. This includes: approval of faculty positions; approval of work plans and budgets; approval of changes to research directions including to work plans, budgets, and faculty and administrative positions; and resolution of scientific and budget conflicts. Members of CAR are the CFANS Dean or Designee, Heads of all Departments with MAISRC Faculty (both inside and outside of CFANS), and the Director. Meetings are organized by MAISRC’s Associate Director.

The Director will also lead, and be advised by, Technical Committee (MTC). This group of scientific experts will include at least three members from DNR, three from MAISRC, and the possibility of two others outside the University. MTC will provide technical guidance and advice. The Center will also have a Center Peer Review Committee (CPRC) whose primary responsibility will be to implement peer-reviews of proposed research and report this to the Director. This committee will be comprised of 2 MAISRC faculty and one outside member. Ad-hoc reviewers from outside the University will be solicited for each project. Following the peer review process, the Director will make recommendations for subproject funding. These recommendations will need to be approved by the CAR prior to being submitted to LCCMR.

Initially there will be 11 Center sub-projects, each of which is described in this work plan. All will be peer-reviewed within the first year of initiation when new staff will be asked to develop roughly two year sub-project proposals with budgets based on the outline provided herein. Staff will administer their own budgets and sub-project work plans which will be shared (for approval) with the LCCMR staff after being approved by the Center

Administrative Review Board. . Subsequent sub-projects and sub-budgets will then be reviewed at least at three-year intervals depending on what the Director deems appropriate. It is expected that sub-projects will generally follow the outline of outcomes proposed in this work plan; however, changes may be proposed in activity scope, direction (specified outcome), FTE allocation, and budget. New sub-projects or activities may be created or old ones terminated by the Director according to process laid out in the Center's MOU with the department and college. Changes will be managed and implemented as described above.

The Scientific and Administrative Director will administer the facilities and activities of MAISRC. This includes a lab manager, a technician, the AIS holding facilities, a truck and boats. Faculty meetings will be held at least four times a year and a peer review (CPRC) as needed (at least once a year). The technical committee will also meet at least twice a year with the DNR (MTC). There will be a yearly workshop or symposium.

Summary Budget Information for Subproject 1:		
	ENRTF Subproject 1 Budget:	$1,372,730
	Subproject 1 Amount Spent:	$1,351,424
	Subproject 1 Balance:	$21,306
	Reserve*:	+ $0
	Total balance + Reserve:	$21,306

**The reserve includes reserve balances for all subprojects and will be released during the course of each subproject pending progress and, when applicable, input from peer-review of the particular subproject.*

Outcome	Completion Date
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2013
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2014
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2015
RFP issued; new priority research projects awarded	2016
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2016
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2017
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2018
Advisory group meeting, workshop, LCCMR reports, press releases, etc.	2019

Sub-Project Status as of *February 10, 2014*
SUBPROJECT 1: An administrative and communications assistant has been added and a technician position has been converted to a lab manager for the Engineering and Fisheries Laboratory, which was recently designated for the Minnesota Aquatic Invasive Species Research Center's use as a central holding and research facility. Additional funds were also included in supplies, capital equipment, and repairs in anticipation of MAISRC's increased responsibility for upkeep of this facility. A Subproject Budget is attached.

Sub-Project Status as of *August 31, 2014*
SUBPROJECT 1: No funds have been drawn down from this sub-project, as the Center's administration and care of shared resources continues to be funded through its 2012 ENRTF appropriation. Please see the 2012 workplan and budget for progress reports on these activities.

Sub-Project Status as of *February 28, 2015*
The Center's core operations are now being funded through this, ENRTF 2013, appropriation as the operations portion of the ENRTF 2012 appropriation has been fully spent down.

The workplan is continuing to be implemented as originally laid out by the founder of the Center, with attention to expediting the initiation of sub-projects that had been delayed in the first year and a half of the Center's existence and to launching all of the remaining sub-projects within the estimated timeframe laid out in the February and August 2014 workplan updates. Included in these efforts is hiring new staff to complete the work of sub-projects #8 and #10. The new extension educator (subproject #10) has been hired and started February 26. An initial coordination meeting with Minnesota DNR, Minnesota Sea Grant, MAISRC, and Minnesota Extension was held in January to identify what outreach and education work is being conducted to date, where there are gaps, and what resources are available to assist in filling some of those gaps. As suspected, there is a large focus within the state by DNR, Sea Grant, and others on prevention efforts. There appear to be gaps, however, (among other things) in coordinated and consistent early detection/ rapid response efforts, and in efforts to educate stakeholders on control options for various AIS. It is most likely these will be the focus of outreach and education efforts by the Center's Extension educator, which will be complemented by and will leverage additional efforts from non-MAISRC Extension personnel.

The Extension Specialist position (subproject #8) hiring process has progressed and is on target for filling this now-permanent position by Fall. This position aims to create capacity in an area that is lacking nation—wide: research on the control of freshwater aquatic invasive plants. We are aiming, therefore, to fill this position with the most talented scientist who shows willingness and ability to grow into what will likely be a new area of research for him/her.

In anticipation of all of the Center's ENRTF funded sub-projects soon being underway, the Center has also begun its first systematic research needs assessment to identify top priorities for its next "phase" of research to be undertaken. The ENRTF projects identified by the MAISRC founder and funded through the ENRTF 2012 and this 2013 appropriation have the following breakdown: 9 projects on Asian carp detection, prevention, control or eradication; 1 project on common carp control; 2 projects on zebra mussel detection, prevention, and/or control; 2 projects on Eurasian water milfoil and curly leaf pondweed control; and 1 project on VHS surveillance.

More research is clearly needed for MAISRC to fulfill its mission to find solutions to aquatic invasive species problems in Minnesota. In some cases, more diverse research on these species is needed; in others, research is needed on additional invasive species of concern or on issues that cut across many species. The Center needs to be strategic about where and how best to have the greatest impact for Minnesota. To assist with this, MAISRC conducted a systematic needs assessment to identify and prioritize research related to aquatic invasive species impacting or likely to impact Minnesota. This process used previous research and prioritization documents and it involved seeking expert opinion from researchers within and outside of the University and from AIS managers throughout the state. It also included input from the DNR AIS Advisory Committee and from other stakeholders that was submitted through an online survey. The Center Advisory Board and the Center Faculty are reviewing the results of the process now and are working with the Center Director to develop next steps, which will be communicated in future workplan updates.

Additionally, and related to the research needs assessment, the Center has engaged its board and faculty in a 10-year strategic planning process to identify key issues and strategies for moving the Center forward in its critical work of finding solutions to Minnesota's AIS problems.

The Center's first research and management showcase, during which all Center faculty, staff, and students shared updates, information, and findings affecting AIS Management in Minnesota, was held in November, 2014 and was attended by over 200 people. It is expected this will be an annual event.

Staff and faculty continue to give talks and serve in advisory and other roles outside the University, contributing to sound planning and coordination around Minnesota's collective AIS efforts.

The research and holding facility renovation is now nearing completion of the detailed design phase and construction is still on target to begin in May, 2015 and to wrap up in December.

Sub-Project Status as of *September 24, 2015*

The Center continues to make significant strides forward. The proposal, peer review and workplan development process is now complete for four new research projects (Subprojects 2, 3, 4 and 6). LCCMR has also approved these workplans; please see below for amendment request to transfer funds from the reserve budget into these subprojects.

Dr. Daniel Larkin has been hired and officially began work on August 31, 2015 to develop and implement a new research and outreach program in aquatic plant management and restoration (Subproject 8). We created an initial workplan to support his program development. This has now been approved by LCCMR; please see below for this amendment as well.

The new extension educator (Subproject #10) was hired and began work February 26, however, it was determined that the position was not a match with the hire. We are working closely with Extension to rehire as soon as possible. Meanwhile, development of this program by MAISRC staff has continued in full force. Additionally, Extension has contributed significant time to develop this program and has also committed personnel to help implement it. This project has now completed external review and the workplan is being submitted for approval by LCCMR simultaneous to this overall workplan submission; please see amendment request above. Quarterly coordination meetings have continued with Minnesota DNR, Minnesota Sea Grant, MAISRC, and Minnesota Extension to insure maximum value added by this program.

Work is underway to develop a request for proposals for new research projects that support collaborative teams to address MAISRC's strategic research priorities as defined through its first systematic research needs assessment. Funding to support this research will be made available through cost savings in Subproject 1 as well as from funds on hand from the Clean Water Fund. We will request LCCMR review of the RFP before releasing it.

We have also clarified MAISRC's expectations and evaluation criteria for subprojects and created a process for consideration of continuation of subproject funding at the end of a phase. We had the opportunity to try this new policy for Subproject 11, which is poised to complete its Phase 1 in the coming month. The decision was made to continue funding for Phase 2; a research addendum and workplan has been approved by LCCMR. Please see amendment request above.

An eight month inclusive strategic planning process has culminated in a draft 10 year MAISRC strategic plan that is now being routed for comment by the Center Advisory Board, Center Faculty Group, and all MAISRC students and staff. After incorporating changes received through this comment period, we will bring an updated plan before the CAB for them to consider adoption at our fall meeting.

Demolition is complete and construction is underway at the research and holding facility, washdown facility, and new storage facility. Completion is still anticipated for end of December; if all goes well, MAISRC would like to host a ribbon cutting sometime in January or February.

The MAISRC's second annual research and management showcase, during which all Center faculty, staff, and students share updates, information, and findings affecting AIS Management in Minnesota, was held September 16, 2015. New this year was a selection of trips to see demonstrations of methods used in our research and to teach in-the-field skills.

Staff and faculty continue to give talks and serve in advisory and other roles outside the University, contributing to sound planning and coordination around Minnesota's collective AIS efforts.

Sub-Project Status as of *October 29, 2015*

MAISRC seeks approval to issue a request for proposals (RFP) as discussed in the previous update to fund additional research on priority topics and species as determined through MAISRC's research needs assessment process. We anticipate the amount of ENRTF funds used in this process will range from $250,000- 400,000. Projects awarded ENRTF funding would be added as additional subprojects to this award and reflected in additional workplans and amendment requests. An outcome regarding this RFP has been added to this subproject.

Sub-Project Status as of *February 29, 2016*

All initial subprojects for the Center are now either approved or in the peer review and workplan development stage. The exception is Subproject 9, which was envisioned to be the 2nd phase of zebra mussel work that is currently being funded through the Clean Water Fund through December 2016.

In order to address additional unmet statewide research needs—for example, expanded scope on zebra mussel prevention and control, and beginning research on critical species such as spiny water flea-- a request for proposals was announced in November 2015 to seek collaborations on top priority research needs that had been identified in the 2015-2106 MAISRC Research Needs Assessment process. We received seventeen proposals, totaling $3.2 million. The proposals addressed a range of priority species—Eurasian watermilfoil, curly leaf pondweed, zebra mussels, spiny waterflea, cross-cutting issues, and phragmites. These also included a nice mix of approaches, such as control, preventing spread, risk assessment, and early detection.

These proposals were then vetted by a committee made up of 2 MAISRC researchers, 2 advisory board members, and the MAISRC director based on the level of research need; likelihood the project will contribute to effective, actionable solutions; and scientific rigor. The top three proposals have been advanced to the full proposal stage and are currently under scientific peer review. A new funding proposal is also being developed for submission to LCCMR for its 2017 call that will allow us to conduct additional prioritizations and RFPs to conduct high priority research in the future. The need is great.

The Center's 10 year strategic plan was endorsed by the Center's Fall 2015 Advisory Board Meeting and is now considered final. A new advisory board chair has been elected and the board is now looking at implementation of other key aspects of the plan, including long term funding for the Center's operations.

Construction on the research and holding facility, washdown facility, and new storage facility is complete. Commissioning is now underway at the research and holding facility and researchers will be able to begin populating it once all systems are shown to be in working condition. A ribbon cutting event is scheduled for March 2. In order to help support future operations of the facility, MAISRC staff has developed draft cost share policies and procedures consistent with University of Minnesota policies on Internal Service Organizations and similar to the UMN greenhouses and BSL 2 and 3 Quarantine facilities. This has also been discussed with LCCMR staff.

Director Sue Galatowitch continues to be involved in managing the content and direction of Subproject 10. We have also hired a new Extension Educator who will begin early April to lead the AIS Trackers program.

MAISRC has identified the date for its 2016 Showcase on the St. Paul campus (September 22) and continues to broadcast updates on MAISRC progress and findings via talks, social media, and newsletters, and now also via a revamped website launched earlier this month. The website provides expanded information on research projects under way, the species on which we conduct research, the researchers involved in our work, and it provides links to published work by MAISRC scientists. The site is also designed with our three largest audiences in mind: AIS managers, researchers, and citizens.

MAISRC recently participated in developing the agenda for the Governor's Clean Water Summit on 2/28/16, attended the summit, and will be involved in helping to organize input received by attendees for delivery to the Governor.

Sub-Project Status as of *August 31, 2016*

The Center is continuing to progress in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota. Highlights from the last six months include:

Three subprojects were at or near the end of their project period and were thus ready to be evaluated for continuation, which involved implementing an evaluation process & policy developed with assistance from the Center Advisory Board and Center Faculty Group. PIs submitted progress results as well as proposed Phase 2 plans, which were evaluated by a team as defined by the policy. As a result, two projects (Subproject #7, and #9) will be considered for Phase 2 funding and must now submit a full research proposal for peer review. One project (Subproject #5) will not be considered for Phase 2 funding, however an extension on a portion of the Phase 1 project may be granted pending a proposal to be reviewed and approved by MAISRC before being submitted to LCCMR. Unused Phase 1 and Phase 2 funds will be returned to MAISRC and will be made available for new research via the Center's RFP process. This transfer will occur as an amendment request with the last update for these projects, which is due January 31, 2017.

Significant effort was put into getting the Extension program (Subproject 10) launched, including writing and reviewing science- based materials for six online training modules and the classroom curriculum for the AIS Detectors Program. Each module includes about 1- hour of audio visual content, resource materials, and self-tests of skills and knowledge. The materials were also reviewed by DNR staff and will be piloted with several lake associations in the Brainerd area this October. Staff and program supervision was also provided for the AIS Trackers program which will be more oriented toward AIS control efforts and will be piloted in 2017.

Transition planning and interviews were conducted by MAISRC leadership and staff, which led to the hiring of Dr. Nicholas Phelps to be the new Center Director starting July 1. Nick will spend the first year being co- Director with Dr. Susan Galatowitsch in order to ensure smooth transition. The position is full time director and AIS research. The 50% director salary for Nick is being covered through Subproject 1 of this appropriation except for one year in which 25% of his salary and a grad student will be covered. His 50% research appointment is being covered by the Department of Fisheries, Wildlife and Conservation Biology and the College of Food, Agricultural, and Natural Resource Sciences. The salary previously covered through Nick's Subprojects #7 and #13 have been accordingly removed. Sue's effort as co-director is no longer being covered through this appropriation.

Sue will continue to serve as PI on this overall appropriation for the time being. Nick will assume PI on Subproject 10 (Extension) now in recognition of his leadership and effort on the program.

The second biennial Research Needs Assessment process has begun. The interagency MAISRC Technical Committee (MTC) serves as the core of the Research Needs Assessment Team. Re-appointments and new two-year appointments were made to this eleven-member committee, which then held its first meeting to review priority species and make modifications based on the present science and status of threats. The final draft list of species will be routed to the Center Faculty Group and Center Advisory Board for input before being finalized. Additional members were also selected to join with the MTC and serve on the 2016 Research Needs Assessment Team. This team represents researchers, AIS managers, and stakeholders from around the state. An added emphasis this year is on cross species issues, which will be informed by social scientists, a DNR conservation officer, and others. A request for input on research priorities from the general public as well as from the DNR's AIS Advisory Committee will be made. The results will be fed into the Research Needs Assessment process culminating in a list of research priorities that will be used for an RFP by the end of the calendar year.

The MAISRC director and technicians have continued to be closely involved with the project designers and engineers in the commissioning process of the newly renovated research lab facilities. Our staff were able to identify several malfunctioning systems and equipment which has since prevented us from being able to fully occupy the space. Our continued time, attention, and expertise has been needed. We are now actively working with CFANS, UMN Capital Planning, and the design and construction firms to develop remedies. In the meantime, accommodations have been made to get as much research as safely possible running in the lab. Other research has been relocated or is on hold. The new storage facility is complete and we have facilitated the occupancy of this space for our faculty and their gear.

Additional funding for research and core operations is being pursued. A 2017 ENRTF proposal was submitted and was recommended for funding. If approved by the legislature, this would provide two additional years to operational capacity and would provide funds for approximately 7 new research projects to address existing and emerging threats. Strategies for obtaining long term capacity funding are also being discussed with the advisory board and partners.

The 2016 Showcase is being planned with a committee of MAISRC researchers and staff and will be held on the St. Paul Campus on September 12. Over fifteen research talks will be given, including talks on new projects funded recently on zebra mussels, spiny water fleas and more. Over 225 people are anticipated to attend.

The newly revamped website is live and efforts to educate, inform, and share findings are continuing via the website, Facebook, Twitter and media efforts. Research Center faculty and staff also continue to give talks and meet with stakeholders. MAISRC will host a special session at the upcoming Upper Midwest Invasive Species Conference in addition to supporting several individual's research talks and talks are also being given at the Aquatic Invaders Summit in October.

We are seeking an amendment to budget phase 2 funds for Subproject 1 at this time to secure operational funds through the end of this appropriation and to dedicate unused reserve funds to research and outreach primarily via our new RFP process.

Sub-Project Status as of *February 28, 2017*

The Center is continuing to progress in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota. Highlights from the last six months include:

Two subprojects (Sub-project 7, Developing eradication tools: Exploring whether native pathogens can be used to control AIS- Nick Phelps and Sub-project 9, Zebra mussel investigations: pathways and mechanisms of spread, new molecular approaches for early detection, and methods for estimating population change in response to pesticide treatment.- Michael McCartney, previously funded through Clean Water Fund) were evaluated as part of the MAISRC's "process for review of research progress and consideration for continuation" policy, which involved convening a review team to hear a presentation, conduct Q&A with the investigator, identify ways the project could be improved, and then vote. The teams recommended both these projects submit full proposals and then MAISRC staff facilitated peer review where needed. Workplans and budgets were reviewed by MAISRC and have now been approved by LCCMR. We are requesting funds be moved from the overall reserve to these new subprojects accordingly. Two additional sub projects (Subproject4, Reducing and controlling AIS: Common carp management using bio-controls and toxins—Bajer and Subproject 2, Delaying the spread of AIS: Metagenomic approaches to develop biological control strategies for zebra/quagga mussels and Eurasian watermilfoil- Michael Sadowsky) have also started the continuation process. Following the review team's evaluation, both investigators were invited to submit full proposals, with the second phase of Subproject 4 (i.e. Subproject 4-2) nearing the workplan development stage.

We completed our 2016 biennial Research Needs Assessment, which included soliciting input from a broad range of experts and stakeholders including AIS managers, researchers, and resource- users. We received 383 submissions that were vetted by our 20-member Research Needs Assessment Team and ultimately resulted in a list of 26 priorities that were supported by the Center Advisory Board. New this year was addition of a cross-species and systems team that included a modeler, enforcement personnel, a social scientist, and a county AIS program coordinator. The breakdown of Research Needs Assessment survey responders was: 54 AIS agency staff, 91 lakeshore owners, 3 watershed district board members, 61 lakeshore association board members, 3 county board members, 51 anglers, 61 boaters, and 40 researchers. 39 indicated "other." To reiterate, this survey was to solicit research ideas from the range of people affected by AIS. Research Needs Assessment Team members reviewed input provided by all entities and only advanced project ideas they felt were most worthy of scientific pursuit.

We also announced our 2016- 2017 RFP in order to find and fund scientists to conduct research on these priorities. We sent the RFP directly to ~300 people. This included high potential researchers who were identified through professional networks, at conferences, and by scanning relevant publications. This also included researchers at and directors of departments and centers that potentially hold expertise needed to help solve AIS problems—for example, departments of environmental sciences, biology, and natural resources as well as applied economics, civil, environmental, and geo- engineering, social sciences, and tourism centers at 4 campuses of UMN, 8 other Minnesota colleges and universities, and 10 regional universities. This also included people within 3 divisions of DNR and 5 federal agencies, inside and out of Minnesota. In response, we received 15 pre-proposals that were sent out to a review team for evaluation. The scores from all reviewers were assembled and the projects were ranked. We convened the review team to discuss the merits of the top pre-proposals. Three project teams were then invited to submit full proposals and discussions are underway with two other project teams. Until the projects satisfactorily complete peer review, their names are confidential.

In order to ensure MAISRC can continue to benefit from the research productivity of the Director, we also developed a Conflict of Interest policy and had it reviewed by our faculty, advisory board, and others. It was approved by the College. Two projects, with current MAISRC Directors as PI or Co-PI, were reviewed and considered under this new policy. Both received positive (anonymous) reviews and were invited to full proposal and peer review. These two director projects are in addition to the three projects selected and two projects pending as part of the regular RFP process.

With significant effort by project and MAISRC staff, the AIS Detectors program piloted to a small cohort of DNR staff and citizens in Fall of 2016. Substantial effort by MAISRC communications staff has continued since then in order to revise the curriculum, update the online module, create videos for classroom sessions, and importantly to create a professional, scientifically vetted AIS (and look-alikes) identification book that will set a new standard in the state for this kind of publication.

For example:

- Unlike individual business- card sized identification cards created by Sea Grant, this is a collated book that is intended for use by citizens doing active detection monitoring for a host of AIS species likely to be found in Minnesota
- Unlike other books in Minnesota, this book provides identification of not just aquatic plants, but of fish and invertebrate AIS as well
- This book provides identification of key AIS along with top look-alikes and the features to help distinguish between the two
- This book includes maps and habitat descriptions to guide where Detectors should look for AIS
- The book includes colored pictures of live specimens, including of multiple life stages
- This book will also include information on reporting a suspected AIS using EDDMapS

- This book is also expandable-- it has a binding that can open so that Detectors can add or remove pages as AIS threats change and Detectors go through advanced trainings

In developing this book, we worked with partners at University of Wisconsin Extension and Minnesota DNR to build on existing knowledge of what is useful for citizen scientists doing this kind of detection work.

AIS Detectors will receive this ID book, as well as classroom companion guide, as part of their registration for one of seven training sessions to be held statewide in 2017.

We held our 2016 Showcase in September, which attracted 171 non-MAISRC attendees and provided 16 presentations spread out among 21 speakers. 90% of attendees rated the event as excellent or very good. MAISRC core staff also attended conferences to stay abreast of current work and research needs around the state and also gave a presentation on MAISRC's Research Needs Assessment process, which has gained attention as an efficient, inclusive, and solutions-oriented model. Efforts to broadcast research progress continue through talks, attendance at statewide AIS Advisory Committee meetings, papers, newsletters, website and other social media formats. We continue to reach larger audiences and receive high engagement from our followers.

We have continued to try to provide the infrastructure needed to support innovate research teams. We have brought the MAISRC Containment Lab ("MCL") online through a difficult commissioning process and began paperwork to create the MCL as an Internal Service Organization and to develop sustainable pricing. Usage policies are currently also being developed. MAISRC technicians continue to manage the facility and trouble-shoot when issues arise. We have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts. This included conducting several large rebudgets for Dr. Sorensen's projects to accommodate changing work plans. We also continue to hold monthly post- docs and donuts meetings and MAISRC faculty meetings to coordinate research, generate new ideas, and ensure smooth center operations.

We have continued transitioning to a new Director, with Nick Phelps and Sue Galatowitsch serving as co-Directors until Nick takes over later this year. In order to better align with our strategic aims, we created a CAB member skills and qualities matrix, expanded and diversified CAB membership, and revised our external MOU with DNR accordingly. With assistance from our newly expanded board, we have also developed an annual MAISRC budget and have begun pursuing funding to sustain the center after 2019. One component of this effort was submitting and testifying on a 2017 ENRTF proposal that has been recommended for funding by the LCCMR.

Sub-Project Status as of *August 31, 2017*

The Center is continuing to make significant advances in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota.

We have completed the continuation evaluation process for two projects (SUBPROJECT 2 and SUBPROJECT 4) and are in the process of closing out these Phase I grants and starting work on Phase 2. These new phases were approved by LCCMR earlier this summer. Details on these awards is provided in their respective project updates.

As a result of our 2016 Research Needs Assessment process and RFP, we reviewed, evaluated, and peer reviewed four new projects (SUBPROJECTS 14, 15, 16, and 18) that were subsequently approved by LCCMR. Two additional projects (SUBPROJECTS 17 and 19) were reviewed through a separate process as guided by our conflict of interest policy. These projects were also approved by LCCMR. Details on these awards is provided in their respective project updates.

We have also been working on two additional needs identified in the RNA: a conference on the ethics and regulations of genetic biocontrol as well as generating white papers that summarize the best known science on the prevention, detection, control of 4 priority species. We are coordinating efforts with the DNR on both fronts.

We have begun implementing Goal 4.2 of the strategic plan, focused on formalizing what it means to be a MAISRC researcher and reorganizing the internal coordination structure, in part to reduce administrative burden. Additionally the College, Department, and MAISRC MOU is required to be reviewed and approved every 4 years, with its first review due in January 2018. These activities are therefore being combined and an update to the MOU is currently being drafted.

An annual review of progress on strategies was conducted by our Center Advisory Board (CAB) this past spring per our Strategic Plan Goal 5.2. Strategy E. and a focused commitment was made for Goal 5.1 Strategy B—to explore and pursue mechanisms for securing stable funds through state appropriation. With support from the College and CAB, we sought additional funds from the legislature this past session to supplement funding received from the ENRTF. While the LCCMR's ENRTF M.L. 2017 recommendation was significant, it would not allow us to maintain our current research levels. The funding effort was also made in an attempt to create a stable year-to-year source of funding on which to plan longer term programs and future investments needed to solve AIS problems. While we were successful at securing funding, its stability is uncertain.

Also as part of the strategic plan review was a renewed commitment to update the communications plan and to create a development plan for the Center. Both efforts are underway with the aim of completion and obtaining support from the advisory board by year-end.

We have also been preparing for our 2017 update to the MAISRC species and research priorities lists, which is a less involved process than the biennial comprehensive effort completed last year. This update will take place in September with help from the Center's Technical Committee in anticipation of another RFP being announced in late October. We anticipate using remaining funds from 2013 combined with new funds from ML 2017 in these awards.

The AIS Detectors program rolled out statewide over the summer, with 125 new detectors having passed their tests after taking online and in person training. As part of their certification, detectors commit to volunteering certain number of hours each year. One such opportunity included the first annual Starry Trek held on August 5. Over 200 volunteers participated in this search at 211 public accesses on 178 lakes across the state for Minnesota's most recent invader. MAISRC staff played an essential role in the planning this event, including creating media tools for local rendezvous sites to draw attention to the event and increase participation. We also coordinated with DNR on creating the announcement of the one new confirmed finding and created template releases for rendezvous sites to thank volunteers and report on results.

The AIS identification book MAISRC staff created is also now available online as a free download or for purchase, the latter of which includes a spiral bound copy printed on waterproof paper that is expandable as needed. We have been in conversation with DNR to incorporate additional pages and to adapt the book for multiple uses.

We have been putting in a considerable effort to prepare for our 2017 Showcase in September, which will have the largest number of speakers to date and will also include a poster session at the end of the day.

Efforts to broadcast research progress continue through talks, meetings, papers, newsletters, website and other social media formats. MAISRC post docs and staff collaborated on a review paper of AIS in the Great Lakes with emphasis on the research conducted at MAISRC. The paper has been accepted in *Reviews in Fisheries Science & Aquaculture.* Another particularly noteworthy effort included working with Start Tribune reporters on in-depth two-issue article of zebra mussels in Minnesota and how "science is fighting back." Additional details on this work are included in the dissemination section of the workplan update.

We continue efforts to get the MAISRC Containment Lab ("MCL") online and have worked to overcome obstacles related to proper functioning of the water decontamination system. We are also continuing to work with the college and the Agricultural Experiment Station to create user policies, reservation and pricing systems, and maintenance procedures for its operation. We plan to go "live" with accounting for the new Internal Service Organization starting January 1, 2018. MAISRC technicians continue to manage the facility and trouble- shoot when issues arise.

Also as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

The transition to a new Director completed when Nicholas Phelps became the sole Director as of July 1, 2017. We are seeking an amendment below to change the Project Manager from Dr. Galatowitsch to Dr. Phelps. In the meantime, Dr. Galatowitsch has continued to assist with workplan review and approval.

Sub-Project Status as of *February 28, 2018*

MAISRC is continuing to make significant advances towards implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota. MAISRC is currently supporting 15 subprojects from ML 2013 – all workplans have been approved by LCCMR and summarized within this overall report.

Every other year, MAISRC conducts and in-depth species prioritization and research needs assessment. This was done in 2016 and will be done again in 2018. However, in the off years (e.g. 2017) we evaluate the current species and priorities and update as needed based on recent research findings and changes in management needs. To do this, MAISRC coordinates a committee of ten technical experts, half researchers and half AIS managers. This Technical Committee recommends priority species and research needs that are then vetted by MAISRC's Faculty Group, MN DNR, and the MAISRC Advisory Board. Ultimately, the MAISRC Director finalizes a list of up-to 40 high priority species and a list of 20-25 high priority research needs based on their recommendations and we offer a competitive request for proposals (RFP). The full list of high priority research needs is available on the MAISRC website or upon request. We encourage other funding agencies to review this list when setting their own AIS research priorities.

There were minor modifications to the high priority species list in 2017, including:
Fish: Removed – Zander; Added – Goldfish/Prussian Carp
Harmful microbes: Removed: *Cyilindrospermopsis raciborskii*; Added: Cyprinid Herpes Virus-3
Plants: Removed: Water soldier; Added: Brittle naiad

We opened our most recent RFP in November 2017 with the intention to fund $1.0 million worth of projects from the reserves of ML 2013 and new funds from ML 2017. MAISRC disseminated the announcement via social media and emailed directly to approximately 100 researchers and relevant programs to encourage proposal submission. In addition, we made an increased effort this funding cycle to 'match-make' research needs with specific researchers that have expertise in those topics and match-make researchers who are proposing to work on similar topics.

In total, 20 pre-proposals were submitted in January 2018 requesting a total of $4.2 million, with one more submitted through a separate process as guided by our conflict of interest policy. All projects were reviewed by a committee and recommendations were made to the Director. A meeting was also held with LCCMR staff to discuss the preproposal selections prior to investigator notification. Full proposals have now been requested from six selected preproposals.

In an effort to promote a culture of collaboration and inclusion, on and off campus, MAISRC created an administrative structure for the affiliations of MAISRC Research Fellow (PhD level scientists) and MAISRC Graduate Research Fellow (Students). The concepts were vetted with a small group of MAISRC faculty, the entire faculty group and several off-campus PIs. These affiliations provide a win-win for the Center and researchers. This was formally launched in December and we now have 29 Research Fellows and 13 Graduate Research Fellows.

We continue efforts to offer the MAISRC Containment Lab as a unique and fully functional AIS research facility and have worked to overcome obstacles related to proper functioning of the water decontamination system, water heating and alarm systems. We have also worked with the college and the Agricultural Experiment Station to finalize user policies, reservation and pricing systems, and maintenance procedures for its operation. We went "live" with accounting for the new Internal Service Organization starting January 1, 2018. MAISRC technicians continue to manage the facility and trouble-shoot when issues arise.

To support the hard work of MAISRC researchers and staff, we have given out awards at our biannual All-MAISRC meetings. The most recent award was given to Dr. Dan Larkin and his team for leading the highly successful AIS Detectors program.

As part of our strategic plan we created a development plan for the Center. A finalized plan was reviewed and supported by the College and the Center Advisory Board. We are now working on revising our communications plan to incorporate strategies from the development plan.

Each year we host an annual Research and Management Showcase and the event continues to grow. In 2017 we had more 260 attendees – than ever before. This year we included a student/post doc poster session during a networking social hour. This was very popular with attendees and presenters alike and will be included in years to come. Importantly, nearly half of the attendees attended for the first time, an indication of MAISRC's expanding reach and credibility.

We have also spent considerable effort with communicating the outcomes of our research. This is discussed in more detail in the dissemination section. We also presented at the International Conference on Aquatic Invasive Species on MAISRC's process for research prioritization, which is quickly becoming a model for other research organizations.

Also, as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

In October 2017, Becca Nash left MAISRC. We hired Cori Mattke as the new Associate Director in January 2018.

Sub-Project Status as of *August 2, 2018*

MAISRC is continuing to provide leadership toward solving AIS problems in Minnesota. We continue to work closely with our Center Advisory Board, Fellows Group, and Technical Committee to ensure high quality and high priority research and outreach is being conducted through MAISRC projects and programs. In addition, MAISRC staff works in collaboration and coordination with many state and regional organizations, for example the Minnehaha Creek Watershed District, Itasca County, MN DNR, MN Sea Grant, US Fish and Wildlife Service and the Great Lakes ANS Panel.

We are currently supporting 14 subprojects from M.L. 2013 – summaries of the progress of these subprojects are included below. Subproject 13 – Eco-epidemiological Model to Assess Aquatic Invasive Species Management – has completed and a final report has been submitted to LCCMR. Additionally, we are in the process of approving seven new research projects that will be funded on M.L. 2013 until funding concludes on June 30, 2019. New subprojects are listed in the update table above and are included in the project summaries below.

This year marks the five-year anniversary of MAISRC and earlier this year, our staff created a comprehensive five-year report that highlights MAISRC's innovative AIS work and big-wins from our research teams. Hard copies of the report were provided to key stakeholders and LCCMR members. An online version of the report was broadly shared through MAISRC's communication channels and has been viewed ~23,000 times. To download a copy of the report, visit:
https://www.maisrc.umn.edu/sites/maisrc.umn.edu/files/maisrc_five_year_report.pdf

Aquatic invasive species are a threatening and impacting waters throughout the state of Minnesota and therefore, MAISRC research and outreach teams are working across the state to advance our understanding AIS and help find solutions. To help visualize MAISRC's statewide focus, our team put together an interactive map of MAISRC research and citizen science sites. With 850 locations included, the map highlights MAISRC's comprehensive approach to AIS research and citizen science. We have found this to be an engaging way for the public to see the impacts of research in their own backyard or favorite waterbody. To view the map, visit: https://www.maisrc.umn.edu/maisrc-map

The AIS Detectors program (Subproject 10) trained its second cohort of volunteers earlier this year, bringing the total number of certified AIS Detectors to 217 throughout the state. Locations of AIS Detectors are included in the MAISRC map, linked above. Building off of this success, MAISRC researchers launched the pilot season of the AIS Trackers program – an additional volunteer program that trains citizens to monitor changes in populations of AIS over time and generate data that can be used for adaptive management. These programs have been recognized with national awards for innovation – a testament to the project team and the importance of engaging the pubic in citizen science.

Expanding knowledge and understanding of AIS is an important part of MAISRC's work and to advance this part of our mission, we have begun to formalize a Communications Plan. Through the process of drafting the plan we will learn more about AIS audiences, how to communicate about AIS and research effectively, and define a communications strategy for MAISRC that aligns with our strategic plan and mission/vision.

Delivering research findings into the hands of managers is at the core of MAISRC's work. This spring, MAISRC staff worked with one of our research teams (Subproject 17) to develop management recommendations for non-native *Phragmites* and make them available online. To view the *Phragmites* website, visit: https://www.maisrc.umn.edu/phrag-management.

We have also worked with one of our affiliated researchers (Clean Water Fund project) to prepare a white paper titled "Treatment options for zebra mussels at various water temperatures". The final white paper is currently being reviewed by the MN DNR to ensure it is recommendations for chemical control are consistent with state permitting guidelines. It will be widely shared to provide science-based recommendations to control zebra mussels.

Part of the value of a research center like MAISRC is the ability to bring together diverse stakeholders to prioritize research needs to ensure that funding and effort align with management goals. Every other year, MAISRC works with our Technical Committee and Research Needs Assessment (RNA) Team to review and revise our list of priority species and generate research questions that will guide our work going forward. We began the species review process with our Technical Committee in July 2018 and anticipate changes to our list of priority species – this list will be available on our website and sent to LCCMR staff when it is available. A survey will be sent out to all MAISRC stakeholders to generate research questions that will be evaluated by our RNA team. We will be meeting with the RNA Team in October to identify and prioritize research needs. These recommendations will be vetted by the MAISRC Advisory Board, Fellows Group and MN DNR AIS leadership, before being finalized by MAISRC staff for our 2018 Request for Research Proposals, which we plan to announce in November 2018.

To continue providing leadership in the AIS research field and to ensure proper stewardship and accessibility to MAISRC research data, we have begun the process of setting up a publicly accessible data repository ("DRUM") in collaboration with the University Digital Conservancy. Beginning this fall, all MAISRC subprojects will contribute their data, publications, and meta data to the DRUM as a part of their project close out.

As a part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

Sub-Project Status as of *February 28, 2019*

MAISRC is currently supporting 20 subprojects on M.L. 2013. Summaries of the progress of these subprojects are included below. Subproject 9 – *Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol* – has completed and a final report is being drafted for submission to LCCMR. MAISRC is currently working with the Minnesota Super Computing Institute (MSI) and the University of Minnesota Genomics Center (UMGC) to coordinate the announcement and public release of the zebra mussel genome that was completed as a part of Subproject 9.

Providing information and tools that have real-world management impacts continues to be a central part MAISRC's research focus. This fall, the AIS risk models that were developed as a part of Subprojects 13 and 19 were made available to local county AIS managers. The risk classification model is being used by several counties and programs to inform early detection and surveillance programs. Using the risk model and boater networks to optimize decision-making of watercraft inspection locations is currently being piloted with Crow Wing, Ramsey and Stearns Counties. The responses we have received about the local use of the models have been positive and we expect their use will likely expand in the future.

The AIS Detectors program (Subproject 10) hosted on-the-water workshops over the summer – on Moose Lake in Beltrami County, Lake Koronis in Stearns County, and on the Mississippi River. The workshops provided opportunities for the public to learn more about starry stonewort identification, biology, and impacts. All three sessions were well attended and reviewed.

In order to share highlights from MAISRC's work over the last year, our staff created a *2018 Research Report* that includes project updates and big-wins from our research teams. Hard copies of the report were provided to key stakeholders and all LCCMR members. An online version of the report was broadly shared through MAISRC's communication channels. To download a copy of the report, visit:
https://www.maisrc.umn.edu/2018-researchreport

In addition, MAISRC partnered with a local videographer to create a series of videos about our research. Video topics include:

- The AIS Detectors program
- Starry stonewort research
- Spiny waterflea research
- The impact of zebra mussels and spiny waterflea on walleye
- Using pathogens to control invasive carp
- Novel methods for controlling common carp

Collectively, the videos have been viewed more than 36,000 times online. While these videos were not produced with ENRTF funds, they play an important role in keeping legislators, managers, and interested members of the public informed by explaining our research in a new and different ways. Videos can be viewed on our website, visit:

https://www.youtube.com/channel/UCrAIM9ZX86P4jlHxKVOaNNg/featured

In September, MAISRC hosted our annual Research and Management Showcase and the event continues to grow – in 2018 we had more than 270 attendees. Importantly and for the second year in a row, nearly half of the participants attended for the first time – a continuing measure of MAISRC's expanding reach and credibility. Presentations from the Showcase are available online, visit:
https://www.maisrc.umn.edu/news/showcase-presentations-1

We are also continuing to work on our Communications Plan. Two key, preliminary activities were accomplished in the fall of 2018 – (1) analysis of current audiences that receive MAISRC communications and (2) a survey of communication preferences of current MAISRC stakeholders. This background information will feed into the development of larger communication goals and activities over the next few months.

Over the last six months, MAISRC has been working with our Technical Committee and Research Needs Assessment (RNA) Team to review and revise our list of priority species and generate research questions that will guide our work going forward. We completed the species review process with our Technical Committee in July 2018, resulting in a few modifications to the high priority species list for 2018:

- Vertebrates: Added Yellow Bass (*Morone mississippiensis*) to the evaluation list
- Invertebrates: Removed Caspian mud shrimp (*Chelicorophium curvispinum*) and added bloody red shrimp (*Hemimysis anomala*) to the priority list
- Microbes: Removed *Piscirickettsia salmonis* and added Rickettsia-like organisms (RLOs) to the priority list

Following final updates to the priority species list, we distributed a survey to all MAISRC stakeholders to generate research questions. In total we received over 400 submissions to the survey. In October, we convened the RNA Team to review potential research questions and identify and prioritize research needs. These recommendations were vetted by the MAISRC Advisory Board, Fellows Group, and MN DNR AIS leadership, before being finalized by MAISRC staff and included in our 2018 Request for Research Proposals (RFP) in November.

We continue to work closely with our Center Advisory Board, Fellows Group, and Technical Committee to ensure high quality and high priority research and outreach is being conducted through MAISRC projects and programs. MAISRC staff continues to work in collaboration and coordination with many state and regional organizations including local watershed districts, county agencies, Minnesota DNR, MN Sea Grant, State AIS Advisory Committee and the Great Lakes ANS Panel. We also continue to spend considerable effort on communicating the outcomes of our research, which is discussed in more detail in the Dissemination section.

Final Report Summary:
This project successfully established the Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota, a vibrant and durable research program that develops research-based solutions to Minnesota's aquatic invasive species (AIS) problems. MAISRC has quickly become a global leader in the field and a go-to resource for managers, the public and researchers. In total, 32 subprojects were supported from this project – significantly advancing our scientific understanding and ability to manage AIS. New tools have been developed and knowledge gaps filled on many of Minnesota's most important AIS, including: zebra mussels, bigheaded and common carps, starry stonewort, non-native *Phragmites*, Eurasian watermilfoil, curlyleaf pondweed, Heterosporosis, and spiny waterflea. The results of this work have been broadly disseminated to end-users via research reports, peer-reviewed manuscripts, fact sheets, white papers, news media, newsletters and presentations (available here: www.maisrc.umn.edu). An annual Research and Management Showcase has been held since 2014, with 700+ unique attendees in total. MAISRC has also created an award-winning and sustainable citizen science program ("AIS Detectors") that has trained hundreds of people from across the state. This project supported efforts to ensure effectiveness and efficiency of a Center-based research model, including a 10-year strategic plan, a comprehensive process for prioritizing research needs, increased collaboration and

coordination between researchers and managers, an annual competitive and peer-reviewed request for proposals, the formation of external and internal advisory boards, research dissemination and outreach, support of a world class research facility, and creation of communication and development plans. Minnesota is much better equipped to address our AIS problems than we were prior to this project – MAISRC has significantly advanced the science of AIS management and engaged thousands of stakeholders and partners from across the state and world. This project will continue with Phase II and III appropriations awarded in 2017 and 2019.

SUB-PROJECT 2-1: Delaying the spread of AIS: Metagenomic approaches to develop biological control strategies for zebra/quagga mussels and Eurasian watermilfoil.

Project Manager: Michael Sadowsky
Description: Aquatic invasive species (AIS) pose a common threat to the health, and the structure and function, of aquatic ecosystems. AIS are recognized as one of the greatest threats to biodiversity, second only to habitat destruction. There are 38 aquatic species that are established or invading Minnesota's waterways, including Eurasian watermilfoil (EWM), quagga and zebra mussels, curly-leaf pondweed, and common carp. Limited options are available to manage AIS established in Minnesota waterways. Microorganisms are closely associated with AIS, and these may include harmless commensal bacteria as well as enteric bacteria and pathogens. This project aims to characterize the total microbial community structure associated with AIS, including zebra/quagga mussels and EWM, in Minnesota waterways across time and space. This will be done using next-generation DNA sequencing approaches of all the microbes associated with specific AIS (termed metagenomics analyses). Sequencing approaches will allow for the characterization and definition of AIS-associated microbes (their microbiota), both within and on AIS, and provide information useful for the potential development of effective biological control agents for their management (a potential Phase II proposal). This will not only provide information on microbes that are symbiotically or pathogenically associated with AIS, but also indicative of potential human health hazards. These studies will put Minnesota at the forefront of this important area of aquatic invasive species research. Project outcomes will provide more insights into conservation practices of native aquatic wildlife and ecological effects of AIS on water quality. We also believe that one of the best approaches to protect and restore native species in Minnesota is to engage the public through outreach programs done in collaboration with The Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota and the MN Department of Natural Resources (DNR).

Summary Budget Information for Sub-Project 2:		
	ENRTF Budget:**	**$299,364**
	Amount Spent:	**$299,364**
	Balance:	**$0**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome Activity 1	**Completion Date**
1. *Sampling collection and water quality monitoring*	September, 2016
2. *Identify microbial community associated with the zebra/quagga mussel using 16S rDNA amplicon-based sequencing approaches*	January, 2017
3. *Correlations of the microbial community to biological characteristics of the zebra/ quagga mussels and aquatic environment*	July, 2017
Outcome Activity 2	Completion Date
1. Sampling collection and water quality monitoring	September, 2016
2. Identify microbial community associated with EWM using amplicon-based 16S rDNA sequencing approaches	January, 2017
3. Correlations of the EWM microbial community to biological characteristics of the EWM and water quality parameters	June, 2017

Sub-Project Status as of *February 10, 2014*
No progress to report as project is not anticipated to start until December 2014

Sub-Project Status as of *August 31, 2014*
The proposal process for this subproject has begun with an estimated start in early 2015.

Sub-Project Status as of *February 28, 2015*
It was hoped that this project could be accelerated to start in December 2014, however this was not possible due to health issues of the PI. The project proposal has now been received and is currently undergoing peer review. Anticipated start time is July, 2015 with a focus on using metagenomics to develop biocontrol strategies for AIS.

Sub-Project Status as of *September 24, 2015*
This subproject was approved to begin June 20, 2015.

Sub-Project Status as of *February 29, 2016*
Work began on this project in earnest. A postdoctoral associate, (Prince Mathai) was hired starting August 31 and an undergrad (Hannah Dunn) has been assisting him with field sampling and processing. Routine EWM sampling was performed at three different sites in Cedar Lake. Sampling commenced in May and ended in October. Field samples were processed in lab for downstream physicochemical and microbiological analyses. DNA extracts from all field (EWM and water) samples were submitted for high-throughput sequencing. Sequencing data analysis will be performed in spring. A 3-month milfoil decay experiment is underway using plants collected from Cedar Lake in November. Cultivation-based experiments will be performed during the spring using frozen EWM glycerol stocks. Zebra and quagga mussels were collected from six lakes (Lake Pelican, Pike Lake, Pepin Lake, Prior Lake, Lake Minnetonka and Lake Michigan) between July and November. Mussels were aseptically dissected in lab and DNA extractions were performed on whole tissues. Protocols have been optimized to ensure maximum recovery of microbial DNA from mussel tissues. DNA samples from mussels will be submitted for high-throughput sequencing in February. New staff have been assigned to the project and updated information has been provided in column A on the attached budget. No amendments are necessary, however, to accommodate these changes.

Sub-Project Status as of *August 31, 2016*
Significant progress has been made in this project and sampling has commenced for this year (Jun - Nov). Sequence analyses have been completed for the samples procured last year and the results look promising. In addition, culture-based experiments were performed on EWM samples from last year. The project has been expanded this year and samples are being collected from 10-15 lakes across Minnesota. Survey trips were made to multiple lakes (35+) in Minnesota to identify sites infested with EWM and zebra mussels (ZM). Ten lakes (Josephine, Vadnais, White Bear, Phalen, Cedar, Minnetonka, Bush, Lower Prior, Holland, and Nokomis) were selected for EWM sampling and 15 lakes (Victoria, Le Homme Dieu, Miltona, Carlos, Cowdry, Lower Prior, Upper Prior, Minnetonka, Vadnais, Ossawinnamakee, Rice, Pelican, Lower Hay, Gull, and Round) for ZM sampling. Field sampling commenced in June and will continue till November. Water and sediment are also being collected from each site. All samples were processed in lab within 24 hours of collection for physicochemical (e.g., nutrients) and microbiological (molecular and culture-based) analyses. DNA extracts (from samples obtained this summer) have been submitted to the UMGC for bacterial (16S rRNA) and fungal (ITS) based high-throughput sequencing. Hannah Dunn was hired as a full-time researcher starting June 1, 2016 to assist the postdoctoral associate (Dr. Prince Mathai) in this project. This information has been updated in column A in the attached budget. No amendments are necessary to accommodate these changes.

Sub-Project Status as of *February 28, 2017*

The project has made significant progress since the last project update and is on schedule for completion in July. Field sampling commenced in June 2016 and continued until November (total six months). EWM, native macrophytes, zebra mussels, sediment, and water were sampled from 25 lakes (ZM project: 15 lakes, EWM project: 10 lakes). Field samples were processed, DNA extracted and high-throughput DNA sequencing of bacteria and fungi (16S rRNA and ITS2) was performed on all samples. Sequencing results showed a distinct clustering of microbes by each sample type. Irrespective of sampling time and location, the greatest number of operational taxonomic units (OTUs) was observed in sediment samples, and the lowest in EWM and ZM samples. Several OTUs were identified that were either specific- or present in higher relative abundance in EWM and ZMs, as compared to sediment and water samples. In addition, culture-based and molecular techniques revealed that EWM harbored elevated levels of fecal indicator bacteria, such as *E.coli* and *Enterococcus*. This means not only are these masses of aquatic plants a nuisance, but they can be human health hazards as well.

Sub-Project Status as of *August 31, 2017*
This project has completed and a final report will be submitted by 9/30/17. We are seeking an amendment to return the remaining balance of $3854 to the MAISRC reserve so that it may be redistributed to other priorities.

Final Report Summary:
Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and invasive mussels pose a serious threat to the health, structure, and function of aquatic ecosystems. Traditional approaches for AIS control, including the use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could potentially be exploited to develop microbe-mediated AIS management strategies. As a first step in identifying potential biocontrols, this project (Phase I) had proposed to characterize the microbial communities (bacterial and fungal) associated with invasive mussels and EWM, across time and space, using amplicon-based high-throughput sequencing approaches. To accomplish this, zebra mussels (ZMs), water, and sediment samples were obtained from 15 lakes twice a year, whereas EWM were sampled from 10 lakes, once a month for six months. Field samples were processed, DNA extracted and high-throughput sequencing was performed on all field samples using the Illumina platform. Sequencing analysis (188 million reads) showed a distinct clustering of each sample type, irrespective of sampling time and location. Core microbial communities were characterized and several taxonomic groups were identified that were either specific or present in high relative abundance in ZMs and EWM, when compared to sediment and water samples. This gives us a promising lead on microbes to purse in Phase II of this study, which will evaluate potential pathogenic characteristics and species- specificity of any pathogens. In addition, our results also indicated that EWM was associated with elevated concentrations of fecal indicator bacteria, such as *E. coli* and *Enterococcus*. This means that not only are these aquatic plants a nuisance, but they may present a hazard to human health as well, especially if they harbor known human pathogens in addition to fecal indicator bacteria. Overall, the results obtained in Phase I have helped to define the distribution of microbes associated with these AIS, and will be useful for the development of future microbiological control strategies (Phase II).

SUBPROJECT 2-2: Delaying the spread of AIS: Metagenomic approaches to develop biological control strategies for zebra/quagga mussels and Eurasian watermilfoil.

Project Manager: Michael Sadowsky
Description: Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and zebra/quagga mussels (ZM/QM), pose a serious threat to the health, structure, and function of aquatic ecosystems. Traditional approaches for AIS control, including the use of chemicals and manual removal, have been mostly ineffective. This problem requires the use of innovative management and eradication tools, such as (micro)biological control strategies. Some microorganisms have evolved to live in close association with aquatic organisms, and these interactions may be commensal, symbiotic, or pathogenic in nature. Such relationships could potentially be

exploited to develop microbe-mediated AIS management strategies. During the first phase of this project (years 1 & 2), we used high-throughput sequencing approaches to characterize the total microbial community (bacterial and fungal) structure associated with ZM/QM and EWM, in Minnesota waterways across time and space. This has provided a distributional map of microbes specifically associated with AIS and these will be key for the development of microbiological control strategies for AIS.

The work proposed in Phase II (years 3 & 4) will build upon the results obtained in Phase I. Specific objectives in Phase II are to: (1) identify and isolate microbes that are potentially pathogenic to AIS, and, (2) evaluate the specificity and effectiveness of potential biocontrol agents in laboratory microcosms. The following activities will be performed to accomplish these objectives: (1) AIS sample collection and processing, (2) isolation and characterization of potential pathogens, (3) challenge/infectivity experiments. The proposed work is about 40% basic, 55% applied research, and 5% outreach in nature. These studies will put Minnesota at the forefront of this important area of AIS research. Project outcomes will provide important information for conservation practices of native aquatic species and management of natural resources in Minnesota.

Summary Budget Information for Sub-Project 2:

ENRTF Budget:**	**$303,217**
Amount Spent:	**$286,610**
Balance:	**$16,607**

Outcome Activity 1	**Completion Date**
1. Collect and process 150 native mussel and macrophyte samples	December 2018
2. Collect and process 100 samples from ZM/QM mortality events	December 2018
3. Collect and process 150 diseased and weevil-infected EWM	December 2018
Outcome Activity 2	
1. Submit 1,200 DNA samples for high-throughput sequencing	December 2018
2. Complete bioinformatics and statistical analyses for 1,200 samples	December 2018
3. Complete targeted cultivation of at least 10 potential AIS-specific pathogens	June 2019
Outcome Activity 3	
1. Test the specificity of at least 10 isolated microbes on select macrophytes and mussels in microcosms	June 2019
2. Test the effectiveness of at least 10 isolated microbes on ZM/QM and EWM in microcosms	June 2019

Sub-Project Status as of *February 28, 2018*
Work began on this project in earnest. Field sampling commenced in July and ended in October. Native plants (seven different species), EWM, water and sediment were collected from the same nine lakes (Josephine, Vadnais, White Bear, Phalen, Cedar, Minnetonka, Bush, Lower Prior, and Nokomis) that were extensively sampled in 2016. Meta data were also measured at each site. DNA was extracted from all samples (n=315) and were sequenced at the University of Minnesota Genomics Center. A few native mussels have also been collected with the help from collaborators at St Anthony Falls Laboratory (SAFL).

Sub-Project Status as of *August 31, 2018*
Significant progress has been made in this project sampling for the 2018 field season commenced in June. Sequence analyses have been completed for all the samples (which included invasive and native macrophytes) procured during the 2017 field season and the results look promising. Targeted cultivation of select microbes has begun based on information obtained from Phase 1. The experimental setup for zebra mussel stress experiments have been completed, which are currently underway. A junior researcher (Jonathan Bertram) was hired on April 16 and replaced Hannah Dunn.

Sub-Project Status as of *February 28, 2019*

Significant progress has been made since the last project update. In particular, several stress experiments were performed on ~2,500 zebra mussels that were collected during the 2018 field sampling season. This was done to develop a disease model for zebra mussels to test the affect of potential biocontrol microbes. Several aquaria were maintained under controlled conditions, and the effect of temperature and salinity on zebra mussel survival was examined. Work is currently underway to elucidate changes within microbial communities associated with these invasive mussels under stressed conditions.

Final Report Summary:
Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and zebra mussels (ZMs) pose a serious threat to the health and function of aquatic ecosystems. Traditional approaches for AIS management, including use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could be exploited to develop microbe-mediated AIS management strategies. As the first step towards the identification of potential biocontrol strategies, microbial communities associated with 'healthy' AIS were compared with that of 'diseased' AIS or to native species. Since no natural diseased mussels were available, we opted to develop an experimental model system, which allowed for the application of different intensities of stress – heat (17, 25, 33℃) and salinity (1.5, 13.5 ppt), to promote the proliferation of opportunistic pathogens. High-throughput DNA sequencing of 414 samples (providing 32 million DNA reads) resulted in the identification of several potentially 'pathogenic' microbial groups that were strongly associated with ZM mortality. These included *Aeromonas*, *Chryseobacterium*, *Flavobacterium*, *Acidaminobacter*, *Clostridiaceae* 1 sp., *Rhodobacteraceae* sp., *Acinetobacter*, *Shewanella*, and *Clostridium sensu stricto* 13. For the identification of EWM-specific microbiota, high-throughput DNA sequencing was performed on 315 samples (46 million reads) derived from leaf and root compartments of EWM and six native macrophyte species. This resulted in the identification of taxa that were significantly enriched in EWM leaves and roots compared to native plants. Though several AIS-associated microorganisms were isolated that could be pathogenic to invasive mussels (e.g. *Aeromonas*) - none of them met our safety requirements for further testing. Future studies must isolate and evaluate the efficacy of 'host-specific and pathogenic' biocontrol candidates that will only infect invasive mussel species.

SUB-PROJECT 3. Reducing and controlling AIS: Attracting carp so their presence can be accurately assessed

Project Manager: Peter Sorensen
Description: The Sorensen lab group is currently developing a scheme to prevent adult bigheaded (invasive) carp from migrating upstream from the lower Mississippi River in numbers sufficient to create a self-sustaining population in Minnesota waters. This scheme relies on deterring adult carps from moving through lock and dam structures by developing acoustic deterrents that can be added to locks while developing an understanding of carp behavior and water flows sufficient to guide changes in gate operations to create water velocities that can hold carp back without affecting other fishes or dam scour. This scheme relies on having extremely accurate and precise information on the abundance of adult invasive carps in the immediate vicinity of the locks and dams because altering gate operation needs to be as strategic and efficient as possible. Information on the abundance of invasive carp could of course, also eventually be used by the DNR for possible removal efforts. Our ongoing work also shows that while current monitoring technologies for carps are all extremely poor (unquantifiable), measurement of the DNA released by fish (eDNA) has excellent potential if problems associated with its current inability to measure scattered carp located even modest distances away from sample points because of rapid dilution and degradation could be solved. eDNA alone is also limited because it cannot provide information on carp sexual maturity, information of critical importance at the invasion front. This proposal will attempt to remedy these deficiencies by developing new techniques to cause predictable aggregations of adult invasive carps to facilitate their accurate measurement using a combination of measurement techniques that include eDNA and pheromones, the latter of which could provide information on

fish maturity to compliment the former. Research examines the potential of using sexual and feeding cues to cause aggregations. We examine both the possibility of using live sterile carp releasing sexual cues ("Judas fish") and sex pheromones to locate and drive aggregations. Food and food chemicals will also be tested. They have promise because carps have unique food preferences that differ from native fishes. Research uses common carp locally to develop concepts with additional, complimentary studies of Bigheaded carp planned out of the state where such test are possible. While several approaches will be examined initially, the project will be modified to focus on the most promising attributes if appropriate. A possible second phase of this project could explore implementation of the most promising option(s) in 2018.

Summary Budget Information for Sub-Project 3:

ENRTF Budget:**	**$682,969**
Amount Spent:	**$663,719**
Balance:	**$19,251**

***This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.*

Outcome Activity 1	**Completion Date**
1. *Establish a pheromone baiting and tracking system in a lake for common carp that might also be used bigheaded carps.*	Jan 2016
2. *Complete sample collection for common carp sex pheromones and eDNA in a lake and conduct initial analyses.*	July 2016
3. *Determine to what extent sexual stimuli (Judas fish and/or sex pheromones alone) can reliably induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds.*	Jan 2017
4. *Identify specific approaches by which sex stimuli might be used to induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds that can be measured.*	July 2017
5. *Final report that describes a recommended scheme for using food-based and/or sex based attractant system that can reliably induce carp aggregations and then measure them using eDNA, sex pheromones and/or other techniques (matches Outcome #5 in Activity #2)*	Jan 2018
Outcome Activity 2	**Completion Date**
1. Establish a food baiting and tracking system in a lake for common carp that might also be used for bigheaded carp.	Jan 2016
2. Develop a baiting strategy using feeding stimuli to induce aggregations of common carp that can be measured.	July 2016
3. Determine to what extent feeding stimuli (food and/or its odor) can reliably induce aggregations of common carp and bigheaded carp in lakes and/or ponds that can be measured.	Jan 2017
4. Identify specific approaches by which food stimuli might be used to induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds that can be measured.	July 2017
5. Final report that describes a recommended scheme for using food-based and/or sex based attractant system that can reliably induce carp aggregations and then measure them using eDNA, sex pheromones and/or other techniques (matches Outcome #5 in Activity #1)	Jan 2018

Sub-Project Status as of *February 10, 2014*
No progress to report as initial work is being funded with 2012 ENRTF funds through June 2015

Sub-Project Status as of *August 31, 2014*

The proposal process for this subproject has begun with estimated project start in Summer 2015 after related ENRTF 2012 project funds have been spent down.

Sub-Project Status as of *February 28, 2015*
This project proposal has been received and is currently undergoing peer review. This sub-project was envisioned to build upon and continue research being conducted as part of the ENRTF 2012 work plan, once those prior phases were complete. Work on subproject 3 would therefore begin July 2015 or as soon as work is completed and ENRTF 2012 funds for activities 3, 4, 5 and 6 are spent down. The outcome table above will be revised once a final workplan for this sub project is approved.

Sub-Project Status as of *September 24, 2015*
This subproject was approved to begin July 9, 2015.

Sub-Project Status as of *February 29, 2016*
Experiments were conducted late summer 2015 in two local lakes to test food and pheromones as attractants to drive common carp aggregation, so that carp density might be measured more accurately using DNA and/or pheromones. While data is still being analyzed, it is clear that food was able to drive large aggregations of common carp, especially at night. We have been able to measure these aggregations using both eDNA and a pheromone using novel techniques and with greatly enhanced sensitivity. We tested ways to add pheromones by implanting female carp with pheromone precursor (a hormone) and tracking them and males using radio-tags. This data look promising but are still being evaluated. Means to add cues, track fish and measure their presence is largely established; work is ahead of schedule. Plans for next summer will be formulated once we have all the data analyzed.

Sub-Project Status as of *August 31, 2016*
Work is ahead of schedule. Water samples collected for eDNA and pheromone evaluation were completely analyzed and a baiting scheme perfected. Experiments conducted last summer to test whether food and pheromones could be used as attractants to drive common carp aggregation have now been analyzed; both were highly successful. In one experiment, we were able to attract a third the population of mature common carp to a specific location within a lake using food while measuring carp abundance using both eDNA and a sex pheromone with a level of sensitivity, precision and accuracy previously unseen. Pheromone-releasing Judas carp were also attractive. A third study successfully measured common carp mating pheromones in waters near mating carp. Finally, a pilot study using food to attract Bigheaded carp was completed in Illinois with the University of South Illinois as collaborators. Whether this behavior enhanced our ability to measure them using eDNA or pheromones (as shown with carp) is presently being evaluated. In sum, experiments are promising and work is ahead of schedule and we likely will be able to determine whether food stimuli or pheromones are most promising for use in invasive carp control by the next report when an amendment with a possible rebudget may be requested.

Sub-Project Status as of *February 28, 2017*
Work is on schedule. An experiment was conducted to determine whether adult male common carp can be attracted to pheromones in small ponds (Activity 1). Pilot data suggest that they can so a final experiment is now planned for spring 2017. Analyses of common carp induced to aggregate around pheromone-implanted Judas fish are also nearly complete. Another experiment was conducted to determine whether adult silver carp can be attracted to food in small ponds (Activity 2). Once again the results were positive so this experiment will be repeated as well next spring. As eluded to in the previous report, a re-budgeting and amendment is proposed and is pending. A meeting to discuss the update with LCCMR has been set.

Sub-Project Status as of *August 31, 2017*
Research is proceeding well and is on schedule. Three specific approaches to use sex pheromones as attractants have now been identified while two approaches have been identified for using feeding stimuli. Experiments on

these approaches are nearing completion. Briefly, for Activity #1 (tests of pheromones) since our last update (April 2017), we conducted a new experiment using pheromones for silver (invasive carp) in Illinois which while promising, suggests food stimuli might work best for attracting this species. Data is also now fully analyzed showing pheromone-implanted common carp can be used as Judas fish. One more field experiment is planned with common carp pheromones this summer. Meanwhile, for Activity #2 (tests of food stimuli), we have now identified using a food reward/training strategy as the most promising and have completed all experiment for common carp and most of the data analyses for this successful experiment, and recently completed a new final experiment for silver carp in Illinois. Data will be analyzed by the next report on this project in a year during which time we may (if reasonable) examine training and pheromone identity to allow data to be fully understood. Our new Activity #3 on sound deterrents started 3 weeks ago (no data to report yet).

Sub-Project Status as of *February 28, 2018*
Research is proceeding well and is ahead of schedule. Work on using sex pheromones and food as attractants carp (Activities 1 and 2, respectively) is now complete and a final report is being prepared which will be formally described in the next update as scheduled. Meanwhile, Activity #3 is proceeding very well. We have now finished testing the effects of linking two different sounds to an air curtain to determine how well they function as a single unified deterrent. Remarkably, unified systems are consistently able to stop close to 99% of all bighead and common carp in the laboratory with no indication of habituation (diminished efficacy with time). A sweeping (pulsed) sound (provided by Fish Guidance Systems Ltd) is more effective than a continuous broadband sound (outboard motor). Full descriptions of this work will be submitted for publication in a peer-reviewed journal within the month and have also been thoroughly vetted by the US Fish and Wildlife Service which is now making plans for full implementation of an integrated system in a large river(s). Meanwhile, the LCCMR has recommended that the state legislature fund tests of the sound we have identified as having greatest promise in Minnesota waters (Lock and Dam #8). With the submission of the final activity report on pheromone and food attractants next June, we will likely request re-budgeting and an amendment to move any possible residual funds to Activity 3 where they can be used to accelerate this important work and test more native species.

Sub-Project Status as of *August 31, 2018*
Work is ahead of schedule. Final reports were submitted for both Activity 1 (Sex attractants) and Activity 2 (Food attractants). Briefly, these studies demonstrate that while sex attractants (pheromones) have promise for attracting (and controlling) male common carp when they are present at low densities, food attractants have exceptional promise to attract and control both male and female carps when they present are at high densities. Further, food can be deployed at relatively low cost. A manuscript has been published in a peer-reviewed journal about food attractants and has been favorably received. Meanwhile, work for Activity 3 is ongoing and showing that light is a strong repellent for carp. A manuscript has been submitted on earlier sound work. Plans are now proceeding to test the sound-air curtain-light deterrent we have developed in the laboratory with ENRTF funds. Tests are planned both in Minnesota waters of the Mississippi River (ENRTF funding) and in the Tennessee River (US Fish and Wildlife Service funding) in 2019. Both field studies would benefit greatly from increased understanding of native fish responses to these stimuli (we are getting many requests from the MN DNR and USFWS).

Sub-Project Status as of *February 28, 2019*
Work is on schedule. Activities 1 and 2 are complete and focus is now on Activity 3. Studies show that both native lake sturgeon and bluegill sunfish are little affected by a sound stimulus alone (unlike carp which are deterred by sound) but are deterred by sound when combined with bubbles. Initial additional tests with strobe lights alone are promising as they show species specific effects dependent upon background lights levels.

Final Report Summary:
This project developed several tools that can manage and control all species of invasive carp species in Minnesota. First, we developed ways using both food and sex pheromones to attract and measure the presence

and density of carp using the environmental DNA (eDNA) they release to the water. This technique is superior to traditional netting because it can be performed in any habitat or water of any depth, including at low densities that are otherwise unmeasurable. eDNA can also determine carp gender. Second, we developed a deterrent system comprised of sound, light and air curtain that is 97% effective in the laboratory and could safely and effectively prevent invasive carp from swimming upstream through navigation locks in Mississippi River. If this deterrent system were to be paired with attractant-based eDNA surveillance methods in specific lock-and-dams whose gate was also adjusted to stop carp, it is extremely likely that enough carp could be prevented from passing through these lock-and-dams that the remainder could be removed by targeted commercial fishing. Field tests of the deterrent system are now underway.

SUB-PROJECT 4-1: Reducing and controlling AIS: Common carp management using bio-controls and toxins

Project Manager: Przemek Bajer
Description: Common carp (*Cyprinus carpio,* or 'carp'), an invasive fish from Eurasia, dominates lakes of south-central Minnesota. The carp 'flip' shallow lakes into turbid, non-vegetated basins and by doing so destroy feeding and breeding grounds that were once used by waterfowl. The carp also reduce recreational use of lakes by increasing water turbidity. Attempts to control carp in Minnesota date back to 1930s when large seine nets, or rotenone were used to rid lakes of carp. Those simplistic effort brought dissappointing results, however, as they were not backed by solid science on processess that drive carp abundance. Currently, carp are managed in only a handful of waterfowl lakes that can be drained and frozen to the bottom. No management is conducted in recreational lakes to improve water quality for swimming or fishing.

The last decade resulted in several studies that rekindled the hope for managing common carp using more sustainable approaches. Bluegill, a very abundant native fish, was shown to consume carp eggs and larvae and suggested to function as a carp biocontrol agent in Minnesota lakes. Patterns in young-of-year carp abundance throughout the state lead to a hypothesis that bluegills (along with other native fish) might be able to control carp's reproductive success in most lakes, except those that winterkill (and lack bluegills) or those that are extremely productive where carp larvae might grow fast enough to escape predation. We propose whole-lake experiments to test whether bluegills might indeed be an effective biocontrol agent for the common carp in moderately-productive and very productive lakes (Objective 1).

In lakes where biocontrol strategies are less likely to be successful (e.g. winterkill-prone lakes where bluegill densities are chronically low), carp could be managed using a different approach. The unique diet of carp (plant seeds such as corn) and the fact that these fish can be trained to aggregate in baited areas creates an opportunity for management using toxins that could be delivered specifically to carp by placing them inside pellets that only carp consume. Further, such pellets could be placed in on-demand feeders such that they would only be dispensed if actively consumed by carp. It has already been shown that carp can be trained to aggregate in specific areas of lakes using corn. Once trained, the carp come to the baited sites at night and consume large quantities of corn, which does not attract native fish. These fish could potentially be controlled by then switching the bait for one that contains a fish toxin that the carp are unable to detect. Antimycin a, a natural fish toxin (a fungicide produced by bacteria) discovered in 1940s and currently used in aquaculture and investigated for Asian carp control, could be used as an active ingredient of common carp pellets. Antimycin is seemingly undetectable by carp and could be incorporated into corn-pellets allowing for "bait and switch" strategies. We propose a pilot study in collaboration with USGS in LaCrosse, WI to test the feasibility of such control strategy in laboratory tanks and experimental ponds (Objective 2).

Summary Budget Information for Sub-project 4-1:	**ENRTF Budget**:**	**$384,231**
	Amount Spent:	**$384,231**
	Balance:	**$0**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome Activity 1	Completion Date
1a. Study lakes for bio-control experiment selected. 1.b List of winter aeration lakes compiled.	2/28/16
2a. Bio-control Experiment started in 4 lakes. 2b. Winter aeration data set developed to select complete-case lakes.	7/31/16
3a. Biocontrol experiment completed in 4 lakes, preliminary analysis completed. 3b. Model selection analysis of common carp recruitment in lakes with or without winter aeration completed.	2/28/17
4a. Biocontrol experiment in 2-3 additional lakes in-progress or completed (the completion date will likely be 9/30/17). Data from both seasons analyzed. Report written. 4b. Analysisof comon carp recruitment in lakes with winter aeration completed, report written.	7/31/17
Outcome Activity 2	**Completion Date**
1. Experimental ponds selected. Experimental design finalized.	2/28/16
2. Experiments 1-4 in progress	7/31/16
3. Experiments 1-4 finished	2/28/17
4. Data from Experiments 1-4 analyzed. Report written.	7/31/17

Sub-Project Status as of *February 10, 2014*
No progress to report as project is not anticipated to start until approximately March 2015

Sub-Project Status as of *August 31, 2014*
The proposal process for this subproject has begun with estimated start Spring 2015.

Sub-Project Status as of *February 28, 2015*
Dr. Przemek Bajer has been identified as the project manager to lead this subproject. Due to existing common carp control research commitments, the PI elected to submit his proposal in January, 2015. The proposal has now been received, is currently undergoing peer review, and is anticipated to start in July 2015. The topic of the proposal is developing control approaches for common carp in shallow lakes, including use of a species-specific toxin for common carp in hypoxia- prone lakes. Previous work by the PI and other team members has focused on control approaches for larger lakes. The outcome table above will be revised once a final workplan for this sub project is approved.

Sub-Project Status as of *September 24, 2015*
This subproject was approved to begin July 7, 2015

Sub-Project Status as of *February 29, 2016*
Research continues to progress and outcome goals have been achieved. Experimental lakes were selected for experiment 1a. Monitoring will continue over the winter and planning will be done for stocking and monitoring these lakes in the spring. For activity 1b, winter aeration data from aeration permit surveys were compiled. Surveys were paired with DNR fish assessments. The number of fish assessments that paired with aeration surveys proved to be too few in number to analyze. A higher-resolution case-study approach is now being pursued. For activity 2, experimental design has been finalized for activity and experimental ponds have been selected at the USGS facility. Activity 2 experiments will begin in the spring. A detailed account of each activity follows.

Sub-Project Status as of *August 31, 2016*
Research continues to advance and outcome goals have been achieved. Experiments are underway for activity 1a. Common carp has been stocked in all four ponds and bluegill sunfish has been stocked in two of the four ponds as planned. Carp spawning has been observed in all ponds. Egg enclosures were used to assess egg density in all lakes. Larval tows have been taken to assess larval density. Backpack electofishing surveys have been done and continue to be conducted to get catch-per-unit-effort estimates for young-of-year carp. Water quality assessments have continued throughout the project to document productivity and zooplankton abundance (food for larval carp). Activity 1b has been adapted to allow analysis of a higher quality dataset. After compiling and assessing the winter aeration dataset, it has been concluded that the data is not of high enough quality to allow for statistical analysis. Instead, we will use a new dataset (MN DNR lake surveys) to assess lake characteristics (depth, size, productivity, etc.) that affect bluegill sunfish densities, especially the ones that cause low densities. This will determine which lakes are capable of supporting bluegill populations to control common carp. This analysis will indicate the extent to which the findings from Activity 1a can be used in lake management. Corn-based bait containing antimycin has been formulated for Activity 2, and has been shown to be lethal to common carp through preliminary gavage studies. Leaching experiment has been conducted by the USGS lab and showed that leeching is occurring at rates higher than expected. The bait is currently being re-formulated. Fish have been stocked for the species specificity trials and are currently acclimating to tanks. A detailed account of each activity follows.

Sub-Project Status as of *February 28, 2017*
The 2016 field season has ended and data are currently being analyzed. Outcome goals have been achieved, or exceeded. Activity 1a has concluded in all four experimental ponds. We used electrofishing, trap netting and seining to obtain mark-recapture estimates of the young-of-year (YOY) carp in each pond. We found that the two ponds without bluegill sunfish had approximately 6.5 times more YOY carp than ponds with bluegill. Preliminary analyses are completed. For activity 1b, the analysis of bluegill sunfish abundance (carp biocontrol) in lakes of southern Minnesota is currently underway. A linear model and a random forest analyses have been conducted to determine which lake types have strong carp biocontrol in Minnesota. For activity 2, control of common carp using antimycin-laden bait, has concluded. All four experiments have been conducted, and data has been analyzed. A manuscript that we anticipate submitting in February is in preparation. Our results suggest that ANT-impregnated bait has potential to target carp without harming most native species. A detailed account of each activity follows.

Final Report Summary:
Two practical control methods for the common carp were explored in this project. First, the ability of bluegill sunfish to control carp populations was tested in whole-lake systems (6 small lakes). All lakes were stocked with adult carp and every other lake was also stocked with bluegill sunfish to create a control/treatment design. Carp offspring survival was assessed in each pond at the end of the season through backpack electrofishing surveys and mark-recapture analyses. Results indicated that lakes containing bluegills had, on average, 11 times fewer carp offspring than ponds lacking bluegills. Our results indicate that biocontrol by bluegill is an important element of common carp control strategies. This might require efforts to strengthen bluegill populations, for example by aeration if feasible, in shallow lakes that are prone to winter hypoxia. Second, strategic use of oral toxicants could allow for practical control schemes for common carp if a toxicant selectively targeted the carp and not native species. In this study, we incorporated antimycin-a (ANT-A), a known fish toxicant, into corn-based food pellets and conducted a series of experiments to determine its toxicity, leaching rate, and species-specificity. First we determined that the bait caused no mortality among carp or native fish due to toxin leaching into the water, which was the desired outcome. Then we conduced lab species-specificity trials where carp were stocked with native species representing families that often occur with carp in our study region: the fathead minnow, yellow, and bluegill. These trials showed high mortality of carp (46%) and fathead minnows (76%) but no significant mortality of perch or bluegill. Finally, a pond study, which used the same species composition except for fathead minnows, resulted in 37% morality among adult carp and no mortality among perch or bluegill. Our results suggest that corn-based bait that contains ANT-A could be used to selectively control carp in

ecosystems dominated by bluegill or perch, such as most lakes in south-central Minnesota. However, further work is needed to ensure that native minnows are not affected by this control strategy. Bait size, texture and application (e.g. only in places and times of day when carp were trained to aggregate) could all be used to further increase species-specificity of this promising control method.

Phase 1 is now completed. We are requesting that the remaining balance of $29,016 be moved back into the MAISRC reserve to be reallocated to other priorities.

SUB-PROJECT 4-2: Reducing and controlling AIS: Common carp management using bio-controls and toxins

Project Manager: Przemek Bajer
Description: This project aims to develop two new strategies to control the invasive common carp (*Cyprinus carpio,* or 'carp') in Minnesota. First, we will determine if carp can be controlled by native fish that consume carp eggs and larvae. Second, we will assess whether an existing fish toxin (Antimycin – A) could be incorporated into food pellets (bait) readily consumed by carp but not by native fish to selectively target carp populations.

Common carp (or 'carp') is one of the world's most invasive fish. This species is very abundant across south-central Minnesota where it has been causing extensive damage to lake ecosystems by uprooting aquatic vegetation and increasing water turbidity. Due to its pervasiveness, carp is an important driver of the decline in the abundance and biodiversity of aquatic plants, insects, waterfowl, amphibians, and possibly also fish across south-central Minnesota. The carp can also reduce recreational use of lakes in Minnesota by increasing water turbidity and stimulating blooms of cyanobacteria. Carp management has been traditionally conducted using large nets that are deployed to remove under-ice aggregations of these fish. While this can be effective, it alone is not able to affect sustainable management in most ecosystems. Rotenone (toxin that is pumped to lakes to kill all fish not just carp) and water draw-downs have also been used to eradicate carp, but these efforts are usually short-lived, very expensive, harmful to native biota and possible in only a small number of lakes.

Research on common carp over the last decade suggested new possibilities for sustainable management. Studies in lakes in Minnesota suggested that many populations of carp can be controlled by native fishes, such as bluegill, that consume large quantities of carp eggs and larvae. For example, lake surveys showed lack of yearling carp in systems dominated by bluegills and high abundance of yearlings in winterkill marshes that lacked bluegills. Experiments in artificial enclosures showed that bluegills can reduce production of young carp by ~ 5-fold. These findings led to Phase I of this project, which used whole natural lakes to test if bluegills could indeed act as biocontrol for common carp. We began testing this hypothesis in four small natural lakes (~ 1 ha) in 2016. These tests were quite promising and showed that lakes stocked with bluegills produced 5-7 times fewer yearling carp than control lakes. We will continue this work in Phase II by conducting experiments in 4 to 6 more small lakes (Activity 1).

A second very promising control strategy is to develop toxic bait that can be delivered selectively to carp and not the native fish or other organisms. The unique diet of carp (plant seeds such as corn) and the fact they can be trained to aggregate in areas baited with corn creates an opportunity for managing carp using oral toxicants incorporated into corn-based bait. Antimycin A (ANT-A), which is a natural toxin produced by soil bacteria, has been identified as a toxicant that could be used for such purpose. ANT-A is highly toxic to fish (including carp), but less so to higher vertebrates that might consume dead fish (see risk considerations below). If unused it breaks-down relatively quickly in the environment (see below), has non-toxic metabolites, and low leaching rate. In Phase I, we conducted four pilot experiments to test the hypothesis that carp could be selectively targeted by using a corn-based bat with ANT-A. We conducted a gavage experiment that showed that a concentration of >=4 mg/kg of ANT-A was toxic to carp. Leaching trials showed no fish mortality and suggested that less than 0.01% of ANT-A leached into the water over 72h. Laboratory trials with mixed species resulted in 46% carp mortality after single feeding, but no significant mortality among bluegill or yellow perch. However, fathead minnows—a member of the cyprinid family —also died in the lab experiment because their diet is similar to carp's. Finally, pond trials with mixed species showed mortality among carp (37%) but not among perch or bluegills. Overall, these results were positive and suggested that corn-based pellets with ANT-A could be used to selectively control carp. In Phase II, we propose expansion of these experiments into larger ponds and lakes by conducting three activities (Activities 2-4). Activity 2 will use a lab experiment to determine if carp can detect presence of ANT-A in bait. Activity 3 will use large earthen ponds to test if carp, and not native fish, can be selectively targeted using bait containing ANT-A. Activity 4 will be conducted in a natural lake to determine if carp, and not native fish, can be selectively attracted to bait/food pellets (without ANT-A) to optimize the delivery of toxic bait in future real-life applications.

Summary Budget Information for Sub-project 4-2:

ENRTF Budget:**	**$406,000**
Amount Spent:	**$348,913**
Balance:	**$57,087**

Outcome Activity 1	**Completion Date**
1. Biocontrol experiment completed in 4 to 6 additional lakes; carp recruitment quantified in bluegill and control treatments using CPUE and mark recapture. Experiment concludes, preliminary data analysis completed.	January 31, 2018
2. Final data analysis for biocontrol experiment completed. Report written. Activity completed.	July 31, 2018
Outcome Activity 2	**Completion Date**
Lab test verifies whether carp can detect presence of lethal concentrations of ANT-A in corn pellets	January 31, 2018
Results analyzed, final report written.	July 31, 2018
Outcome Activity 3	**Completion Date**
Pond experiments conducted to test species-specific control of common carp	January 31, 2018
Results of pond experiment analyzed. Final report written. Publication in preparation or submitted.	July 31, 2018
Outcome Activity 4	**Completion Date**
A list of potential study lakes compiled.	January 31, 2018
Study lake selected for Objective 4. Implanting fish with radiotags and PIT tags under way.	July 31, 2018
Lake experiment finished to test if carp can be targeted in species-specific manner	January 31, 2019
Results analyzed. Final report written. Publication in preparation or submitted.	July 31, 2019

Sub-Project Status as of *February 28, 2018*
All activities are proceeding as planned. To address Activity 1, we conducted an experiment in 6 lakes in 2017. The experiment showed that the abundance of post-larval carp (life stage directly affected by bluegills) was ~ 10 times lower in lakes stocked with bluegills than in control lakes. To address Activity 2, we conducted a laboratory experiment using 34 young-of-year carp that were fed either control pellets (cracked corn) or pellets containing a lethal amount of toxin (corn and Ant-A). The carp consumed control pellets at the same rate as the toxic pellets suggesting that they cannot detect the presence of ANT-A in the pellets or do not show adverse behaviors towards it. To address Activity 3, we conducted an experiment in six ponds at USGS, La Crosse. In these ponds, carp were stocked with three species of native fish (bluegills, yellow perch, white suckers). All fish were implanted with electronic tags to monitor whether they visited a site where carp bait (corn pellets) was placed daily. The bait was then replaced with one that contained toxin (ANT-A) for 2 days and mortality among all fish was recorded. Our preliminary results suggest that only carp (~ 25%) perished in each treatment pond, with the exception of three white suckers that also perished but for reasons that are most likely unrelated to the use of toxic bait because they had no trace of bait in their intestines. This suggests that carp could be targeted with relatively high specificity using corn pellets that contain lethal amounts of ANT-A. To address activity 4, we compiled a list of lakes to conduct an experiment in the summer of 2018.

Sub-Project Status as of *August 31, 2018*
Activities 1, 2, 3 are completed. Data analyses have been finished and manuscripts are in final stages of preparation. We expect to submit two manuscripts (Activity 1, and Activity 2 and 3 combined) by the end of the summer. Activity 1 (experiment in 6 small lakes) showed that bluegill sunfish can suppress (8-fold difference) the production of young common carp in shallow lakes. Activity 2 (laboratory experiment) showed that common carp are unable to avoid food pellets that contain a toxin (Antimycin A). Thus, such pellets could be used for carp control. Activity 3 (toxin experiment in 6 earthen ponds) showed that corn-based food pellets that contained antimycin A might be used to selectively target common carp as no evidence was observed that native fish (white suckers, yellow perch and bluegills) consumed the pellets, while carp did.

Activity 4 (test of corn-based carp bait in a whole lake) is just beginning. This experiment will start in August and will run through the end of October 2018. We are currently in the process of finalizing lake selection, manufacturing experimental arenas (PIT antennas) and are getting ready to install them in our study lake. We will then tag carp and native fish in early August and the experiment will commence.

Sub-Project Status as of *February 28, 2019*
Results of Activity 1 (biocontrol experiment in 6 small lakes) have been submitted for publication to PLoS One and accepted pending revisions. Manuscript summarizing the results of Activity 2 and 3 is complete and has been submitted to USGS (our co-authors) for internal review before submitting to a journal.

To address Activity 4 (test of corn-based carp bait in a whole lake), we conducted an experiment in Long Lake during last summer and fall. Over 400 carp and over 800 native fish were implanted with passive integrated transponders (PIT tags). We then selected a site in the lake that was baited with cracked corn for over a month while electronic antenna positioned at the bait continuously monitored which fish visited the bait and when (another un-baited site was used as control). Underwater camera was also installed at the bait. The response of carp to baiting was immediate. The number of carp at the bait increased over 10 folds within 48h. We were attracting ~1,600 carp to the bait each day (10% of population). Native fish were not attracted to the bait (<1% of fish detected by PIT antennas or seen on the camera). Our results suggest that corn can be used to selectively attract large numbers of carp. Toxins could be incorporated in corn-based food pellets to control carp (Activity 2 and 3). Alternatively, the carp that aggregate at the bait could be captured in nets.

While Activity 4 has been progressing as scheduled, the Long Lake experiment revealed unexpected findings about the behavior of individual carp. At the onset of the experiment we hypothesized that once carp find the bait, they would return to it consistently. That was not the case. 68% of the carp returned to the bait less than 3 times and only 7.9% or carp returned to the bait consistently. We concluded that even though some carp learned the location of the bait, they were not willing to compete for restricted access to bait with other carp - underwater videos showed 100s of carp competing for access to the bait. Only the “boldest” carp were willing to access the bait each day and compete with other carp for access. The hypothesis that carp populations are comprised of “bold” and “shy” individuals is strongly supported by the literature. We hypothesize that increasing access to bait (multiple and larger baited sites vs. one small site) might result in consistently attracting larger numbers of carp to the bait, which has strong management implications. Further, once management (removal) begins, it might be beneficial to release the bold carp back to the population, because those fish may be key in bringing other carp that are yet unfamiliar with the bait to the baited site using group learning strategies.

Final Report Summary:
This project aimed to test new management tools for the common carp, Minnesota’s most abundant invasive fish. We used a whole lake experiment to test if bluegill sunfish can reduce production of carp fry in shallow lakes (Activity 1). We also used a series of lab, pond and lake experiments to test if corn-based food pellets that contain a toxin can be used to selectively target carp without harming native fish (Activities 2, 3, 4). Activity 1 (bluegill experiment in 6 small lakes) showed that bluegills can suppress the production of carp fry in shallow

lakes by 8-fold. Thus, maintaining healthy bluegill populations in lakes would serve as an important biocontrol strategy for carp in Minnesota.

Activities 2, 3, and 4 showed that common carp readily consume corn pellets that contain a toxin (Antimycin-A, ANTA) and cannot distinguish between pellets with or without the toxin. Further, in a pond experiment with carp and three native species (white sucker, bluegill, yellow perch), only carp ate the toxic pellets and perished. Finally, in a natural lake experiment where we tagged nearly 500 carp and 900 native fish, only carp were attracted to corn-based pellets (we did not use toxin in the lake experiment). This was further verified using underwater cameras. Overall, corn-based food pellets appear to be very powerful and relatively species-specific attractant for carp. Toxins, such as ANTA, could be incorporated into such pellets to target carp. Our work also showed that corn (without toxin) can be used as bait to train carp to form large feeding aggregations that could be targeted using simpler and safer means than toxins, such as nets.

Future directions might include: 1) Focusing on risks and costs associated with using corn-based pellets that contain ANTA or other toxins to control common carp, 2) Focusing on how baiting with corn can be used to induce large feeding aggregations of carp than could be removed with nets. This is being addressed in Phase III.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants.

Project Manager: Ray Newman
Description: University of Minnesota professor and invasive plant expert, Dr. Ray Newman (0.08 FTE for 5.5 years), will work with the DNR to evaluate extant and new strategies to control submersed invasive plants selectively in ways that will also restore native plant communities. This work can start as soon as peer-review is complete (2013) because Dr. Newman is on staff. A full time postdoctoral fellow (1.0 FTE for 5.5 years) or equivalent will be hired to assist with this sub-project along with part-time undergraduate student(s) (0.25 FTE for 5 years). The Center truck and boat will also be available. Strategies proposed for invasive plant control will include use of native herbivorous insects, integrated management with selective chemical or mechanical controls, and techniques to enhance native plant communities. Working with the DNR, at least one chemical treatment to control a species of invasive plant will also be examined and ecological effects will be evaluated. The focus will be a large-scale, multi-lake manipulation to determine if altering fish community structure can be accomplished to enhance the biological control of Eurasian water milfoil with milfoil weevils, a species of native herbivorous insect. Previous research funded by ENRTF has shown weevils can control water milfoil if sunfish do not consume the weevils. Our bio-control experiment will determine if we can reduce sunfish populations and enhance herbivore populations to control milfoil. The sub-project will proceed in several steps, with tentative outcomes listed below. Specific details will be determined by Center-led peer-review process. This description and the outcomes below will be updated following approval of a more detailed subproject work plan and budget.

Summary Budget Information for Sub-Project 5:	**ENRTF Budget**:**	**$194,415**
	Amount Spent:	**$194,415**
	Balance:	**$0**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome Activity 1	Completion Date
1. *Obtain, collate and compile existing data on curly leaf pondweed*	15 April 2015
2. *Analyze factors influencing curly abundance among years and lakes*	15 April 2016

3. *Identify other collaborative projects on integrated control of submersed macrophytes for future development*	31 July 2016
4. *Write final report or article for publication on factors influencing the abundance and successful selective control of curlyleaf pondweed*	31 Dec 2016
Outcome Activity 2	**Completion Date**
1. *Sample survey lakes to determine relationships between herbivores and milfoil and to identify candidate lakes for future manipulations*	August 2016
2. *Conduct enclosure experiments to determine effect of sunfish density on herbivores and milfoil abundance*	September 2016
3. *Submit proposal for phase 2 research to manipulate sunfish populations to enhance biocontrol of milfoil in several lakes*	September 2016
4. *Analyze results and produce final report on the effects of sunfish on herbivore density and recommendations for methods to enhance herbivore density and biological control of Eurasian watermilfoil*	December 2016

Sub-Project Status as of *February 10, 2014*
A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of *August 31, 2014*
A workplan and budget for this subproject were approved July 31, 2014 and initial work is now underway.

Sub-Project Status as of *February 28, 2015*
As reported in the sub-project's January 31, 2015 update: Project planning is underway. The postdoctoral position was advertised internationally and an offer has been made to a postdoctoral candidate. Progress has been slow due to a delay in hiring the postdoc. Once the postdoc is onboard we will be able to more aggressively collect and collate data sets on curlyleaf pondweed (Activity 1) and to begin planning, permit and equipment acquisition for the summer fieldwork and experiments in Activity 2.

Sub-Project Status as of *September 24, 2015*
Postdoc Adam Kautza was hired and started work in March. Queries for curlyleaf pondweed data sets were sent out and we have identified at least 40 lakes that have potentially suitable surveys. We will follow up again with non-respondents and partial respondents this fall after the 2015 field season wraps up to obtain and collate all available data for analysis this winter. Undergraduate assistants were hired in May and field equipment and supplies were acquired and assembled. Weevil/herbivore surveys have been conducted on 14 lakes and point intercepts on three lakes. Early summer weevil densities appear lower this year than in some previous years but mid-summer surveys will provide a better assessment of trends this year. Enclosures have been deployed in Peltier Lake (Anoka County) and Cedar Lake (Hennepin County) and sampling for sunfish diet assessments has begun.

Sub-Project Status as of *February 29, 2016*
We have received and collated curlyleaf pondweed datasets for 57 lakes from state and county agencies, watershed districts and consultants. We are still waiting on several important data sets before beginning analysis. Data that have been received are organized and we have had several preliminary discussions regarding analytical approaches.

Eight of the 14 lakes surveyed for weevils/herbivores were resurveyed in August and/or early September. The trend of lower than average weevil densities this year continued in 5 of the 8 resurveyed lakes; only 3 lakes showed an increase in weevil densities in mid- to late-summer.

Enclosures and adjacent control plots were surveyed for weevils and plants, from late July/early August through early October. Diets were collected from sunfish at Peltier and Cedar, and four additional lakes.

Sub-Project Status as of *August 31, 2016*
Compilation of the curlyleaf pondweed data sets and ancillary data was completed and analyses conducted. An abstract was submitted and accepted, and a talk was given on the analysis and results at the Aquatic Plant Management Society meeting in Grand Rapids, MI in July. After resolution of some analysis questions, a manuscript will be developed for submission to an aquatic plant or lake management journal. The technician (Researcher 1) joined the project in mid-May to lead field activities.

After reconnaissance of several lakes we decided to again use Cedar and Peltier Lakes for enclosure experiments. The enclosures are installed, stocked with fish and pre- and mid-experiment samples have been collected. Fish diets were obtained from the fish collected for stocking in Cedar and Peltier and fish diets are now being collected from other lakes. Herbivore surveys have been conducted in 14 lakes and additional lakes are being selected for surveys in August.

A summary of research progress for Phase I and a preliminary proposal for Phase II research was presented to the MAISRC Director and review team in July. They decided to not fund Phase II of the project based on the complexities and unclear results with the Eurasian watermilfoil biocontrol work (Activity 1) and the uncertainty of getting a conclusive determination of the feasibility of manipulating sunfish to enhance milfoil control within the Phase II time frame. They invited a proposal for an extension of the current project to complete additional analysis of the curlyleaf pondweed research (Activity 2) based on anticipated remaining funds from the Phase I project. A proposal will be submitted to MAISRC in September.

Sub-Project Status as of *February 28, 2017*
Field work was completed in fall and all data were entered and analyzed. Eighteen lakes were assessed for milfoil weevil densities, which ranged from none found to 0.27/stem, lower than for most lakes in 2015. Densities were lower in 6 lakes in 2016 compared to 2015, and 2015 generally had lower densities than in previous years. Sunfish stomach contents were analyzed from over 300 sunfish from ten lakes. Benthic and macrophyte associated invertebrates were common in the diets but only one milfoil weevil was found. Enclosure experiments were completed in August. Despite methodological improvements and an earlier start in June we were unable to get definitive results from the enclosure experiments. Herons likely removed stocked sunfish and poor water clarity in both enclosure lakes affected milfoil densities.

Curlyleaf analysis was continued and the mid-summer plant data sets provided with curlyleaf data were organized and systematized to allow an analysis of the effects of curlyleaf and curlyleaf control on the associated native plant communities. The final report and abstract was submitted on 2/28/17. Revisions are underway.

Final Report Summary:
Curlyleaf pondweed (*Potamogeton crispus*) and Eurasian watermilfoil (*Myriophylum spicatum*) are the most widespread and problematic invasive aquatic plants in Minnesota. Approaches to improve their management are needed to reduce economic and ecological costs of invasive control. We collated and analyzed pre-existing data on curlyleaf pondweed from 60 lakes across Minnesota to provide an analysis of factors affecting curlyleaf abundance. For untreated lakes, productivity (prior summer Secchi depth) and over winter conditions were important with greater abundance in lakes with higher productivity and milder overwinter conditions (shorter duration of ice cover and lesser snow depth). For herbicide treated lakes, consecutive years of treatment was also important; abundance decreased with more years of treatment. There were diminishing returns from repeated treatment and populations can rebound quickly once treatment stops. Mild winters will likely result in more abundant populations that spring.

Potential biological controls are available for Eurasian watermilfoil and we focused on assessing factors liming the milfoil weevil and other herbivores. We conducted enclosure experiments to assess the effect of sunfish predation on herbivore and milfoil abundance. Enclosures were placed in two lakes and stocked with 0, 5 and 20 sunfish. Weevil populations developed in the enclosures but there were no differences in weevil abundance or milfoil biomass due to fish stocking. We were unable to recover stocked fish from the enclosures and suspect that predation by herons removed the fish. We assessed herbivore abundance in metro lakes and found milfoil weevils in 12 of the 19 lakes surveyed. Abundance was higher in 2015 than 2016 but abundance both years was lower than some prior years. Milfoil weevil abundance was negatively correlated (r=-0.44) with sunfish abundance but only 1 weevil was found in over 450 sunfish stomachs examined. Further work accounting for environmental variability is needed to identify factors limiting milfoil herbivores.

SUB-PROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control

Project Manager: Paul Venturelli
Description:
Heterosporosis is a disease of emerging concern in Minnesota. This disease is caused by the parasite *Heterosporis sutherlandae*, which damages the skeletal muscle of susceptible fish and renders them unfit for human consumption. Infection can result in direct mortality, but infected fish are more likely to die from complications related to reduced food consumption, immune function, predator avoidance, and reproduction. *H. sutherlandae* can infect up to 40% of the individuals in a wild population of game or bait fish and there is no known treatment. Infection rates are higher in systems with close contact.

Heterosporosis was first discovered in Leech Lake, Minnesota, in 1990, and has since been detected in ~30 Minnesota waterbodies. These include Leech Lake (Cass County), Mille Lacs (Mille Lacs County), Gull Lake (Cass/Crow Wing), Lake Winnibigoshish (Cass County), and Vermillion (St. Louis County). These waterbodies are some of the most ecologically, economically, and recreationally important in the state. Heterosporosis has also been detected in Wisconsin, Michigan, and Ontario. In response to heterosporosis, the Minnesota Department of Natural Resources (MN DNR) has stopped using feeder fish in its hatcheries (resulting in increased per fish production costs).

The list of susceptible fishes is long and growing, and includes a number of economically important species such as yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), northern pike (*Esox lucius*), lake whitefish (*Coregonus clupeaformis*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), koi (*Cyprinus carpio*), and baitfish. *H. sutherlandae* is a regulated pathogen in many states (Minnesota, Wisconsin, Michigan, Utah, Maine, Illinois) and is a disease of concern for the Great Lakes Fisheries Commission. *H. sutherlandae* was identified as a high-priority aquatic invasive microbe by the 2014 MAISRC Research Needs Assessment because little is known about its pathology, epidemiology, and population-level effects. Population-level effects are particularly important for understanding the impact of heterosporosis on harvestable biomass.

The objectives of this project (Phase 1) are to: (1) Provide an initial estimate of threat that heterosporosis poses to the harvestable biomass of yellow perch in Minnesota, and establish timelines for population-level impacts; (2) address 'low hanging' yet critical knowledge gaps in support of Objective 1; and (3) prioritize lab and field research that will improve the accuracy of model prediction by addressing the remaining gaps in our knowledge of *H. sutherlandae* ecology (Phase 2; see Section VII.B for a description).

We will develop a population model of yellow perch and couple this model with a disease model that describes *H. sutherlandae* dynamics as well as a generic population model that describes the dynamics of other fish hosts. We will base model parameters on current knowledge, and fill any gaps using related species, professional opinion, simple lab experiments, and field observations. We will use the model to estimate the threat that heterosporosis poses to yellow perch harvest in Minnesota, and prioritize future empirical research for improving model predictions. The overall project (Phases 1 and 2) will generate advice related to heterosporosis spread prevention, monitoring, control, and management; and establish a framework for approaching other invasive species that are relevant to Minnesota.

Summary Budget Information for Sub-Project 6:

ENRTF Budget:**	**$111,889**
Amount Spent:	**$111,889**
Balance:	**$0**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome Activity 1	**Completion Date**
1. A assembled list of the parameters that are needed for the aggregate model, the value and source of each of these parameters	31 July 2016
2. A working aggregate model (i.e., coded and debugged)	31 January 2017
3. An estimate of the timing (years since introduction) and impact of heterosporosis on the harvestable biomass of yellow perch	31 July 2017
Outcome Activity 2	**Completion Date**
1. Estimated effect of heterosporosis on consumption, activity, growth	31 January 2017
2. Estimated rates of heterosporosis infection and recovery	31 January 2017
3. Estimated heterosporosis frequency and seasonality in the wild and the degree to which heterosporosis affects the susceptibility of fish to angling	31 January 2017
Outcome Activity 3	**Completion Date**
1. Future lab and field work prioritized via sensitivity analysis	31 July 2017

Sub-Project Status as of *February 10, 2014*
This sub-project has been delayed to more appropriately sequence it after additional empirical data has been gathered by the Center. It is anticipated that this project will move ahead with a project proposal and start after July 1, 2015.

Sub-Project Status as of *August 31, 2014*
The proposal process for this subproject has begun with estimated project start summer 2015.

Sub-Project Status as of *February 28, 2015*
The project proposal has been received and is currently undergoing peer review, with an aim to start research July 2015. The proposal aims to address key knowledge gaps by providing, through modeling, an initial estimate of the threat caused by the parasite Heterosporis to the harvestable biomass of yellow perch in Minnesota. The outcome table above will be revised once a final workplan for this sub project is approved.

Sub-Project Status as of *September 24, 2015*
This subproject was approved to begin June 15, 2015

Sub-Project Status as of *February 29, 2016*
We are on pace with model development (Activity 1) and field and lab work (Activity 2), and are already interacting with stakeholders through a fact sheet and presentation at the 2015 MAISRC Showcase. We will submit our first paper in February. No work has been completed on Activity 3 because we first need to complete Activities 1 and 2. Model development is well under way. We have collected a quarter to a third of necessary parameter values, and beginning to code the subroutines that simulate disease and energy dynamics. In

collaboration with the MN DNR, we collected 1,221 yellow perch and other fishes from Cass, Leech, and Winnibigoshish lakes in September. Preliminary results from the lab suggest that ~8% of fish are infected. Most of these fish were yellow perch. Winter gill netting is now under way so that we can determine if the frequency and intensity of heterosporosis infection is seasonal or temperature-dependent. To determine if infected fish are more or less susceptible to angling, we have also distributed to log books to resorts on all three lakes. Finally, we have obtained ~1100 yellow perch for laboratory experiments. We spent 4-6 weeks training these fish to feed on pellets, and will move them into the fish lab to begin experiments when construction of the MAISRC containment facility is complete. To help with the lab work, we recruited and trained two undergraduate students and one high school student. They are assessing heterosporosis infection rates and working with laboratory fish (e.g., health checks, husbandry, water quality testing, feeding procedures).

Sub-Project Status as of *August 31, 2016*
We have a working model that combines bioenergetics and population dynamics to model perch in the absence of heterosporosis, and are beginning to couple this model with the disease sub-model (Activity 1). Outcome 1 of this activity (parameter list including values and sources) is complete except for the parameter values that we are obtaining from the field and lab work. The list and values are available upon request, but also subject to change as we work toward Outcome 2 (a working aggregate model). We have completed one cycle of field work (Activity 2). In addition to our fall sample of 1,221 fishes from Cass, Leech, and Winnibigoshish lakes, we have also sampled Leech Lake in winter (270 fishes), spring (341 fishes), and summer (210 fishes) so that we can determine if heterosporosis varies seasonally or with size, sex, or species. We are processing these samples. Preliminary results suggest that ~3% of fish are infected with heterosporosis, which is consistent with the 2% reported by the two resorts with which we are working. These resorts have agreed to keep any infected fish that they find in order to increase the culture of spores within living fish at the MAISRC lab. We will use this culture to infect perch for our experiments. We are on pace with model development and field work, but not lab experiments. Unfortunately, lab experiments (Activity 2) will be delayed at least 9 months because the MAISRC laboratory is not yet operational due to unforeseen construction delays. As a result of these delays, we i) will have to purchase new experimental fish (the batch that we obtained in fall have grown too large), ii) have cancelled the experiment to determine if perch can recover from heterosporosis, and iii) have adjusted the timelines and sample sizes of the remaining experiments. We are also using the perch that we have to culture *Heterosporosis* and test our experimental protocols. We have recruited and trained a third undergraduate student to help with lab work and experiments. In the last 6 months, we have also interacted with stakeholders directly during field work, via two local media interviews, and an award-winning presentation at the 57th Annual Western AFS-Fish Health Section conference. Our first paper has yet to be submitted because we needed to conduct additional analyses. We have not worked on Activity 3 (sensitivity analysis in support of a second phase of the project) because we first need to complete Activities 1 and 2.

Sub-Project Status as of *February 28, 2017*
We are on pace with model development, but not lab experiments. We have a working aggregate model that uses bioenergetics, population and disease modeling to predict perch dynamics in a system with varying degrees of disease prevalence and virulence (Activity 1). We are now parameterizing this model with lab and field experiments so that can generate predictions and perform a sensitivity analysis (Activity 3). We have finished microscope analysis on field samples from the fall and winter, resulting in a 6% and 1% prevalence of heterosporosis in Leech Lake, respectively. We are still processing samples from the spring and summer. We also have completed another sample for the fall season in order to more accurately detect heterosporosis visually than was possible in the fall of 2015, as well as collect infected tissue for laboratory experiments. Visual detection of heterosporosis resulted in less than 1% prevalence. We are behind on lab experiment due to delays in facility construction and difficulties in finding and culturing *Heterosporis*. We were able to run a small experiment in which we exposed 19 perch twice to heterosporosis by feeding infected tissue. Only one fish tested positive for the disease. Given our remaining timeline and the challenges associated with infecting perch in the lab, we are cancelling experiments to determine heterosporosis effects on consumption, activity or recovery, and will instead focus on lab experiments to determine heterosporosis transmission rates via direct

contact among fathead minnows (which are highly susceptible to heterosporosis and easier to work with than perch). We have recruited and trained two new undergraduate students to help with lab work and experiments. In the last 6 months, we have interacted with stakeholders directly during field work and during the annual Minnesota Aquatic Invasive Species Research Center Showcase. Our first paper has yet to be submitted, but is in the final stages of internal review. We have initiated work on Activity 3, and have started planning and structuring the model to best implement the sensitivity analysis.

Sub-Project Status as of *August 31, 2017*
This project has completed. A final subproject report will be submitted by 9/30/17.

Final Report Summary:
Heterosporosis has been an emerging disease of concern in Minnesota that is caused by the parasite *Heterosporis sutherlandae*. It damages fish muscle and renders it inedible. Heterosporosis was discovered in Leech Lake in 1990 and confirmed in 2000 and has since been detected in ~30 Minnesota waterbodies and over a dozen species. Heterosporosis was identified as a high research priority by the 2014 MAISRC Research Needs Assessment because it can infect up to 40% of fish, there is no known treatment, and we knew little about the disease or population-level effects. Our objectives were to collect field and lab data to better understand heterosporosis, and to estimate its threat to perch harvest. We collected perch and other fishes from Leech Lake seasonally from fall 2015 to winter 2017, and from Cass and Winnibigosish lakes in fall 2015 and 2016. Heterosporosis was rare among all species in all seasons and lakes. We detected heterosporosis in only 10% of perch, and only 20-30% of these had visible muscle damage. Low prevalence compared to 2004 samples may be due to immunity or low environmental stress. Heterosporosis infection did not vary seasonally, and healthy and infected perch were equally susceptible to angling. Our experiments found low rates of infection due to inoculation (32%) and transmission due to exposure to diseased fish (2% and 17%, minnow to minnow and perch to minnow, respectively). A population model based on this and other information suggested that heterosporosis can have short-term impacts on perch harvest (e.g., in a naïve population or after a stressful year), but that long-term impacts are unlikely. There was no significant difference between infected and uninfected individuals in terms of their growth rate or survival probability. Based on the results of this project, we do not consider heterosporosis to currently be a significant threat to Minnesota fish populations. However, we recommend monitoring future outbreaks and long-term trends as the climate changes and an assessment of the threat to aquaculture and laboratory fish.

SUB-PROJECT 7. Developing eradication tools: Exploring whether native pathogens can be used to control AIS

Project Manager: Nick Phelps
Description: Although ambitious, eradication is our ultimate goal. Only three techniques presently appear capable of achieving it: 1) introduction of exotic predators, 2) introduction or promotion of species-specific pathogens, 3) genetic-engineering and release of AIS with lethal genes. We presently believe the second option has the most promise in Minnesota and also poses the least risk. However, using infectious agents to target specific species is still a high-risk, high-reward approach that must be evaluated carefully. Viruses threaten native populations as well and have not been well characterized. This activity will initially be led by a part-time assistant professor (Dr. Nick Phelps [0.08 FTE for 5 years]) who will initially focus on the first step of this evaluation: identifying native pathogens of both native fishes and the carps. Focus is placed on two native virus *(Picornavirus*, *Orthomyzovirus)*. A postdoctoral fellow (1.0 FTE per year for 5.5 years), or equivalent, will provide assistance. This work can start as soon as peer-review is complete (2013) because Dr. Phelps is on staff. Because there has been little research on infectious agents that control, or even might control fishes in Minnesota, we must first perform a survey to identify endogenous infectious agents of native fish and carps. Specific details of this sub-project will be determined by Center-led peer-review. If successful, new funding would be requested from the LCCMR and other agencies to develop the technology to apply identified

pathogens to AIS control (i.e. we do not ask for that here). This description and the outcomes below will be updated following approval of a more detailed subproject work plan and budget.

Summary Budget Information for Sub-Project 7-1:	**ENRTF Budget**:**	**$206,754**
	Amount Spent:	**$206,754**
	Balance:	**$0**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

<u>Outcome</u>	<u>Completion Date</u>
1. Conference (AFS-FHS Annual meeting) presentation	Aug 2014
2. Manuscript prepared for publication	June 2015
3. *Obtain 240 silver carp from Illinois and Mississippi River systems*	Dec 2015
4. *Obtain suitable fish from 15-20 invasive carp mortality events*	Dec 2015
5. Database of characterized viruses of carp created	May 2016
6. Determine disease causing potential of selected virus	May 2016
7. Manuscript prepared for publication	June 2016
8. Conference (AFS-FHS Annual meeting) presentation	June 2016
9. Survey summary of koi herpes virus in Minnesota	Dec 2016

Sub-Project Status as of *February 10, 2014*
A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of *August 31, 2014*
As reported in the project's July 31, 2014 update: As of July 1, 2014 Dr. Sunil Mor was hired as a post-doctoral associate to perform biological and molecular characterization of viruses. Laboratory equipment is currently being purchased to begin sample processing. No fish have been collected yet, however two sample events are planned in the coming weeks.

This project's budget as shown below in VI a 2 and in the attached Overall budget and the outcomes listed above have been updated to reflect those in the approved SUBPROJECT 7 workplan and budget.

Sub-Project Status as of *February 28, 2015*
As reported in the sub-project's January 31, 2015 update: The first six months of Phase I have been focused on building capacity and collaboration to describe the virome of invasive carp species in the Upper Midwest. Several essential pieces of equipment were purchased to conduct the laboratory work and increased communication with the MN DNR, USFWS, USGS, and various field biologists from across the region will provide opportunities for additional sample collection soon. In the fall of 2014, common carp were collected from five bodies of water in Minnesota as part of ongoing research within MAISRC. The common carp did not have an active infection of koi herpes virus at the time of sampling, however diagnostic tests needed to determine prior exposure were not available at that time. Tissue samples from the fish have been archived for culture and molecular testing in the coming months. The importance and approach used in Phase I, along with some related findings of a novel virus in cyprinid fish, were presented at a scientific conference and are currently being prepared for peer-review publication. The project is progressing as expected. The outcome table above has been revised to reflect those in the approved workplan for this subproject.

Sub-Project Status as of *September 24, 2015*

Significant progress has been made to perform diagnostic tests on the previously collected common carp. To date, 316 common carp have tested negative for a variety of potential viral pathogens (cyprind herpes viruses 1-3, carp edema virus, and spring viremia of carp virus). However, a still unknown virus was isolated by cell culture. Confirmatory tests are currently pending. Two novel viruses have been identified from common carp and grass carp mortality events: novel picornavirus and novel paramyxovirus. The previously known grass carp reovirus (GCRV) was also confirmed. This was the first report of GCRV associated with fish mortality in the United States. Efforts are underway with new partners at Purdue University and the Illinois Department of Natural Resources to collect silver carp this summer/fall. An update on this project was invited to be presented at the Great Lakes Fisheries Commission – Great Lakes Fish Health Committee meeting held in July 2015. The project is progressing as expected.

Sub-Project Status as of *February 29, 2016*
Significant progress has been made to collect new common carp samples from different sites. Total of 94 common carp were collected from three different sites in Minnesota. In addition, 120 silver carp from the Fox and Illinois rivers were collected. Significant progress had been made to perform diagnostic tests on the previously and recently collected common carp as well as silver carp. Bighead carp samples were also collected from mortality even from US Geological Survey, Columbia Environmental Research Center, Columbia, MO. Samples have been processes for virus isolation and molecular diagnostic. Multiple novel viruses have been isolated and are currently being characterized by next generation sequencing from common carp collected this last fall.

Unfortunately, there have been two unforeseen challenges that have affected the proposed activities. Due to delays in the construction of the MAISRC biocontainment facility, Activity 3 will no longer be completed during this project period. Adding this again in Phase II is being strongly considered. Due to the unavailability of the commercial ELISA kit for testing prior exposure to KHV we have relied on the PCR test that has been validated for use in our laboratory. While this does not give us as much information as planned, it is still a useful and first-ever attempt to survey common carp in Minnesota for this important virus.

We are currently in the progress of organizing and analyzing data to propose the continuation of this project in Phase II.

Sub-Project Status as of *August 31, 2016*
Significant progress has been made and recent findings have greatly informed ongoing and future efforts. Samples from apparently healthy invasive carp and those from mortality events were screened by virus isolation, targeted PCR and next generation sequencing (NGS) Illumina MiSeq for molecular identification of viruses. Novel RNA viruses belonging to six different families were identified since the previous update, including three picornaviruses, two reoviruses, hepatovirus, astrovirus, hepatitis E virus, and betanodavirus. The analysis of DNA Miseq sequences from all samples and both RNA and DNA sequences from a recent mortality event will be complete in the coming weeks. Analysis of complete NGS work will fulfill the aim of Activity 2 in Phase I, which is to generate baseline data of local invasive carp pathogens. The manuscript on RNA viruses of invasive carp populations in Minnesota is in preparation.

Activities 1, 2, 4, and 5 are complete and all outstanding balances will be reconciled with unused funds being returned to MAISRC at the January 31, 2017 update and a final report summary for all activities will be provided shortly thereafter. Activity 3 is still in progress pending amendment approval. The amendment was withdrawn and replaced on October 7, 2016 to reflect completion of the project with a possibility for including the unfinished Activity 3 work in Phase 2 of the project.

Final Report Summary:
Although ambitious, eradication of aquatic invasive species is an ultimate goal of the MAISRC. One possible method would be through the introduction or promotion of species-specific pathogens. This high-risk, high-

reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. As a first step in Phase I of a multi-phase project, invasive carp species were surveyed to identify viruses circulating in these populations. Nearly 700 common carp were collected from Minnesota lakes, 120 silver carp from the Fox and Illinois Rivers, and a variety of carp species from eight mortality events. All fish were negative for cyprinid herpes viruses 1, 2, and 3, carp edema virus, and spring viremia of carp virus. However, advanced molecular approaches and virus isolation detected several known and unknown viruses of significance. This included novel viruses from at least seven RNA virus families: picornavirus, reovirus, hepatovirus, astrovirus, hepatitis virus, betanodavirus, and paramyxovirus. The novel carp paramyxovirus was associated with a mortality event and shows particular promise for further evaluation as a biocontrol agent. The standard operating procedures developed during Phase I will be essential to advance future work on this and related pathogen discovery research. Unfortunately, Phase I was met with several unforeseen challenges that hindered completion of all proposed activities, including laboratory renovation progress, service provider availability and delays, and access to mortality events. In spite of these setbacks, this project has significantly advanced our understanding of invasive carp viruses and positioned us well to for future research efforts. Phase I of this project provided researchers and managers with baseline data on viruses circulating in invasive carp populations in the region. These data have been broadly disseminated at scientific conferences, peer-reviewed and lay publications, and through MAISRC communications. Continued efforts to build upon this line of research will commence in Phase II of this long-term effort.

SUB-PROJECT 7-2. Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents

Although ambitious, eradication of aquatic invasive species is the ultimate goal of many aquatic invasive species. One possible approach would be through the introduction or promotion of species-specific pathogens. This high-risk, high-reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. Phase I (Years 1-2.5) of the long-term project provided initial baseline data on viruses of carp species in the region. Phase II (Years 2.5-6) will build upon this work for carp species and now include zebra mussels to utilize newly developed techniques to more strategically identify viral biocontrol candidates for control of invasive carp and zebra mussels. More specifically, Phase II will 1a) Collect apparently healthy invasive carp and mussel species in the Midwest region; 1b) Collect samples from mortality events of native and invasive fish and mussel populations in the Midwest region; 2) Conduct virus discovery by next generation sequencing and culture potential pathogens; 3) Determine the disease causing potential of two selected viruses, one for native and invasive fish and the other for native and invasive mussels; and 4) Communicate findings to scientific, management, and public stakeholders. This will provide the scientific foundation to begin to evaluate specific pathogens for invasive species control. Furthermore, understanding the virome of invasive species will serve as a potential early indicator for the movement and distribution of pathogens that may threaten native species. Phase II will largely be basic research (60%) generating baseline data on the virome diversity of invasive and native species. Significant effort will also be in applied research (40%), whereby diagnostic and disease challenge findings will be used to inform the health management of fish populations.

Summary Budget Information for Sub-Project 7-2:	**ENRTF Budget**:**	**$445,210**
	Amount Spent:	**$422,667**
	Balance:	**$22,543**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome	Completion Date
1-1. *Collect 600 common carp from 10 locations in Minnesota*	December 2018
1-2. *Collect 240 silver carp from 4 locations in the Illinois and Mississippi Rivers*	December 2018
1-3. *Collect 1,200 zebra mussels from collaborating researchers*	December 2018

1-4. *Collect samples from 40 fish or mussel mortality events in the Midwest region*	December 2018
2-1. *Database and isolate archive of viruses of fish*	June 2019
2-2. *Database and isolate archive of viruses of mussels*	June 2019
3-1. *Determine disease causing potential of selected fish virus*	December 2018
3-2. *Determine disease causing potential of selected mussel virus*	June 2019
4-1. *Three peer-reviewed manuscripts submitted*	June 2019
4-2. *Three scientific conference presentations*	June 2019
4-3. *Dissemination of research findings via MAISRC communications*	June 2019

Sub-Project Status as of *February 28, 2017*
This subproject was approved in February 2017. An updated project description, budget, and outcomes are provided above.

Sub-Project Status as of *August 31, 2017*
During the first part of the project, we have focused our efforts on sample collection. We have collected samples from six fish kill events of invasive and native fish. Koi Herpes Virus (KHV) was identified from a large common carp mortality event in Lake Elysian. This is a significant finding since this is the first report of KHV in wild fish in Minnesota and the candidate biocontrol agent for common carp in Australia. We are working with the MN DNR and hope to conduct follow up surveys in the coming months to estimate viral persistence, mortality rates and prevalence in surrounding lakes. Sampling of healthy and sick/dead fish and mussels will continue in the coming months.

We have made changes within our personnel category due to the promotion of Dr. Sunil Kumar Mor. Dr. Mor is now an Assistant Professor with the Minnesota Veterinary Diagnostic Laboratory and head of the Molecular Development section. Although his percent effort will be lower, the capacity and value he brings with this new position will be highly beneficial to the project. In addition, the official start dates of Dr. Mor and Dr. Alex Primus has been delayed to 7/1/17. With the cost savings we have hired Dr. Soumesh Kumar Padhi to be a full time post-doctoral associate starting in August 2017. We have also hired Dr. Todd Knutson, a bioinformatics specialist to assist part-time with the project. Lastly, we have added Isaiah Tolo to the team. Isaiah received the competitive University of Minnesota Diversity Scholars Fellowship for the 2017-2018 academic year and will be at no cost to the project until Year 2. The descriptions in Column A of the Subproject budget spreadsheet have been updated accordingly. These changes in personnel do not affect the overall budget, but have delayed spending, hence a full balance on this budget line. In the meantime, Meg Thompson has provided assistance on the project by collecting and processing samples. She is currently being paid from a non-ENRTF source of funds.

We learned from Phase I of this project (MAISRC SubProject 7-1) that an increased communication effort was needed to generate collaboration on sample collection. We have presented at the joint meeting of the American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop and the Great Lakes Fish Health Committee to present on this project. The presentations were titled: “Investigating fish kills: Looking back, looking deep and looking forward” and “Understanding the virome of invasive carp: What it could mean for biocontrol”. These presentations resulted in an active discussion on the potential use of viruses for biocontrol, interest to submit samples for the project and potential collaborations for future research efforts related to this project. In addition, we have invited a world leader on the use of viruses for biocontrol, Dr. Ken McColl (Commonwealth Scientific and Industrial Research Organization, Australia), to present at the 2018 iCOMOS meeting to be held at the University of Minnesota, more information: http://icomos.umn.edu. We expect that as part of Dr. McColl’s visit, we will host meetings with members of state and federal agencies to socialize this approach and generate ideas for future research needs.

Sub-Project Status as of *February 28, 2018*
The project is progressing as expected. Dr. Soumesh Kumar Padhi has joined the project as post-doctoral associate on September 11, 2017. The last quarter of this project was focused on healthy common carp and

silver carp sampling along with fish kill events of native and invasive species. We have also collected zebra mussels from different lakes in Minnesota. A work flow, starting from sample homogenization, sample pooling, nucleic acid extraction by targeting viral particle concentration, removal of host genome contamination in the NGS process, detection of KHV, SVCV and CEV from samples using qPCR are currently being optimized. Based on these optimized protocols we will process all the sampled tissue for virus analysis. The communications efforts were increased by giving presentations at the MAISRC showcase and Minnesota Veterinary Diagnostic Laboratory. The project was also presented at the 20th International Conference on Aquatic Invasive Species entitled "Understanding the Carp Virome: What Could It Mean for the Control of Invasive Carp?".

An amendment was approved by LCCMR on 02/06/2018 to move the moderate cost savings from a capital equipment purchase to a new service category for shipping samples from collaborating labs. We expect no additional expenses related to capital equipment. We could now use the extra funds to improve sample collection for fish kill events from other states. This amendment does not change the scope of the project, timeline or overall budget.

Sub-Project Status as of *August 31, 2018*

We have made significant progress in the last six months and are on schedule. All fish kill and healthy fish tissue samples from the 2017 season were processed and screened for the presence of KHV, CEV and SVCV. We confirmed that all carp kills investigated as part of this project were associated with KHV. This is a major finding and getting international attention. Interestingly, we have detected CEV in two different lakes, co-infected with KHV. This is a very unique infection and the first time CEV has been detected in wild common carp in Minnesota and the second time in the USA. The thymidine kinase and partial p4a genes were amplified by conventional PCR from KHV and CEV positive tissues, respectively. Sanger sequencing was performed to get the nucleotide sequences of these amplified genes and determine the relationship of KHV and CEV present in MN to the other international variants. These results are still pending but promise to provide an understanding of genotypic distribution of KHV and CEV viral populations in the region. Sampling of ongoing fish kills continues for the 2018 field season – as of this report, 15 mortality events have been investigated, with results pending.

A more complete picture of the viral communities present in healthy carp, fish kills and zebra mussels is moving at a good peace. The viral RNAs were eluted using a newly developed and optimized RNA extraction protocol from all the tissues collected in 2017 field season. These RNA samples were submitted to University of Minnesota Genomics Centre (UMGC) for RNA-Hiseq next generation sequencing. The optimization of DNA-NGS protocol is under process.

Based on our research and consultation with others, we have decided move forward with the investigation of KHV as a potential biocontrol agent in our experimental challenge study. We have received two specific cell lines required for isolation of KHV and are currently growing those cells for subsequent in vitro culture.

Members of our research team presented at the Eastern Fish Health Workshop, Aquatic Invader Summit and a special meeting of the Freshwater Mollusk Conservation Society focused on the health of native and invasive mussels. The presentations were all well received and garnered significant interest by attendees. We are preparing a manuscript on the KHV and CEV outbreaks we observed during the 2017 season. Dr. Ken McColl from Commonwealth Scientific and Industrial Research Organization, Australia has visited MAISRC in 3rd May 2018 during iCOMOS-2018 to present "Use of virus as a biocontrol agent". Our research group had a meeting with him to discuss the different approaches and future research needs towards the development of current biocontrol projects.

Sub-Project Status as of *February 28, 2019*

We have continued to make good progress in the previous six months of the project period. We are nearly complete with collection of fish kills and healthy common carp – we plan to work with commercial fishermen in the coming months to collect common carp from the final two lakes. We have spent considerable time this project period working to process, sequence, analyze and finalize the results for viral discovery. While still in progress, we have already confirmed the detection of 11 novel viruses from common carp mortality events and five novel viruses from mortality events of native fish species. Results for healthy common carp, silver carp and zebra mussels are still pending. We have also confirmed six additional lakes positive for KHV, two lakes with CEV, and two lakes with both KHV and CEV. These results continue a trend of detections that first started in 2017 of this project. We are currently finalizing the phylogenetics to better determine the origin of the viral strains detected in Minnesota. Culture of the KHV remains a challenge for our project team (and other researchers around the world). We continue to discuss with collaborators and are working to modify and optimize are methods to improve isolation. However, we have begun experiments to grow the virus in vivo (in live fish) and are hopeful this strategy will prove effective in the coming months. Lastly, we have continued to communicate project progress at scientific conferences and with local/federal stakeholders.

Final Report Summary:
One possible component to an effective integrated pest management plan for aquatic invasive species would be through the introduction or promotion of species-specific pathogens. This high-risk, high-reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. In Phase II of this long-term effort, we characterized the virome invasive and native fish species and zebra mussels. *We achieved our ultimate goal of this project and identified a candidate virus (koi herpes virus) that caused high mortality in common carp and was not detected in native fish species – this virus will be the focus of Phase III.* We also identified many other novel and undescribed viruses in health and dead fish, however the implications of these results are unknown and warrant additional research to better understand the threat to native species and/or potential as biocontrol agents. The virome of zebra mussels was also interesting with lower viral diversity than the fish species investigated; however, no viruses emerged as potential zebra mussel biocontrol candidates from field samples or laboratory trials.

This study emphasized the value of advanced molecular approaches to unbiased viral discovery and diagnostics. The methods we developed and optimized for sample collection, processing, and sequence analysis (all together called a 'pipeline'), have informed testing protocols at the Minnesota Veterinary Diagnostic Laboratory. We have also elevated awareness among managers that viral diversity is much higher than currently known and deserves more attention as early indicators of potential threats.

The project team spent considerable time during Phase II engaging with managers, scientists, and the public in multiple formats. It is important that this type of research is transparent and understandable to all stakeholders. To that end, we held formal in person meetings, attended local-national-international scientific conferences, published a peer-review manuscript, networked with internationally-renowned experts, produced two videos, and provided interviews for print, radio and TV media.

SUB-PROJECT 8. Risk assessment, control, and restoration research on aquatic invasive plant species.

Project Manager: Dan Larkin
Description:

Aquatic invasive plants are a major threat to Minnesota's lakes, rivers, and wetlands. AIS plants can grow densely and form surface mats, reducing space and light available to other plant species. This can lower native plant diversity, reduce habitat quality for fish and other animals, and change the way lakes function. Aggressive growth of AIS plants also interferes with boating, recreation, and other human uses. AIS plants can thus harm biodiversity, habitat quality, and human activity.

Despite strong interest and investment in preventing new invasions, controlling existing infestations, and supporting the recovery of impacted waterbodies, there are still key gaps in scientific knowledge needed to support effective management. To help address these gaps, this subproject will involve applied research on four high-priority aquatic plant species that are invasive or potentially invasive in Minnesota lakes. These species are at different stages of invasion in Minnesota. Because of this, management priorities and associated research needs differ, from evaluating risk of future invasion and spread, to improving the toolkit available for control, to identifying strategies for aiding recovery of lakes affected by AIS:

(1) (Discontinued)

(2) *Nitellopsis obtusa* (starry stonewort) is a charophyte (green alga) that is a new invader in Minnesota, having been found in Lake Koronis (Stearns Co.) in summer 2015. Starry stonewort is native to Europe and Asia. It appears to be spreading rapidly in northern-tier lakes, after first being found in the St. Lawrence River in 1978. We will assess risk of further spread of starry stonewort in Minnesota based on climate and environmental factors and by testing how long starry stonewort can remain viable out of water—mimicking potential movement by boaters. We will also test methods for controlling starry stonewort, which has proven difficult and on which there has been almost no scientific research. For now, herbicides/algaecides are the most promising tool for controlling starry stonewort. To ensure that control efforts are as effective as possible while minimizing harm to native species, we will conduct laboratory experiments to test the efficacy and selectivity of different herbicides. This information is urgently needed during this window of opportunity to minimize impacts of starry stonewort to Minnesota lakes.

(3) *Myriophyllum spicatum* (Eurasian watermilfoil) is native to Europe and Asia, was first found in Minnesota in 1987, and now occurs in 322 Minnesota lakes in 40 counties.

(4) *Potamogeton crispus* (curly-leaf pondweed) is native to Europe, Asia, Africa, and Australia; has been in Minnesota since at least the early 1900s; and is now in 750 Minnesota lakes in 70 counties. Eurasian watermilfoil and curly-leaf pondweed have been a focus of management and research in Minnesota for decades. But there are still limits in our ability to effectively control these species and, following treatment, to support recovery of native plant species. We will analyze existing datasets, perform new field work, and develop a citizen-science monitoring program to improve understanding of factors that drive invasion of these species and influence the effectiveness of management efforts. Eurasian watermilfoil and curly-leaf pondweed are not new to Minnesota, but ≥ 94% of our lakes do not contain these species. Improved ability to manage these species and contain further impacts is needed.

An undergraduate, graduate student, and postdoctoral researcher will be trained under this subproject. Findings will be disseminated through peer-reviewed publications, presentations, and outreach and extension programming for agency staff, lake service providers, lake associations, and other stakeholders.

Summary Budget Information for Sub-Project 8:

ENRTF Budget:**	**$822,000**
Amount Spent:	**$820,251**
Balance:	**$1,749**

***This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.*

Outcome Activity 1	**Completion Date**
1. *Proposal submission to MAISRC for evaluation and peer review*	October 31, 2015
2. *Revisions following peer review submitted to MAISRC*	February 15, 2016
3. *Workplan submission to LCCMR*	March 15, 2016
4. *Aquatic invasive plant project implementation*	April 15, 2016
5. *Final subproject deliverable*	June 30, 2019
Outcomes Activity 3	
A1. Starry stonewort ecological niche modeling completed and paper published	January 31, 2017
A2. Begin lake-level risk assessment for starry stonewort	January 31, 2017
A3. Complete risk assessment and present results to MNDNR and other stakeholders	July 31, 2018
B. Begin laboratory experiments testing starry stonewort climate tolerance	January 31, 2017

C. Begin lab experiments testing starry stonewort desiccation resistance	July 31, 2016
D. Begin laboratory experiments testing starry stonewort control options and non-target impacts to native plant species	January 31, 2017
<u>**E.** Begin field sampling to evaluate outcomes of starry stonewort control efforts in Minnesota lakes</u>	<u>January 31, 2017</u>
B–E. Complete experiments, analyze data, and present results to stakeholders	January 31, 2018
A–E. Complete manuscripts and submit for peer review	July 31, 2018
Outcome Activity 4	**Completion Date**
A1. Compile existing datasets for investigating spread and nuisance growth of Eurasian watermilfoil	July 31, 2017
A2. Analyze data to identify key factors influencing spread and nuisance growth of Eurasian watermilfoil	January 31, 2018
B1. Begin development of Trackers program	July 31, 2016
B2. Begin fieldwork for refinement of sampling methods and data collection	July 31, 2016
B3. Begin Trackers sampling and quality control testing	July 31, 2017
B4. Analyze data collected by Trackers and synthesizes outcomes of Eurasian watermilfoil control efforts	January 31, 2019
A–B. Complete fieldwork and data analysis and present results to stakeholders	January 31, 2019
A–B. Complete manuscripts and submit for peer review	June 30, 2019
Outcome Activity 5	**Completion Date**
A1. Compile existing datasets for investigating spread and nuisance growth of curly-leaf pondweed	July 31, 2017
A2. Analyze data to identify key factors influencing spread and nuisance growth of curly-leaf pondweed	January 31, 2018
B. Analyze data collected by Trackers and synthesizes outcomes of curly-leaf pondweed control efforts	January 31, 2019
A–B. Complete fieldwork and data analysis and present results to stakeholders	January 31, 2019
A–B. Complete manuscripts and submit for peer review	June 30, 2019

Sub-Project Status as of *February 10, 2014*
No progress to report at this time as the project is not anticipated to start until approximately January 2015

Sub-Project Status as of *August 31, 2014*
Through consultation with Center researchers, Center Advisory Board members, MNDNR, and other stakeholders in Summer 2014, it has been determined that the most critical gap in expertise needing to be filled by the new research assistant professor position created through SUBPROJECT 8 is in the area of aquatic invasive plant management.

The Director has since been able to work with the Deans of CFANS and Extension to leverage ENRTF funds to secure this as a full-time tenure track position, with the University committing to fund its salary after this Subproject award expires in 2019. This faculty member will be responsible for developing a new research and extension program aimed at advancing aquatic plant management and restoration approaches for lakes, rivers, and wetlands degraded by invasive species and other human-caused stressors. Research focus may be on control of invasive aquatic/wetland plants through mechanical, biological or herbicidal means, restoration of aquatic and wetland vegetation, and monitoring outcomes of management/restoration actions. Research and adult education efforts will be developed synergistically and in cooperation with stakeholder groups, university research faculty, Extension Specialists and Educators statewide and nationally.

The University is proceeding with the typical University tenure track hiring practices in reliance on past approvals by LCCMR for this subproject. A search committee has been formed with intent to hire by March 2015 and to start in time for Fall Semester, 2015.

Upon hire of this faculty member, a draft work plan and project budget for estimated $145,000 will be submitted to LCCMR. This will provide the faculty member's salary upon his/her start date and $15,000 for travel, equipment, services, and supplies so that this person can develop his/her full research proposal and seek review and approval of the research proposal according to the process laid out in the MOU. Approval according to this process, which includes ultimate LCCMR approval of a workplan and detailed budget, will be required before release of additional research funds beyond the estimated $145,000 mentioned above.

Sub-Project Status as of *February 28, 2015*
As previously reported, Dr. Galatowitsch was able to leverage this position from a term-limited position to a more competitive and permanent tenure- track position within the Department of Fisheries, Wildlife, and Conservation Biology. Per University procedures, a search committee was created, the position was posted, and candidates were interviewed. An offer was made recently; we hope the position will be filled this spring and the new hire will begin in August 2015. The outcome table above will be revised once a final workplan for this sub project is approved.

Sub-Project Status as of *September 24, 2015*
This subproject was approved August 13, 2015 for purposes of Dr. Larkin beginning to develop and implement a new research and outreach program in aquatic plant management and restoration. Specific details of the project will be fleshed out through development of a proposal to MAISRC by Dr. Larkin and a Center-led peer-review process. The above Suproject 8 description, title and outcomes will be updated accordingly following subsequent work plan and budget submission.

Sub-Project Status as of *February 29, 2016*
The full research proposal is currently in peer review. Additionally, an ecological niche model has been developed to determine the threat of starry stonewort spread in Minnesota. The model indicated that this species is persisting in novel habitats – meaning that it is occurring in areas here that are climatically distinct from its native range, and that conditions in portions of the upper Midwest and other regions in the U.S. are ideal for its growth and spread. Additionally, a convening in the next months of researchers and managers with starry stonewort experience is being led by Dr. Larkin to determine current research and management knowledge and gaps.

Sub-Project Status as of *May 2, 2016*
This project has completed peer review, revision, and its workplan and budget have now been approved by MAISRC. And amendment is being requested to move $692,000 from the Budget Reserve of Subproject 8 and allocate it within the project so that the full project budget is $822,000

The project description and outcomes, above, have been updated.

Sub-Project Status as of *August 31, 2016*
This Sub-project was just approved in May with an understanding that its next status update would be provided January 31, 2017

Sub-Project Status as of *February 28, 2017*
This funding has enabled an active research program addressing applied issues in aquatic invasive plant management in Minnesota lakes. Research on starry stonewort has addressed spread risk using ecological niche modeling and ongoing work to predict vulnerability of individual Minnesota lakes to starry stonewort invasion based on environmental characteristics. Culturing of starry stonewort is being refined to enable laboratory experiments addressing starry stonewort climate and desiccation tolerance and chemical control. Field sampling and experimental germination of starry stonewort bulbils from areas treated with algaecides and/or mechanical harvesting revealed high capacity for reinvasion of treated areas. In-lake outcomes of starry stonewort

management efforts are being monitored in collaboration with DNR and other external partners. Research on Eurasian watermilfoil and curly-leaf pondweed has shown that shallow lakes with higher native plant diversity are more vulnerable to invasion, and that these invasive plants are associated with rapid biotic homogenization of vegetation in these lakes (loss of plant community distinctiveness). We are compiling monitoring data from past treatments of Eurasian watermilfoil and curly-leaf pondweed in Minnesota lakes to investigate how management decisions and environmental conditions influence effectiveness of control and capacity for recovery of native plant communities. The curly-leaf pondweed component incorporates and builds upon previously ENRTF-funded work by Dr. Ray Newman (Subproject 9). Finally, our research is being integrated with joint MAISRC-Extension efforts to develop the Trackers citizen science program (Subproject 10). Research related to this project has been presented in peer-reviewed publications (one complete, two in revision, several in preparation), research and outreach talks (13 total, 12 invited), and media coverage (7 total, including print, television, and radio).

Sub-Project Status as of *August 31, 2017*
We have advanced progress of our research on several fronts. The completion dates for some outcomes have been amended and three small budget adjustments have been made. These updates are described below.

In the past 6 months, we have continued to address key applied questions in aquatic invasive plant biology and management in Minnesota lakes. Substantial progress has been made on addressing spread risk of starry stonewort using ecological niche modeling. This work has now advanced into lake-level risk prediction for individual Minnesota lakes based on water chemistry variables; findings from this work are being used to guide a statewide MAISRC/Extension citizen-science starry stonewort search effort (see Subproject 10 workplan update). Research on Eurasian watermilfoil and curly-leaf pondweed are elucidating the role of biotic interactions in risk of aquatic plant invasions and the outcomes of herbicide control efforts through compilation, synthesis, and analysis of large-scale datasets. Our work on Eurasian watermilfoil and curly-leaf pondweed includes cross-cutting collaborations with Drs. Ray Newman (Subproject 9) and Przemek Bajer (Subproject 4).

Michael Verhoeven, a graduate student conducting research under this project, was awarded a highly prestigious Graduate Research Fellowship from the National Science Foundation. Carli Wagner, an undergraduate conducting research on starry stonewort in Dr. Larkin's lab, was awarded first place for her student poster presentation at the annual meeting of the Midwest Aquatic Plant Management Society. Rafael Contreras-Rangel is joining the project as a Master's student advised by Dr. Larkin following positions with MnDNR and Conservation Corps Minnesota; Rafael was awarded a one-year fellowship by the University.

Research under this award has been presented in peer-reviewed publications (two complete, one in revision, three in review, and several in preparation), research and outreach talks (19 total, 16 invited), and media coverage (12 total, including print, television, and radio).

Sub-Project Status as of *February 28, 2018*
Over the past six months, we have made substantial progress on our research addressing aquatic invasive plant biology and management in Minnesota lakes. We performed experiments testing desiccation tolerance of starry stonewort as part of our assessment of spread risk between lakes. We also established long-term, permanent monitoring locations on two infested lakes to evaluate rates of local spread of starry stonewort within lakes. We have continued to compile and analyze statewide aquatic plant survey data to understand the effects of herbicide treatments, environmental factors, and weather patterns on Eurasian watermilfoil and curly-leaf pondweed abundance and diversity of native plant communities. This work has informed and provided guidance for statewide AIS detection and decision-making through collaboration with Extension, lake associations, watershed districts, and MnDNR.

Since the last workplan update, we have disseminated our findings through (1) peer-reviewed publications (one paper has been accepted since the last update and two manuscripts are currently in revision and one is in review); six invited talks to agency staff, other researchers, and the public; two contributed talks at national scientific meetings; and 12 print, television, and radio stories.

An amendment was approved by LCCMR on 02/15/2018 that updated the project budget to balance higher than anticipate costs for *Travel* with lower than anticipated costs for *Professional Services* and *Equipment/Tools/Supplies*. The amendment does not change the overall cost of the project.

Sub-Project Status as of *August 31, 2018*
We continued to publish manuscripts from our research on starry stonewort spread and management (Activity 3) and have initiated laboratory experiments to test effectiveness of different algaecides/herbicides and concentrations for products that are currently being used for starry stonewort treatments in Minnesota but have not been subject to rigorous evaluation through published, peer-reviewed experiments.

We continue to acquire and synthesize monitoring data from statewide treatments for Eurasian watermilfoil (Activity 4) and curly-leaf pondweed (Activity 5). For both of these species, we have also initiated in-lake removal experiments to determine whether effective control of these AIS is sufficient to support recovery of native aquatic plant communities or whether additional management strategies (e.g., water quality improvement, native plant seed addition) are needed to restore native aquatic vegetation.

Over the last reporting period, we have communicated our findings through 3 peer-reviewed journal articles, 7 invited talks, 4 contributed presentations, and over 13 print, radio, and television stories.

Sub-Project Status as of *February 28, 2019*
We continued to publish manuscripts from our research on starry stonewort spread (Activity 3) and are continuing to conduct laboratory experiments testing the effectiveness of different algaecides/herbicides being used for starry stonewort treatments that have not been subject to rigorous evaluation through published, peer-reviewed experiments.

We continue to acquire and synthesize monitoring data from statewide treatments for Eurasian watermilfoil (Activity 4) and curly-leaf pondweed (Activity 5). For both of these species, we have made substantial progress on in-lake removal experiments to determine the extent to which control of these AIS is sufficient to foster recovery of native aquatic plant communities or whether additional management interventions are needed to restore native vegetation.

Over the last reporting period, we have communicated our findings through 2 peer-reviewed journal articles, 6 presentations, and 8 media stories.

Final Report Summary:
Aquatic invasive plants can lower native plant diversity, reduce habitat quality for fish and other animals, and interfere with recreation. To protect Minnesota's water resources, steps need to be taken to prevent new invasions, control existing populations, and support recovery of native biodiversity. These efforts require sound, science-based guidance. To provide such support, we conducted research to predict invasion risk, assess ecological impacts, evaluate control efficacy, and investigate factors limiting post-control recovery of native aquatic plants. This work was applied to three target species at different stages of invasion: (1) *Nitellopsis obtusa* (starry stonewort), first found in Minnesota in 2015 and now known in 14 lakes; (2) *Myriophyllum spicatum* (Eurasian watermilfoil), found in 1987 and established in >300 lakes; and (3) *Potamogeton crispus* (curly-leaf pondweed), here for >100 years and in >750 lakes. For starry stonewort, we developed models to predict risk of further spread and prioritize search locations for statewide volunteer search efforts, experiments to determine how long starry stonewort remains can survive out of water (i.e., remain transportable by boaters), and field and lab-based control experiments to guide management. For Eurasian watermilfoil and curly-leaf pondweed, we investigated relationships with native plant biodiversity, finding that they displace

native species, an effect compounded by lower water clarity, and contribute to "biotic homogenization"—loss of ecological distinctiveness. We are investigating how to better control these invasive species and foster recovery of native vegetation by synthesizing thousands of aquatic plant surveys and management records collected in Minnesota and by conducting in-lake removal and restoration experiments. This work will continue under a follow-up project (MAISRC Subproject 8.2: Impacts of invader removal on native vegetation recovery). Our findings help Minnesotans by highlighting practices needed to protect lake ecosystems and refining approaches for preventing invasions, reducing populations of established AIS, and restoring native species.

SUB-PROJECT 9. Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol

Project Manager: Michael McCartney
Description:
Phase II of this effort focuses on prevention of zebra mussel invasion by developing genetic evidence of spread sources and pathways so that they may be interrupted and also lays the groundwork for potential biocontrol through genetic modification technologies.

The prevention research will result in direct evidence of sources and pathways for zebra mussel invasions in Minnesota and will provide accompanying prevention management recommendations based on these findings. We will use highly variable population genetic markers called microsatellite DNAs, and variable DNA positions in the zebra mussel genome—Single Nucleotide Polymorphisms, or SNPs—to genetically type zebra mussel populations, and assign these populations to the source waters from which they were carried to infest new waters. We will complete this work for approximately 75 waterbodies, while also creating a database that will enable a more powerful analysis of additional waterbodies that may be studied in the future (e.g. new infestations).

While our first focus to reduce zebra mussel spread and impacts in Minnesota should be on well-informed inspection and decontamination programs, prevention cannot stop all new invasions, particularly in MN, with >11,000 lakes and > 4,650 boat ramps (includes DNR + local + private). Phase II therefore also includes a substantial focus on researching zebra mussel control options.

While several MAISRC and other programs are pursuing options related to chemical pesticides and biological controls, including microorganisms and parasites, this Phase II project focuses on rapidly growing genetic biocontrol technologies, including gene silencing by RNA-interference (or RNAi) as well as genome editing using CRISPR/Cas9 systems that have potential for application to zebra and quagga mussels ("dreissenids").
In Phase II, we will lay the groundwork for potential genetic biocontrol by completing the following: producing the first ever complete sequence of the zebra mussel genome; developing a Dreissenid Mussel Genome Collaborative (DMGC) to generate strategies for applying genetic technologies to zebra and quagga mussel biocontrol; and analyzing the zebra mussel genome (and "transcriptomes" of expressed genes) to find genes that could be targets for these technologies.

Summary Budget Information for Sub-Project 9:	**ENRTF Budget**:**	**$380,318**
	Amount Spent:	**$380,318**
	Balance:	**$0**

***This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.*

Outcome Activity 1	**Completion Date**
1. 30 mussels from each of 72 waterbodies genotyped and analyzed using microsatellite DNA markers	August 31, 2018

2. 10-15 mussels from each of 72 waterbodies genotyped and analyzed using SNP markers	December 31, 2018
3. Findings summarized and management recommendations made to DNR; results published	December 31, 2018
Outcome Activity 2	**Completion Date**
1. Long read genome sequencing	February 2018
2. RNA-Seq of transcriptomes (genes expressed in different life stages under various environmental conditions) a. Adult tissues b. Embryos and larvae	December 2017 March 2018 November 2018
3. Bioinformatics: genome assembly and annotation	August 2018
4. Bioinformatics: search for 3 high potential target genes	September 2018
Outcome Activity 3	**Completion Date**
1. Collaborative formed, “White paper” draft choosing target genes for transcriptome sequencing, published	March 2018
2. Collaborative formed	June 2018
4. Manuscript draft: genome assembly and initial analysis	December 2018

Sub-Project Status as of *February 10, 2014*
Project is currently being peer reviewed and will be funded with Clean Water Funds through June 2016.

Sub-Project Status as of *August 31, 2014*
Subproject has been approved and is underway with funding from the Clean Water Funds through June 2016.

Sub-Project Status as of *February 28, 2015*
The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of *September 24, 2015*
The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of *February 29, 2016*
The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of *August 31, 2016*
The preliminary phase of this sub project continues to advance with funding from the Clean Water Fund. Through the continuation process discussed in Subproject 1, the PI has been invited to submit a Phase 2 proposal for consideration and peer review. If selected, MAISRC will recommend it for funding as Subproject 9 of this proposal via a workplan and budget to be reviewed and approved by LCCMR. The above description of this Subproject will be updated accordingly.

Sub-Project Status as of *February 28, 2017*
This subproject was approved in February 2017. An updated project description, budget and outcomes are provided above.

Sub-Project Status as of *August 31, 2017*
For Activity 1 (genetics of spread), we expanded our analysis of Minnesota water bodies and added samples of zebra mussels from the Great Lakes, to produce a more comprehensive study of spread. All Great Lakes samples in our collection from summer 2016 were genotyped with 9 microsatellite markers, and these samples collected through 2016 from MN were analyzed by genetic clustering, assignment and ABC invasion model testing. We also launched the 2017 sampling season, visiting 31 new waterbodies in MN and collecting from 23 of these (out

of 75 new MN sites listed in the research addendum, Appendix 2; 8 water bodies either had too few mussels to collect, or had access issues that we will solve). For Great Lakes samples, it was necessary to develop a test to quickly and reliably distinguish between zebra and quagga mussels, because Great Lakes collections contain both species, and most are dominated by quagga mussels. Our SNP test is refined for zebra mussels, and to avoid the expense of submitting samples of the wrong species, we tested a quick molecular assay (modified from the literature) validated it on sequenced DNA from zebra and quagga mussels, and now use it routinely on these collections.
We processed all samples that were genotyped in Phase I (with microsatellites), as well as the newly extracted Great Lakes samples, and submitted them for genomic SNP analysis [using the University of Minnesota Genomic Center's (UMGC) assay refined for zebra mussels that was completed in Phase I (December 2016)]. We performed initial analyses of SNP data (examining effects of filtering parameters, filtering SNP data, scoring SNP markers, initial clustering analysis…) and found that with conservative parameters 3320 SNPs could be scored for each of 439 mussels, with no missing data); 10 times or more can be scored with less filtering. This important step shows that the SNP analysis generates a very large number of scorable markers (approximately the number expected), and shows the route we can take to increase the number of markers to study relationships between important source water bodies (e.g. Lake Minnetonka, St. Croix and Mississippi Rivers, Great Lakes).

On Activity 2, we completed the bulk of our lab's work, planned for May-July, that was required to launch the sequencing of the genome. For this, we needed new zebra mussel tissue from animals of known gender. This information is critical (e.g. there are male and female specific genes of interest to us) but was lacking from the genome we sequenced in Phase I—that genome was generated simply to help isolate and score SNP markers. We collected large mature zebra mussels from Pelican Brook (Crow Wing Co.), sexed them by microscopy of gonads in our lab, then extracted very high molecular weight DNA using a specialized DNA protocol that we have developed, which generates DNA of an average length of 60,000 bases—ideal for the long-read sequencing being done this summer and fall. Also for Activity 2, we collected specimens and preserved material for the following transcriptomes: [larval (D-stage, umbonal stage, pediveliger), and female adult and male adult (gonad, mantle) from high calcium environments (transcriptomes 3-8 in research addendum)].

For Activity 3, we selected target genes, contacted and have held continued discussions with developmental biologists, CRISPR/Cas9 and RNAi biotechnology experts, a population genomics expert, and with bivalve biologists who are candidates for the genome collaborative.

For dissemination and outreach, we made 7 presentations to public audiences and to MN DNR. Two papers are in press from Phase I work, and we completed revisions and resubmitted a manuscript for *Biological Invasions*: analysis of spread on the entire microsatellite data set from MN.

Sub-Project Status as of *February 28, 2018*
Activity 1: All additional samples collected and extracted this summer and fall have been submitted or will be submitted for Sequence-Based Genotyping of SNP markers by February 2018, so we expect a complete data set to be available April 2018. Analysis is progressing. We have become familiar with the pipeline we use to process and filter the raw data. We have completed a substantial amount of genetic clustering analysis. No invasion model testing has been completed with the SNP data yet but that is next.

Activity 2: Long-read genome sequencing was completed to the depth we used as our first target and initial assemblies were completed. Sequencing quality is very high, which is extremely good news given the many efforts we made to extract long molecules of genomic DNA from March-September. By examining the average length of contiguous assembled genome fragments from this first round of sequencing, we determined that additional sequencing depth is needed and this work was launched January 2018. RNA was extracted from all transcriptome samples. RNASeq libraries were created and samples are in the cue to be sequenced for all other transcriptomes, including the shell formation transcriptomes that we added. Since we did not obtain a full set of larvae and embryonic stages in 2017, those transcriptomes will be postponed to 2018.

Activity 3: We made substantial progress on genetic biocontrol technology. We launched a collaboration with M. Smanski's lab at UMN. Smanski has investigated the use of technology to engineer promoter sequences of genes that are regulatory "switches" during embryonic development. Release of animals containing these engineered genes could lead to embryonic lethality or infertility—when engineered animals mate with resident animals with the non-engineered wild type promoters. We will pilot some work on this technology this spring, and use our genome sequence data to obtain promoter sequences. Smanski will join the Genome Collaborative, along with G. Wessel from Brown University who will provide advice to help us identify developmental genes. We have also made contact with E. Hendrickson at the UMN Genetic Engineering Shared Resource to examine potential research directions for CRISPR/Cas 9, and we contacted Stanley Burgiel in Washington DC for information on the status of the US regulatory process concerning gene drives in invasive species.

An amendment was approved by LCCMR on 02/16/2018 that moved the project completion date to February 2018.

Sub-Project Status as of *August 31, 2018*
All samples collected for Activity 1 have been genotyped using Sequenced Based Genotyping. At present, we have a data set scored for 6092 markers per mussel, 91 sampling sites, 70 water bodies and 1,445 mussels. We have completed genetic clustering analyses that demonstrate the increased power of these markers compared to microsatellites. We have drafted a manuscript that compares the power of these genomic markers to the older markers for studies of zebra mussel invasions. We are working on testing invasion models.

Activity 2: The zebra mussel genome has been sequenced and a high-quality assembly has been prepared using the software Canu, 1 month ahead of schedule. Our next steps are to scaffold the assembly to map the sequences to chromosomes. Late summer and fall will be taken up with running the homology searching to find target genes within this genome, name them and characterize them. Transcriptome RNA sequencing is complete, although we will add a few this summer (to include adult gonad). We are on schedule for a draft genome to be completed by December 31.

Activity 3: The "white paper" on the zebra mussel genome project is in review at the journal *Conservation Genetics.* We have found other scientists who have interests in working on this genome—including new contacts at the University of Göttingen (D Jackson), McGill University (M Harrington) and the University of Toronto (E Sone) who work on embryonic development, shell formation and byssal threads, and offer expertise in biochemistry, developmental biology and materials science. We also have a growing collaboration with the population genomics group at the University of Montana. We developed 2 proposals on genetic biocontrol but have not yet secured funding for that work.

Sub-Project Status as of *February 28, 2019*
This project ended on December 31, 2018. A final report is currently being drafted and will be submitted to LCCMR before the February 28, 2019 deadline. An amendment request in included in this report to transfer unspent funds back into MAISRC reserves.

Final Report Summary:
Since arriving in Duluth Harbor in 1989, zebra mussels have infested more than 150 inland lakes and 17 rivers and streams in MN, with rising ecologic and economic costs. Efforts to block new invasions must be focused strategically on major sources of spread. To help achieve this, we used direct, forensic-like analyses to genetically identify waters from which mussels were carried to infest MN lakes. Using our new genome sequences and methods, we genetically classified mussels from more than 70 water bodies, with more than 6,000 DNA markers per mussel (compared to 9 markers/mussel in Subproject 9.1) – providing significantly increased clarity in the analysis. We found that lakes in the Detroit Lakes, Brainerd and Alexandria regions form large, unique genetic clusters found nowhere else. Additionally, mussels from the Mississippi and St. Croix

Rivers, Lake Superior, and Lake Minnetonka (4 highly-likely source waters) are distinguishable from the clustered invasions with 6,000 genomic markers, but with our previous analysis of 9 markers, they were not. More research is needed across a larger, more regional landscape to determine the original sources of zebra mussels into Minnesota, but results reinforce the management message that prevention can work – there is no genetic information to support the hypothesis of a "super spreader" lake. Early and high profile infestations of zebra mussels appear to have been contained (e.g. Lake Millle Lacs). However, vectors that are moving mussels locally within lake-rich regions, need to be identified and blocked.

For the first time, we sequenced the entire zebra mussel genome, using state of the art technology that allowed mapping of genes to chromosomes with great confidence. We sequenced and measured expression of genes in tissues that control shell formation, byssal thread attachment, and survival in high temperatures—each are strong candidates for targeted gene modification. The results include a publicly accessible genome: a powerful tool for invasion biology and biocontrol researchers in Minnesota and worldwide.

SUB-PROJECT 10. Implementing Findings: An educator-outreach position.

Project Manager: Dan Larkin
Description:
Aquatic invasive species (AIS) pose a growing threat to Minnesota's health, economy, and environment. Consequently, there is an increasing need to expand the effort to detect and respond to AIS. Although Minnesota has many well-designed and executed AIS outreach and educational programs, critical gaps exist: no organized statewide surveillance programs exist to target high risk areas with trained observers and no monitoring system is in place to collect and share AIS treatment response data that could inform both research and management. This project will fulfill these needs.

A network of citizen scientists and professionals will be developed to enhance reporting and management of AIS. This will be achieved by:

1) Developing and implementing a program to train observers to rapidly identify and report possible AIS,
2) Training participants to work with AIS agency professionals who are responsible for evaluating and verifying AIS reports;
3) Developing and implementing a program for monitoring populations of AIS in conjunction with treatment efforts, to help advance management strategies and decision making, and;
4) Developing and launching an interactive data base for AIS population survey data.

In partnership with the Minnesota Aquatic Invasive Species Research Center (MAISRC), University of Minnesota Extension will offer two programs, AIS Detectors and AIS Trackers. The AIS Detectors program will train citizen scientists and professionals to make credible AIS reports in coordination with MnDNR, allowing agency AIS staff to more efficiently focus on verifying new infestations. The AIS Trackers program will train citizen scientists and professionals to monitor changes in populations of AIS over time in specific locations (i.e., a lake or river reach) and to generate data useful for adaptive management, which includes assessing treatment options and evaluating response to treatment efforts. Together these programs will implement 17 actions identified as priority needs in Minnesota's Management Plan for Invasive Species (2009), developed by the Minnesota Invasive Species Advisory Council.

Both programs will recruit and train professionals (i.e., AIS managers and service providers) and citizen scientists (lake association leaders, county AIS task forces members, Master Naturalists and other motivated citizens). Successful completion of these programs will be recognized by certification. To maintain their status as a certified AIS Detector or AIS Tracker, volunteers must perform a minimum level of service and maintain and increase their expertise through continuing education opportunities offered by the programs. Annual service will include activities that are self-initiated as well as those that are organized by the programs, such as surveys of high risk lakes for new AIS occurrences or providing outreach related to reporting AIS.

An interactive AIS database, A-DRUM (AIS Data Repository – University of Minnesota) will be developed to manage the information collected by AIS Trackers. This information will be fully accessible to certified trackers, to DNR AIS managers, and to MAISRC researchers. AIS Detectors, AIS Trackers, and A-DRUM will be designed so that the work of the trained citizen scientist is coordinated with professional managers, notably Minnesota Department of Natural Resource (DNR) AIS specialists, so that it can effectively extend their reach for surveillance, monitoring, response, and management. The aim of this project is to have a fully-functioning network of 240 AIS Detectors and initial groups of AIS Trackers contributing to Minnesota's AIS efforts by 2019.

Summary Budget Information for Sub-Project 10:	**Revised ENRTF Budget:**	**$525,389**
	Amount Spent:	**$520,850**
	Balance:	**$4,539**

*****This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.***

Outcome Activity 1	**Completion Date**
1. Draft web based course for review	August 22, 2016
2. Draft classroom course for review	August 22, 2016
3. Run peer test training (~20 University and state agency staff)	October 12, 2016
4. Pilot train ~20 master volunteer detectors	October, 2016
Outcome Activity 2	Completion Date
1. Master detector volunteer support	March 30, 2016
2. Provide web-based and 1 classroom basic training sessions per year (4 years)	May 1, 2019
3. Develop advanced trainings	March 30, 2017
3. Provide 1-2 advanced training sessions per year (4 years)	June 30, 2019
Outcome Activity 3	Completion Date
1. Develop introductory field session curriculum, including training aids	May 31, 2017
2. Develop online training curriculum, including training aids	April 30, 2017
3. Develop classroom and second field session curriculum, including training aids	June 30, 2017
4. Offer Pilot training	July 31, 2017
Outcome Activity 4	Completion Date
1. Create and review finalized list of adjustments to existing Software	December 31, 2017
2. Modify Software	June 30, 2017
3. Test usability of software, refine as needed	March 30, 2018
4. Populate A-DRUM, as data is gathered by A-Trackers; add other available data suitable for statewide comparisons	August 31, 2018
5. Analyze collected data to identify trends in AIS abundance and effectiveness of management actions	June 1, 2019
Outcome Activity 5	Completion Date
1. Develop and launch social networking site	July 1, 2019
2. Develop 1 additional species modules	July 1, 2018
3. Offer basic course 3 times -- 50 person total enrollment	July 1, 2019
4. Create and deploy survey gear kits for regional check-out	July 1, 2018
5. Offer 2 refresher trainings (1 in person, 1 webinar)	July 1, 2019
6. Offer 2 advanced training classes—20 person total enrollment	July 1, 2019

Sub-Project Status as of *February 10, 2014*
No progress to report as project is not anticipated to start until approximately March, 2015

Sub-Project Status as of *August 31, 2014*
An Extension Educator position has been approved by the Center Advisory Board, the Deans of CFANS and Extension, and the Center Administrative Review Committee. This person will be responsible for planning, developing, implementing, and evaluating educational programs that help local governments, lake associations, and citizens groups plan, develop and implement science-based programs that prevent, monitor, and control the establishment and spread of aquatic invasive species. A letter agreement has been executed with the MDNR and Sea Grant to identify unmet needs, avoid redundancy, and ensure this position creates added capacity in AIS education efforts.

MAISRC is proceeding with hiring process for this position with the intent to have someone on board by March 2015. Prior to this new hire starting, we will submit a workplan with a request to approve an initial budget that will be used to pay salary, fringe, and program costs once the new person is hired. Since this project is a non-research position, no project proposal and peer review will be conducted. Updates will be reported as part of the Overall project workplan.

Sub-Project Status as of *February 28, 2015*
Danielle Quist started work February 26, 2015 as the new Extension Educator for the Center. Ms. Quist is meeting with key partners and stakeholders while she works with Extension and MAISRC to develop a detailed program plan in the next few months. This program plan will be focused on outreach and programming related to AIS control, which is consistent with the programming gaps identified by DNR, Minnesota Sea Grant, MAISRC, and Extension in preliminary outreach coordination meetings. Dr. Galatowitsch will continue to serve as project manager of this Subproject, with Ms. Quist as the key implementing staff. Ms. Quist will be paid from Sub-project #1 until a program plan is approved by MAISRC and funds released to this sub-project.

Sub-Project Status as of *September 24, 2015*
The new extension educator was hired and began work February 26, however, it was determined that the position was not a match with the hire. We are working closely with Extension to rehire as soon as possible. Meanwhile, development of this subproject by MAISRC staff has continued in full force as part of Subproject #1.

Additionally, Extension has contributed significant time to develop this program that will have three components: 1) an “AIS Detectors” program to train 400 citizen scientists and professionals to rapidly identify and report AIS, increasing capacity for AIS response 2) an “AIS Trackers” program to train 100 citizen scientists and professionals to survey and monitor populations of AIS using standardized protocols in order to guide and evaluate effectiveness of AIS management; and 3) development of an interactive, web based date repository for collecting and sharing standardized data for improved AIS management.

Further, Extension has committed approximately 50% of Eleanor Burkett’s time and 5% of Faye Sleepers time over the next four years to implement the AIS Detector portion of the program, which will be considered in-kind support from Extension.

This project has now completed external review and the workplan is being submitted for approval by LCCMR simultaneous to this workplan submission; please see amendment request above.

Sub-Project Status as of *February 29, 2016*
An online template for the online portion of the AIS Detectors course has been designed and created in Moodle, the University of Minnesota’s course delivery platform. The course information for the online portion of the course was organized into six modules and for each module, the specific learning outcomes were developed.

The AIS Detectors course will initially focus on ten AIS species (4 fish, 3 plants, and 3 invertebrate and their native "look alikes"). These species were chosen in consultation with MAISRC's technical committee. Work on AIS Trackers has not yet begun because we are currently hiring the Extension Educator, who will provide leadership for this initiative.

Sub-Project Status as of *August 31, 2016*
The two part curriculum for the AIS Detectors program has been developed and is ready for pilot-testing. Part 1 is an online course consisting of 8 modules and will be pilot-tested by citizens and agency professionals in September 2016. Part 2 is an all-day classroom session, which will be pilot-tested in October 2016. Based on feedback received, we will revise the online and classroom sessions, so the program is ready for a statewide launch in Spring 2017.

An updated timeline was created for the AIS Trackers program to achieve the given outcomes by the end of the grant cycle. As part of this update various assessments and reviews have been completed that are needed to help build the A-DRUM database, develop curriculum and training materials, and select methods needed to monitor AIS population changes and identify trends from AIS treatments.

Sub-Project Status as of *February 28, 2017*
Progress was made in several key areas of Detectors and Trackers. The full web-based Detectors course was pilot-tested by U of M faculty and staff, MnDNR staff, and by an initial cohort of citizen volunteers. These groups then participated in and evaluated a full-day workshop, feedback from which is currently being used to revise the curriculum. Groundwork has been laid for full implementation of the AIS Detectors program in spring of 2017 with six all-day workshops scheduled throughout the state. Advanced training opportunities are being developed, including a coordinated, statewide search effort for starry stonewort scheduled for August 5, 2017. Development of the Trackers program is in progress, with a detailed plan for program roll-out and preparation of sampling protocols that have undergone technical review by external partners. In addition, progress has been made in refining the scope of the Trackers database and we have met with a vendor and agreed on a timeline for development of the data management system.

Sub-Project Status as of *August 31, 2017*
The AIS Detectors program has fully launched since our last workplan update and we have made progress in development of the AIS Trackers program. Following 8 Detectors workshops being held around the state in spring 2017 (7 for new participants, 1 refresher training for pilot participants), 125 citizen scientists have now completed Detectors training, of which 121 have completed all steps necessary to become certified AIS Detectors. Our first Detectors advanced training opportunity (Starry Trek) will take place August 5, 2017.
For the AIS Trackers program, program and monitoring protocols have been reviewed by MnDNR and revised based on their input, detailed learning objectives have been developed for online training modules, field and workshop components for training have been outlined, a contract is in place for development of the A-DRUM database and web-entry system, and initial testing and revision of the system is underway.

Sub-Project Status as of *February 28, 2018*
The AIS Detectors program has completed its first full field season, including the launch of the first advanced training opportunity, and we have made progress in developing AIS Trackers since the last workplan update. Following the completion of their training, our 121 certified AIS Detectors recorded 1,899 volunteer hours in 2017. We are currently working to update the AIS Detectors curriculum based on feedback from the first full cohort of AIS Detectors and discussions with MnDNR and our other agency partners following the field season. We have scheduled six AIS Detectors workshops for spring 2018.

On August 5, 2017, 200 volunteers and over 20 local host coordinators throughout the state participated in Starry Trek, our first AIS Detectors advanced training opportunity. Our volunteers discovered what was, at the time, the tenth known population of starry stonewort in Minnesota (Grand Lake, Stearns Co.). Early detection of this small, likely recent infestation through Starry Trek enabled a rapid response plan to be developed by the Grand Lake Association, MnDNR, and MAISRC and implemented by MnDNR, whose AIS Specialists dove and hand-removed all visible plants.

For AIS Trackers, we are currently developing the online course curriculum and training modules based on the learning objectives described in the previous workplan update. We are continuing to test and review the online database and web-entry system. We have recruited a pilot group from the Lake Demontreville-Olson Association (Washington Co.) to pilot-test the AIS Trackers curriculum and monitoring protocols in 2018.

An amendment was approved my LCCMR on 02/06/2018 to Activities 1–3 to account for: (1) changes to project staffing, (2) to balance higher than anticipated costs associated with Office and General Operating Supplies and Services with lower than expected costs for Professional Services and Non-Capital Lab & Field Equipment/Supplies, (3) an accounting correction for Travel – Domestic, and (4) Room Rental fees being needed for Activity 2 but not for Activity 1.

Sub-Project Status as of *August 31, 2018*
The AIS Detectors program trained its second cohort in 2018 (96 participants), for a total of 217 certified Detectors throughout the state from the first two years of the program. The 2018 training featured online and in-person curricula updated based on feedback from the 2017 cohort. We offered an Advanced Training opportunity in plant identification in June 2018 and are offering four additional Advanced Training opportunities in the remainder of summer 2018.

Starry Trek will again be held in 2018 (August 18) in partnership with MnDNR, University of Wisconsin-Extension, and the River Alliance of Wisconsin. Volunteer registration is currently underway; we will have 25 rendezvous sites thought Minnesota, up from 20 in 2017.

Our pilot launch of AIS Trackers is currently underway. A pilot group from the Lake Demontreville-Olson Association (Washington Co.) has completed and provided feedback on the online curriculum and we will provide hands-on training in monitoring methods over the remainder of the summer.

Sub-Project Status as of *February 28, 2019*
Following the completion of the educational season for the second cohort of AIS Detectors, we offered four Advanced Training opportunities throughout the summer, including three new training opportunities (Advanced Aquatic Plant ID, AIS on the Water, and Emerging Threats) and the second annual Starry Trek. Our 217 certified AIS Detectors recorded 5,278 volunteer hours in 2018.

Starry Trek was held on August 18th in partnership with MNDNR, University of Wisconsin-Extension, and the River Alliance of Wisconsin. Over 225 volunteers registered and participated in Starry Trek 2018 (up from 200 volunteers in 2017) at 23 rendezvous sites statewide.

We continued to work with our pilot group for the AIS Trackers program (Lake Demontreville-Olson Association). After they completed the curriculum, we held a focus group with them to solicit feedback on course content and structure. This discussion focused on the goals of the program, the needs of participants, the level of difficulty of the material, feasible expectations, and other topics related to AIS Trackers. As a result of this feedback, we are revising the course design of AIS Trackers. The AIS Trackers core curriculum will now focus on providing more comprehensive web-based education in fundamentals of aquatic plant management: its underlying science, methods, and goals. The prior emphasis on training participants to perform their own monitoring of management efforts will be reduced, though we will offer advanced training opportunities for groups that have completed the core training and want to perform their own monitoring. We will pair the revised training with continuing to reach out to lake groups and professionals to solicit relevant management, vegetation monitoring, and water quality data.

During the last reporting period, our AIS Extension programs were featured in 1 peer-reviewed publication, 4 talks, and 19 media stories.

Final Report Summary:
Early detection of invasive species is critical. However, there are few professionals addressing aquatic invasive species (AIS) in Minnesota relative to our state's vast water resources. Furthermore, while many efforts each year seek to control AIS, there are gaps in synthesizing treatment outcomes. These gaps limit our ability to improve management and contribute to uncertainty for lake associations and others tasked with management decision-making. We developed AIS citizen science and training programs to address these challenges. Specifically, AIS Detectors trains volunteers as "eyes on the water" for AIS detection and response, and AIS Trackers educates non-professionals on AIS management and leverages monitoring data to refine management guidance. Over 820 Minnesotans have participated; more have been reached through presentations, media, and publications. To date, 299 people have become certified AIS Detectors and gone on to contribute >10,000 hours to outreach, stewardship, citizen science, and other volunteer activities, a service value >$273,000. Outgrowths of Detectors have led to additional service, including "Starry Trek", which annually draws ~200 volunteers statewide for targeted searches for the invasive alga starry stonewort. This event, in partnership with the Minnesota DNR and colleagues from Wisconsin, has led to identification of two new starry stonewort populations and associated opportunities for rapid response; over 500 people have participated. Through AIS Trackers, we developed a new online course to educate people about AIS management and new mechanisms for analyzing AIS treatment outcomes. Over 70 people have piloted this program, which will open in 2020 to a wide audience in Minnesota and beyond. Minnesotans benefit from our work through enhanced capacity for AIS surveillance and robust training that helps professionals and non-professionals alike make better-informed management decisions. Results show that natural resources benefit when we empower Minnesotans to contribute to AIS prevention efforts through rigorous, science-based training and service programs. These programs are now well-established and will continue to be implemented under support from MAISRC, UMN Extension, and program revenue.

SUB-PROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods.

Project Manager: Dave Andow
Description: Simulation models are an efficient and low-cost means of developing and evaluating control Working with the DNR, we will also use risk analysis to prioritize management actions based on cost/benefit trade-offs. This activity will be led by Professor David Andow (head of the University of Minnesota's NSF risk assessment training program [0.8 FTE for3 years]) who will have a postdoctoral fellow (1.0 FTE for 3 years), or equivalent. He is prepared to start immediately and expected to work with the DNR on evaluating the relative risks of Asian carp invading different Minnesota rivers so that systems can be selected for possible barrier construction. Specific details and costs of this project will be determined by Center-led peer-review. This description and the outcomes below will be updated following approval of a more detailed subproject work plan and budget.

Summary Budget Information for Sub-Project 11:	**ENRTF Budget*:**	**$93,343**
	Amount Spent:	**$93,343**
	Balance:	**$0**

This value is projected; it may be adjusted during the course of the project pending progress and input from peer-review of this particular sub-project.

Outcome	Completion date
1. Analyze management goals of Asian carp	Sept 30, 2015
2. Analyze adverse ecological effects of Asian carp	Sept 30, 2015

Sub-Project Status as of *February 10, 2014*
A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of *August 31, 2014*
As reported in the sub-project's July 31, 2014 update: As of this sub-project status update, we have adhered to our initial milestones and have completed a variety of background research. Specifically, we have gathered and reviewed key publications on Asian carp and have conducted informational interviews with 11 people involved with Asian carp efforts in Minnesota from state and federal agencies, academia, and non-governmental organizations. This background research has provided the base of knowledge that will inform our subsequent research activities.

Sub-Project Status as of *February 28, 2015*
As reported in the sub-project's January 31, 2015 update: we have further refined our research design based on feedback from informational interviews, obtained Institutional Review Board approval for the study, and started data acquisition and analysis. We have conducted four focus groups and the final one is scheduled. In each of these focus groups we had participants produce a list of potential adverse effects given the establishment of invasive Asian carp in Minnesota and discuss the importance of each potential adverse effect. In addition, we had participants discuss the existing and potential management of invasive Asian carp in Minnesota. The results of this work will inform a report on potential adverse effects for distribution, will inform the subsequent in-depth interviews and survey, and will inform the analysis stage of a risk assessment to be conducted in Phase 2 of this project. The project objectives above have been revised to reflect those in the approved work plan for the sub project.

Sub-Project Status as of *September 24, 2015*
As of this sub-project status update, we have finished the research for both parts of Phase 1, have finished the report on the potential adverse effects, and are in the process of analyzing and writing the report on the management interviews. First, we conducted the fifth and final focus group on the potential adverse effects that could result from the establishment of silver and bighead carp in Minnesota, and we completed the report summarizing the findings from these interviews. The adverse effects gathered in these focus groups were associated with 26 valued and potentially affected entities that were grouped into 9 categories: Native fish species; Plankton/Cyanobacteria; Other aquatic organisms; Birds and other animals; Ecosystems; Diseases/Parasites/Pathogens; Commercial fishing/Commercial bait/Commercial aquaculture/Commercial transportation; Tourism/Recreation; and Public perception and relationship to water resources. These findings will inform the risk assessment to take place in Phase 2 of this project and were used to inform the in-depth interviews on the management of Asian carp. Second, we conducted 16 in-depth interviews with agency officials, scientists, and stakeholders involved with the existing management of Asian carp in Minnesota. These interviews were used to better understand and help address the conflicts and tensions that exist surrounding the management of Asian carp. Preliminary findings reveal that management is hampered by uncertainties surrounding the likely impact of Asian carp in Minnesota and the impacts of barriers on Asian carp and native fish species. In addition, management and research efforts are hindered by decision making that is based on apathy or fear – two common responses to Asian carp and invasive species, more broadly.

Final Report Summary:

Individual Asian carp continue to be found in Minnesota waters, and there remains pressure for sound statewide management to address this potential threat. To help advance the management of Asian carp in Minnesota and inform the initial problem formulation step in a risk assessment, this project conducted focus groups and in-depth interviews to: 1) identify potential adverse effects from Asian carp to inform a subsequent risk assessment, and 2) characterize the tensions and conflicts that are hampering Asian carp management. First, we conducted 5 focus groups with 20 individuals, including MN-DNR managers and stakeholders involved with Asian carp. During these focus groups, participants created a list of potential adverse effects that could occur if Asian carp were to establish in Minnesota and discussed the importance and potential causes of these adverse effects. The resulting potential adverse effects were associated with 26 valued and potentially affected entities. Focus group participants also discussed what could and should be done to manage Asian carp, including where improvements in existing management efforts are needed. The results from this work were summarized in the report *Potential adverse effects and management of Silver & Bighead carp in Minnesota: Findings from focus groups*, informed the in-depth interviews on management, and will inform the risk assessment to be conducted in Phase 2 of the project. Second, to study and help address the tensions and conflicts impeding management we conducted 16 in-depth interviews with individuals who have been involved with Asian carp management in Minnesota, including state and federal agency officials, University researchers, and representatives from non-governmental organizations. As presented in the report *Exploring tensions and conflicts in invasive species management: The case of Asian carp*, we found three areas of tension and conflict impeding Asian carp management: 1) scientific uncertainty (concerning the impacts of Asian carp in Minnesota and the impacts of barriers on Asian carp and native fish species), 2) social uncertainty (concerning the divergent views of what, if anything, should be done to manage Asian carp), and 3) the needed approach to Asian carp research and management. Findings point to the need for the right relationship to uncertainty and for reflexive deliberation on the judgments informing research and management decisions.

SUB-PROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk Analysis

Project Manager: Dave Andow
Description: A growing body of work, including this project's Phase 1 research, has identified a few key issues surrounding Asian carp management in Minnesota. First, there is a need to determine which areas of the state should be prioritized for management. Second, management is hampered by uncertainties surrounding how Asian carp will impact Minnesota's waterways and whether barriers do more good than harm. Third, soundly addressing these first two points is complicated by the existence of, and concerns about, apathy and fear based responses to Asian carp. Furthermore, there exist conflicting views about how to advance in the face of these points, based partially on differences over risk adversity, judgments about the scientific literature, and differing goals for Minnesota's waterways. In the face of these complexities, there is a need for a science-based tool, such as risk analysis, to guide decision making.

To help address this complicated situation, the project will conduct a risk assessment to prioritize issues and areas for Asian carp management and to reduce the uncertainty about how Asian carp will impact Minnesota's waterways. The risk assessment will assume that silver and bighead carp will arrive in all Minnesota waterways and will focus on determining which potential adverse effects are most likely and consequential in the different watersheds of Minnesota. It will first use a qualitative approach to determine which adverse effects are most salient and then work towards quantifying their likelihood and consequence of the most salient effects. The risk assessment will follow a deliberative process involving a variety of relevant experts and expert stakeholders, and will be designed in coordination with the MNDNR. Important areas of disagreement, remaining uncertainties, and additional research needs will be characterized so as to foster a productive discussion about them. This project will also include a risk communication component to share findings and foster a conversation about the findings' implications for management. The risk communication process will involve organizing a meeting where those involved with the risk assessment will discuss its results and implications with the relevant and interested parties who were not involved with the risk assessment, including a broader set of stakeholders, researchers, managers, and decision makers from relevant state and federal agencies.

The findings from this project are greatly needed, as Minnesota progresses through the many challenges that arise as it seeks to manage Asian carp. This project will help prioritize the management of Asian carp in Minnesota by thoroughly gathering the existing knowledge on Asian carp and using it to assess how they will impact Minnesota. In addition, by furthering deliberation on and characterizing important areas of disagreement, remaining uncertainties, and additional research needs, this project will help identify ways to make management progress despites these limitations. Finally, by fostering conversation among researchers, managers, stakeholders, and decision makers, this project will promote needed dialogue and communication to support decision making in the face of complexity and uncertainty.

Summary Budget Information for Sub-Project 11-2:	**ENRTF Budget:**	**$126,677***
	Amount Spent:	**$126,677**
	Balance:	**$0**

* This includes remaining funds from Phase 1, transfer of which was approved by LCCMR in November 2015.

Outcome	**Completion Date**
1. *Risk assessment report*	Dec 30, 2016
2. *Risk communication report*	May, 2017

Sub-Project Status as of *February 29, 2016*
As of this project update, the planning for the risk assessment is advancing as scheduled. A small committee including this project's research staff and one person from the Minnesota Department of Natural Resources (DNR) and one from the US Fish and Wildlife Service was formed to guide the planning of the risk assessment. The two-day meeting where a majority of the risk assessment will be conducted has been scheduled for March 8^{th} and 9^{th}, 2016 on the Minneapolis campus of the University of Minnesota. Twenty-eight people have agreed to participate in the risk assessment including individuals from: the Minnesota DNR, stakeholder groups, DNRs from 5 other Midwestern states, 5 federal agencies, and 5 academic institutions. An online survey to help determine the most salient potential adverse effects for the risk assessment has been designed and will be administered one month before from the risk assessment meeting.

Sub-Project Status as of *August 31, 2016*
Since the last project update, the two day risk assessment workshop took place and writing has started on the resulting report. Twenty-three experts on bigheaded carps and Minnesota's waterways participated in the risk assessment workshop including individuals from 5 federal agencies, 5 academic institutions, the MN DNR, DNRs from 2 other states, and a stakeholder group. In advance of this workshop, an online survey was conducted with workshop participants and Phase 1 focus group participants to select the potential adverse effects to focus on in the risk assessment. As a result of the survey, the risk assessment focused on the impacts to game fish, non-game fish, species diversity/ecosystem resilience, and recreation (from the silver carp jumping hazard). Four watersheds were chosen to be studied, selected to be both geographically diverse and relevant to the current decision making context. During the workshop, risk assessment participants characterized the likelihood that bigheaded carps would establish in each watershed, the resulting abundance of bigheaded carp in each watershed, and the severity of each potential adverse effect in each watershed. The risk assessment report is being written by project researchers and a subset of the workshop participants and the report writing is ongoing.

Sub-Project Status as of *February 28, 2017*
Since the last project update, the report for the Minnesota Bigheaded Carps Risk Assessment has been drafted and reviewed by risk assessment workshop participants. In working on the risk assessment report, project

researchers: 1) transcribed key documents from the risk assessment workshop and provided them to volunteer authors from each watershed small group for their use in drafting the section of the report on their watershed, 2) calculated the overall risk by combining the establishment likelihood and adverse effect consequence level for each watershed, 3) drafted the introduction, methodology, overall risk characterization, and discussion sections of the overall report, and combined them with the sections from each watershed to arrive at the overall risk assessment report, and 4) sent the full draft report to all risk assessment workshop participants for their review. Comments have been received and the risk assessment report is in the process of being revised. Planning for the risk communication meeting has also begun. The project's completion date has now been extended from December 30st, 2016 to May 31st, 2017, however still within the appropriation timeframe.

Sub-Project Status as of *August 31, 2017*
This project is complete. A final subproject workplan will be submitted by 9/30/2017. We are asking that the remaining balance of $13,294 be returned to the MAISRC reserve to be distributed to other priorities.

Final Report Summary:
Bighead and silver carps (bigheaded carps) pose a threat to Minnesota's waterways and there is a need to better understand their potential impacts to inform management actions. Towards this end, project researchers designed and conducted a risk assessment for bigheaded carps in Minnesota. Results from previous (Phase 1) research and a survey with risk assessment participants were used to focus the scope of the risk assessment on four potential adverse effects: impacts to game fish, non-game fish, species diversity/ecosystem resilience, and recreation (from the silver carp jumping hazard). Four watersheds were focused on, selected to be both geographically diverse and relevant to the current decision-making context. The risk assessment was conducted with the participation of twenty-three experts on bigheaded carps and Minnesota's waterways. A workshop was held to discuss the risk assessment findings and their implications for the management of bigheaded carps in Minnesota, and 50 people attended including stakeholders, researchers, managers, decision makers, and members of the public. Insights garnered from this workshop informed the final version of the risk assessment report, "Minnesota Bigheaded Carps Risk Assessment" which was released in May 2017. This risk assessment represents the first systematic analysis of the risks posed to Minnesota from bigheaded carps and will both justify and inform future management efforts. Specific findings from this report include that the risk from bigheaded carps varies greatly depending on the watershed and potential adverse effect considered. The risk was higher for the species diversity/ecosystem resilience and recreation potential adverse effects and for the Minnesota River-Mankato and Lower St. Croix River watersheds. These findings emphasize the need for a timely management response to protect watersheds identified as most at risk, while ensuring that any collateral damage from management actions leads to less ecological harm than bigheaded carps are likely to cause.

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology

Project Manager: Donn Branstrator
Description: Spiny water flea (*Bythotrephes longimanus*) is a major threat to the lower food webs in Minnesota lakes, yet how the invader's establishment and proliferation impact native game fish remains a critical unanswered question. Fish are generally long-lived, their populations are often dominated by one or two cohorts, and their growth and survival are influenced by multiple environmental factors, making it challenging to link changes in fish populations and health to particular stressors such as spiny water flea invasion. To address the problem, one promising approach being pursued by staff at the Minnesota Department of Natural Resources (MNDNR) and Voyageurs National Park is to use long-term gill net and seine surveys to assess the type, chronology, and magnitude of fishery changes in response to spiny water flea invasion in Rainy Lake and Kabetogama Lake. The fish surveys being used are recognized as some of the longest-running, most complete data bases of fish in inland Minnesota lakes and represent excellent opportunities to test impacts of spiny water flea on higher trophic levels. Their utility, however, hinges in part on our ability to resolve joint historical

timelines of spiny water flea presence, abundance, and ecological impacts in the lower food webs in order to ascertain meaningful time periods for analyses of anticipated, cascading impacts on fish.

A well-recognized tool available to aquatic scientists for reconstruction of long-term environmental histories is dated (e.g., via $_{210}$Pb) lake-sediment cores. This approach has enabled the collection of time-continuous records of a wide variety of past environmental events including lake eutrophication, acidification, species invasions, and climate change. Crustacean zooplankton, including the spiny water flea, are among the best preserved organisms in lake sediments and numerous studies have used their subfossils to reconstruct past food-web dynamics.

We recently used dated ($_{210}$Pb, $_{137}$Cs) sediments from four sites in Island Lake Reservoir and demonstrated that spiny water flea first appeared in the lake sediments eight years before its first detection in the water. Logistic growth models fit to subfossil accumulation rates showed that spiny water flea population growth was slow during the first five years, and required one to two decades to achieve an annual equilibrium. Post-invasion, *Daphnia mendotae* became proportionally the most abundant daphnid in the lake, but the timing of the switch coincided more with the proliferation of spiny water flea than with its arrival to the lake. This pattern in early temporal dynamics of spiny water flea during colonization, and delayed response in the lower food web, suggest that sound evaluation of spiny water flea impacts on fish will require synchronous sets of high resolution records of populations.

The goal of this project is to describe the long-term historical trends, dating from before invasion to present, in the lower food webs of three Minnesota lakes invaded by spiny water flea. The results will quantify the types, chronologies, and magnitudes of changes occurring in populations of several key crustacean zooplankton species, and changes in phytoplankton pigment deposition, bridging the period of spiny water flea invasion from 1970 to present. The target lakes are Rainy (surface area = 921 km2), Mille Lacs (536 km2), and Kabetogama (104 km2). These are recognized by the MNDNR (Jodie Hirsch; personal communication) as high priority lakes and are three of 10 lakes included in the MNDNR “Large Lake Program”, where there is annual fish data from as early as the 1980’s, and extensive and ongoing zooplankton data. The results will serve a wide array of management and non-management groups in Minnesota working on and impacted by invasive species, particularly stakeholders whose economic and recreational interests align with the game fish industry.

Summary Budget Information for Sub-Project 12:	**ENRTF Budget*:**	**$212,266**
	Amount Spent:	**$211,708**
	Balance:	**$558**

******This value is approximate; it may be adjusted during the course of peer review and will be updated after approval of a subproject workplan and budget.*

Outcome	Completion Date
1. Produce raw material from 15 sediment cores for analyses	March 2017
2. Produce dated profiles of zooplankton and pigments in sediment cores during 1970s- present	June 2019
3.Produce descriptions of historical changes in lower food webs of invaded lakes that will inform understanding of spiny water flea impacts on game fish in Minnesota Lakes	June 2019

Sub-Project Status as of *May 2, 2016*
This project is currently undergoing revision and workplan development following peer review. The Subproject workplan and budget will be submitted to LCCMR following approval by MAISRC, at which point the above budget, description, and outcomes will be revised as needed.

Sub-Project Status as of *August 31, 2016*
This Sub-project was just approved in August with an understanding that its next status update would be provided January 31, 2017

Sub-Project Status as of *February 28, 2017*
We have been preparing for the field season (February and March, 2017) when we will collect sediment cores from the 4 study lakes (Kabetogama, Leech, Mille Lacs, and Winnibigoshish) on this project. This preparation has included the hiring of an undergraduate research assistant (Mr. Ben Block), application for a permit to remove lake bottom sediment from Lake Kabetogama in Voyageurs National Park (a federally protected area), ordering of additional supplies for the field work, and the collection and interpretation of information from the MNDNR and Voyageurs National Park on suitable coring locations (latitude, longitude) in the study lakes based on historical work that these organizations have done related to spiny water flea presence. During an upcoming meeting of the research team (Branstrator, Reavie, Kennedy), final coring locations will be chosen. Preliminary coring locations in two of the lakes are indicated in the table below under Activity 1.

We have also made progress on outreach goals. Branstrator gave two 50-minute presentations at the MAISRC Annual Showcase (September 12, 2016) in St. Paul and conducted four 10-minute laboratory demonstrations during an afternoon workshop at the Annual Showcase. During the presentations, the goals and general methods of this project were described.

Sub-Project Status as of *August 31, 2017*
We completed a successful field season during February and March when we collected 13 sediment cores including 7 cores from Lake Mille Lacs and 6 cores from Lake Kabetogama. We also began laboratory preparation and examination of core contents. All 13 cores were sectioned. Water and organic content was done on 3 cores from Lake Mille Lacs and subsamples from one of the cores was prepped (freeze dried) and sent to the St. Croix Watershed Research Station for Lead-210 and Cesium-137 dating. We recruited a graduate student, Nichole DeWeese, into the Water Resources Science Graduate Program. She will assist with fossil analysis of spiny water flea and other zooplankton in the core material, and use this project as the centerpiece of the MS degree.

We met methodological challenges that prevented us from collecting sediment cores from all of the field sites this winter. On Lake Mille Lacs we encountered problems locating firm sediment at times and had to abandon one of the four sites. We will return to Lake Mille Lacs this coming winter (2017-2018) to complete the field work. Due to an early spring thaw and poor, thinning ice conditions, we were unable to collect sediment cores from all four sites in Lake Kabetogama. We will return to Lake Kabetogama this coming winter to complete field work. Due to an early spring thaw, we were also unable to collect sediment cores from Leech Lake and Winnibigoshish Lake, and we will return to both lakes this coming winter to conduct field work. These delays will not affect the pace of data collection on the project because there is plenty of work to be done on the 13 cores that were collected. Funds remain in the budget for the remaining field work.

Sub-Project Status as of *February 28, 2018*
We completed a successful start to the laboratory analyses. Two of the sediment cores were processed for dates (measured for age by depth) based on Pb-210 at the St. Croix Watershed Research Station. One of these two cores was also processed for algae pigments (measured as concentrations and types of pigments by depth) at the University of Regina. We processed this same core for zooplankton remains (measured as subfossil numbers and types by depth) in our lab at UMD. We worked out a variety of sample preparation methods prior to processing the sediment for zooplankton remains. The Minnesota Department of Natural Resources staff shared some of their data with us on zooplankton abundance in Lake Mille Lacs that we will use to construct calibrations to help us infer abundances of zooplankton remains in the sediment samples from that lake.

Sub-Project Status as of *August 31, 2018*

During this period we collected the final sediment cores for the project. All 25 sediment cores have now been collected, bringing Activity 1 to a close. We continued to process the sediment cores for water and organic content, isotopic aging, zooplankton subfossils, and algae pigments, all under Activity 2. We adopted a technique to help predigest unwanted organic material in the sediments before we search them for subfossils. This necessitated an amendment to the proposal that will allow us to purchase enough of the chemical to complete the work. We hired three undergraduate students at UMD who are assisting us in the laboratory this summer. We are making good progress and we are on schedule to meet our outcome deadlines.

Sub-Project Status as of *February 28, 2019*
During this period we worked mainly on the outcomes under Activity 2. We continued to analyze sediment cores for age and have completed that outcome (#3) for 11 of 12 cores. We continued to analyze sediment cores for zooplankton subfossils back to 1970 and have completed that outcome (#4) for 6 of 12 cores. We continued to analyze sediment cores for algae pigments back to 1970 and have completed that outcome (#5) for 2 of 6 cores. We are generally on or near schedule to meet our outcome deadlines for Activity 2 and 3 as specified in the work plan. The only exception is Activity 2 (outcome #3, sediment dating, deadline December 31, 2017) but this outcome should be completed in the next month. Under Activity 3 (outcome #2), we gave a poster presentation at the Upper Midwest Invasive Species Conference (Rochester, Minnesota) on this project.

An amendment request is included in this report to provide an additional $4,500 in funding to Subproject 12, to enable the project team to extend their search for subfossil evidence of spiny water flea to earlier time periods, with the objective of finding the transition between presence and absence.

Final Report Summary:
Although aquatic invasive species threaten Minnesota's environment, economy, and recreation, we still know little about the colonization histories and ecosystem impacts of some of the state's invaders such as spiny water flea. This project made large advances in understanding the colonization and impact of spiny water flea in Lake Mille Lacs, Lake Kabetogama, Lake Winnibigoshish, and Leech Lake through the collection and analysis of organism remains in lake bottom sediments over about a 120 year period from present (2017 or 2018) back to the year 1900. The results provide replicated evidence that spiny water flea was resident continuously in Lake Mille Lacs and Lake Kabetogama since the 1930s, or about 80 years before it was first detected in the open waters of either lake. Evidence demonstrates that spiny water flea had a prolonged history of low abundance in both lakes before about the year 2000 at which time it began to increase rapidly. Zooplankton that are prey and competitors of spiny water flea often declined in abundance after spiny water flea increased in abundance. There was no evidence of spiny water flea in the sediments of Lake Winnibigoshish. There was evidence of a small population of spiny water flea in the sediments of Leech Lake that dated to the year 2001, possibly representing a failed invasion. To date, Leech Lake has never been known to contain this organism. The data allow us to test hypotheses about the timing and impact of spiny water flea on the food webs of Minnesota lakes. The results re-cast our understanding of the timeline of spiny water flea invasion in Minnesota and underscore the value of lake sediments to study invasive species. The results suggest that traditional methods of spiny water flea detection with nets, as carried out by academic units and management agencies in Minnesota, may be inadequate to detect spiny water flea when it is low or transient in abundance.

SUB-PROJECT 13: Eco-epidemiological model to assess AIS management

Project Manager: Nicholas Phelps
Description: New evidence-based decision-making tools developed using robust and updated information are needed to generate effective intervention strategies, predict impacts, test what-if scenarios, increase stakeholder buy in, and design cost-effective surveillance programs to mitigate and prevent AIS spread. To that end, we will develop a first of its kind eco-epidemiological model to forecast the potential risk of AIS spread in Minnesota. Our risk model will focus on three high-priority AIS, including Zebra mussel (*Dreissena polymorpha*),

Heterosporis (*Heterosporis sutherlandae*), and Eurasian watermilfoil (*Myriophyllum spicatum*), and will be composed of three main risk-components, including environmental suitability, pathways for potential translocation, and levels of management interventions. We will integrate these components into three model-compartments as follows: [SRi,j = TRi,j + ER i,j + MR i,j], where SR i,j is the cumulative risk value of AIS spread for the AIS *i* in waterbody *j*, TRi,j is the risk of translocation to waterbody *j*, ERi,j is the risk of establishment, and MRi,j is the intervention scenario by management agencies. When available, a measure of species impact (IRi,j) will be incorporated into each cumulative model based on complimentary ongoing or proposed research for each species. The collaborative process and resulting information will build upon ongoing AIS research, provide immediate value to the design of evidence-based AIS control plans in Minnesota and will significantly advance future AIS research.

Summary Budget Information for Sub-Project 13:	**ENRTF Budget*:**	**$195,249**
	Amount Spent:	**$195,249**
	Balance:	**$0**

**This value is approximate; it may be adjusted during the course of peer review and will be updated after approval of a subproject workplan and budget.*

Outcome	**Completion Date**
Activity 1	
1.Validated next generation ecological niche model for Zebra mussel	*July 2016*
2. Validated next generation ecological niche model for Eurasian watermilfoil	*Nov 2016*
3. Validated next generation ecological niche model for Heterosporis	*Feb 2017*
Activity 2	
1.Validated network model of lakes and rivers	*Nov 2016*
2. Validated network model of boater movement	*Mar 2017*
Activity 3	
1. First workshop: Categorization of management strategies	*Nov 2016*
2.Final cumulative risk model for the three AIS selected	*June 2017*
3. Second workshop: Evaluation of final cumulative risk model	*Sept 2017*
Activity 4	
1.Scientific and public presentations (n=6; i.e. MAISRC Showcase, research meetings, etc)	*March 2018*
2. Publication of peer-reviewed manuscripts (n=6)	*March 2018*

Sub-Project Status as of *May 2, 2016*
This project is currently undergoing revision and workplan development following peer review. The Subproject workplan and budget will be submitted to LCCMR following approval by MAISRC, at which point the above budget, description, and outcomes will be revised as needed.

Sub-Project Status as of *August 31, 2016*
This Sub-project was just approved in September with an understanding that its next status update would be provided January 31, 2017

Sub-Project Status as of *February 28, 2017*
The ecological niche model for Heterosporosis was developed to achieve outcome 1 from Activity 1. Thus, we were able to identify the geographic areas in Minnesota with suitable conditions for the establishment or presence of this fish disease and produce risk maps for use by managers and researchers. These findings will be submitted for peer-review in late January to the open access journal *Frontiers in Veterinary Science* (Working title: "Novel methods in disease biogeography: A case study with Heterosporosis").

The early results of this project were presented at the 2016 MAISRC showcase, to more than 200 participants (https://goo.gl/atJ1Zm). The audience was interested in the project's outputs and requested future presentations showing how the suitability and network models will identify lakes where preventive measures should be implemented and prioritized. A second manuscript is currently under review in the scientific journal *Reviews in Fisheries Science and Aquaculture,* with a broad overview of MAISRC studies, including this project, ("Aquatic invasive species in the Great Lakes region: An overview.").

Data for the zebra mussels risk maps were collected and cleaned and models are under development. Data for the network models is currently being organized and cleaned by Dr. Huijie Qiao, the visiting researcher involved with the project. This status provides us confidence to achieve the results according to our schedule.

Sub-Project Status as of *August 31, 2017*
The project attempts to forecast invadable areas for an invasive pathogen, a plant, and an animal, assessing risk of invasion and establishment in Minnesota. The ecological niche model for the pathogen Heterosporosis has been completed and was published. Thus, results are currently available to the international scientific community and the managers in Minnesota (Escobar, L. E., Qiao, H., Lee, C., & Phelps, N. B. D. (2017). Novel methods in disease biogeography: A case study with Heterosporosis. Frontiers in Veterinary Sciences doi:10.3389.fvets.2017.00105). The second manuscript of the project ("Aquatic invasive species in the Great Lakes region: An overview.") has received the first round of reviews. We expect to publish this manuscript as a guide for students and citizens about the state of aquatic invasive species in Minnesota, including the gaps in the knowledge and the ongoing research at the Minnesota Aquatic Invasive Species Research Center at the University of Minnesota (MAISRC). The ecological niche model for zebra mussel was completed and predictions to Minnesota were done at a fine spatial resolution. We are now working on the forecasts for the invasive plant starry stonewort.

A second part of this project includes the exploration of pathways for the spread of invasive species to suitable lakes in which species can establish populations. For this component, a visiting scholar, Dr. Huijie Qiao, worked at MAISRC from December 2016 to June 2017. During his collaboration, Dr. Qiao developed a first of its kind database with spatial distances between lakes and the connection of lakes via streams/rivers. These databases are essential to the development of network models and will likely have value in many other water resource issues.

A workshop was hosted in August to present the current status of this project to key stakeholder groups. This will result in the development of management scenarios that, when hypothetically implemented in the models in the coming months, could affect the risk of AIS establishment.

Sub-Project Status as of *February 28, 2018*
The project is progressing nicely and has made significant progress. For Activity 1, we have spent considerable effort cleaning the massive boater survey database provided by the MN DNR. We developed a data cleaning algorithm that improved inclusion of available data from 21.1% to 99%, a significant increase and fills in a much more complete assessment of boater movement. This now includes 1,690,613 total boater movements among 2,588 unique lakes during the 2014-2017 survey years. We have also created a network of water connectivity in the state – also the most detailed dataset of its kind at a statewide scale. These networks, along with geographic proximity, are now being integrated to evaluate the risk of AIS introduction based on historical invasion patterns.

For Activity 2, we hosted a workshop with AIS stakeholders to develop and evaluate hypothetical (but realistic) management scenarios that could be integrated into our risk models. The group ultimately came to consensus on likely effectiveness of 12 management options that ranged from not effective (but easy to implement) to very effective (but difficult to implement). These will be used to modify our risk models and be presented back to the same group for reaction in May of 2018.

Results of this project have been presented at the International Conference on Aquatic Invasive Species, as well as regional and local meetings to a wide range of AIS stakeholders. We have also published two manuscripts highlighting the results of this project.

Final Report Summary:
Aquatic invasive species (AIS) are spreading at an alarming rate in Minnesota, putting the urgent need for prevention at odds with limited budgets and capacity. To inform decision making, we have developed a series of integrated models that provide the cumulative risk of introduction and establishment of zebra mussels and starry stonewort in all Minnesota lakes. We first answered the question of 'can the species get there?' using network models to describe lake connections. The watercraft network was built with 1.6M MN DNR watercraft inspections from 2014-2017, with gaps and biases accounted for with a variety of statistical approaches. The water connectivity network was created at a finer resolution and larger geographic area than currently available using multiple sources of GIS data and satellite imagery. Next, we answered the question of 'will the species survive?' using advanced methods of ecological niche modeling. With current species distribution of the invaded and native ranges, paired with local environmental data, we projected suitability at the lake level. These three massive data sources fed into the development of an integrated model that quantified the risk of AIS invasion for each waterbody from 2018-2025. Not surprisingly the results suggest the number of infested waterbodies will increase in the years to come. However, with the integration of hypothetical management scenarios developed and incorporated during two project workshops, we demonstrated the value of this approach to assess management effectiveness by determining the number of new infestations averted. While the model is not perfect (no models are), the results are robust and provide useful information from which to make decisions. When considered across a watershed, county or state, the ability to rank waterbodies based on actual, not perceived, risk is a game changer for the prioritization of intervention strategies.

SUB-PROJECT 14: Cost-effective monitoring of lakes newly infested with zebra mussels

Project Manager: John Fieberg
Description: Our objective is to develop recommendations for underwater survey methods and methods for estimating population abundance and distribution of zebra mussels, accounting for imperfect detection, which can be used to monitor newly infested lakes.

Advice regarding appropriate survey methods is desperately needed by Minnesota Department of Natural Resources' (MNDNR) staff, citizen groups, MN Counties, watershed districts, and lake managers confronted with new infestations of zebra mussels. The earliest stages of lake colonization are difficult to monitor because abundance is low, mussels are sparsely distributed, and they are hard to locate and count. In 2015, the MN DNR initiated a Pilot Project Program to evaluate effectiveness of pesticide treatments, focusing on water bodies where zebra mussels have been determined to be "limited in size and localized" using "an established monitoring protocol" (http://www.dnr.state.mn.us/invasives/aquaticanimals/zebramussel/pilot_project.html). This program issues treatment permits and provides protocols for survey and monitoring of zebra mussel larvae, juvenile recruitment, adult densities and pesticide mortality to evaluate outcomes following treatment efforts (http://files.dnr.state.mn.us/natural_resources/invasives/aquaticanimals/zebramussel/zebra_mussel_monitoring_2015-09-10.pdf). Lakes in the program must be surveyed for 3 successive years post-treatment, but the Pilot Project Program currently lacks guidelines for allocating survey effort (e.g., through a valid statistical sampling design), which makes extrapolation to unsampled areas and comparisons over time problematic. Additionally, no guidelines exist to account for imperfect detection (i.e., mussels present but not observed) when sampling.

Sampling designs for zebra mussels must be feasible to implement by SCUBA divers and result in data that allow for efficient estimation of abundance and spatial distribution patterns while also accounting for imperfect detection. Methods must also be standardized to allow comparisons across lakes. We will take advantage of recent methodological advances for collecting and modeling spatial data using line-transect surveys. Line-transect sampling designs are appealing for several reasons: 1) divers can quickly survey large contiguous areas; 2) methods for estimating and correcting for imperfect detection are well developed; and 3) recent advances in spatial modeling can be used to estimate the distribution of mussels throughout the lake.

We will survey lakes in 2017 and 2018 using a variety of line-transect sampling designs. In addition, we will conduct an extensive simulation study to evaluate the efficiency of alternative survey designs and to provide recommendations regarding appropriate sampling effort. We plan to select lakes that were first listed and confirmed infested in years 2015 and 2016 from a publicly available database maintained by the MN DNR Invasive Species Program (http://www.dnr.state.mn.us/invasives/ais/infested.html: updated 12/29/16). We will draw untreated reference lakes from 2015 and 2016 to bracket a range of initial densities, and will select lakes that have been treated with pesticides from MN DNR's Pilot Project Program. We will estimate abundance and distribution patterns by fitting density surface models to the resulting data. These density estimates will also allow us to develop realistic simulation scenarios for comparing alternative sampling designs and to evaluate how sampling effort affects our ability to detect changes in abundance and distribution over time and therefore the efficacy of pesticide treatments.

This work will result in the following outcomes:

1. Recommended, cost-effective monitoring programs for estimating distribution and abundance of mussels that can be implemented in recently infested lakes, allowing for targeted control efforts.
2. Estimates of population distribution and abundance patterns in 10 newly infested lakes.
3. Comparisons of mussel abundance and distribution in lakes that are and are not treated with pesticides as part of MNDNR's Pilot Project Program.

Summary Budget Information for Sub-Project 14:	**ENRTF Budget*:**	**$266,500**
	Amount Spent:	**$225,553**
	Balance:	**$40,947**

Outcome	**Completion Date**
Activity 1	
1. *Survey up to 10 lakes in 2017*	November 1, 2017
2. *Survey 5 lakes in 2018, test feasibility of adaptive line-transect design*	November 1, 2018
Activity 2	
1. *Report preliminary estimates of distribution and abundance patterns from lake surveys conducted in 2017*	January 31, 2018
2. *Report final estimates of distribution and abundance patterns from lake surveys conducted in 2017 and 2018*	June 20, 2019
Activity 3	
1. *Senior capstone project, simulation study to compare alternative sampling designs*	*June 1, 2018*
2. *Develop recommendations for monitoring newly infested lakes*	*June 30, 2019*

Sub-Project Status as of *February 28, 2018*
We visited a total of eleven lakes reported to have low/moderate-density zebra mussel populations and conducted SCUBA surveys in six of them. Five lakes were excluded because zebra mussel populations were too high or, in one case, there was an active algae bloom that prevented the survey from occurring. Zebra mussels are a cryptic species so we knew they would be difficult to detect, even with SCUBA surveys. We surveyed lakes using two different methods that can, if certain model assumptions are met, provide estimates of the number of mussels encountered but not observed within the surveyed area. We had two dive teams survey the same areas in Lake Burgen in Douglas County. This "double-observer" dive allowed us to evaluate important assumptions of

our approach and to quantify differences in detection ability of the divers. Our estimates of the probability of detecting a mussel within 1 meter of a diver was between 3% and 30% depending on who the observer was and the environmental conditions (e.g., water clarity) near the mussel. When averaged across surveyed areas and environmental conditions, we estimated divers detected 16% (diver 1) and 28% (diver 2) of the mussels present in the surveyed area. Thus, we may expect low detection probabilities even with experienced divers. Our data also suggest that divers are likely to miss zebra mussels that are on (or very near) the transect line. This result challenges a critical assumption of conventional survey designs, namely that observers are able to detect all objects on the transect line. We can get around this assumption by conducting surveys with multiple dive teams, but the additional personnel will increase survey costs and may reduce the total amount of area that can be surveyed. Our initial results suggest that to estimate zebra mussel densities accurately, we need to implement double-observer surveys.

An amendment was approved by LCCMR on 02/06/2018 to reduce the number of lakes to be surveyed in 2018 from 10 to 5. By concentrating on fewer lakes, we can save time and money allocated to travel and devote it to increased survey efforts on the 5 lakes we choose. This proposed change in sampling effort will ensure we are able to collect sufficient data to evaluate the assumptions of our survey methods and will also better facilitate comparisons among survey methods (e.g., single and double observer dives). In particular, we would be able to resurvey lakes multiple times, using different survey methods, and compare results. The disadvantage of this shift in survey effort is that we would estimate zebra mussel density and distribution patterns in a smaller number lakes.

Sub-Project Status as of *August 31, 2018*
We have developed initial plans for sampling lakes this summer (2018). To increase time spent in lakes with appropriately low densities, we have decided to sample lakes in 3 “phases”. The first 2 phases will be used to quickly assess relative abundance and spatial distribution of mussels in a set of candidate lakes without attempting to estimate detection probabilities or correct for imperfect detection. The third phase will be used to more rigorously compare alternative survey methods useful for estimating abundance (i.e., correcting, as necessary, for mussels not observed in the surveyed area) in a small number of low density lakes.

In June, we visited 18 lakes and sampled 15 of these using 20-minute timed surveys (phase 1). Based on these initial surveys, we chose 6 lakes for phase 2 sampling, in which we surveyed 15 transects spread throughout the lake. In July and August, we plan to compare 3 different survey methods in a subset of these 6 lakes (phase 3 sampling).

We have analyzed the data from last year’s surveys and recently completed a first rough draft of a manuscript describing these methods and results. Lastly, students from Carleton College completed a simulation study to explore the efficiency of different survey designs using simulations. Their results support the use of distance sampling for estimating density of zebra mussels in lakes, but point to the need for increased sampling effort to reduce uncertainty associated with density estimates.

Sub-Project Status as of *February 28, 2019*
We completed our field surveys associated with Activity 1. In particular, we implemented 3 different survey techniques (double-observer surveys with and without distance sampling, quadrat counts) in three lakes capturing a range of zebra mussel densities: Lake Florida in Kandiyohi County, Lake Burgan in Douglas County, and Little Birch Lake in Todd County.

We developed two approaches for analyzing the data from our first field season in Lake Burgan, a straightforward approach that can be implemented with existing open-source software and a more refined approach that can be used to explore the effect of covariates (e.g., plant presence, substrate) on detection probabilities and zebra mussel density. Both methods produced density estimates that were 3 times larger than the observed densities (uncorrected for detection). These results demonstrate the importance of estimating and adjusting for detection probabilities <1 rather than relying on observed counts when comparing densities over time or space.

We compared estimates of detection probabilities and zebra mussel density from data collecting during our second field season using 3 different survey methods (double-observer with and without distance sampling, quadrat counts). We found that estimates of detection probabilities were fairly similar in all three sampled lakes (Lake Burgan, Lake Florida, and Little Birch Lake), and the different survey methods all gave similar estimates of density. The estimated detection probability using double-observer surveys without distance sampling was 0.94, suggesting we may be able to achieve near perfect detection, provided we use 2 observers and survey a smaller width transect. However, we detected a pattern of slightly lower density estimates when using this approach (compared to double observer surveys with distance sampling and quadrat counts). Preliminary comparisons of the 3 survey methods suggest that double-observer surveys with distance sampling may be most efficient at low densities and quadrat or double-observer surveys (without distance data) may be more efficient when densities are high. This spring, we will further evaluate relative efficiencies of these methods using simulated data across a range of zebra mussel densities.

Final Report Summary:
The current lack of standardized methods for surveying zebra mussels during their earliest stages of lake colonization limits our ability to track changes in density over time or to evaluate effectiveness of treatment programs (e.g., as required by DNR permits). We evaluated 5 different survey designs for estimating zebra mussel density (2 designs in 2017 and 3 designs in 2018), employing methods that utilize counts by two divers to estimate the probability of detecting mussels in the surveyed area. We also compared survey designs in terms of their density estimates, associated measures of uncertainty, and sampling efficiencies (time required to complete a survey), using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data. In 2017 in Lake Burgan, we estimated that a diver could detect between 5% and 41% of the mussels present in the surveyed area, depending on the specific diver and on whether the lake bottom was vegetated, with vegetation having the larger effect on detection. Accounting for low detectability of zebra mussels led to an estimate of density over three times higher than the observed density. Thus, for every zebra mussel detected by our divers, approximately two were missed. Using the data collected in 2018 and further simulation and analytical work, we found that double-observer survey designs that allow for imperfect detection are optimal when surveying lakes at low density, whereas quadrat counts that assume perfect detection are optimal at higher densities. We developed a training video, data collection worksheets, and an analysis tutorial so that others may implement our proposed survey designs in newly infested lakes. These tools benefit Minnesotan's by providing better ways to monitor lakes infested with zebra mussels and to evaluate the effects of treatment options on zebra mussel density.

SUB-PROJECT 15: Determining Highest Risk Vectors of Spiny WaterFlea Spread

Project Manager: Valerie Brady
Description: Spiny water flea is a predatory species of zooplankton that represents a serious threat to the ecology and recreational value of Minnesota waters. As of 2015, spiny water flea (SWF) was reported in 36 lakes in Minnesota, including some of the largest basins (Superior, Kabetogama, Lake of the Woods, Mille Lacs, Rainy, Vermilion) that now unfortunately serve as potential source populations to uninfested waters. A major potential risk for the health of Minnesota lakes is that spiny water flea is a carnivore that feeds aggressively on native herbivorous zooplankton, a food resource that is shared as prey by many species of young fish including walleye, northern pike, and yellow perch. This potential competitive interaction with young fish could slow the growth and health of many native fish species in Minnesota. A second potential risk for the health of Minnesota lakes is that herbivorous zooplankton play key roles as grazers on algae, the microscopic plants that form the base of aquatic food webs. Higher concentrations of algae are directly related to lower water clarity. Thus, through removal of herbivorous zooplankton, spiny water flea threatens to reduce the health of fish through

competition and to reduce water clarity through eliminating native grazers. These impacts could bring changes to Minnesota lakes that have serious implications for recreation and wildlife. Estimates are that >40% of northern Minnesota lakes provide suitable habitat for spiny water flea, indicating that management programs that foster best practices for containment are critical.

Human recreational activity is believed to be the primary vector of spread; however, little is known about the specific pathways by which dispersal occurs. Current best management practices direct recreationalists to clean, drain, and dry their equipment before moving it to another water body (this is the core message of the "Stop Aquatic Hitchhikers!" [SAH!] campaign). While this message should be effective if followed stringently, it is broad and fails to draw attention to what may be high risk equipment where decontamination effort could be focused or whose usage could be minimized or avoided altogether. Hence, while we have an opportunity to prevent further spread of spiny water flea in Minnesota, clear evidence-based educational messages and policies are urgently needed. A key aspect of spiny water flea behavior is that it migrates closer to a lake's surface at twilight to feed. This behavior increases its potential contact with surface-based equipment (e.g., boat live wells, bait buckets) that could boost the likelihood of a transport event. To increase the effectiveness of the SAH! campaign against the spread of spiny water flea, we need answers to two critical questions: 1) What forms of recreational equipment pose the highest-risk pathway for spiny water flea? 2) Does usage of recreational equipment at twilight (dusk) increase the dispersal risk of spiny water flea over midday equipment usage?

Goal: The goals of this project are 1) to measure and rank recreational (mostly fishing) gear in its ability to spread the adult free-swimming spiny water flea using Lake Mille Lacs as the test lake; and 2) to widely disseminate the results, our recommendations, and gear-cleaning tips both in the Mille Lacs area and throughout the state to anglers, the tourism industry, AIS managers, agency staff and legislators, and lake associations.

How: The goal will be accomplished by deploying commonly-used forms of recreational equipment including anchor ropes, angling lines, bait buckets, downrigger cables, and live wells and then cleaning them and comparing the "load" (total number) of spiny water flea relative to the flea's natural abundances in surrounding Mille Lacs lake water. We will use NRRI's boats to test the different types of gear in Lake Mille Lacs. We will set out three different types of anchor rope and have three fishing poles each rigged with a different type of fishing line, with a hookless weight on the end. One boat will also be set up for downrigging gear to determine the numbers of spiny water flea that accumulate on the steel cable and the monofilament line. One of the boats will also have a bait bucket in the water and be running water into a live well.

At the same time as the fishing gear are in the water potentially encountering and being fouled by spiny water flea, we will determine the fleas' abundance in the water using zooplankton nets. Spiny water flea will be cleaned from all gear being tested, and will be collected out of the plankton nets to determine ambient flea densities. Collected spiny water flea will be preserved and returned to the laboratory for microscopic analysis.

Field work will be done from July to September 2018 in Lake Mille Lacs. Lake Mille Lacs has supported spiny water flea since 2009 and is a major sport-fishing and recreational destination in the Midwest, elevating its potential threat as a source population for new infestations in other lakes. For statistical rigor, we plan to collect 30 samples per type of gear during daylight and again during twilight (evening). We anticipate collecting approximately 1000 samples total from the recreational gear and the sampling nets. Analyzing spiny water flea numbers on each gear type versus the spiny water flea densities in the lake at the same time will allow us to create a ranking of the threat that each type of gear poses for spiny water flea spread to other water bodies. We will use this information to create specific outreach messages for the public, including reminder stickers with gear cleaning tips. We will provide this information to lake associations, lake managers, anglers, and recreationalists.

Our long-term goal is to provide science-based information that will improve the effectiveness of current best management practices used in Minnesota to minimize pathways for AIS introduction. **Our long-term outcome** is to help slow the spread of spiny water flea to uninfested lakes.

Summary Budget Information for Sub-Project 15:	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
	Subproject Budget:	**$92,932**	**Subproject Budget:**	**$26,581**
	Amount Spent:	**$92,756**	**Amount Spent:**	**$7,456**
	Balance:	**$176**	**Balance:**	**$19,125**

Outcome	**Completion Date**
Activity 1	
1. Test anchor ropes, angling lines, bait buckets, downrigger cables, and live wells in Lake Mille Lacs for entanglement with spiny water flea on 6 different daylight and evening trips, as well as collect water column samples of spiny water flea.	Fall 2018
2. Microscopically examine samples in the lab and count the number of spiny water flea on each gear type.	Dec. 2018
3. Determine spiny water flea transfer risk from each gear type using appropriate statistics.	April 2019
4. Write detailed report of results and conclusions; provide report to agency AIS personnel.	June 2019
5. Write peer-reviewed manuscript for submission to a scientific journal to inform other AIS researchers of findings.	June 2019
Activity 2	
1. In collaboration with MAISRC, U Extension staff, and Wildlife Forever, create up to 10,000 waterproof, UV-protected stickers with plain-English outreach messages for anglers and boaters on gear cleaning. For example: “Clean, Drain, Dry, and don’t forget your anchor rope!" Stickers will be placed at bait shops, gas stations near boat launches, and where fishing licenses are sold.	March 2020
2. In collaboration with MAISRC, the Aquatic Nuisance Species taskforce, and Sea Grant outreach staff, we will create radio and TV PSA-type ads highlighting what anglers should do; purchase spring/summer ad time for the Mille Lacs area.	April 2020
3. Presentations to AIS managers, agency staff, lake associations, tourism industry (esp. Dock Boys and Girls), policy makers, and fishing groups. Also, social media outreach messages targeted to connect with anglers and boaters.	May 2020
4. Outreach article for Minnesota Sportsman (or similar) magazine.	June 2020
5. Service for MAISRC, including participation in the 2018 and 2019 Showcase Events and participation on 1-2 committees.	June 2019

Sub-Project Status as of *February 28, 2018*
This project (by design and approval of MAISRC) has not yet started. We will begin planning for our first field season within the next couple of months with fieldwork to start in July. However, our companion project to do similar work in Island Lake Reservoir, near Duluth, funded by St. Louis County, had a full and successful sampling season last year. In the process of working on the St. Louis County-funded project we have been able to test and

refine our sampling methods to ensure that they will work. These changes are detailed below under Activity 1. None of these changes affects the budget.

Sub-Project Status as of *August 31, 2018*
This project (by design and approval of MAISRC) is just getting started. We have planned for our fieldwork and will start sampling by July 23 on Lake Mille Lacs using methods we tested and refined during our companion project on Island Lake funded by St. Louis County.

Per an approved amendment request, we are postponing our outreach activities (Activity 2) to ensure that we are able to craft an outreach message that is supported by project data being collected in the 2018 summer season. To avoid confusion and increase the effectiveness of our outreach campaign, we need to carefully word and test our message about preventing the spread of spiny water flea. An unclear or inconsistent message about AIS prevention could actually decrease the likelihood that anglers will be motivated to carefully clean and dry their gear. Delaying our campaign and testing our message increases the effectiveness and likelihood of compliance.

Under this new timeline, we will target the 2020 fishing season (beginning with walleye opener) and will purchase TV and radio ad time in the late winter/early spring of 2020. We will be coordinating our outreach on this project with the outreach on our companion St. Louis County funded spiny water flea project. Combining the outreach efforts on these companion projects will allow us to generate more outreach for the same amount of money since we will not have to pay designers twice for similar products. All efficiency savings will go into purchase of more outreach materials, particularly TV and radio ads. St. Louis County has agreed to provide a no-cost extension to Activity 2.

Sub-Project Status as of *February 28, 2019*
This past summer we completed all fieldwork associated with this project by conducting 7 sampling events on Lake Mille Lacs for spiny water flea entanglement on fishing gear, as described in Activity 1. This sampling resulted in collection of 718 samples. Samples collected included zooplankton tows, spiny water flea on fishing gear (downrigger, surface lines, bait bucket, and live well), and spiny water flea on anchor ropes. In the lab, we have counted and aged spiny water flea in 195 of the 718 samples collected. The remaining samples will be processed in February and March.

MAISRC staff hired a videographer and drone operator to come with us on one sampling trip. The resulting video has been used for a number of presentations to great reviews. PIs Brady and Branstrator participated in the 2018 MAISRC showcase event. In addition, graduate student Nicole DeWeese gave a presentation on this project at the Upper Midwest Aquatic Invasive Species Conference in Rochester, MN, in October 2018.

An amendment request is included in this report to transfer $3,127 of surplus funds from Subproject 15, back into MAISRC reserves.

Sub-Project Status as of *August 31, 2019*
This past winter we completed processing of the samples collected during the 7 sampling events on Mille Lacs during the summer of 2018. Processing involved counting and aging all spiny water flea in samples. In total we processed 360 zooplankton tows; 36 braided nylon, 36 twisted nylon, and 36 polypropylene anchor ropes; 35 bait bucket samples; 21 livewell samples; 36 downrigger steel cable samples; 35 downrigger monofilament lines; and 36 braided, 36 monofilament, and 35 fluorocarbon fishing lines.

All data from samples was entered and QC'd. We ran data analyses and summaries for each gear type and have presented the findings at a number of meetings and conferences. We are currently crafting and testing our outreach message for distribution in the spring of 2020. We have tested potential messages with different user groups to determine which short phrase will best convey our message most effectively.

Activity 1 and Activity 2, Part I were funded on M.L. 2013, which ended on June 30, 2019. Activity 2, Part II will continue on M.L. 2017 funding.

Subproject Status as of ***January 31, 2020*****:**
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 16: Sustaining walleye populations: assessing impacts of AIS

Project Manager: Gretchen Hansen
Description: Minnesota's walleye fisheries are vulnerable to ecosystem changes following the introduction of invasive species such as zebra mussels and spiny water fleas. For example, zebra mussels reduce zooplankton, limiting the amount of food available for fish in the open water zone of lakes. At the same time, the high filtering capacity of zebra mussels creates an "energy shunt" that moves food and energy from the water column into the bottom of the lake and nearshore areas, changing the structure of the food web by providing extra resources for fish that feed primarily in nearshore areas. Spiny water fleas are large predatory zooplankton that also reduce the abundance of other, smaller zooplankton. They themselves are inedible to some fish species and life stages due to their long protective tail spine. The zooplankton declines associated with both of these invaders are likely to affect predatory fish such as walleye, because both young walleye and many of their prey species rely on zooplankton as a food source. However, the impacts of zebra mussels and spiny water fleas on sport fish populations are not well understood.

The impacts of zebra mussels and spiny water fleas on fish likely depend upon the ability of fish to switch to alternative food sources if and when invaders cause zooplankton to become scarce. This ability to switch food sources likely depends on lake characteristics including size, depth, productivity, and fish community composition. Determining how these invasive species affect walleye, and identifying characteristics of walleye populations that can withstand these invasions with minimal effect, will allow managers to set realistic goals for future walleye production and harvest. Managers will also be able to assess the impacts of current and future invasions, and separate these effects from other potential causes of walleye population changes.

In this collaborative effort among the Minnesota Department of Natural Resources (MNDNR), the Natural Resources Research Institute, University of Minnesota-Duluth (NRRI), and Voyageurs National Park (VNP), **we will quantify the impacts of zebra mussels and spiny water fleas individually and together on walleye and their food webs in Minnesota's large lakes.** Minnesota's nine largest walleye lakes (all greater than 15,000 acres) are at different stages of invasion by zebra mussels (Cass, Winnibigoshish, Leech), spiny water fleas (Kabetogama, Lake of the Woods, Rainy, Vermilion), both (Mille Lacs), or neither (Red). Notably, we have an unprecedented opportunity to track the effects of each invader on walleye populations throughout all stages of invasion by tracking impacts early in the invasion. Zebra mussel veligers (larvae) were first discovered in Leech Lake in 2016 and no adult zebra mussels have yet been found. Similarly, spiny water fleas were discovered in Lake Vermilion in 2015 but have not reached high abundances and currently only occur in one of the lake's two major basins. Each of the nine study lakes will be sampled once in either 2017 or 2018.

We will use two approaches to evaluate the impacts of zebra mussels and spiny water fleas on walleye and food webs in Minnesota's large lakes. First, we will determine which habitats and food resources support walleye and other fish species in each lake by examining stable isotopes in their bodies. Naturally occurring stable isotopes show what a fish has been eating in the past few weeks to months. This analysis will allow us to determine the amount of food resources various fish species and ages (young or adult) are eating from different habitats (nearshore or open water), and at what trophic level they are feeding (their position in the food web). The results of this analysis in each lake will tell us to what degree walleye and their prey rely on zooplankton in the open water as a food source to sustain their populations. This will allow us to assess how likely it is that walleye could switch to other food sources if zooplankton abundances are greatly reduced by zebra mussels or spiny water fleas.

We will also assess the effects of reduced zooplankton abundance due to zebra mussels and/or spiny water flea invasion on the growth rates of walleye and yellow perch in their first year of life. These young fish rely on small zooplankton prey in their early life stages, but they also can eat invertebrates (for example, insects, snails, small mussels) that are less likely to be reduced by zebra mussels or spiny water fleas. We will assess whether young fish may be less affected by the negative impacts of zebra mussels and spiny water fleas if they can successfully switch to other prey even as zooplankton food resources decline. Growth rates will be compared both among lakes with and without zebra mussels and/or spiny water fleas, and within lakes pre- and post-invasion using historical data collected by the Minnesota DNR.

The MNDNR will serve as lead and project manager, ensuring that timely and accurate reporting on the project is completed. MNDNR is also responsible for coordinating and carrying portions of Activities 1 - 3 as described in each section below with a focus on describing whole lake food webs. Funds requested here will support benthic invertebrate sampling for all 9 lakes; fish sampling from all 9 lakes in coordination with existing MNDNR sampling programs; stable isotope analysis for each trophic level; and organizing historical data for pre- invasion comparison. The MNDNR budget includes fieldwork conducted under contract by VNP on Rainy and Kabetogama lakes as well as other project activities. The MNDNR's funds will be provided through a subaward with MAISRC. MNDNR co-PI salaries, as well as additional sampling work already planned through the MNDNR's Large Lakes Program, are provided in-kind.

NRRI will be responsible for portions of Activities 1-3 as described below with a focus on fish diet sampling in 6 lakes; and age-0 fish sampling in 2 of the 9 lakes. NRRI will receive $81,116, which will be awarded internally through a subproject child account similar to other MAISRC projects.

This project will provide a greater understanding of the impacts of zebra mussels and spiny water fleas on food webs and fish in Minnesota lakes, and will facilitate better walleye management in the face of these invasions. Quantifying how these invaders disrupt food webs supporting walleye will allow managers to project realistic levels of walleye production. Additionally, understanding the most important prey supporting walleye will allow us to assess the vulnerability of each population to the impacts of invasion. This project will provide a critical supplement to the existing MNDNR Large Lakes program by incorporating the community and ecosystem-level data required for understanding the lake-wide impacts of AIS.

Summary Budget Information for Sub-Project 16:

Sub-Project Budget: $198,700
DNR Portion: $88,139
NRRI Portion: $29,445
UMN Portion: $81,116

Amount Spent: $197,568
Balance: $1,132

Outcome	**Completion Date**
Activity 1	
1. Collect benthic macroinvertebrates from nearshore and deepwater lake bottom areas to quantify baseline isotopic positions to determine which fish feed on these invertebrates. To be done in Mille Lacs, Red, and Leech lakes in 2017 and in Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018. Co-Lead: MNDNR and NRRI	10/2018

2. Collect muscle tissue from fish sampled during fall gillnetting (part of MNDNR large lakes core sampling) of Mille Lacs, Red, and Leech lakes in 2017 and in Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018. Fish targeted from this sampling include walleye, yellow perch, northern pike, cisco (where present), black basses, and other Centrarchids such as bluegill, black crappie, and rock bass (where present). Lead: MNDNR	10/2018
3. Collect age-0 walleye, age-0 yellow perch, and littoral prey fish in summer for isotopic analysis for food web assessment via seining in Leech and Red lakes in 2017 and Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018. Lead: MNDNR (including subcontract to NPS)	10/2018
4. Collect age-0 walleye, age-0 yellow perch, and littoral prey fish in summer for isotopic analysis via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI	10/2018
5. Process fish and invertebrate samples from Mille Lacs, Red, and Leech lakes in 2017 and in Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018 to prepare samples for stable isotope analysis. Processing includes dissecting muscle tissue from small fish and combining invertebrate taxa across sites and taxonomic groups as appropriate to ensure sufficient biomass is available for stable isotope analysis. Lead: MNDNR	12/2018
6. Process zooplankton samples from Mille Lacs, Red, and Leech lakes in 2017 and from Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018 (part of MNDNR large lakes core sampling) to prepare for stable isotope analysis. Processing includes separating major taxonomic groups (spiny water flea, large native predatory zooplankton, large herbivores, small herbivores, etc.) Lead: MNDNR	
7. Quantify stable isotope composition of Carbon and Nitrogen of each trophic group collected by MNDNR in all study lakes using an external lab. This will provide the data for the food web analysis. Lead: MNDNR via subcontract to an external stable isotope laboratory.	2/2019
8 Determine how much food/energy is coming from nearshore versus open water habitats contributing to walleye production in each study lake, and how this varies with invasion status. Lead: MNDNR	6/2019
Activity 2	
1. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Leech and Red lakes in 2017 and Kabetogema, Lake of the Woods, Leech, Rainy, Red, Vermilion, and Winnibigoshish in 2018. Lead: MNDNR (including subcontract to NPS)	10/2018
2. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI	10/2018
3. Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI	10/2017
4. Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS)	2/2018
5. Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS)	3/2019
Activity 3	
1. Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR	6/2019
2. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI	6/2019
3. Dissemination of findings – at least one peer-reviewed publication in preparation. Co-lead: MNDNR (including subcontract to NPS) and NRRI	6/2019

Sub-Project Status as of *February 28, 2018*
We successfully collected fish, benthic macroinvertebrates, and zooplankton from the three lakes targeted for 2017 (Mille Lacs, Red, and Leech lakes). Multiple species were obtained from multiple sites in each lake, which will allow us to characterize the food webs of these lakes with a high degree of accuracy. A total of 1,481 tissues samples were collected and are ready for stable isotope analysis (Activity 1).

We collated hundreds of thousands of historical fish records for historical data analysis of growth rates of age-0 walleye and yellow perch. We also collected additional age-0 fish from Mille Lacs. These data will be used to assess whether any changes have occurred in the growth rates of young fish corresponding to invasion by zebra mussels or spiny water flea. Diets of age-0 walleye and yellow perch were also collected and analyzed to ensure that our sampling of the food web included important diet items.

Finally, we have delivered three presentations describing our work in progress to MNDNR staff, stakeholders, and at the MAISRC showcase. Our project has been featured in the popular press and the University media.

An amendment was approved by LCCMR on 02/06/2018 to amend the sampling design based on the results of the first field season. In the original proposal, we planned to sample three of our nine study lakes in each of two study years. The remaining six study lakes were to be sampled only once. Under this proposed amendment, we would sample each of our nine study lakes one time. This proposed change will allow us to more fully characterize the food web of each lake to better understand ongoing and future impacts of zebra mussels and spiny water fleas. Additionally, the amendment changes the lab with which we will contract for our stable isotope analysis. We are pursuing permission to send our samples to the Cornell University Stable Isotope lab, which can analyze samples in our desired timeline and at lower cost. The amendment requires that funds are shifted between budget categories, though the overall budget remains the same.

Sub-Project Status as of *August 31, 2018*
We sent our fish, invertebrate, and zooplankton samples from Leech, Mille Lacs, and Red Lakes to the Cornell stable isotope laboratory for analysis. We have received a subset of results from these samples and begun developing a workflow for analysis to facilitate analysis of the complete dataset when it becomes available.

We analyzed age-0 walleye and yellow perch data from each of the 9 lakes. Preliminary results suggest changes in growth associated with zebra mussel invasion, but these results are heavily influenced by data from a single lake. We will collect additional data from Cass Lake that will provide further data to test the hypothesis that zebra mussel invasion negatively affects the growth rates of young of year fish.

Finally, we have delivered 3 presentations since January describing our work in progress to MNDNR staff and interested stakeholders.

Sub-Project Status as of *February 28, 2019*
We have collected fish, invertebrate, and zooplankton samples from all 9 lakes and sent them to the Cornell stable isotope laboratory for analysis. We have received most of our stable isotope composition results from these samples and have initiated preliminary analysis of the large lake food webs and how energy sources supporting walleye differ among lakes.

We analyzed age-0 walleye and yellow perch data from each of the 9 lakes through 2018. Our results demonstrate slower growth of walleye in their first year of life in lakes invaded by zebra mussels and spiny water flea. Yellow perch growth rates were somewhat slower in lakes invaded by zebra mussels, but these differences were not statistically significant. We detected no changes in yellow perch growth associated with spiny water flea invasion. We are writing a manuscript reporting these results to be submitted before the completion of this project in June 2019.

Finally, we have delivered 3 presentations since July describing our work in progress at the MAISRC showcase, a professional scientific conference, and one lake association meeting.

Final Report Summary:
Minnesota lakes experience ecosystem-level changes following the introduction of aquatic invasive species (AIS), specifically zebra mussels and spiny water fleas. However, the effects of these AIS on fish are poorly understood and vary among lakes. We evaluated the impacts of zebra mussels and spiny water fleas on walleye and yellow perch in Minnesota's nine largest walleye lakes. We compared age-0 walleye and yellow perch growth over 35 years, including pre- and post-invasion. Age-0 walleye were >10% smaller at the end of summer following invasion by either AIS. Age-0 yellow perch growth decreased following zebra mussel invasion, although this effect was not statistically significant. Smaller length at the end of the growing season was associated with decreased survival to later life stages for walleye in 7 of the 9 study lakes.

We used stable isotope analyses to understand which habitats and food resources support walleye and other fish and to assess their position in the food web in each lake. We documented a high degree of variability in the resources supporting all life stages of walleye. In general, juvenile walleye relied on offshore prey resources in invaded lakes. Combined with reduced growth rates, these results suggest that as zooplankton food resources decline following invasion, young walleye are not sufficiently accessing alternative prey resources to maintain pre-invasion growth rates. Variability in walleye diets among lakes may reflect differences in lake productivity or morphology, not necessarily the presence of AIS.

Our results demonstrate that zebra mussels and spiny water flea influence the growth rates of age-0 walleye and that a wide range of food resources and habitats support walleye in these lakes. Declines in growth rates of young walleye are an early signal of potential negative effects on walleye. This information can guide managers on the most effective and sustainable walleye harvest and stocking strategies in invaded lakes.

SUB-PROJECT 17: Building scientific and management capacity to respond to invasive Phragmites (common reed) in Minnesota

Project Manager: Daniel Larkin
Description: European strains of common reed (*Phragmites australis*), a highly invasive wetland grass, have been introduced to multiple locations in Minnesota and appear to be spreading. Invasive populations of *Phragmites* can have strong negative impacts on biological diversity, wildlife, habitat quality, and recreation. Thus far, there have been no systematic attempts in Minnesota to map and monitor spread of invasive *Phragmites* and develop coordinated control efforts. **The aims of this project are to: 1) Map the current distribution of invasive *Phragmites* in Minnesota, 2) Determine its capacity for further spread in Minnesota, and 3) Formulate and disseminate model management protocols for this species.** The products of this work will support a comprehensive statewide response to this aquatic invasive species (AIS).

Like many AIS, *Phragmites* does not quickly spread immediately after introduction. The initial barrier to rapid spread is overcome when *Phragmites* can produce viable seed—in addition to its ability to spread vegetatively. This occurs when there is enough genetically diverse *Phragmites* on the landscape to support sexual reproduction. In Minnesota, seed production may also be limited by climate because of our relatively short growing season. Once viable seeds start spreading by wind and water, eradication is no longer feasible and control is much more difficult and expensive. Compared to other Midwestern states, we have relatively little invasive *Phragmites*, but this is changing. The window of opportunity to limit invasion in Minnesota is now. For this reason, it is crucial to map the current distribution of invasive *Phragmites* in Minnesota, assess its potential for further spread, and promote coordinated control and spread prevention efforts.

The distribution of invasive, European *Phragmites* in Minnesota is unknown because it is not easy for non-experts to distinguish it from native *Phragmites*. *Phragmites* is a "cryptic" invader in the U.S. because there are both native and non-native lineages here. Native *Phragmites* is an important component of wetlands that can be displaced by invasive *Phragmites* and harmed by indiscriminate control efforts that do not distinguish invasive from native forms. Resource managers need support in distinguishing and targeting the invasive.

An efficient statewide response to *Phragmites* requires effective management techniques for different invasion scenarios found in Minnesota. For example, treating a large infestation in a high-quality wetland presents different challenges than a new infestation along a roadside. We will develop management protocols that identify and communicate optimal responses to different scenarios. These protocols will consider different factors, such as: How large is the population? Is it producing seed? Is the invaded site connected to other water bodies? Is the population a threat to resources of special concern such as wild rice waters?

The proposed project will generate critical data on statewide distribution and reproduction of invasive *Phragmites*. We will collaborate with external partners to use findings to respond to *Phragmites* invasion. We will also leverage a separately funded workshop for managing *Phragmites* in Minnesota. This workshop will engage resource managers from state, federal, and other agencies and will inform the proposed project by helping us identify invasion scenarios in the state and key areas of uncertainty. Project partners will also help us focus capacity-building efforts on solutions that are feasible within the context of their agencies' broader missions. Management protocols will be developed for different *Phragmites* invasion scenarios and disseminated to partner agencies and other stakeholders through in-person meetings, webinars, and online resources. While this project is focused on invasive *Phragmites*, this approach to research-management collaboration will serve as a model that could be applied to other invasive species issues. In particular, use of a collaborative network ("crowdsourcing") for sampling statewide distribution and development of custom response protocols for different invasion scenarios will be applicable to other invasive species.

Summary Budget Information for Sub-Project 17:	**Revised ENRTF Budget:**	**$283,568**
	Amount Spent:	**$269,773**
	Balance:	**$13,795**

Outcome	**Completion Date**
Activity 1	
1. Adapt GLEDN (EDDMapS) portal and develop submission system	August 15, 2017
2. Morphological identification and genetic fingerprinting	December 15, 2018
3. QA/QC crowdsourcing/identification approach	May 1, 2018
4. Publish/update distribution map for non-native Phragmites	November 15, 2018
Activity 2	
1. Microsatellite results to quantify genetic diversity of subset of statewide populations	December 15, 2018
2. Collection of seed heads from subset of populations	February 15, 2018
3. Evaluation of seed viability from subset of populations	June 15, 2018
Activity 3	
1. Project website	*May 1, 2018*
2. Project webinars	*April 15, 2019*
3. Decision making resources and meetings	*June 30, 2019*
4. Assessing potential for landscape-scale *Phragmites* control	*June 30, 2019*

Sub-Project Status as of *February 28, 2018*

Our invasive *Phragmites* early detection and response effort ("MNPhrag") engaged 155 volunteer observers to assist us in searching for populations of invasive *Phragmites* throughout Minnesota (Activity 1). This crowdsourcing approach, combined with our project staff's own search efforts throughout the state, resulted in more than 290 populations of *Phragmites australis* (both non-native and native) being documented in fall 2017. Plant samples and/or reports were submitted by 50 observers and project staff. Morphological and genetic analyses were then used to confirm the identification of the samples as either native or non-native. Of the submitted reports, 188 have already been confirmed or are suspected to be invasive *Phragmites*. Our project has identified populations of invasive *Phragmites* in 28 different counties to date. More than 100 of the occurrences of invasive *Phragmites* are closely geographically associated with rural wastewater treatment plants permitted to use non-native *Phragmites* in their dewatering basins. In general, most populations of non-native *Phragmites* occurred in roadside and/or wetland habitats.

In the next phase of the project (Activity 2), we will assess seed viability of invasive *Phragmites* populations from 9 regions throughout the state that differ in growing season length and other climatic factors that may influence potential for development of viable seed. Seed heads from 48 populations were collected in December 2017 and January 2018 for this assessment by project staff.

We have also initiated work related to Activity 3 (building response capacity). A graduate student research assistant has been hired for the spring 2018 semester to work on a literature review/synthesis of management strategies in preparation for a structured decision making workshop scheduled for April 9-11, 2018.

Sub-Project Status as of *August 31, 2018*
We continued to accept reports and vouchers of invasive *Phragmites* throughout the winter and have sent out an update with a request that our volunteers continue to report new populations of invasive *Phragmites* in summer and fall 2018 (Activity 1). The MNPhrag mailing list continues to grow as more agency staff and citizen scientists learn about the effort to document invasive *Phragmites*. In addition, we were invited to speak to agency staff at both DNR and USFWS regional meetings and presented at the State of Water conference, which targeted lake association members and lake managers. Through our project, we have documented more than 200 populations of invasive *Phragmites* in 33 counties.

We performed initial testing of seed viability in relation to climatic factors (Activity 2). Most of the 33 populations tested produced viable seed. There was a significant effect of latitude, with populations further south having greater reproductive potential in terms of both seed numbers and seed viability. We will perform additional seed viability assessment in fall 2018 to increase the robustness of these results.

We created resources for *Phragmites* control efforts (Activity 3) through development of management recommendations, convening of a structured decision-making workshop with agency staff, and launch of a new website providing information on invasive *Phragmites* identification, impacts, and control.

MASIRC and LCCMR also approved an amendment request and budget adjustment to add an additional task (Outcome 4) to Activity 3 and hire an additional person to complete the work.

Sub-Project Status as of *February 28, 2019*
The MNPhrag program continued to accept reports and vouchers of invasive *Phragmites* throughout the 2018 growing season and has encouraged its volunteers to continue to document and report new populations of invasive *Phragmites* through the end of the project (Activity 1). Additional reporters have been engaged and added to the MNPhrag mailing list during the last reporting period. We had several opportunities to speak to citizens, agency staff, and researchers at workshops, meetings, and conferences. To date, we have documented and verified nearly 400 populations in 38 counties.

Seed head samples have been collected from the subset of invasive *Phragmites* populations that were sampled in January 2018 in order to repeat the seed viability assessment conducted last winter (Activity 2). We are processing seed heads at this time. This additional seed viability assessment will increase the robustness of our results, showing whether the patterns we observed previously are consistent year to year, i.e., under a different annual climate. In addition, leaf tissue was collected in August 2018 from the same populations to assess their genetic structure and diversity, which has important implications for sexual reproduction potential. These results are forthcoming.

We are currently drafting an assessment of capacity and needs for a strategic response to invasive *Phragmites* and have held meetings with several partners critical to collaborating on and supporting such a response effort (Activity 3). Our assessment highlights 12 distinct "*Phragmites* regions" of the state—based on current distribution of invasive *Phragmites*, stakeholder capacity, and potential for coordinated regional partnerships. It describes for each region its *Phragmites* invasion context; suggests opportunities for coordination between local, regional, and state entities; funding sources; control approaches and cost estimation; and training needs. We expect to have a complete draft by late winter and will be hosting a webinar and engaging partners in the coming months to solicit feedback. We have also met with partners at MNDNR and MPCA to share project findings and begin discussing response approaches, and are providing information to the Noxious Weed Advisory Committee to fill gaps in knowledge about invasive *Phragmites* that were identified as key areas of uncertainty during the last review of *Phragmites*' noxious weed classification, which is expected to be revisited during the next reporting period.

Final Report Summary:
MnPhrag is an early detection and response effort targeting invasive *Phragmites australis* (common reed) (www.mnphrag.org), with the goal of supporting landscape-scale, strategic management throughout Minnesota. We mapped the distribution of invasive *Phragmites*, investigated its spread potential, and developed strategies for coordinated response in collaboration with agency staff and other resource managers. We engaged professionals and citizen scientists in reporting suspected populations; conducted intensive search efforts in under-sampled regions; and revisited unverified reports from a web-based invasive species reporting system. Over 70 active observers helped us identify 435 invasive *Phragmites* populations statewide, and we showed that non-experts can reliably distinguish invasive from native *Phragmites* using an identification guide we developed (www.maisrc.umn.edu/identifying-phragmites). The value of this "crowdsourcing" approach to surveillance is reflected in most invasive stands we identified being small populations (90% are <0.25 acres), for which effective control is much more feasible. Invasive *Phragmites* is producing viable seed in Minnesota, which increases spread risk; however, the extent of seed production varies across populations, and there is still time to prevent further spread through sound, sustained control efforts. We are working closely with diverse stakeholders to support coordinated response efforts. Our work has also brought state agencies together to address crosscutting issues related to invasive *Phragmites*' regulatory status, including its use in some wastewater treatment facilities in "reed beds" for removing water from biosolids. We recently published an action plan outlining how *Phragmites* spread could be stopped and reversed in Minnesota; this assessment includes management recommendations, cost estimates, and region-specific response guidance (www.maisrc.umn.edu/reversing-spread). Our findings reveal a window of opportunity to slow and reverse spread of invasive *Phragmites*, which would benefit Minnesotans by protecting vital natural resources. This approach to statewide surveillance, and framework for a coordinated, landscape-scale response, are strategies that could be applied to other invasive species issues in Minnesota.

SUB-PROJECT 18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota

Project Manager: Ray Newman

Description: Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most troublesome aquatic weeds in North America. In addition to suppressing native plant communities, inhibiting recreation and use and suppressing property values, hundreds of millions are spent annually on its control, with over $2 million per year in Minnesota. Recently concern has arisen for hybrid watermilfoil, which may respond differently to management or be more invasive than pure Eurasian. This study will determine the distribution and extent of the hybrid milfoil problem in Minnesota to define the scope of the problem and develop specific hypotheses that can be tested with future studies to improve management.

In Minnesota, Eurasian watermilfoil was first found in Lake Minnetonka in 1987 and White Bear Lake in 1988. It now occurs across the state in more than 300 waterbodies in 35 counties. Permits are issued for larger scale control of Eurasian watermilfoil on 80 to 100 lakes per year in Minnesota, and most control efforts are with auxinic herbicides: 2,4-D and triclopyr.

Eurasian watermilfoil hybridizes with the native northern watermilfoil (*M. sibiricum*). Hybrids are difficult to distinguish from Eurasian watermilfoil, and as a result, populations identified as "Eurasian watermilfoil" may be composed of "pure" Eurasian watermilfoil, hybrids, or both. Although managers and aquatic botanists increasingly recognize Eurasian and hybrid watermilfoil as distinct taxa, they are not frequently distinguished when it comes to operational management strategies, control tactics, or evaluations of management actions. As a result, there is still uncertainty regarding whether, and to what extent, hybrid watermilfoils may exhibit unique ecologies and/or pose distinct challenges for management (e.g., will they exhibit faster growth and/or herbicide tolerance?).

However, there is increasing concern that hybrid watermilfoil might be more invasive than Eurasian watermilfoil. A laboratory study found that hybrid watermilfoils in Michigan had faster vegetative growth rates and increased tolerance to 2,4-D, on average, compared to Eurasian watermilfoil. Similarly, a field study found that efficacy of the auxinic herbicides 2,4-D and triclopyr were much greater on pure Eurasian compared to hybrid watermilfoil in Houghton Lake, MI (93% versus 44% reduction, respectively). Overall, the number of quantitative comparisons of Eurasian watermilfoil and hybrids is low, and more comparisons are needed to determine whether generalities exist in terms of differences between Eurasian watermilfoil and hybrids.

Recent molecular genetic studies demonstrate that genetic diversity is much higher in watermilfoils than previously recognized. Although clonal reproduction is common, sexual reproduction is also common, as indicated by genetic diversity, including evidence for sexual reproduction by hybrid watermilfoils. Genetic variation is generally higher for hybrid and northern watermilfoil compared to Eurasian watermilfoil. It is therefore possible that differences among Eurasian watermilfoil and hybrids depend on the specific genotypes being compared.

Several studies have identified clear tolerance by some hybrid genotypes to some herbicides, including fluridone and the auxin mimics 2,4-D and triclopyr, whereas studies on other genotypes have not found any evidence for tolerance. Because the properties of populations likely vary as a function of their genetic composition, an important first step in being able to predict the growth and control response of populations is to delineate and quantify genetic variation within and among populations. These observations regarding hybrid watermilfoil illustrate the need for a structured effort to document the occurrence and distribution of hybrid milfoil in Minnesota.

Although hybrid watermilfoil has been documented in Minnesota since the early 2000s and additional occurrences have since been reported, a comprehensive assessment of the distribution and genetic diversity of hybrid watermilfoil in Minnesota has not been conducted. We have identified 12 lakes with verified hybrid watermilfoil (out of 330 + waterbodies with verified Eurasian, which includes hybrids). All of these lakes are in the Twin Cities Metro Region (Anoka, Dakota, Hennepin, Ramsey and Washington counties), but few lakes outside the Metro Region have been genetically analyzed. Furthermore, analysis for specific genotypes has only been conducted on Christmas Lake and several bays in Lake Minnetonka and these analyses showed considerable diversity. Hybrid watermilfoil had 34 distinct hybrid genotypes compared to nine Eurasian genotypes and 24 northern watermilfoil genotypes. One hybrid genotype appeared to be more prevalent after bay-wide herbicidal control. There was also evidence that northern watermilfoil was restricted to shallower sites and Eurasian and hybrid were found in deeper water. The distribution and occurrence of hybrid milfoil is unknown around the state and even less is known about distribution of milfoil genotypes.

To address this gap, we will assess the distribution and occurrence of hybrid watermilfoil in Minnesota and determine relations to factors that may affect its ecology and management. Specifically, our project has the following objectives:

Objective 1: Describe the frequency of occurrence and the geographic distribution of hybrid watermilfoil in Minnesota in order to determine the extent of this AIS problem and evaluate factors that are relevant to its biology and management. Specifically, test whether it is a) geographically widespread versus restricted to the Metro Region, b) more likely to occur in lakes with native northern watermilfoil, or c) more likely to occur in lakes with a longer invasion history.

Objective 2: Delineate and quantify genetic variation in hybrids in order to determine the role different genotypes and genetic diversity might play in its distribution and management. Specifically, A) assess whether specific genotypes are associated with a) geography and distribution extent, b) invasion history, or c) management history. B) Determine whether genetic diversity or the occurrence of specific genotypes is related to a) local environment and aquatic plant communities or b) management history or actions.

To address these objectives, we will conduct a statewide survey of lakes infested with Eurasian watermilfoil to determine the occurrence and distribution of hybrid milfoil across the state. We will use molecular genetic techniques to identify hybrids and genotypes of hybrid, Eurasian and northern watermilfoil. Finally, we will conduct more detailed study on a small subset of lakes to determine the relationship of local scale factors such as depth and plant community with hybrid genotypes, and the influence of management actions to hybrid milfoil genetic diversity.

With the results of this study, we will be able to determine if hybrid watermilfoil is a widespread or limited problem, if there are few or many genotypes that are of potential concern, and if specific approaches will be needed to manage hybrid watermilfoil. We will be able to identify specific genotypes or populations in need of further study and develop specific hypothesis for future studies to test to improve management and effectively deal with hybrid milfoil in control programs.

Summary Budget Information for Sub-Project 18:	**ENRTF Budget*:**	**$221,375**
	Amount Spent:	**$220,412**
	Balance:	**$963**

Outcome	**Completion Date**
Activity 1	
1. *Select and sample 50-60 lakes across the state for milfoil, process and preserve samples and send material to Thum for genetic analysis.*	August 2018
2. *Extract DNA and identify plant taxa with internal transcribed spacer DNA sequence (ITS).*	December 2018
3. *Analyze distribution of hybrid and co-occurring milfoils across state.*	March 2019
4. *Develop a manuscript describing the distribution of hybrid milfoil and addressing the relationship of hybrid and Eurasian milfoil with geographic location, time since invasion, depth, and co-occurrence with northern milfoil.*	June 2019
Activity 2	
1. *Decide whether to use microsatellites and AFLPs versus SNPs to genotype plants.*	January 2018
2. *Analyze 25-100 DNA samples from each lake for identification of genotypes.*	January 2019
3. *Analyze distribution of genotypes and genetic diversity across lakes in relation to geography, invasion history and management*	March 2019
4. *Develop a manuscript describing the distribution of genotypes and genetic diversity.*	June 2019
Activity 3	
1. *Select and sample 10 lakes for intensive study*	September 2018
2. *Analyze DNA samples for identification of genotypes.*	January 2019
3. *Analyze intensive study lakes for relationships of genotypes and genetic diversity to depth, plant community and management actions.*	April 2019
4. *Develop a manuscript that addresses local scale factors associated with genotype occurrence or the response of hybrid genotypes to management actions.*	June 2019
Activity 4	

1. *Disseminate preliminary results at MAISRC showcase 2017, 2018 and coordinate with MAISRC Extension Specialist Dan Larkin and communicator to address hybrids and milfoil genetics on MAISRC website.*	December 2018
2. *Host meeting with stakeholders to present results and discuss management strategies*	April 2019
3. *Submit one or more manuscripts to peer-reviewed scientific journal(s)*	June 2019

Sub-Project Status as of *February 28, 2018*
The project got started in summer 2017 and we were able to collect milfoil samples from 33 lakes. Due to the somewhat later than anticipated start date, we mostly sampled lakes in the Twin Cities Metro region, but we sampled a good coverage of lake types and age of infestation. At most lakes, we sampled 100 points for milfoil (Eurasian, northern or hybrid); we found no milfoil at one lake (previously known to be infested) but got a good distribution of samples at most of the lakes. Samples of all taxa were processed and have been shipped to the Thum lab for genetic analysis. Thum has started DNA extractions and completed ITS identifications on a subset of lakes. These results indicate that our visual determinations of milfoil taxon (hybrid, Eurasian or northern) are not always correct and corroborate the need for genetic analysis. Thum will complete the taxonomic identifications this winter and the genotyping by April. At that time, we will host a meeting with the DNR and cooperators to determine lakes to sample in summer 2018, including lakes for intensive analysis.

An amendment was approved by LCCMR on 02/06/2018 to re-budget resources to poster printing and publication charges. We did not budget for poster printing and publication charges but these are important to our outreach and scientific publication efforts. We will allocate $200 out of the current Services – Office and General Operations that was for mailing and shipping. We currently have spent less on shipping than anticipated. If we later need more resources for shipping or publications we will request a re-budget from another budget category.

Sub-Project Status as of *August 31, 2018*
Genetic identifications of plants with ITS has been completed for up to 20 plants for each lake sampled in 2017. Eurasian watermilfoil was found 19 lakes, hybrid in 18 lakes, northern in 10 lakes and all three taxa in just one lake. A comparison of our visual identifications with the genetic IDs indicated that overall our visual IDs were correct 80% of the time but most of the miss-matches were hybrid misidentified as Eurasian or vice versa. Although we can often visually detect hybrids, genetic analysis is needed for certain identification. Genotypic characterization with microsatellites has also been completed for the samples identified with ITS. Northern watermilfoil was most diverse with different genotypes in each lake and generally several different genotypes within a lake. Only three genotypes of Eurasian watermilfoil were found; one that was widespread and two others that occurred each in a different lake. Hybrid watermilfoil showed intermediate diversity; most lakes with hybrid had only one genotype of hybrid but several bays in Lake Minnetonka had 5 to 7 different genotypes. One hybrid genotype was found in 6 lakes in the northeast metro; most other lakes had unique hybrid genotypes. The Thum lab will process additional samples from lakes that had more than one taxa or different genotypes this summer.

We selected and sampled 5 treatment and 5 control lakes with point intercept surveys (generally 150 or more points) to characterize the genetic composition and plant community structure. Treatment lakes were subjected to a range of herbicide treatments including fluridone, 2,4-d, and ProcellCOR. We will resample these lakes in August to assess changes in relation to management or changes over time.

We have presented our results at several local and national meetings addressing lake users, managers and scientists and have been interacting with the DNR, consultants and applicators in lake selection. We will start surveying additional lakes for the presence of hybrids in July to further characterize the distribution of taxa and genotypes in the state.

Sub-Project Status as of *February 28, 2019*
Genetic identifications of plants has been completed for up to 20 plants from 31 lakes sampled in 2018. Across both years we sampled 62 lakes and found Eurasian in a total of 43 lakes, hybrid watermilfoil in 27 lakes, and northern in 23 lakes; all three taxa were found in four lakes. Overall most lakes tend to either contain just EWM (29%) or just hybrid (21%). This indicates that a lake does not necessarily have to have Eurasian or northern in order to have hybrid present.

Amongst the three taxa, EWM was the least diverse. Overall we have identified 8 Eurasian genotypes, 76 northern genotypes, and 57 hybrid genotypes in Minnesota. For EWM most of the lakes sampled in 2018 (21 lakes) contained the same genotype that was the dominant genotype within the lakes sampled in 2017. A total of 37 lakes overall contained this same genotype. There was no within-lake diversity for EWM, and overall we have found seven EWM genotypes that were different from the common widespread genotype. Hybrid watermilfoil showed intermediate diversity in comparison to EWM and NWM. Ten lakes had multiple hybrid genotypes, with there being particularly high diversity in one lake and in three bays of Lake Minnetonka. A few lakes shared common genotypes, which indicates some clonal spread of hybrids in Minnesota. There are numerous hybrid genotypes that could become problematic, but there are relatively few hybrid genotypes that have been more widely distributed. Northern watermilfoil was the most diverse, with most lakes having multiple different genotypes within lakes and no genotypes shared between lakes.

Ten lakes were intensively sampled based on recommendations by the DNR, consultants and applicators. Five treatment lakes and five reference or control lakes were surveyed in 2018 to characterize the plant community and milfoil genotypes to assess the response to herbicide treatment and characterize the native plant community. The lakes with a lake-wide fluridone application both had significant decreases in milfoil abundance following treatment, with almost complete elimination of milfoil (<2% frequency remaining). The lakes with 2,4-d and ProcellaCor had more focused treatments and less overall control. One lake treated with ProcellaCor needed a second treatment in the fall to further target the milfoil population. It is unknown if the poor response to it was due to application issues or the presence of tolerant genotypes.

We presented our results at several local and national meetings addressing lake users, managers and scientists and have been interacting with the DNR, consultants and applicators in lake selection. We had a productive meeting with applicators, consultants and DNR staff to discuss results and strategies to address key management questions during the MAISRCshowcase and users are keenly interested in our results.

Final Report Summary:
Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most problematic invasive aquatic plants in Minnesota. It can hybridize with the native northern watermilfoil (*M. sibiricum*) and reproduce sexually. Previous studies show that some genotypes of hybrid are resistant to specific herbicides and some may be more invasive. We determined the distribution of hybrid, Eurasian, and northern watermilfoil in Minnesota and assessed factors related to this distribution. We also assessed genetic variation (diversity) and distribution of specific genotypes and began an assessment of the response of watermilfoil and genotypes to management with herbicides. We sampled 64 lakes across the state stratified by county, size, and duration of infestation and collected milfoil from random points. The DNA from the milfoil samples was analyzed to determine taxon (Eurasian, northern or hybrid) and specific genotypes.

We found Eurasian in 43 lakes, hybrid in 28 lakes, and northern in 23 lakes. Hybrid was much more common in the metro, whereas Eurasian was broadly distributed. Northern watermilfoil was the most diverse with 84 genotypes, none shared across lakes. In contrast, we found one widespread genotype of Eurasian and six others found in indivdual lakes. Hybrid was intermediate in diversity with 53 genotypes; most lakes had only 1 unique genotype but 40% had multiple hybrid genotypes. Several genotypes were found in multiple lakes indicating clonal spread. The high diversity of hybrid watermilfoil indicates there is much potential for selection of problematic genotypes that are resistant to herbicides or that are competitively superior. There are numerous hybrid genotypes that could become problematic, but few have been widely distributed. We have not yet identified any clearly problematic genotypes in Minnesota but lakes with unexplained treatment failures, and populations with high diversity should be assessed. We will implement a strategy to identify and test problematic genotypes in Phase II of this project – MAISRC Subproject 18.2: Genetics to improve hybrid and Eurasian watermilfoil management.

SUB-PROJECT 19: Decision-making tool for optimal management of AIS

Project Manager: Nicholas Phelps
Description: Effective management of aquatic invasive species (AIS) in complex and dynamic systems, considering variable needs, values, and constraints, has proven difficult. AIS managers at the local and state levels urgently need science-based tools to inform planning and decision-making. For example, mathematical and optimization models using robust and updated information can be used for developing effective intervention strategies, predicting impacts, testing what-if scenarios, increasing stakeholder buy in, and designing cost-effective surveillance programs to mitigate and prevent AIS spread. We have been moving in this direction with previous and ongoing research led by the Project Manager and collaborators to describe environmental suitability and pathways of spread for high priority AIS. We have reached a point where the previously developed risk maps could be incorporated into dynamic system models to visualize risk and evaluate optimization approaches for management.

The aim of this proposal is to build upon and refine previous research to develop and deploy a decision-making tool for optimal management intervention on a county and statewide scale to minimize the spread of high priority AIS.

Based on the dynamics of AIS and the systems in which they live and move, we will develop models to forecast the invasion of zebra mussels and Eurasian watermilfoil in Minnesota at the lake level. These models will be subjected to strict verification and cross-validation to ensure confidence in model predictions. The risk scores for each waterbody will then be used to inform AIS management optimization models at the county level. Optimization models are a useful approach to identify a set of actions that make the best use of available resources while achieving a desired outcome. Therefore, in addition to the risk scores, values and management objectives such as types of lakes to prioritize for prevention (e.g. All lakes equally? Large/popular lakes?) will be incorporated to recommend the allocation of available funds and strategic locations for prevention and control activities to reduce the risk of new AIS introductions within each county. Similarly, cumulative risk models will be developed to help inform statewide allocation of the County AIS Prevention Aid, compared to the current approach of total boat ramps and parking spots. Local and state AIS managers will be engaged throughout the project to ensure consistency with management goals and realities. Ultimately, the models will be visualized through a user-friendly and interactive application for online or mobile viewing to empower AIS management stakeholders.

Summary Budget Information for Sub-Project 19: **ENRTF Budget*:** **$172,465**
Amount Spent: **$80,469**
Balance: **$91,996**

Outcome	Completion Date
Activity 1	
1. *Development and validation of multiplex network metacommunity (MnM) model*	May 2018
2. *Result dissemination: MAISRC communications, scientific presentation, peer-reviewed publication*	August 2018

Activity 2	
1. *Development of county-based AIS management optimization models*	September 2018
2. *Development of risk-based statewide funding allocation model*	September 2018
3. *Deploy models at AIS manager workshops*	October 2018
4. *Result dissemination: MAISRC communications, scientific presentation, peer-reviewed publication*	January 2018
Activity 3	
1. *Development of visualization tool for AIS management*	April 2019
2. *Deployment of visualization tool to AIS managers*	June 2019
3. *Result dissemination: MAISRC communications, peer-reviewed publication*	June 2019

Sub-Project Status as of *February 28, 2018*
Project is progressing as expected, despite a small delay in data availability. The first step in developing AIS risk estimates for each lake in Minnesota is complete, with the creation of a hydromorphological network models. As hypothesized, the model suggests that while water connectivity is important (explains ~35% of distribution for ZM and EWM), other factors are clearly influencing the spread of AIS. In the coming months, we will be adding other variables, such as environmental suitability and boat movement, to increase complexity and predictability of the models. In addition, theoretical optimization model has been created to conceptually evaluate AIS management tradeoffs, considering prevention (focus on uninfested lakes), containment (focus on infested lakes), or a mix of the two. We have found with early conversations that the DNR's strategy has been largely focused on containment, while most local groups have largely focused on prevention. We will continue to explore various scenarios with two counties (likely Ramsey and Crow Wing) in the coming months.

An amendment was approved by LCCMR on 1/31/2018 to reduce one service contract identified in the budget and add another service contract. Under the new workplan, funding will be split $15,000 to TheBlackTechGuy for app development and $10,000 to SMART Solutions for Questions and Decisions model website and web-service in connection to the dynamically updated predictions of the multiplex network metacommunity model. This update does not change the scope of the project, timeline or overall budget.

Sub-Project Status as of *August 31, 2018*
This has been a productive phase of the project, with additional data made available with the completion of MAISRC Subproject #13. Significant progress has been made with the multiplex metacommunity model development. With the application of the model, we verified the importance of the Hydrologic Network (HN) to be higher for Zebra Mussel (ZM) than Eurasian Watermilfoil (EW); the latter seems more affected by local environmental variability and characterized by a more confined dispersal. ZM and EW fluctuate more proportionally to systemic runoff and local rainfall, respectively. Thus, runoff as an output from lakes informs a more dynamic risk determinant of species invasion vs. local lake features. Certainly, it is clear that it is not sufficient to consider only the environment as a determinant of a higher or lower chance of species invasion downstream or upstream an invasive population. Furthermore, these results emphasize once again the importance to consider physical basin boundaries rather than political lines for effective management. This paradigmatic shift creates some tension with the management of AIS because a basin can belong to different counties and decisions are typically taken at the county scale. These models are being incorporated into a new application that can be used to visualize risk of AIS.

We have also begun to evaluate 'optimal management scenarios' based on the data available for lake connectivity and suitability. We evaluated Ramsey and Washington counties to inform the location of a limited number of watercraft inspection sites to intercept the largest number of 'at-risk' boats. The mathematically optimal results have been counter-intuitive to some, demonstrating this as a valuable exercise for managers. We will continue to develop these models for other counties and a statewide approach in the months to come.

Sub-Project Status as of *February 28, 2019*

The January 31, 2019 status update for this subproject has been delayed due to the federal government shutdown from December 2018 – January 2019. A status report is currently being drafted and will be included in the next update.

Final Report Summary:
Understanding the patterns of historic AIS invasion can provide the framework for forecasting future invasions. To that end, we used a big data approach to combine hydrologic connectivity and boat movement to create a multiplex metacommunity model for both zebra mussel and Eurasian watermilfoil. We found that the hydrological corridors are important pathways of spread, even more so that previous research has suggested. While overland dispersal of AIS via boater movement is still a significant factor, additional management strategies should be developed to include intervention of hydrological pathways.

Using connectivity networks of boater movement, we developed county-based AIS management optimization models that prioritize inspection locations that will intercept the highest number of 'risky boats' (e.g. moving from infested to uninfested lakes). We piloted the models in Crow Wing, Ramsey, and Stearns Counties and had a very productive collaboration with county managers and citizen advisory boards during the development and evaluation for each. Ultimately, the application of this approach was well received and helped inform allocation of their inspection hours at the county level (for example: https://www.crowwing.us/1004/Aquatic-Invasive-Species-AIS).

Dissemination and usability of the models was a priority of this project. We created online tools to 1) visualize the spread risk for zebra mussels and Eurasian watermilfoil based on model predictions made in Activity 1, and 2) visualize and modify the decision optimization model at the county level based on management thresholds or funding availability. These tools and more detailed descriptions of the project has been disseminated through in-person stakeholder meetings and presentations to diverse audiences, including managers, researchers and the public.

SUB-PROJECT 20: A Novel Technology for eDNA Collection and Concentration

Project Manager: Abdennour Abbas
Description: In a very recent informal survey of Minnesota Department of Natural Resources (DNR) managers and researchers, it became evident that a major need for aquatic surveys is not developing new detection methods but improving the sampling tools. A number of promising techniques are available today including environmental DNA (eDNA) amplification using PCR and LAMP assays or metagenomics sequencing. However, the major problem is that the results obtained from eDNA techniques do not always correlate with traditional netting data (e.g., some species are missed, or abundance relationships are weak) in part due to sample size and quality. Current attempts to use eDNA for detecting species typically require numerous samples from each site, especially when detecting rare species such as a newly invading aquatic invasive species (AIS). Improving detection probability or precision of abundance estimates by increasing the number of samples leads to high costs using current sampling methods. To convert these techniques into reliable species detection tools and enhanced quantitative tools (offering a good correlation between eDNA copies and species abundance) new efficient and cost-effective sampling methods need to be developed.

Environmental DNA (eDNA) is the genetic material (genomic DNA) obtained directly from environmental samples such as soil and water. The collection of eDNA is an emerging cost-effective alternative or complement to traditional sampling (mostly nets and electrofishing for fish, visual surveys or net tows for inverts). When combined with DNA sequencing technology or quantitative PCR (qPCR), eDNA could represent a cost-effective and reliable tool for biodiversity monitoring, including species detection and abundance. However, current eDNA sampling methods may result in significant false positives or negatives that prevent wide-spread adoption for management purposes. To avoid failure to detect a species across an entire site of interest (e.g., lakewide, stream reach), several to tens of individual water samples are typically collected. The need for a large number of samples is greatest when targeting rare species, such as a newly invading AIS where limited concentrations of DNA may be present in the water. Our improved sampler aims to reduce these per sample costs directly but could also provide savings elsewhere, including reduced staff time per site and ability to sample more locations in a single trip.

This proposal aims at developing a novel aquatic eDNA collection and concentration technology for more efficient, reliable and cost-effective screening for not only invasive aquatic organisms and pathogens but also native and endangered species. The technology would significantly enable and empower aquatic ecosystem survey and management programs in Minnesota.

Specific aims: The proposed eDNA aquatic sampling technology will be developed and tested in three major steps:

1. Develop an eDNA nanofilter that specifically and rapidly captures nucleic acids (DNA, RNA) from water
2. Develop a housing system for the nanofilter to allow field deployment and continuous sampling of large water volumes or large areas
3. Verify increased eDNA sampling efficiency of the new device in field settings (proof-of-concept)

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
Summary Budget Information for Subproject 20:	**Subproject Budget:**	**$94,599**	**Subproject Budget:**	**$96,264**
	Amount Spent:	**$90,263**	**Amount Spent:**	**$39,876**
	Balance:	**$4,336**	**Balance:**	**$56,388**

Outcome	Completion Date
Activity 1	
1. Development of eDNA nanofilter using a polymeric membrane modified with nanotechnology	March 2019
2. Development of a housing system for the eDNA nanofilter	July 2019
3. Evaluation of the performance of the eDNA nanofilter	November 2019
Activity 2	
1. Collection of eDNA from selected locations	April 30, 2020
2. Sample analysis: quantitative PCR of collected samples	April 30, 2020
3. Dissemination of research findings to AIS managers, policy makers, and planners, including at the annual Showcase event; coordination with MAISRC and Extension on media efforts and communications; and participation on 1-2 committees	June 30, 2020

Subproject Status as of *January 31, 2019*:

The project is currently progressing as expected and no amendment is needed. A full-time researcher (category 4), Mr. Akli Zarouri and one undergrad student were hired to work on the project. Mr. Akli started his position on December 20, 2018. Both hires received on week-long research and safety training.

Currently, we are working on Phase 1 of Activity 1, related to the development of an efficient eDNA filter. This phase will be completed in March. Details of the technical progress of the development of an eDNA filter is provided below in the Activity 1 summary below. Activity 2 will be initiated early April 2019 as planned.

Subproject Status as of *July 31, 2019*:

The project is progressing as expected. We have successfully developed a new eDNA filter that captures > 90 % of DNA (our objective was 50%) within 10 seconds. The filter is a cellulose membrane functionalized with a polysiloxane polymer and put in contact with eDNA solution with concentration ranging from 10 ng/L to 1000 ng/L. The loading capacity of the new filter is up to 5 mg/g, meaning that 1 g of filter can capture up to 5 mg of DNA. This is a record-breaking capacity that enables the filtration of large volumes of water with one filter, knowing that surface water contains usually 10 ng/L of eDNA.

We are currently working on Phase 3 of Activity 1 that involves the development of a housing system for the eDNA filter to enable field use. This is expected to be completed as planned in November 2019.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of *January 31, 2020***:**
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 21: Early detection of zebra mussels using multibeam sonar

Project Manager: Jessica Kozarek
Description: Zebra mussels (*Dreissena polymorpha*) pose a serious threat to water supply and power plant infrastructure, and to Minnesota lake and river ecosystems, including native mussel species (Baker and Hornbach 1997). Current methods for detection and quantification of zebra mussel colonies rely on time consuming and expensive diving surveys, video imaging, or sampling of veligers (larvae) in the water column. Survey sampling design would be made much more efficient given spatially extensive information on the on the presence/absence of zebra mussel beds. Such remote sensing technology would also be useful for early detection and warning in rivers, lakes and reservoirs through routine monitoring, or to follow changes in zebra mussel density (boom or bust cycles).

This study will test the utility of swath mapping systems such as multibeam sonar for detecting and quantifying the abundance of invasive mussels at a very large scale. Multibeam sonar can map tens to hundreds of square kilometers of river or lake bed in a single day from a moving vessel. Ostensibly an instrument for bathymetric mapping, each sounding from a multibeam sonar also records the echo from the bed surface, which can be analyzed to provide information about the roughness and composition of the ensonified bed. This echo can be used to reliably distinguish among various substrates (Brown et al., 2011). Acoustics are also increasingly being used to map and monitor shellfish (e.g. Sanchez-Carnero et al. 2014) and submerged vegetation (e.g. Buscombe et al., 2017). There is a strong likelihood that mussels have a distinct acoustic response (echo) compared to their surrounding substrate. If so, this acoustic signature can be readily used to detect and map zebra mussel beds at cm to m resolution in any navigable waterway of sufficient water depth.

This study will define the methodology needed to detect, distinguish and quantify mussels from a moving vessel by studying backscattering of sound by mussels and common mussel-supporting substrates. Mussels are soft-bodied invertebrates with hard shells. The acoustics of backscattering by mussels might depend on many physiological and morphological factors such as size, shape, shell thickness/roughness/composition, and the composition of soft tissues. In concert, these factors manifest as differences in scattering due to differences in roughness and hardness. It should therefore be possible to discriminate between different species of mussel (zebra mussels vs. native species) using acoustics alone, or acoustics in combination with measurable environmental variables that govern the spatial distributions of mussels. In lakes and rivers, this methodology will enable the scanning of large areas for the early detection of zebra mussel colonies. In river systems, it could be applied to detect longitudinal changes in zebra mussel populations downstream from a source population to evaluate the role of downstream drift in zebra mussel spread.

The first phase of this study, laboratory experiments, is designed as a proof-of-concept to utilize multibeam sonar to distinguish amongst substrate, native and zebra mussels in a controlled setting. We will study the acoustic backscattering properties of zebra mussels (*Dreissena polymorpha*) and native mussels, Threeridge (*Amblema plicata*), under controlled laboratory settings. Experiments in self-contained tanks at the St. Anthony Falls Laboratory will be used to determine the acoustic parameters that will maximize the discrimination between mussels and substrates. Following this study, a second research phase is planned to validate and further develop methodology in the field. Field measurements will allow the incorporation of a larger range of variables (mussel density, mixed substrates, water depth, etc.), once methodology has been tested in carefully controlled laboratory conditions.

Summary Budget Information for Subproject 21:

Subproject Budget:	**$96,549**
Amount Spent:	**$96,175**
Balance:	**$374**

Outcome	Completion Date
1. Acoustic parameters to detect zebra mussels	June 2019
2. Acoustic parameters to detect native mussels	June 2019
3. Effect of substrate on detection	June 2019

Subproject Status as of *January 31, 2019*:
We successfully completed the planned lab experiments over 4 weeks in September 2018. Using the data, we have developed machine-learning-based substrate classifiers hypothetical situations of abiotic (bare) and biotic (mussel-supporting) substrates. The input into each model is measured backscattering strength of the bed over prescribed combinations of several acoustic frequencies and pulse lengths. The model output is the likelihood of each substrate class. Each model is trained only on distributions of uncalibrated acoustic backscatter measured in the lab over ten unique substrates, namely: 1) sand, 2) mix sand-gravel (MSG); 3) gravel; 4) sand-supported *A. plicata*; 5) MSG-supported *A. plicata*; 6) gravel-supported *A. plicata*; 7) sand-supported *D. polymorpha* (low density); 8) sand-supported *D. polymorpha* (high density); 9) gravel-supported *D. polymorpha* (low density); and 10) gravel-supported *D. polymorpha* (high density). Phase I, experiments to examine the feasibility of using multibeam sonar to detect zebra mussels, is considered complete when the following objectives have been met:
** indicates objective has already been met*

1. Conduct lab experiments (summer 2018)*
2. Develop an empirical substrate classifier based on measured uncalibrated backscatter (fall/winter 2018)*
3. Develop an analytical substrate classifier based on measured calibrated backscatter (spring 2019)
4. Develop a prototype field protocol for zebra mussel detection (spring/early summer 2019)
5. Write and disseminate findings (spring/early summer 2019)

Final Report Summary:
Zebra mussels pose a serious threat to Minnesota lake and river ecosystems. However, monitoring zebra mussel populations is challenging because current methods for detecting and counting zebra mussel colonies rely on time consuming and expensive diving surveys, video imaging, or sampling of veligers (larvae), which limits the areas surveyed. Remote sensing techniques have been shown to quickly and efficiently gather spatially extensive information. Using this technology to detect zebra mussels would likely be much more efficient and more effective than traditional methods and could be used for early detection and warning in rivers, lakes and reservoirs and to track changes in zebra mussel density.

This project was the first phase of research designed to test the utility of a swath mapping system, multibeam sonar, for detecting the presence and abundance of invasive mussels. Laboratory experiments were conducted to test the feasibility of using multibeam sonar to distinguish zebra mussel containing substrates. Acoustic backscatter data were collected in a two meter deep tank over sand, gravel, and mixed substrate containing high and low densities of zebra mussels and with native mussels using combinations of different sonar settings (frequencies and pulse lengths). Machine-learning was used to differentiate the acoustic backscattering signatures in a data-driven substrate classifier approach. Using these methods, we were able to classify substrate by size and mussel density. Classification errors decreased with more sonar settings. For minimum errors of less than 20%, 8 sonar settings are required, and for minimum errors of 10% or less for all substrates, 12 sonar settings. Each sonar setting corresponds to a separate boat survey of an area with a multibeam sonar in the field. Therefore, the next phase of this research is to further develop and test multibeam sonar monitoring approaches in the field (MAISRC Subproject 21.2: Field validation of mulitbeam sonar zebra mussel detection).

SUB-PROJECT 22: Copper-based control: zebra mussel settlement and non-target impacts

Project Manager: James Luoma
Description: Development of population level management techniques that have potential to reduce the environmental and economic impacts of zebra mussels while also protecting and preserving native species and habitats are critically needed. Targeting treatments to kill zebra mussel larvae and prevent their settlement also has potential use for zebra mussel containment or eradication in small, hydrologically isolated inland water bodies. Potential users include the MN DNR, local governmental units, and water infrastructure owners/users.

This project builds upon previous work (McCartney 2016) which identified the susceptibility of larval zebra mussels to much lower doses of copper compared to adult zebra mussels. This project will involve a 10-day, low-dose (60-ppb) copper treatment of an entire enclosed bay in Lake Minnetonka. St. Albans Bay (treated bay) and Robinson's Bay (control bay) will be sampled before and after application to determine treatment-related impacts on zebra mussel veliger abundance and settlement success. Treatment-related impacts to adult zebra mussels, algal, zooplankton, benthic invertebrates, and fish communities will be assessed. The three main objectives in this project are: 1) evaluate the efficacy of low-dose copper treatments to control populations of zebra mussel veliger larvae, 2) evaluate the use of low-dose copper treatments to suppress zebra mussel larval settlement, and 3) evaluate the effects of low-dose copper treatments on native aquatic animals and algal biomass.

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
DRAFT Summary Budget Information for Subproject 22:	**Subproject Budget:** (UMN Portion: $54,438) (USGS Portion: $12,428)	**$66,866**	**Subproject Budget:** (UMN Portion: $26,670) (USGS Portion: $121,790)	**$148,460**
	Amount Spent:	**$62,436**	**Amount Spent:**	**$106,457**
	Balance:	**$4,430**	**Balance:**	**$42,003**

Outcome	Completion Date
Activity 1	
1. Refine methods to assess zebra mussel settlement	December 2018
2. Complete acquisition contract for EarthTec QZ	May 2019
3. Develop project protocol and obtain necessary permits for application and test cages	May 2019
Activity 2	

1. Conduct pretreatment collection of veliger/zooplankton tows, benthic invertebrate samples, water chemistry samples, secchi disk readings, and chlorophyll samples.	July 2019
2. Placement of buoys, nontarget fish and unionid mussels, adult zebra mussels, and zebra mussel plate samplers in control and treated bays.	July 2019
3. Entire bay applications of EarthTec QZ over 10 days, consisting of 5 independent applications.	August 2019
Activity 3	
1. Conduct post-treatment collection of veliger/zooplankton tows, benthic invertebrate samples, water chemistry samples, secchi disk readings, and chlorophyll samples.	August 2019
2. Conduct survival assessments of adult zebra mussels, unionid mussels and fish	August 2019
3. Complete assessments of settlement success on plate samplers	December 2019
4. Complete data entry, proofing, and summarization	January 2020
5. Prepare study report and peer-reviewed manuscript	June 2020

Subproject Status as of *January 31, 2019*:
Since work plan approval in November 2018, the project teams at the USGS and MAISRC have been working on administrative set-up for project budgets and subawards and refining methodology for the 2019 field season. Project activities detailed in the workplan and spending have not yet begun.

Subproject Status as of *July 31, 2019*:
The project teams at the USGS and MAISRC have completed action items under Activity 1 and have initiated action in Activity 2 to include buoy and settlement sampler placement and all preparations leading up to application of the EarthTec QZ. Pretreatment sampling is scheduled to begin on July 18, 2019 and test animals will be placed within the treated and control bays by July 21, 2019. Treatment applications are scheduled to begin on July 22, 2019 and be completed on July 30, 2019.

Additional non-sponsored funding was secured by MAISRC to enhance the data collection for the study. The additional labor provided by a graduate student will allow for more robust water sampling to allow for water copper concentration profiling and test animals will be analyzed for tissue residues after the exposure is completed. More information is provided in section VI.B.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of *January 31, 2020*:
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 23: Public Values of Aquatic Invasive Species Management

Project Manager: Amit Pradhananga
Description: Emerging evidence shows that Aquatic Invasive Species (AIS) management can be used to restore ecosystem services. For example, management of the invasive common carp (*Cyprinus carpio*) can lead to increases in water clarity and declines in nutrient concentrations in a more cost-effective manner than other management practices (Vilizzi et al. 2015; Bartodziej et al., 2017). Yet, management of AIS is often not considered an option when planning ecosystem restoration. Even if the direct costs of AIS management are known, lack of information about the potential benefits of AIS management makes informed decision making

difficult. With an accurate assessment of the costs and benefits of AIS management strategies, as well as information on public perception, resource managers will be better prepared for the efficient investment of management resources. The overall goal of this project is to quantify and analyze the ecological and economic value of AIS damages and AIS management as they relate to ecosystem services (e.g., fishing, swimming, biodiversity, navigability). The specific objectives of this project are to:

1. Assess the use and non-use values assigned to ecosystem services impacted by AIS. Use values are those values generated from using a resource, such as recreation values. Non-use values are those values generated even when a resource is not directly used-- the value a person has for a resource they never visit and never will visit. An example would be existence value—valuing a resource just for existing, or bequest value—valuing a resource for the benefit of future generations.
2. Investigate the costs and effectiveness of carp management as a strategy for water clarity restoration
3. Develop a flexible ecological and economic optimization modeling framework to inform AIS management decisions

We will employ a multi-pronged approach with five activities: estimating public benefits of AIS management (Activities 1 and 2), analyzing costs of carp management (Activity 3), and the development of a broad AIS analysis framework (Activity 4) which we will use to estimate efficient carp management (Activity 5). The main goal of Activities 1 (mail survey of residents and lakeshore owners) and 2 (onsite survey of recreationists) is to produce data which can be used to estimate the lost public value attributed to AIS. The on-site surveys will target recreationists to generate use values related to boating, fishing, swimming, and general hiking/wildlife viewing/enjoyment of nature. The third activity, a cost analysis, will focus on common carp, an established AIS with long management history. This activity will generate cost and effectiveness information for various methods of carp management, potentially including removal, prevention, and barriers. Activities 4 and 5 include the development of a programming framework both to analyze the data generated in activities one, two, and three, and to provide guidance for AIS management in other regions of the state.

This project will provide multiple benefits to stakeholders and natural resources throughout Minnesota, as well as other areas with AIS concerns. This project will provide both natural resource managers and water quality regulators with information that will help to prioritize AIS and water quality management projects, permitting them to make more effective use of limited conservation dollars. This project will quantify the dollar value of the public benefits of AIS management, as well as the costs of managing a specific AIS (i.e., common carp) for water quality outcomes. Expected outcomes of this project include a decision support tool that will help resource managers assess the costs and benefits of AIS management. Specific outcomes of the study include a comprehensive AIS valuation data compilation for use by other researchers, and an eco-economic programming model to predict the economic and ecological repercussions of using AIS prevention and control initiatives.

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
Summary Budget Information for Subproject 23:	**Sub-Project Budget:**	**$131,845**	**Sub-Project Budget:**	**$110,245**
	Amount Spent:	**$131,149**	**Amount Spent:**	**$50,656**
	Balance:	**$696**	**Balance:**	**$59,589**

Outcome	**Completion Date**
Activity 1	
1. Develop survey questionnaire for residents and lakeshore owners	January 31, 2019
2. Administer survey to 2,000 MN residents and lakeshore owners	July 31, 2019
Activity 2	
1. Develop the survey questionnaire for recreationists (e.g. boaters, anglers), sampling plan, and sampling schedule	April 30, 2019

2. Administer onsite surveys to recreationists at boat docks	September 30, 2019
Activity 3	
1. Compile list of management cases and supporting lake and watershed data in MN	January 31, 2019
2. Conduct preliminary cost-benefit analysis and identify data gaps	July 31, 2019
3. Finalize the database by scouring out-of-state data and conducting global literature review	January 31, 2020
4. Finalize cost-benefit analysis, submit manuscript, present the results to stakeholders (e.g. Minnesota Association of Watershed Districts (MAWD))	July 31, 2020

Subproject Status as of *January 31, 2019*:
We have made substantial progress in Activity 1 (general resident survey), Activity 2 (onsite survey of recreationists), and Activity 3 (cost-benefit of carp management). Because this is the first phase of this study, we conducted literature review to identify survey topics and questions (for Activities 1 and 2) from past research. We are currently developing the questionnaire that will be administered with Minnesota residents and lakeshore owners. We have also collected secondary data on lakes and AIS establishment from multiple sources (e.g., DNR, USGS). We developed, piloted, and revised a carp management questionnaire that will be used for data collection in Activity 3.

Subproject Status as of *July 31, 2019***:**
We have made progress in Activity 1 (general resident survey), Activity 2 (onsite survey of recreationists), and Activity 3 (cost-benefit of carp management). For Activity 1, we developed a draft survey that will be administered with 2,000 residents across Minnesota. The survey is currently being reviewed by experts in survey design. For Activity 2, we developed the survey questionnaire, sampling plan, and sampling schedule. We have also hired and trained field surveyors. The survey is being administered at 6 lakes across Minnesota. For Activity 3, we developed and administered a questionnaire with watershed districts and other carp management agencies to collect information about cost estimates (for each management action) and water quality (clarity and Phosphorus) before and after AIS management.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of *January 31, 2020***:**
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 24: Genetic method for control of invasive fish species

Project Manager: Michael Smanski
Description: Invasive fish species present an estimated $5.4 billion burden on our domestic economy, and much of that extends to the lakes and rivers of Minnesota. For example, the foraging habits of the invasive common carp, *Cyprinus carpio*, diminishes water quality, reduces vegetative cover and waterfowl numbers, and reduce the ability of lakes to absorb nutrients that enter water systems through agricultural runoff. Current control methods have not been able to stem the tide of invasive carp and other fish species, so improved strategies are needed. The overall goal of this project is to demonstrate a novel approach for controlling aquatic invasive species using invasive carp species as proof-of-concept. Success of this project would lead to its implementation in other aquatic invasive species (AIS), including Asian carp and zebra mussels.

We have three activities in this subproject. Activity 1 aims to develop state-of-the-art carp transgenesis capabilities at the MAISRC Containment Lab. Obtaining freshly laid eggs and fertilizing them with freshly collected sperm is a prerequisite for generating the young carp embryos needed for carp transgenesis. In Minnesota, wild carp only spawn during late spring/early summer, creating a very short window of opportunity for performing genetic engineering experiments. A serious effort towards developing new biocontrol methods in carp requires year-round access to young carp embryos, and we will achieve this be maintaining several independent tanks of captive carp that have been slowly 'trained' to be on different annual cycles.

Activity 2 aims to transition our new genetic biocontrol strategy into carp. We have done proof-of-concept experiments in simple laboratory organisms to demonstrate the feasibility of our approach. In this aim, we begin engineering these genetic components in carp. The complete engineering effort will require more time than is funded in this current subproject, but we have listed milestones that will demonstrate substantial progress towards our engineering goals.

Activity 3 accomplishes two tasks. First, we use computer modeling to predict the efficacy of our approach when combined with existing strategies for carp management. Second, we engage the public to develop a better understanding of their attitudes and opinions on using genetically engineered organisms as one part of an integrated pest management plan.

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
Summary Budget Information for Subproject 24:	**Subproject Budget:**	**$110,112**	**Subproject Budget:**	**$140,004**
	Amount Spent:	**$109,000**	**Amount Spent:**	**$36,693**
	Balance:	**$1,112**	**Balance:**	**$103,311**

Outcome	**Completion Date**
Activity 1	
1. Begin husbandry of 4 separate carp populations synced to unique annual cycles	July 2018
2. Demonstrate the ability to harvest and fertilize carp eggs/sperm from laboratory carp during Summer, Fall, and Winter (seasons when wild carp are not actively spawning)	December 2019
3. Generate transgenic carp expressing the genes needed to engineer our biocontrol system	June 2020
Activity 2	
1. Assess genetic diversity in wild populations of common carp	June 2019
2. Generate and validate point mutations in promoters of GATA5, SSH1, and ERN, which are three genes in carp that we need to modify for our genetic biocontrol approach.	June 2020
3. Transfer sex-ratio biasing construct to the *C. carpio* chromosome	June 2020
4. Introduce genetic components into carp that will drive the incompatibility between wild carp and engineered fish. These components will not be toxins but will cause natural carp genes to be turned on at the wrong time during development and lead to inviable offspring.	June 2020
Activity 3	
1. Complete optimal IPM plan based on agent-based simulation models	July 2019
2. OUTREACH: Survey state-wide Watershed District Managers about GMO technologies	September 2018
3. OUTREACH: Oral presentation at MAISRC open houses	September 2018/19
4. OUTREACH: Public survey via MAISRC Detectors volunteers and 2019 MN State Fair	September 2019

Subproject Status as of *January 31, 2019*:

We have made significant progress towards developing a first-of-its-kind biocontrol approach to combat invasive carp using Sterile Male Accelerated Release Technology (SMART) carp. Since we received notice of the LCCMR-MAISRC award in August 2018, we have created protocols for creating and rearing transgenic carp at the MAISCR Containment Facility. We have built genetic constructs encoding components of our technology and prototyped them in model laboratory fish. Lastly, we have designed and conducted a survey concerning the public perceptions surrounding genetic biocontrol of invasive carp. He learned that the public is more likely to embrace genetic biocontrol compared to alternative options, although there are major knowledge gaps concerning the potential risks and benefits of this technology.

Subproject Status as of *July 31, 2019***:**
We have made significant progress towards developing a first-of-its-kind biocontrol approach to combat invasive carp using Sterile Male Accelerated Release Technology (SMART) carp. Since our last status update, we have successfully spawned carp during 'off-cycle' calendar periods. We have tested several genetic constructs in the model laboratory fish, *Danio rerio*. We have not yet found a genetic design that is suitable for introduction to carp. Lastly, we have organized a second iteration of our public engagement survey that will be administered at the 2019 Minnesota State Fair.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of *January 31, 2020***:**
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 25: What's in Your Bucket? Quantifying AIS Introduction Risk

Project Manager: Nicholas Phelps
Description: The use of baitfish for recreation angling results in billions of farm-raised and wild-caught fish (and accompanying hitchhikers) being moved long distances overland and intentionally introduced into new environments. As a result, baitfish movement has been considered a high-risk activity for the spread of aquatic invasive species (AIS), with potentially major economic, ecological, and societal consequences. Consequently, state legislatures and management agencies across the country, including Minnesota, are considering dramatic overhauls of their baitfish regulations. This has put supporting a multimillion-dollar bait industry at odds with conserving a multibillion-dollar recreational fishery. The lack of a structured framework to evaluate risk in the face of differing perceptions and great uncertainty (ie. minimal data) for many aquatic hazards is limiting our collective ability to understand and mitigate the risk that baitfish movement could spread potentially devastating AIS.

While the baitfish trade has the potential to move all varieties of AIS, perhaps most vexing are invasive pathogens that can move as passengers undetected at high prevalence, have little or no management options, and can cause long lasting population-level impacts on important fish species. In Minnesota alone, numerous novel baitfish viruses have been discovered in recent years, highlighting the limited information we have regarding the health status of baitfish. There is a clear need for a rigorous risk analysis, but the lack of an informed framework to do so has limited our ability to quantify the risk and make risk-based decisions. The goal of this study is to assess the risk of introduction of important fish pathogens through the recreational use of baitfish. We will synthesize existing knowledge to identify priority hazards for the baitfish trade, develop a risk analysis framework, and characterize the volume, patterns, and complexity of baitfish use by anglers in Minnesota, to develop a tool for estimating risk of AIS introduction via the baitfish pathway. The tool will be tested with three pathogens of concern to estimate the number of likely introductions to wild fish populations - a useful metric when considering trade-offs for risk management.

This work builds upon, and will be informed by, an ongoing baitfish risk assessment led by the MN DNR, previous baitfish hazard assessments, and previous and ongoing research by members of the project team. By quantifying the actual, not just perceived risks, we will help to facilitate discussions among agency, industry, and public stakeholders, inform risk-based management decisions, and ultimately lead to better outcomes that support the state's bait and fishing industries while protecting natural resources. This project aligns with MAISRC High Priority Research Needs (Research Priority A.8), builds upon existing MAISRC research, forms a new collaborative team, and will fill critical knowledge gaps identified by managers and industry alike.

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
Summary Budget Information for Subproject 25:	**Subproject Budget:**	**$111,642**	**Subproject Budget:**	**$88,142**
	Amount Spent:	**$101,540**	**Amount Spent:**	**$25,556**
	Balance:	**$10,102**	**Balance:**	**$62,586**

Outcome	Completion Date
Activity 1	
1. Identification of 2-4 priority pathogen hazards for further research (Activity 4) and to create an overall Hazard Report.	November 2018
2. Finalization of the hazard prioritization matrix	January 2019
Activity 2	
1. Create process model for the baitfish supply chain and points of risk that will feed in to the design of angler survey (Activity 3).	December 2018
2. Development of initial introduction risk assessment framework to assess the risk of baitfish as a pathway for pathogen entry into MN waters.	March 2019
Activity 3	
1. Finalization of survey design and initial contact for mailed survey	March 2019
2. Survey coding and data analysis of survey responses	November 2019
3. Final boat launch surveys administered and evaluated	December 2019
4. Technical report on angler bait-related behaviors and peer reviewed manuscript	March 2020
Activity 4	
1. Updated risk assessment framework to inform decision making on AIS in the baitfish trade	June 2020
2. Peer-reviewed manuscript and policy brief	June 2020

Subproject Status as of *January 31, 2019*:
We have made substantial progress on the project, including the completion of Activity 1 and laying the groundwork for Activities 2 and 3. We completed our hazard prioritization matrix, which selected viral hemorrhagic septicemia virus (VHSV), *Ovipleistophora ovariae,* and the Asian tapeworm from among 30+ pathogens initially considered. Selection criteria included the pathogen's ability to evade detection, the impact of its establishment, and its current distribution in the state. We also outlined a conceptual model designating the steps in the bait pathway that will be evaluated for their contribution to overall risk by our quantitative model in Activity 2. Finally, we began development of angler survey questions, the answers to which will provide quantitative data to inform the risk model.

Subproject Status as of ***July 31, 2019*****:**

We have made substantial progress, particularly for Activity 3. After finalizing a design for the mailed paper survey in consultation with our project advisory team and our survey design collaborators at UMN Liberal Arts Technology and Innovation Services (LATIS), we completed the mailing procedures for the written survey protocol. We mailed invite letters, paper questionnaire surveys, and reminder postcards to 4,000 anglers across the state between May and June 2019. To date we have received approximately 600 completed mail surveys and expect more to come (see amendment request). We have also distributed 1,000 postcard surveys to trained MAISRC AIS Detector volunteers who are in the process of administering them at boat launches and other accesses around the state during the summer of 2019. We have been recording data from the surveys as they arrive as well as monitoring the online portal by which some survey participants responded. Once the data from these two methods have been recorded we can begin analysis and parameterization of the risk assessment model. Finally, we are drafting a manuscript explaining the process and importance of our risk ranking exercise in Activity 1, which we expect to submit September 1, 2019.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of ***January 31, 2020*****:**
Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:
Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 26: Updating an invasive and native fish passage model for locks and dams

Project Manager: Anvar Gilmanov
Description: Bighead and silver carps (together known as Bigheaded carps (*Hypophthalmichthys spp.*) and sometimes “Asian carp”) were introduced to the Arkansas in the 1970’s and are now threatening to enter Minnesota waters of the Mississippi River from Iowa where they presently exist as self-sustaining populations. This would become a significant problem for Minnesota aquatic ecosystems which are already burdened with high populations of invasive Common carp (*Cyprinus carpio*), which were introduced over a century ago. To preserve the Mississippi and St. Croix Rivers ecosystems, it is crucial to stop this invasion. One way to accomplish this is to use existing Mississippi River lock and dams (LDs), through which all fish must pass to go upstream. Existing data and numeric models suggest that carp passage through the spillway gates of these LDs systems is already hindered by the high velocities the gates create. Of course, it would highly desirable to avoid hindering native fish passage, and if possible even improve it, while stopping invasive carp passage through gates. Because of the complexity of LDs, and the high costs of conducing field work, a numeric model is the best way to achieve these goals in the immediate future. It is important that this model be as accurate as possible.

This project aims to create an updated version of Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model using new field data that can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams. These new field data presently being generated by an ongoing Sorensen laboratory field study of fish behavior and passage at Lock and Dam 2 (LD2) will be analyzed. Parameters on fish behavior will then be updated in the CFD-AB fish passage model already developed by [Zielinski et al., 2018] to improve it. We will then use this updated CFD-AB model to predict fish passage for invasive carp (silver carp, common carp) and two native fishes (channel catfish, lake sturgeon) at two model lock and dams (LD2, LD8). If the updated model predicts better than the old one, we will then determine new optimum spillway gate positions to stop carp for these sites and will share these new data with the US Army Corps of Engineers (USACE) and the MN DNR.

It is crucial to protect the freshwater ecosystems of Minnesota by stopping the invasion of bigheaded carp from Asia and promoting native fish passage through Mississippi River locks and dams. We have the opportunity to do this by altering operating procedures for spillway gate openings at existing lock and dam structures. The CFD-AB has already been developed to do this and is being implemented at LD8 but new field data on fish movement suggest that there are some divergences from the model. These finding contrast with the CFD-AB model and suggest that improvement of this computational model must be developed. This project will do that. Application of our updated proposed model to LD8 could prevent invasive species such as silver and bighead carp from colonizing Minnesota.

Summary Budget Information for Subproject 26:	**Subproject Budget:**	**$90,827**
	Amount Spent:	**$88,296**
	Balance:	**$2,531**

Outcome	**Completion Date**
Activity 1	
1. Developed and validated updated version of CFD-AB model based on LD2 experimental data.	November 2018
2. Provide numerical simulations of invasive and native fish passage through LD2 based on the updated version of CFD-AB model.	December 2018
Activity 2	
1. Provide numerical simulations of invasive and native fish passage through LD8.	April 2019
2. Prepare 1 papers for submission to an engineering/biological journal.	May 2019
3. Organize meeting with all interested agencies: MN Department of Natural Resources, US Fish and Wildlife Service, US Army Corps of Engineers to report our progress and take into account any critical remarks.	May 2019
4. Give recommendations to USACE to improve gate regulation at LD8 to block invasive fish passage and to help native fish.	May 2019
5. Final Report to MN Department of Natural Resources	June 2019

Subproject Status as of *January 31, 2019*:
The following progress has been made so far. For Activity 1, the code development and validation of Computational Fluid Dynamics – Agent Based (CFD-AB) model has been done:

(a) The current CFD-AB code used the nodes of the fluid grid to locate fish position. We have changed the algorithm so that the new approach would allow the fish to be at any spatial location (vs only at fluid grid nodes). The accuracy of fish swimming calculation in the modified version of the CFD-AB model has increased, which was demonstrated on a test problem of fish swimming in a channel.

(b) Numerous simulations with common carps, which were trying to pass through LD2, have been performed. In contrast with our previous simulations (Gilmanov et al., 2017, 2018), a new approach with actual initial fish distribution as described by the experimental data from Lock and Dam 2 (Finger J., Riesgraf A, and Sorensen P., 2019, unpublished) has been prepared. These simulations provided excellent comparisons between the percentage of passing common carp of computational results and the experimental field data.

(c) A recent modification of the CFD-AB model which considers fish swimming up and downstream the Mississippi River has been finished. Presently, work on debugging of the code is performed. In order to validate the modification of the CFD-AB model, we have proposed an idea of "Attractive Zones" (resting, migration, feeding zones, etc.). We get the positions of resting zones from the field data of (Finger J., Riesgraf A, and Sorensen P., 2019, unpublished).

Final Report Summary:
The main purpose of the project was to develop an updated version of the Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model (Zielinski, et al., 2018) using the field/experimental data of fish passage through Lock and Dam #2. This updated CFD-AB model can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams.

The subproject has been fulfilled for all the goals that were declared:

1. The computational code CFD-AB directed to enhance the simulation of swimming fish trying to pass through the navigation dams was updated/developed. The analysis of different fish passage index (FPI) showed that the values of FPI for the modified algorithm for a model channel (Gilmanov, et al., 2019, Water, under review) were greater than the FPI of the original algorithm at about 16%. At this moment, no essential differences in fish passage index FPI for the original and modified model at LD2 and LD8 have been found. This effect can be explained by the special gate adjustments, which generate a rather high fluid flow prevented fish to pass through the dams. In other words, in case of blocking invasive species, the modified algorithm does not change the final results of FPI at LD2 and LD8. But the modified algorithm could play a positive role to help native fish to pass through the navigation dams in the case of changing gate adjustments leading to decrease flow velocity.
2. The modified algorithms now account for more realistic fish behavior, including placement of "attraction points", such as resting zones characterized by low recirculating fluid flow. These parameters have been informed by the literature and unpublished field data collected on other projects.
3. Based on investigations of (Larson, et al., 2017, Kokotovich et al, 2017) it was reported that the "Invasive Front" is currently positioned in southern Iowa between Pool 14 and Pool 16. Therefore, the strategy of blocking bigheaded carp at Lock and Dams of Minnesota should be reconsidered. It is well documented that the navigational dams have significantly altered the movement, spawning, feeding and other activities of native fish (Wilcox et al. 2004). Hence, managers should consider alternative strategies whereby navigation dams are adjusted to *help* native fish pass, instead of *blocking* invasive fish. This strategy could help with ecosystem restoration efforts and potentially improve natural resistance to invasion by bigheaded carps. To evaluate this strategy, simulations of walleye passing through LD2 have been executed. It has been shown that by changing gate adjustments, FPI=4% is for the original algorithm and FPI=12% for the modified algorithm. We have to note, that for current gate adjustments from USACE the FPI=0% for original and modified CFD-AB models. By utilizing active monitoring data of bigheaded carp managers could *instantly* change gate adjustments at LD2-LD8 by using our CFD-AB approach if the invasion front threatens Minnesota.

V. DISSEMINATION:

Description: Findings will be disseminated by annual public workshops organized by the Center, the Center's web site, collaborative meetings with our advisory boards, peer-reviewed publications and student theses.

Status as of *February 28, 2015*
Updates and research findings continue to be published in a (roughly) bi-monthly e-newsletter and through the MAISRC website, Facebook, and Twitter.

MAISRC organized and hosted the "2014 Minnesota Aquatic Invasive Species Research and Management Showcase" on November 19, 2014. This public workshop was attended by over 220 people from around the state and included 13 talks and demonstrations given by 23 MAISRC-affiliated researchers, an Extension educator and DNR scientist. Participants saw demonstrations of methods used to advance the science of AIS detection and control, gained some basic skills for working on AIS issues in their communities, and learned about some of the current research on invasive carps, zebra mussels, aquatic invasive plants, and harmful fish diseases.

An anonymous participant survey showed 98% of respondents found the information presented at the Showcase relevant or extremely relevant to their work on AIS; 92% said they learned new skills and information that will help their efforts to prevent and control AIS; and 90% reported they plan to take at least 3 actions as a result of something they learned at the Showcase. A press release was disseminated about the Showcase event.

The Center initiated its first systematic research needs assessment to determine state priorities for the next "wave" of research projects and disseminated information about the process and ways to provide input. The process included consideration of 33 different species of fish, plants, invertebrates, and harmful microbes and involved input from UMN scientists, agency biologists, statewide AIS managers, and the public. In addition to emails, the newsletter, and Facebook and website postings, a press release was disseminated to solicit input from the public. The process in still underway; results will be likely be shared with the public later in 2015.

Three candidates were interviewed for the Extension Specialist position during the month of March with each candidate providing research seminar and outreach seminar. The DNR, the public, and professional stakeholders were invited to attend these seminars in person or by Webex, to provide evaluations, and to meet one-on-one with the candidates as well. These opportunities were advertised by email, Facebook and on the MAISRC websites.

Status as of *September 24, 2015*
MAISRC organized and hosted the "2015 Minnesota Aquatic Invasive Species Research and Management Showcase" on September 16, 2015. This public workshop was attended by 175 people from around the state and included 16 talks and demonstrations given by MAISRC-affiliated researchers, an Extension educator and two DNR staff. Participants received updates on current research and saw demonstrations of methods used to advance the science of AIS detection and control, including through on-campus talks, lunch with researchers, and field trips to nearby lakes and research sites.

Updates and research findings continue to be published in a (roughly) bi-monthly e-newsletter and through the MAISRC website, Facebook, and Twitter.

Status as of *February 29, 2016*
MAISRC has identified the date for its 2016 Showcase on the St. Paul campus (September 22) and continues to broadcast updates on MAISRC progress and findings via talks, social media, and newsletters, and now also via a revamped website launched earlier this month. The website provides expanded information on research projects under way, the species on which we conduct research, the researchers involved in our work, and it provides links to published work by MAISRC scientists. The site is also designed with our three largest audiences in mind: AIS managers, researchers, and citizens.

Status as of *August 31, 2016*
Efforts to educate, inform, and share findings are continuing via the website, Facebook, Twitter, media efforts, and our annual Showcase event. Research Center faculty and staff also continue to give talks and meet with stakeholders.

Planning began for the 2016 Showcase and involved recruiting a committee, finding a date, securing facilities, sending out a save- the- date, and beginning to rough out a program. The event will be held on the St. Paul Campus on September 12.

After a significant effort designing, editing, and creating new content, the newly revamped website is live. It is continually updated with descriptions of research projects underway, progress and results, MAISRC events, researcher information, and opportunities for input by our stakeholders. Our average monthly views have grown from approximately 400 to over 1,000.

Newsletters continue to be written every other month, which includes seeking input from researchers, drafting stories, getting them reviewed by scientists, taking photographs, and formatting materials for dissemination. We now have over 1,700 subscribers with an even mix of agency personnel, non-governmental and lakeshore association members, private industry, and higher ed. We have a consistently high open rate (30-40% versus industry average of 18%). We also leverage Facebook and Twitter to get our messages out and have consistently high reach and engagement there as well.

MAISRC has also planned a special session at the upcoming Upper Midwest Invasive Species Conference taking place in October.

Status as of *February 28, 2017*
Efforts to broadcast research progress continue through talks, attendance at statewide AIS Advisory Committee meetings, papers, newsletters, website and other social media formats. We continue to reach larger audiences and receive high engagement from our followers.

We held our 2016 Showcase in September, with attracted 171 non-MAISRC attendees and provided 16 presentations spread out among 21 speakers, including 5 grad students and 4 postdocs, and faculty and non-Twin Cities campus- based researchers. Copies of most of these presentations can be found on our website. Tours of the lab were also provided. 90% of attendees rated the event as excellent or very good.

MAISRC core staff also attended conferences to stay abreast of current work and research needs around the state and also gave a presentation on MAISRC's RNA process, which has gained attention as an efficient, inclusive solutions-oriented model. We have also submitted an abstract to present at the 20th International Conference on Aquatic Invasive Species in October, 2017.

Status as of *August 31, 2017*
Efforts to broadcast research progress continue through talks, attendance at statewide AIS Advisory Committee meetings, papers, newsletters, website and other social media formats. We continue to reach larger audiences and receive high engagement from our followers.

Since our last update, we have had 56 news stories published about MAISRC, the work we are conducting, and the results our work is generative. We have also had 13% growth in followers on Facebook, 15% growth in followers on Twitter, 20% growth in newsletter subscribers, and have had 10,170 unique visitors to our website. We consider these to be positive indicators of more people being engaged in the issue of AIS, becoming informed on the science, and at some level supporting the investment in research to help solve our state's AIS problems.

We are currently planning for our 2017 Management and Research Showcase, scheduled for September 13. Approximately half of the people registered this far have never attended a Showcase before—another indication of our expanding reach. 18 talks are scheduled by 31 MAISRC researchers plus lab tours with demonstrations, including by Whooshh Innovations, a collaborator in an ENRTF- funded carp project. New this year will be a poster session during the end of day reception. We were accepted to present at the 20th International Conference on Aquatic Invasive Species in October 2017.

Status as of *February 28, 2018*
MAISRC is continuing its efforts to educate, inform, and share our research findings. Key outreach and communications activities include:

MAISRC currently has a social media following of 1,500 and an e-newsletter list with 2,700 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories on our research projects.

Since the last workplan update, MAISRC has been featured in the news approximately thirty times, with stories on Asian carp, zebra mussels, and pathogens, as well as a podcast from Montana Public Radio that focused specifically on zebra mussels and featured many MAISRC researchers and stakeholders. We recently worked with Minnesota Public Radio for a story about our invasive plants research which will be appearing soon.

To mark MAISRC's fifth anniversary in late December 2017, staff put together a comprehensive five-year report that includes key findings and accomplishments, big wins, and plans for the future for each of MAISRC's twelve species of research. It also includes an overview of our outreach programs and our strategic plan process. It was mailed to numerous MAISRC stakeholders and pushed heavily through e-newsletter and social media. It has now been viewed online over 21,000 times.

Since the last workplan update, over 10,500 unique visitors have visited the website a total of 15,520 times; viewing 30,940 pages. These statistics are routinely increasing and we view this as a sign that MAISRC is growing in name recognition and being seen as an important resource.

We held the 2017 AIS Research and Management Showcase on September 13 and hosted just under 200 attendees, not including anyone affiliated with MAISRC. Three legislators attended. Planning is now beginning for the 2018 Showcase, to be held on Sept. 12.

Many MAISRC researchers are giving talks around the state, including the Aquatic Invaders Summit, the New Brighton Sportsmen's Club, the State of Water Conference, the Itasca Area Business Water Summit, the Pelican Lakes Association of Crow Wing County annual meeting, the Cass County watercraft inspection conference, and more.

Status as of *August 2, 2018*
MAISRC currently has a social media following of over 1,700 and an e-newsletter list with just under 3,000 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

Since the last workplan update, MAISRC has been featured in 48 stories in the press. Stories have included our AIS Detectors program, invasive carp research, invasive plants research, a full feature on Minnesota Bound, and an op-ed from members of University administration.

Staff continued to push out the five-year report that was created in early 2018. We followed it up with an interactive online map that shows all points of MAISRC research and outreach activities. It can be seen online at www.maisrc.umn.edu/maisrc-map.

Since the last workplan update, over 18,000 unique visitors have visited the website a total of 24,000 times; viewing 41,500 pages. This is a significant increase over the last reporting period. We feel that our consistent growth in these communications areas is a sign that MAISRC is growing in name recognition and being seen as an important resource around the state, nation and world.

Planning is underway for the 2018 AIS Research and Management Showcase, which is scheduled for September 12, 2018. Registration is moving quickly and we expect to have 200+ attendees.

This spring and summer, many MAISRC researchers gave talks around the state, including the Pelican Lakes Association of Crow Wing County, the AIS Roundtable (organized by the Whitefish Area Property Owners Association and attended by members of 17 lake associations), an all-day event with MAISRC speakers in Detroit

Lakes, and more. Several researchers are slotted to speak at the Upper Midwest Invasive Species Conference in October.

Lastly, MAISRC is partnering with a videographer this summer to create a series of videos about our research. The videos will cover:

- The AIS Detectors program
- Starry stonewort research
- Spiny waterflea research
- The impact of zebra mussels and spiny waterflea on walleye
- Using pathogens to control invasive carp
- Novel methods for controlling common carp

These videos will help us keep legislators, managers, and interested members of the public informed by explaining our research in a new and different way.

Status as of *February 28, 2019*
MAISRC currently has a social media following of just under 2,000 and an e-newsletter list with just under 3,250 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

Since the last workplan update, MAISRC has been featured in 35 stories in the press. Stories have included research updates on starry stonewort, zebra mussels, common carp, spiny waterflea, as well as the Showcase and the AIS Detectors program.

Staff created a 2018 Annual Report in late 2018. An electronic version was sent to all newsletter subscribers and shared on social media, and a print version was sent to donors and other interested stakeholders.

In late summer, MAISRC released its first-ever white paper, *Treatment options for the eradication of limited-scale zebra mussel infestations at various water temperatures*. This white paper was shared at the Showcase and distributed through our newsletter, website, and social media.

Since the last workplan update, 22,500 unique visitors have visited the website a total of 31,000 times; viewing 56,000 pages. This is an increase of 25%, 23%, and 35%, respectively, over the last reporting period. This consistent growth shows that MAISRC is growing in name recognition and being seen as an important resource for different stakeholders around the state.

The 2018 AIS Research and Management Showcase had over 200 attendees (who were not affiliated with MAISRC). Roughly half of these attendees had never attended the event before.

In summer 2018, MAISRC created a series of videos about our research which were very well-received. The videos covered: the AIS Detectors program, starry stonewort research, spiny waterflea research, the impacts of AIS on walleye, using pathogens to control invasive carp, and novel methods for controlling common carp. In total, the videos were viewed 36,000 times.

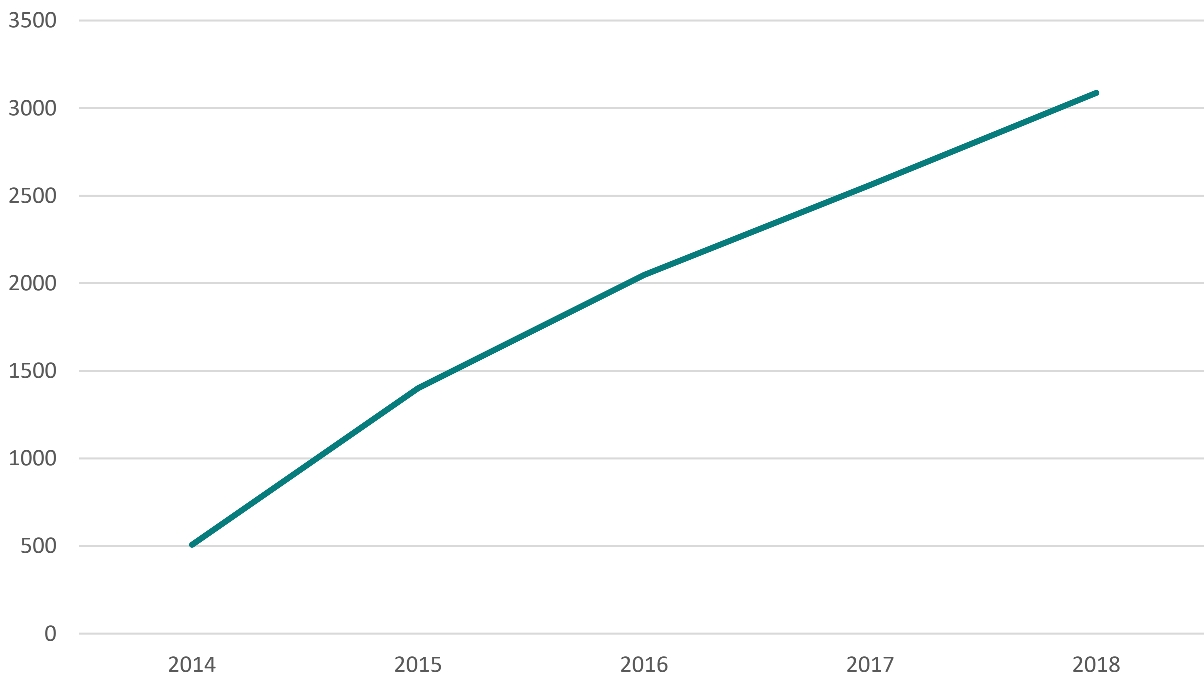
MAISRC staff will coordinate in-person talks from the MAISRC Director and other MAISRC researchers around the state this spring and summer, and will share these event announcements through the newsletter and social media.

Final Report Summary:

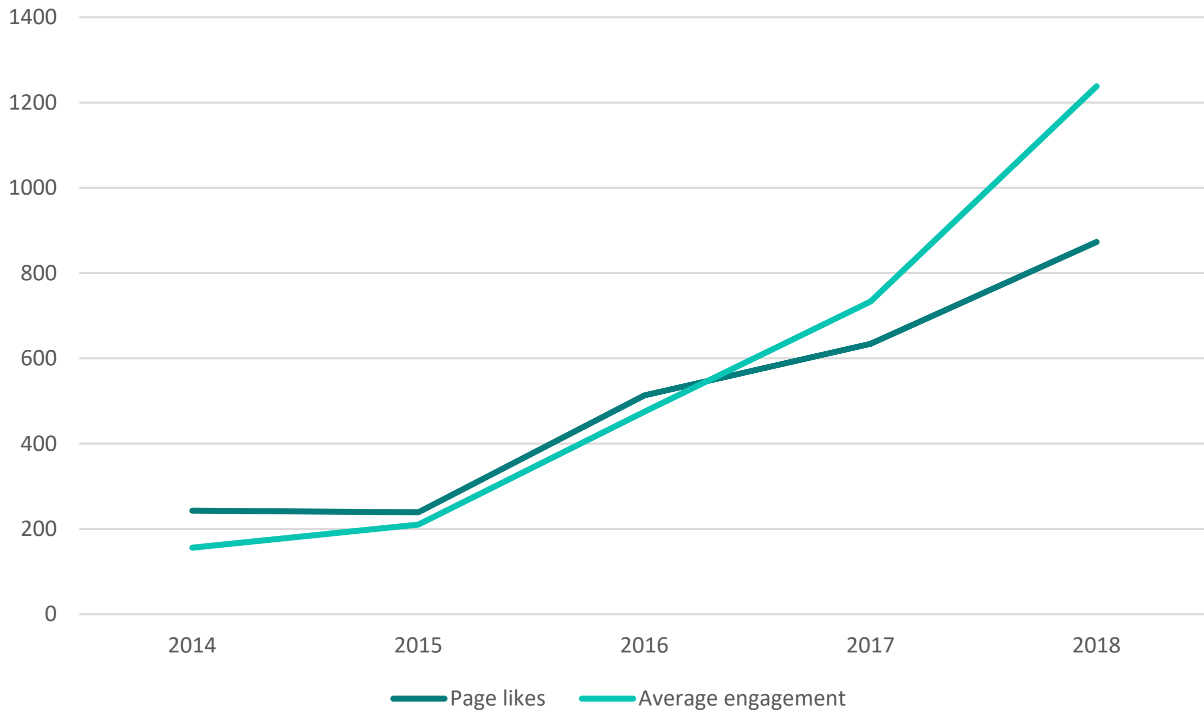
Social media and e-newsletter
MAISRC currently has a social media following of just under 2,300 and an e-newsletter list with just under 3,500 recipients. Social media posts about research findings, events, AIS Detector workshops, and general invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

MAISRC's Facebook, Twitter, and e-newsletter accounts were all created after the start of this workplan in July 2013.

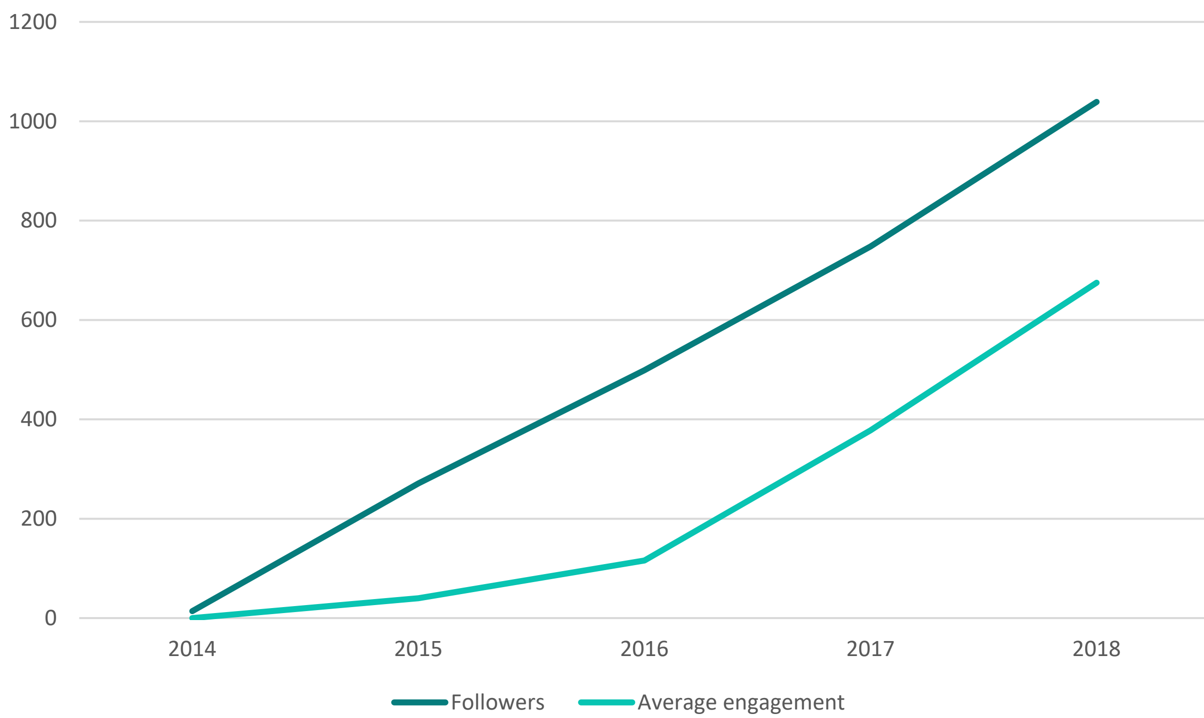
Newsletter list growth, 2014 – 2018:



Growth in followers and average engagement on Facebook:



Growth in followers and average engagement on Twitter:

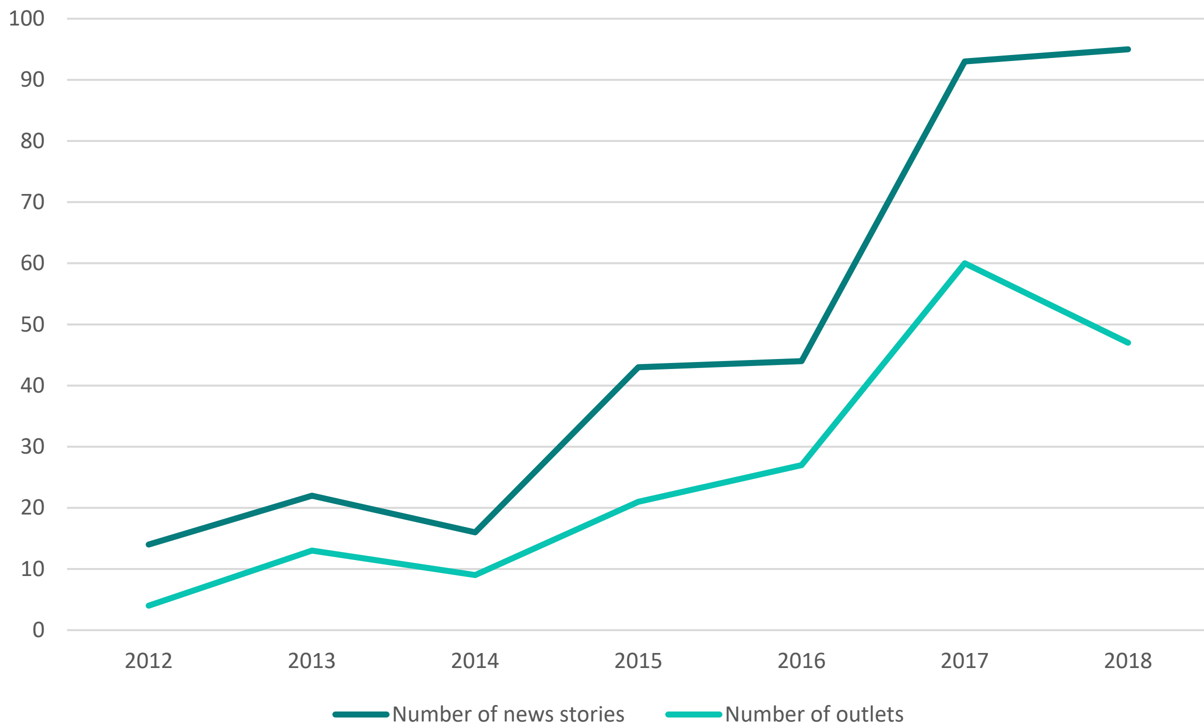


Media relations

Since the last workplan update, MAISRC has been featured in 62 stories in the press. Stories have included research updates on zebra mussels, the annual Starry Trek event, invasive carp, starry stonewort, and more.

Over the course of the last six years, MAISRC has been in approximately 350 news stories in roughly 117 different outlets. The most common outlets have been the *Star Tribune*¸ Minnesota Public Radio, and KSTP-TV. Other notable outlets include *The New York Times, The Washington Post,* and Minnesota Bound.

News stories featuring MAISRC research:

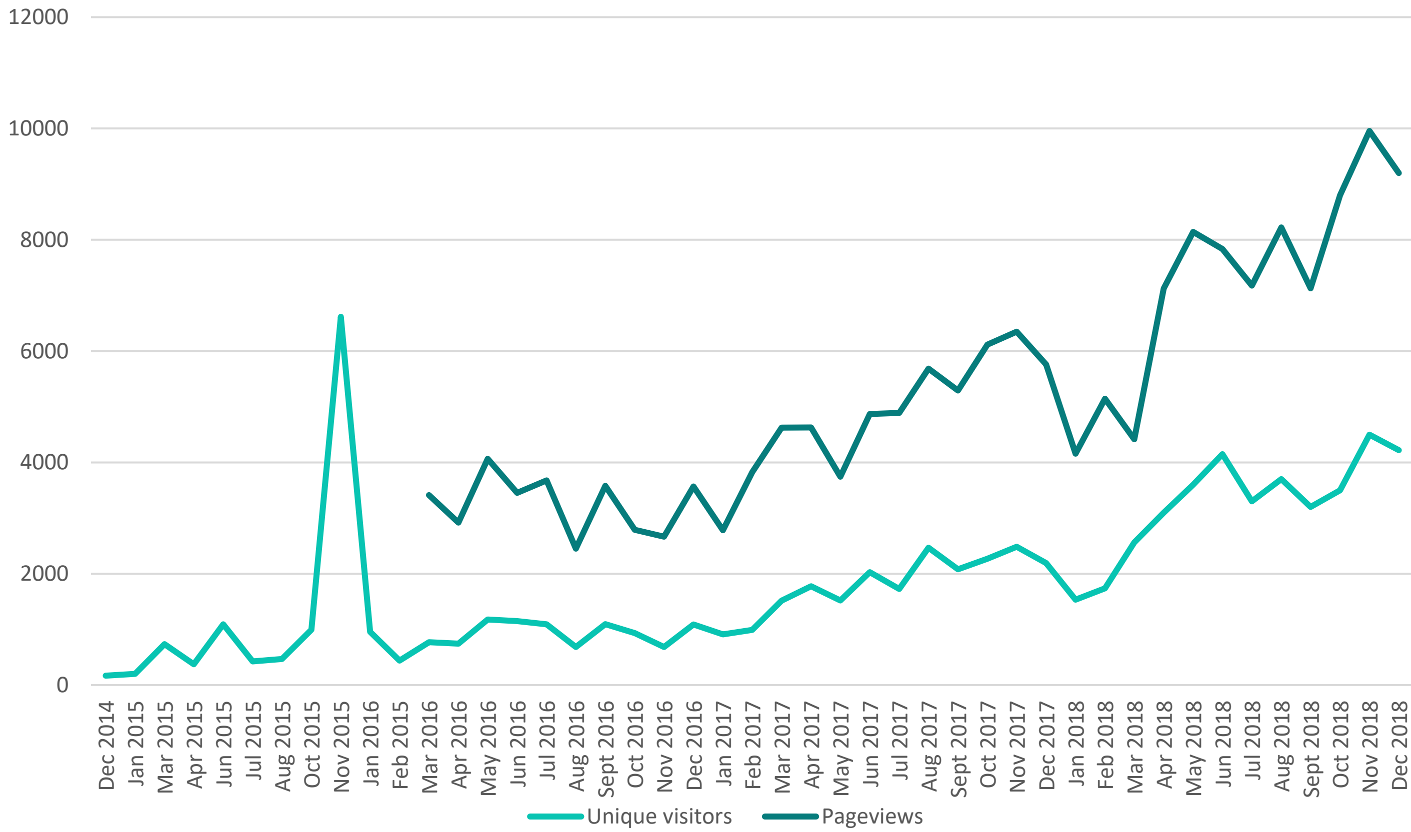


MAISRC website

Since the last workplan update, 26,584 unique visitors visited the MAISRC website a total of 35,660 times; viewing 62,645 pages. This is an increase of 18%, 15%, and 12%, respectively. This consistent growth shows that MAISRC is growing in name recognition and being seen as an important resource for different stakeholders around the state.

Average number of unique users and pageviews per month:

Pageview information unknown prior to launch of new MAISRC website in February 2016.



MAISRC Showcase

The 2019 AIS Research and Management Showcase will be held on Sept. 18, and registration is already at its highest of any year. Over 200 attendees (who are not affiliated with MAISRC) will attend; roughly half of whom have never attended the event before. In total, roughly 700 different people have attended the AIS Research and Management Showcase since 2014.

Videos

In summer 2019, we created three videos about our research which will be released soon. The videos covered the Whooshh fish transport system (project led by Przemek Bajer), evaluating public values of AIS management (project led by Amit Pradhananga) and the genetic biocontrol of invasive fish (project led by Mike Smanski). A MAISRC project on the control of zebra mussels (project led by Jim Luoma) was also chosen by University Relations to be highlighted in upcoming *Driven* campaign. A video will be released and widely promoted in October 2019.

Statewide talks

MAISRC staff also coordinated in-person talks rom the MAISRC Director and other MAISRC researchers around the state this spring and summer, including the Stillwater Rotary Club, the Bay Lake Improvement Association, the Clamshell-Bertha Lake Association, the Pelican Lakes Association of Crow Wing County, and the Whitefish Area Property Owners Association.

Summary of notable MAISRC communications and outreach activities

Summer 2013 – summer 2019

Events and trainings

- Have held six AIS Research and Management Showcases with roughly 700 different attendees
- Held a lab ribbon-cutting ceremony in March 2016
- Hosted new University President Gabel for a lab tour and research demonstration in September 2019

- Have held three Starry Trek events, through which volunteers have found new infestations of starry stonewort, Eurasian watermilfoil, and Chinese mystery snails
- Formally launched the AIS Detectors program in March 2017; have now certified 299 Detectors around the state

Videos

- Created nine videos, highlighting MAISRC subproject research:
  - AIS Detectors
  - Starry stonewort research
  - Spiny waterflea research
  - Impacts of AIS on walleye
  - Using pathogens to control invasive carp
  - Novel methods for controlling common carp
  - Valuing AIS management
  - Genetic control of invasive carp
  - Using the Whooshh fish transport system (not released yet)
- Featured in U of M Driven campaign in summer 2018
- Featured in U of M Driven campaign in fall 2019

Reports and other materials

- Treatment options for the eradication of limited-scale zebra mussel infestations at various water temperatures
- An assessment to support strategic, coordinated response to invasive *Phragmites australis* in Minnesota
- 2018 Research Report
- Five years of AIS Research | 2012 – 2017
- Interactive map: MAISRC work around the state
- Aquatic Invasive Species ID Guide

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget: See budget attachments.

Explanation of Use of Classified Staff: *n.a.*

Explanation of Capital Expenditures Greater Than $5,000:

SUBPROJECT 1: MAISRC portion of a new electrofishing boat purchased in partnership with the Fisheries, Wildlife, and Conservation Biology (FWCB) Department at the University of Minnesota ($65,000). The new electrofishing boat will be available for use by any MAISRC funded or MAISRC partnership project. MAISRC use of the boat will be in proportion to the percent investment by MAISRC/LCCMR in its purchase. MAISRC staff will also provide oversight of the management of the boat, to ensure that it is being used proportionally for the purpose of advancing AIS research in Minnesota. This oversight will continue throughout the useful life of the boat. If for some reason the use of the boat changes, MAISRC will pay back the Environment and Natural Resources Trust Fund an amount equal to the proportional residual value (approved by the director of the LCCMR), or the proportional cash value received if it is not sold.

For capital expenditures made by MAISRC subprojects, see the subproject final reports.

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:

Subproject 1: 3.3 FTE
Subprojects 1-26: 74.45 FTE

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:

Subproject 1: 0 FTE
Subprojects 1-26: 2.58 FTE

B. Other Funds (related projects that can synergize this one):

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
National Science Foundation	$234,000	$232,520	Radio-tags for Judas fish
USGS	$129,646	$124,343	Preliminary work with Asian carp
Riley Purgatory Bluff Watershed District	$2,728,771	$2,728,771	Preliminary work on Judas carp
State			
ENRTF –M.L. 2012, chp 264, art4. Sec 3- Aquatic Invasive species (AIS) Cooperative research center	$2,000,000	$2,000,000	Startup funds for Center (eDNA work, facility repair, Judas carp study, administrative costs)
Clean Water Legacy Funds	$1,800,000	$1,794,028	Startup for Center (Zebra mussel position, facility repair, administrative costs)
TOTAL OTHER FUNDS:	$6,892,417	$6,879,662	

VII. PROJECT STRATEGY:

A. Project Partners:
DNR (a full partner and co-lead on CAB with whom the University will have a memoradum of understanding), USGS (LaCrosse WI; and Columbia, MI; former with a memorandum of understanding), Riley Purgatory Bluff Watershed District (Chanhassen, MN), Ramsey Washington Metro Watershed District (Maplewood, MN), Minnehaha Watershed District (Minnetonka, MN)

B. Project Impact and Long-term Strategy: This project will establish a new national center of excellence for AIS in Minnesota that will develop and disseminate new infornation and useful techniques for their control to public agencies and the private sector.

C. Spending History:

Funding Source	**M.L. 2005 or FY 2006-07**	**M.L. 2007 or FY 2008**	**M.L. 2008 or FY 2009**	**M.L. 2009 or FY 2010**	**M.L. 2010 or FY 2011**
ENRTF – M.L. 2008 Chp 367, Sec 2, Subd. 04b - Accelerating plans for integrated control of common carp		550,000			
ENRTF –M.,L. 2005, First Special Session, Chp.1, Art 2, Sec 11, Subd. 05g – Integrated and pheromonal control of the common carp	550,000				

VIII. ACQUISITION/RESTORATION LIST: n.a.

IX. MAP(S): Entire state of Minnesota

X. RESEARCH ADDENDUM: *not applicable (peer review of all activities will be completed by the Center)*

XI. REPORTING REQUIREMENTS: Periodic work plan status update reports will be submitted not later than February 28 and August 31 each from February 10, 2014 through February 28, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019 as requested by the LCCMR.

Environment and Natural Resources Trust Fund

M.L. 2013 Sub-Project Budget of M.L. 2013-06a: Aquatic Invasive Species Research Center

Project Title: Aquatic Invasive Species Research Center Subproject 1: Coordinating, Synergizing, and Promoting Expertise: establishing an Administrative Structure

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

Project Manager: Nicholas Phelps

Organization: University of Minnesota – Minnesota Aquatic Invasive Species Research Center

Subproject Budget: $1,805,859

Subproject Phase 1 Length and Completion Date: *3 years, June 30, 2016*

Project Length and Completion Date: 6 Years, June 30, 2019

Date of Report: November 11, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	***Activity 1: Coordinating, Synergizing, and Promoting Expertise: establishing an Administrative Structure (Phase 1)***			***Activity 2: Reserves***					
BUDGET ITEM	**Activity 1 Budget**	**Amount Spent**	**Activity 1 Balance**	**Activity 2 Budget**	**Amount Spent**	**Activity 2 Balance**	**TOTAL BUDGET**	**TOTAL SPENT**	**TOTAL BALANCE**
Personnel (Wages and Benefits) - Total	**$1,199,487**	**$1,194,619**	**$4,868**	**$0**	**$0**	**$0**	**$1,199,487**	**$1,194,619**	**$4,868**
Associate Director Professional & Admin: $83,000 Salary (66.4%Salary, 33.6% benefits, 1 FTE)									
Scientific Director Professional & Admin: $79,000 (66.4%Salary, 33.6% benefits, 0.5 FTE)									
Name- Post Doctoral Fellow: $Salary; (79.25% Salary, 20.75% benefits) 1.0 FTE									
Undergraduate Student: $6000 (93% salary, 7% benefits) 0.25 FTE									
Admin and Communications Assistant: $28,000 (63.2% salary, 36.8% benefits) 0.75 FTE									
Field Technician (Civil Service): $42,000; (63.2% salary, 36.8% benefits) 1.0 FTE									
Lab Manager (Civil Service): $49,000; (63.2% salary, 36.8% benefits) 1.0 FTE									
Professional/Technical Services and Contracts - Total	**$35,675**	**$32,263**	**$3,412**	**$0**	**$0**	**$0**	**$35,675**	**$32,263**	**$3,412**
Services- office & gen oper. (printing/duplication, mailing, printer repairs, audio visual associated with seminars & conferences, conf. calls, surveys, insurance for pontoon, etc.)	$16,221	$14,241	$1,980			$0	$16,221	$14,241	$1,980
Services- lab & medical (data storage, sequencing, biochemistry, microscopy, well permits, discharge licences and fees, preventative maintenance and maintenance of lab facilities)	$49	$49	$0			$0	$49	$49	$0
Professional Services & contracts- (fees or honoraria for guest lecturer and speakers, etc)	$165	$165	$0			$0	$165	$165	$0
Repairs- lab & field (vehicle, EFL holding facility, or other shared equipment)	$19,240	$17,808	$1,432			$0	$19,240	$17,808	$1,432
Rentals- space and facilities for conferences and events (e.g. annual Showcase)	$0	$0	$0			$0	$0	$0	$0
Equipment/Tools/Supplies - Total	**$48,317**	**$39,497**	**$8,820**	**$0**	**$0**	**$0**	**$48,317**	**$39,497**	**$8,820**
Supplies- office & gen oper. (paper, toner, folders, brochures, provisions for meetings, displays)	$22,108	$19,025	$3,083			$0	$22,108	$19,025	$3,083
Supplies- lab & field (piping, glue, hardware and plumbing for facilties, gas, hoses for washdown facility)	$5,005	$4,853	$152			$0	$5,005	$4,853	$152
Equipment- non capital lab & field (primarily equipment for central holding facilities if needed for repair or replacement, pumps for washing down boats, storage containers, etc)	$21,204	$15,619	$5,585			$0	$21,204	$15,619	$5,585
Capital Expenditures Over $5,000 - Total	**$65,000**	**$65,000**	**$0**	**$0**	**$0**	**$0**	**$65,000**	**$65,000**	**$0**
Cap expenditures over $5,000: MAISRC portion of new electrofishing boat, purchased in partnership with UMN Dept of Fisheries, Wildlife, and Conservation Biology	$65,000	$65,000	$0			$0	$65,000	$65,000	$0
Travel - Total	**$23,669**	**$19,496**	**$4,173**	**$0**	**$0**	**$0**	**$23,669**	**$19,496**	**$4,173**
Travel - MN (mileage, meetings, conferences, guest speakers, out of town experts for research needs assessment, travel etc. Field tech mileage will be paid from specific subprojects)	$15,669	$11,890	$3,779			$0	$15,669	$11,890	$3,779
Travel - Domestic (mileage, conferences, mtgs for Center coordination)	$8,000	$7,605	$395			$0	$8,000	$7,605	$395
Other - Total	**$582**	**$550**	**$32**	**$0**	**$0**	**$0**	**$582**	**$550**	**$32**
Telecommunications (voicemail service for MAISRC researchers and staff)	$582	$550	$32			$0	$582	$550	$32
Budget Reserve Pending Progress and Peer Review - Total	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**
Funds for future phases to be allocated to specific budget categories at a future date pending sub-project progress	$0	$0	$0	$0	$0	$0	$0	$0	$0
COLUMN TOTAL	**$1,372,730**	**$1,351,424**	**$21,306**	**$0**	**$0**	**$0**	**$1,372,730**	**$1,351,424**	**$21,306**

Environment and Natural Resources Trust Fund

M.L. 2013 Project Budget - Overall Budget of Aquatic Invasive Species Research Center

Project Title: Aquatic Invasive Species Research Center
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
Project Manager: Nicholas Phelps
Organization: University of Minnesota
M.L. 2014 ENRTF Appropriation: $8,700,000
Project Length and Completion Date: 6 Years, June 30, 2019
Date of Report: November 11, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 1 - Coordinating, synergizing and promoting expertise: Establishing an administrative structure			SUBPROJECT 2 - Metagenomic approaches to develop biological control strategies for aquatic invasive species			SUBPROJECT 2.2 - Metagenomic Approaches to Develop Biological Control Strategies for Aquatic Invasive Species, Phase II: Development of Potential Microbiological Control Agents for AIS		
BUDGET ITEM	Subproject 1 Budget	Amount Spent	Subproject 1 Balance	Subproject 2 Budget	Amount Spent	Subproject 2 Balance	Subproject 2.2 Budget	Amount Spent	Subproject 2.2 Balance
Personnel (Wages and Benefits) - Overall Total	$1,199,487	$1,194,619	$4,868	$226,717	$226,717	$0	$198,070	$198,070	$0
Professional/Technical Services and Contracts - Overall Total	$35,675	$32,263	$3,412	$45,251	$45,251	$0	$54,500	$42,333	$12,168
Equipment/Tools/Supplies - Overall Total	$48,317	$39,497	$8,820	$23,000	$23,000	$0	$37,500	$37,000	$500
Capital Expenditures Over $5,000 - Overall Total	$65,000	$65,000	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$23,669	$19,496	$4,173	$4,396	$4,396	$0	$13,147	$9,208	$3,939
Other - Overall Total	$582	$550	$32	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total	$0	$0	$0						
COLUMN TOTAL	**$1,372,730**	**$1,351,424**	**$21,306**	**$299,364**	**$299,364**	**$0**	**$303,217**	**$286,610**	**$16,607**
		Total spent adjusted to account for rounding discrepancy	*Balance adjusted to account for rounding discrepancy*					*Total spent adjusted to account for rounding discrepancy*	

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 3 - Reducing and controlling AIS: Attracting carpo so their presence can be accurately assessed; Determining if and how a sound-bubble system can be combined with light in the laboratory to deter carp while examining potential impacts to native			SUBPROJECT 4 - Common carp management using biocontrol and toxins			SUBPROJECT 4.2 - Common carp management using biocontrol and toxins, Phase II		
BUDGET ITEM	Subproject 3 Budget	Amount Spent	Subproject 3 Balance	Subproject 4 Budget	Amount Spent	Subproject 4 Balance	Subproject 4.2 Budget	Amount Spent	Subproject 4.2 Balance
Personnel (Wages and Benefits) - Overall Total	$518,950	$518,950	$0	$286,024	$286,024	$0	$257,740	$221,115	$36,625
Professional/Technical Services and Contracts - Overall Total	$57,289	$46,701	$10,588	$59,261	$59,261	$0	$73,460	$62,074	$11,386
Equipment/Tools/Supplies - Overall Total	$90,102	$82,709	$7,393	$13,079	$13,079	$0	$50,000	$49,182	$818
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$5,925	$5,925	$0	$0	$0	$0
Travel - Overall Total	$16,628	$15,359	$1,270	$19,943	$19,943	$0	$24,800	$16,542	$8,258
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$682,969**	**$663,719**	**$19,251**	**$384,232**	**$384,232**	**$0**	**$406,000**	**$348,913**	**$57,087**
			Balance adjusted to account for rounding discrepancy						

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 5 - Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants			SUBPROJECT 6 - Determining Heterosporosis Threats to Inform Prevention, Management, and Control			SUBPROJECT 7 - Developing eradication tools for invasive carp species. Phase I: Understanding the virome of carp species in the Upper Midwest.		
BUDGET ITEM	Subproject 5 Budget	Amount Spent	Subproject 5 Balance	Subproject 6 Budget	Amount Spent	Subproject 6 Balance	Subproject 7 Budget	Amount Spent	Subproject 7 Balance
Personnel (Wages and Benefits) - Overall Total	$160,771	$160,771	$0	$98,836	$98,836	$0	$70,855	$70,855	$0
Professional/Technical Services and Contracts - Overall Total	$4,769	$4,769	$0	$0	$0	$0	$60,403	$60,403	$0
Equipment/Tools/Supplies - Overall Total	$18,265	$18,265	$0	$5,645	$5,645	$0	$47,123	$47,123	$0
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$23,776	$23,776	$0
Travel - Overall Total	$10,610	$10,610	$0	$7,408	$7,408	$0	$4,597	$4,597	$0
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$194,415**	**$194,415**	**$0**	**$111,889**	**$111,889**	**$0**	**$206,754**	**$206,754**	**$0**

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 7.2 - Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents			SUBPROJECT 8 - Risk assessment, control, and restoration research on aquatic invasive plant species			SUBPROJECT 9 - Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol.		
BUDGET ITEM	Subproject 7.2 Budget	Amount Spent	Subproject 7.2 Balance	Subproject 8 Budget	Amount Spent	Subproject 8 Balance	Subproject 9 Budget	Amount Spent	Subproject 9 Balance
Personnel (Wages and Benefits) - Overall Total	$238,650	$227,552	$11,098	$761,604	$761,604	$0	$297,752	$297,752	$0
Professional/Technical Services and Contracts - Overall Total	$114,413	$110,607	$3,806	$14,946	$13,358	$1,587	$73,334	$73,334	$0
Equipment/Tools/Supplies - Overall Total	$70,429	$67,300	$3,129	$25,307	$25,223	$84	$5,609	$5,609	$0
Capital Expenditures Over $5,000 - Overall Total	$7,718	$7,718	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$14,000	$9,490	$4,510	$20,143	$20,066	$77	$3,623	$3,623	$0
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$445,210**	**$422,667**	**$22,543**	**$822,000**	**$820,251**	**$1,749**	**$380,318**	**$380,318**	**$0**
						Balance adjusted to account for rounding discrepancy			

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 10 - Citizen Science and Professional Training Programs to Support AIS Response			SUBPROJECT 11 - Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods, Phase 1: Problem Formulation			SUBPROJECT 11.2 - Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods, Phase 2: Risk Analysis		
BUDGET ITEM	Subproject 10 Budget	Amount Spent	Subproject 10 Balance	Subproject 11 Budget	Amount Spent	Subproject 11 Balance	Subproject 11.2 Budget	Amount Spent	Subproject 11.2 Balance
Personnel (Wages and Benefits) - Overall Total	$394,038	$393,172	$866	$89,274	$89,274	$0	$104,923	$104,923	$0
Professional/Technical Services and Contracts - Overall Total	$48,491	$47,969	$522	$1,400	$1,400	$0	$7,543	$7,543	$0
Equipment/Tools/Supplies - Overall Total	$61,085	$58,349	$2,736	$1,304	$1,304	$0	$1,757	$1,757	$0
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$21,260	$20,846	$415	$1,365	$1,365	$0	$12,454	$12,454	$0
Other - Overall Total	$515	$515	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$525,389**	**$520,850**	**$4,539**	**$93,343**	**$93,343**	**$0**	**$126,677**	**$126,677**	**$0**
		Total spent adjusted to account for rounding discrepancy							

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 12 - Characterizing spiny water flea impacts using sediment records			SUBPROJECT 13 - Eco-epidemiological Model to Assess Aquatic Invasive Species Management			SUBPROJECT 14 - Cost-effective monitoring of lakes newly infested with zebra mussels		
BUDGET ITEM	Subproject 12 Budget	Amount Spent	Subproject 12 Balance	Subproject 13 Budget	Amount Spent	Subproject 13 Balance	Subproject 14 Budget	Amount Spent	Subproject 14 Balance
Personnel (Wages and Benefits) - Overall Total	$159,732	$159,271	$461	$163,351	$163,351	$0	$206,384	$184,302	$22,082
Professional/Technical Services and Contracts - Overall Total	$45,250	$45,248	$2	$17,871	$17,871	$0	$38,563	$19,697	$18,866
Equipment/Tools/Supplies - Overall Total	$2,338	$2,252	$86	$4,124	$4,124	$0	$6,617	$6,617	$0
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$6,817	$6,817	$0	$0	$0	$0
Travel - Overall Total	$4,946	$4,937	$9	$3,086	$3,086	$0	$14,936	$14,936	$0
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$212,266**	**$211,708**	**$558**	**$195,249**	**$195,249**	**$0**	**$266,500**	**$225,553**	**$40,947**
								Total spent adjusted to account for rounding discrepancy	*Balance adjusted to account for rounding discrepancy*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 15 - Determining Highest Risk Vectors of Spiny WaterFlea Spread			SUBPROJECT 1 Populations: As
BUDGET ITEM	Subproject 15 Budget	Amount Spent	Subproject 15 Balance	Subproject 16 Budget
Personnel (Wages and Benefits) - Overall Total	$83,085	$83,085	$0	$16,275
Professional/Technical Services and Contracts - Overall Total	$0	$0	$0	$44,245
Equipment/Tools/Supplies - Overall Total	$1,577	$1,401	$176	$5,370
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0
Travel - Overall Total	$8,270	$8,270	$0	$22,249
Other - Overall Total	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total				
COLUMN TOTAL	**$92,932**	**$92,756**	**$176**	**$88,139**

Environment and Natural Resources Trust Fund

M.L. 2013 Project Budget - Overall Budget of Aquatic Invasive Species Research Center

Project Title: Aquatic Invasive Species Research Center
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
Project Manager: Nicholas Phelps
Organization: University of Minnesota
M.L. 2014 ENRTF Appropriation: $8,700,000
Project Length and Completion Date: 6 Years, June 30, 2019
Date of Report: November 11, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	6 (DNR)- Sustaining Walleye ssessing impacts of AIS		SUBPROJECT 16 (UMN)- Sustaining Walleye Populations: Assessing impacts of AIS			SUBPROJECT 16 (NNRI)- Sustaining Walleye Populations: Assessing impacts of AIS		
BUDGET ITEM	Amount Spent	Subproject 16 Balance	Subproject 16 Budget	Amount Spent	Subproject 16 Balance	Subproject 16 Budget	Amount Spent	Subproject 16 Balance
Personnel (Wages and Benefits) - Overall Total	$16,275	$0	$28,645	$28,644	$0	$71,309	$71,309	$0
Professional/Technical Services and Contracts - Overall Total	$44,245	$0	$0	$0	$0	$0	$0	$0
Equipment/Tools/Supplies - Overall Total	$5,370	$0	$0	$0	$0	$1,130	$1,130	$0
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$21,295	$954	$800	$739	$62	$8,677	$8,561	$116
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total								
COLUMN TOTAL	**$87,185**	**$954**	**$29,445**	**$29,383**	**$62**	**$81,116**	**$81,000**	**$116**
					Balance adjusted to account for rounding discrepancy			

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 17- Building scientific and management capacity to respond to invasive Phragmites in Minnesota			SUBPROJECT 18- Eurasian and hybrid watermilfoil genotype distribution in Minnesota			SUBPROJECT 19 - Decision-making tool for optimal management of AIS		
BUDGET ITEM	Subproject 17 Budget	Amount Spent	Subproject 17 Balance	Subproject 18 Budget	Amount Spent	Subproject 18 Balance	Subproject 19 Budget	Amount Spent	Subproject 19 Balance
Personnel (Wages and Benefits) - Overall Total	$244,663	$243,864	$799	$105,671	$105,671	$0	$117,465	$38,911	$78,554
Professional/Technical Services and Contracts - Overall Total	$15,120	$15,120	$0	$94,704	$94,395	$309	$47,000	$39,774	$7,226
Equipment/Tools/Supplies - Overall Total	$5,025	$2,786	$2,239	$8,000	$7,926	$74	$3,000	$1,784	$1,216
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$13,010	$8,003	$5,007	$13,000	$12,420	$580	$5,000	$0	$5,000
Other - Overall Total	$5,750	$0	$5,750	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$283,568**	**$269,773**	**$13,795**	**$221,375**	**$220,412**	**$963**	**$172,465**	**$80,469**	**$91,996**

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 20 - A Novel Technology for eDNA Collection and Concentration (Year 1)			SUBPROJECT 21 - Early detection of zebra mussels using multibeam sonar			SUBPROJECT 22 (UMN) - Copper-based control: zebra mussel settlement and non-target impacts (Year 1)		
BUDGET ITEM	Subproject 20 Budget	Amount Spent	Subproject 20 Balance	Subproject 21 Budget	Amount Spent	Subproject 21 Balance	Subproject 22 Budget	Amount Spent	Subproject 22 Balance
Personnel (Wages and Benefits) - Overall Total	$66,599	$66,599	$0	$37,743	$37,655	$88	$0	$0	$0
Professional/Technical Services and Contracts - Overall Total	$6,805	$6,805	$0	$48,771	$48,566	$205	$0	$0	$0
Equipment/Tools/Supplies - Overall Total	$21,195	$16,859	$4,336	$10,035	$9,954	$81	$54,438	$50,008	$4,430
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Other - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$94,599**	**$90,263**	**$4,336**	**$96,549**	**$96,175**	**$374**	**$54,438**	**$50,008**	**$4,430**

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 22 (USGS) - Copper-based control: zebra mussel settlement and non-target impacts (Year 1)			SUBPROJECT 23 - AIS Management: An Eco-economic Analysis of Ecosystem Services (Year 1)			SUBPROJECT 24 - Genetic method for control of invasive fish species (Year 1)		
BUDGET ITEM	Subproject 22 Budget	Amount Spent	Subproject 22 Balance	Subproject 23 Budget	Amount Spent	Subproject 23 Balance	Subproject 24 Budget	Amount Spent	Subproject 24 Balance
Personnel (Wages and Benefits) - Overall Total	$12,428	$12,428	$0	$122,603	$121,908	$695	$90,588	$90,588	$0
Professional/Technical Services and Contracts - Overall Total	$0	$0	$0	$0	$0	$0	$6,538	$6,538	$0
Equipment/Tools/Supplies - Overall Total	$0	$0	$0	$4,742	$4,741	$1	$11,020	$10,608	$412
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$0	$0	$0
Travel - Overall Total	$0	$0	$0	$4,500	$4,500	$0	$1,000	$306	$694
Other - Overall Total	$0	$0	$0	$0	$0	$0	$966	$960	$6
Budget Reserve Pending Progress and Peer Review - Overall Total									
COLUMN TOTAL	**$12,428**	**$12,428**	**$0**	**$131,845**	**$131,149**	**$696**	**$110,112**	**$109,000**	**$1,112**

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 25 - What's In Your Bucket? Quantifying AIS Introduction Risk (Year 1)			SUBPROJECT 26 - Updating an invasive and native fish passage model for locks and dams					
BUDGET ITEM	Subproject 25 Budget	Amount Spent	Subproject 25 Balance	Subproject 26 Budget	Amount Spent	Subproject 26 Balance	TOTAL BUDGET	TOTAL SPENT	TOTAL BALANCE
Personnel (Wages and Benefits) - Overall Total	$80,642	$78,592	$2,050	$86,824	$84,309	$2,515	$6,597,698	$6,436,996	$160,701
Professional/Technical Services and Contracts - Overall Total	$11,000	$9,616	$1,384	$0	$0	$0	$1,026,602	$955,141	$71,461
Equipment/Tools/Supplies - Overall Total	$19,000	$12,971	$6,029	$2,492	$2,477	$15	$658,625	$616,050	$42,575
Capital Expenditures Over $5,000 - Overall Total	$0	$0	$0	$0	$0	$0	$109,236	$109,236	$0
Travel - Overall Total	$1,000	$361	$639	$1,511	$1,510	$1	$300,028	$264,327	$35,704
Other - Overall Total	$0	$0	$0	$0	$0	$0	$7,813	$2,025	$5,788
Budget Reserve Pending Progress and Peer Review - Overall Total							$0	$0	$0
COLUMN TOTAL	**$111,642**	**$101,540**	**$10,102**	**$90,827**	**$88,296**	**$2,531**	**$8,700,000**	**$8,383,770**	**$316,230**
							Total Budget adjusted to account for rounding discrepancy	*Total Spent adjusted to account for rounding discrepancy*	*Total Balance adjusted to account for rounding discrepancy*

2013 Project Abstract
For the Period Ending July 31, 2017

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project 2, Phase 1: Metagenomic approaches to develop biological control strategies for aquatic invasive species
PROJECT MANAGER: Michael J. Sadowsky
AFFILIATION: University of Minnesota – Minnesota Aquatic Invasive Species Research Center
MAILING ADDRESS: BioTechnology Institute, 140 Gortner Lab, 1479 Gortner Avenue
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E-MAIL: sadowsky@umn.edu
WEBSITE: www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $299,363
AMOUNT SPENT: $299,363
AMOUNT REMAINING: $0

Overall Project Outcomes and Results

Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and invasive mussels pose a serious threat to the health, structure, and function of aquatic ecosystems. Traditional approaches for AIS control, including the use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could potentially be exploited to develop microbe-mediated AIS management strategies. As a first step in identifying potential biocontrols, this project (Phase I) had proposed to characterize the microbial communities (bacterial and fungal) associated with invasive mussels and EWM, across time and space, using amplicon-based high-throughput sequencing approaches. To accomplish this, zebra mussels (ZMs), water, and sediment samples were obtained from 15 lakes twice a year, whereas EWM were sampled from 10 lakes, once a month for six months. Field samples were processed, DNA extracted and high-throughput sequencing was performed on all field samples using the Illumina platform. Sequencing analysis (188 million reads) showed a distinct clustering of each sample type, irrespective of sampling time and location. Core microbial communities were characterized and several taxonomic groups were identified that were either specific or present in high relative abundance in ZMs and EWM, when compared to sediment and water samples. This gives us a promising lead on microbes to purse in Phase II of this study, which will evaluate potential pathogenic characteristics and species- specificity of any pathogens. In addition, our results also indicated that EWM was associated with elevated concentrations of fecal indicator bacteria, such as *E. coli* and *Enterococcus*. This means that not only are these aquatic plants a nuisance, but they may present a hazard to human health as well, especially if they harbor known human pathogens in addition to fecal indicator bacteria. Overall, the results obtained in Phase I have helped to define the distribution of microbes associated with these AIS, and will be useful for the development of future microbiological control strategies (Phase II).

Project Results Use and Dissemination

Results obtained in this study (Phase I) helped us define the distribution of microbes specifically associated with these AIS, and will be useful for the development of future microbiological control strategies. Experiments that will be performed during Phase II will build upon the results obtained in Phase I.

Oral presentations have been made at the 'AIS Research Management Showcase' each year to update the public on research findings and progress, the next one is September 2017. In addition, project results will be presented at the 20th International Conference on Aquatic Invasive Species at Fort Lauderdale in October. Three manuscripts are currently under preparation and will be submitted for publication in peer-reviewed journals.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 2: Metagenomic Approaches to Develop Biological Control Strategies for Aquatic Invasive Species - Phase II: Development of Potential Microbiological Control Agents for Aquatic Invasive Species
SUBPROJECT MANAGER: Michael J. Sadowsky
AFFILIATION: University of Minnesota – Minnesota Aquatic Invasive Species Research Center
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WEBSITE: http://www.maisrc.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $303,217
AMOUNT SPENT: $286,610
AMOUNT REMAINING: $16,607

Sound bite of Subproject Outcomes and Results
This project evaluated the potential for harnessing natural microbes for use as biocontrol agents against Eurasian watermilfoil and zebra mussels. Several microorganisms were isolated that could be pathogenic to zebra mussels, but none met safety requirements for testing. EWM is associated with elevated concentrations of E. coli and human pathogens.

Overall Subproject Outcomes and Results:
Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and zebra mussels (ZMs) pose a serious threat to the health and function of aquatic ecosystems. Traditional approaches for AIS management, including use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could be exploited to develop microbe-mediated AIS management strategies. As the first step towards the identification of potential biocontrol strategies, microbial communities associated with 'healthy' AIS were compared with that of 'diseased' AIS or to native species. Since no natural diseased mussels were available, we opted to develop an experimental model system, which allowed for the application of different intensities of stress – heat (17, 25, 33°C) and salinity (1.5, 13.5 ppt), to promote the proliferation of opportunistic pathogens. High-throughput DNA sequencing of 414 samples (providing 32 million DNA reads) resulted in the identification of several potentially 'pathogenic' microbial groups that were strongly associated with ZM mortality. These included *Aeromonas*, *Chryseobacterium*, *Flavobacterium*, *Acidaminobacter*, *Clostridiaceae* 1 sp., *Rhodobacteraceae* sp., *Acinetobacter*, *Shewanella*, and *Clostridium sensu stricto* 13. For the identification of EWM-specific microbiota, high-throughput DNA sequencing was performed on 315 samples (46 million reads) derived from leaf and root compartments of EWM and six native macrophyte species. This resulted in the identification of taxa that were significantly enriched in EWM leaves and roots compared to native plants. Though several AIS-associated microorganisms were isolated that could be pathogenic to invasive mussels (e.g. *Aeromonas*) - none of them met our safety requirements for further testing. Future studies must isolate and evaluate the efficacy of 'host-specific and pathogenic' biocontrol candidates that will only infect invasive mussel species.

Subproject Results Use and Dissemination

Our research findings were disseminated via oral and poster presentations at the following (international/ national/ local) conferences: 61st International Association for Great Lakes Research conference (Toronto, Canada), UNC Water Microbiology Conference 2019 (Chapel Hill, NC), 20th International Conference on Aquatic Invasive Species (Fort Lauderdale, FL), 5th Upper Midwest Invasive Species Conference (Rochester, MN), 119th General Meeting of the American Society for Microbiology (San Francisco, CA), and the AIS Research Management Showcase in 2017 & 2018 (St. Paul, MN). Two papers were published in the journals 'FEMS Microbiology Ecology' and 'Science of the Total Environment' during this project period. One manuscript is currently undergoing peer-review and two additional manuscripts are under preparation. All sequencing data generated in this project will be publicly available (via submission to NCBI Genbank) and all publications will list accession numbers to link to short read archive of all samples. Thus far, all sequence data mentioned in current publications is directly linked to a publicly available web site for download.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 3: Attracting carp so their presence can be accurately assessed
SUBPROJECT MANAGER: Peter Sorensen
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WEBSITE: www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $682,969
AMOUNT SPENT: $663,719
AMOUNT REMAINING: $19,251

Sound bite of Subproject Outcomes and Results

A sound deterrent system that is over 98% effective at stopping invasive carp was developed in the laboratory and versions of it have been installed in two rivers. To complement this deterrent system we developed food and pheromone attractants which, when coupled with DNA measurements, detect carp with extreme sensitivity.

Overall Subproject Outcome and Results

This project developed several tools that can manage and control all species of invasive carp species in Minnesota. First, we developed ways using both food and sex pheromones to attract and measure the presence and density of carp using the environmental DNA (eDNA) they release to the water. This technique is superior to traditional netting because it can be performed in any habitat or water of any depth, including at low densities that are otherwise unmeasurable. eDNA can also determine carp gender. Second, we developed a deterrent system comprised of sound, light and air curtain that is 97% effective in the laboratory and could safely and effectively prevent invasive carp from swimming upstream through navigation locks in Mississippi River. If this deterrent system were to be paired with attractant-based eDNA surveillance methods in specific lock-and-dams whose gate was also adjusted to stop carp, it is extremely likely that enough carp could be prevented from passing through these lock-and-dams that the remainder could be removed by targeted commercial fishing. Field tests of the deterrent system are now underway.

Subproject Results Use and Dissemination

The first invasive carp deterrent system in the world is now in place in southern Minnesota using the sensory cues we identified. The USGS is now exploring the pheromone and food attractants we developed in the Great Lakes, and the sound/light stimuli we developed are being used at Barkley Dam in Kentucky by the UAFWS with whom we have partnered with. Sorensen and colleagues have at 5 peer-reviewed scientific publications in high quality journals and several technical reports. A PhD and a MS thesis are being produced. A dozen talks were given as part of this project.

M.L. 2013, Chp. 52, Sec. 2, Subd. 06a **Project Abstract**
For the Period Ending July 31, 2017

PROJECT TITLE: Common carp management using biocontrol and toxins
PROJECT MANAGER: Przemyslaw Bajer
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $ 384,231

Overall Project Outcome and Results

We tested two new methods to control common carp, which are invasive fish that degrade lakes of south-central Minnesota. First, we tested biocontrol, which is the ability of bluegill sunfish (native fish) to control carp reproduction by consuming their eggs and larvae. This was tested in 6 small lakes. All lakes were stocked with adult carp and every other lake was stocked with bluegills. Carp offspring survival was assessed through electrofishing and mark-recapture. At the end of the season, lakes with bluegills had 11 times fewer carp offspring than those without bluegills. This shows that biocontrol by bluegill is an important element of common carp management strategies. Bluegill populations can be strengthened in many shallow lakes by winter aeration to prevent winter fish kills.

Second, we tested if toxic bait could be developed to target carp without impacting native fish. This is important in lakes where biocontrol is unlikely. We incorporated an EPA-approved toxin antimycin-A (ANT-A) into corn pellets, which the carp consume with high specificity and performed 4 experiments: 1) using gavage trials we showed that the bait was toxic at 8 mg/kg; 2) using leaching trials we showed that <1% of ANT-A leached out of the bait and did not cause mortality among native fish; 3) using lab tanks where carp were stocked with three native fish we showed that 46% of carp and 76% of fathead minnows perished after one application of pellets, but perch and bluegill were not impacted; 4) using ponds with carp, bluegills and perch we showed that 37% adult carp perished after 6 days of pellet application, while no perch and bluegill did. Our results suggest that corn-based toxic pellets could be developed to selectively target carp but more work is needed to minimize impacts on native minnows. This is being addressed by ongoing work.

Project Results Use and Dissemination

Information collected in these experiments were disseminated and will continue to be disseminated in a variety of ways. Presentations were given at MAISRC showcases, the Minnesota and National American Fisheries Society meetings, and will be given at the International Conference for Invasive Species. We anticipate publishing 3 papers, one of which is in revisions, another written, and one to be completed. We have also shared this work with colleagues, watershed association, and MAISRC extension.

Biol Invasions (2018) 20:1809–1820
https://doi.org/10.1007/s10530-018-1662-y

ORIGINAL PAPER

Assessing the efficacy of corn-based bait containing antimycin-a to control common carp populations using laboratory and pond experiments

Joshua R. Poole · Blake W. Sauey · Jon J. Amberg · Przemyslaw G. Bajer

Received: 7 May 2017 / Accepted: 5 January 2018 / Published online: 15 January 2018

Abstract Strategic use of oral toxicants could allow for practical and sustainable control schemes for the invasive common carp (*Cyprinus carpio,* or 'carp') if a toxicant selectively targeted carp and not native species. In this study, we incorporated antimycin-a (ANT-A), a known fish toxicant, into a corn-based bait and conducted a series of experiments to determine its toxicity, leaching rate, and species-specificity. Our results showed that ANT-A was lethal to carp at doses $\geq$ 4 mg/kg and that the amount of ANT-A that leached out of the bait in 72 h was not lethal to carp or bluegill (*Lepomis macrochirus*). Species-specificity trials were conducted in 227 L tanks, in which carp were stocked with three native species representing families that occur sympatrically with carp in our study region: the fathead minnow (*Pimephales promelas*), yellow perch (*Perca flavescens*) and bluegill. These trials showed high mortality of carp (46%) and fathead minnows (76%) but no significant mortality of perch or bluegill. Finally, a pond study, which used the same species composition except for fathead minnows, resulted in 37% morality among adult carp and no mortality among perch or bluegill. Our results suggest that corn-based bait that contains ANT-A could be used to selectively control carp in ecosystems dominated by percids or centrarchids, such as lakes across the Great Plains ecoregion of North America, where carp are especially problematic.

Keywords *Cyprinus carpio* · Toxins · Toxicants · Invasive fish · Management · Species-specific

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-018-1662-y) contains supplementary material, which is available to authorized users.

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Introduction

The Common carp (*Cyprinus carpio,* or 'carp') is one of the world's most invasive and ecologically harmful species (Lowe et al. 2004). Invasions of freshwater ecosystems by carp are commonly associated with severe declines in aquatic macrophytes, causing a loss of habitat for waterfowl and other biota (Crivelli 1983; Haas et al. 2007; Bajer et al. 2016). Due to their feeding behavior, carp also stir up sediment, reduce water clarity, and increase nutrient concentrations, which often promote nuisance blooms of cyanobacteria (Weber and Brown 2009; Vilizzi et al. 2015). The search for sustainable control strategies for carp has continued for the last several decades, first in North America and later in Australia (Marking 1992; Koehn 2004). Physical removal has been used frequently to control carp populations, especially in temperate North America, because carp form tight winter aggregations that can be located by tracking radio-tagged fish and removed via netting (Bajer et al. 2011; Armstrong et al. 2016). This strategy is believed to be sustainable mainly in systems with abundant egg and larval predators that control carp's reproductive success (Lechelt and Bajer 2016). In systems with poor predatory communities, removal has not been very effective due to density-dependent compensatory responses in recruitment (Colvin et al. 2012; Weber et al. 2016). Non-specific toxicants dispersed into lake water and water draw-downs have also been used to eradicate carp populations, but they have been used sporadically because they are expensive, impact native biota, and can primarily be used in lakes that are isolated with barriers to prevent reinvasion (Hanson et al. 2017). Viruses and genetic technologies have been proposed for carp control in Australia; however, carp are likely to develop resistance to viruses within a few generations, (McColl et al. 2014), and genetic technologies remain at the developmental stage and are associated with social concerns and uncertainties (Thresher et al. 2014a, b).

Strategic use of toxicants has been instrumental in developing arguably the only successful integrated pest management strategy for an aquatic invasive species to date, the control of the sea lamprey (*Petromyzon marinus*) in the Great Lakes (Hubert 2003). Toxicants might similarly be used to manage common carp populations in a selective and effective manner. Currently, four compounds are registered in the United States (U.S.) for use as piscicides: 3-Trifluoromethyl-4-nitrophenol (TFM) and niclosamide, which are used to control sea lamprey, and rotenone and antimycin-A (ANT-A), which are used in the control of bony fishes (Bettoli and Maceina 1996; McDonald and Kolar 2007). ANT-A shows substantial promise over the other piscicides for the purposes of controlling populations of common carp. It is highly toxic to fishes (more so than rotenone; Marking and Bills 1981; Finlayson et al. 2002), but much less toxic to higher vertebrates (Herr et al. 1967; Finlayson et al. 2002). In the aquatic environment, ANT-A degrades into compounds that are not known to pose a risk (Turner et al. 2007; Environmental Protection Agency 2007), which might be particularly desirable to prevent the accumulation of unused toxin in the environment. Finally, unlike rotenone, it appears that fish, including carp, are unable to detect and avoid ANT-A (Bonneau and Scarnecchia 2001; Gehrke 2003; EPA 2007; Rach et al. 2009). Although ANT-A is often applied directly to water to affect fish mortality, existing evidence suggests that ANT-A could be incorporated into bait and delivered to carp as an oral toxicant, which would make its application more targeted (Rach et al. 1994; Kroon et al. 2005). Feeding experiments conducted in laboratory arenas and in natural lakes showed that common carp possesses the ability to quickly learn and remember the location of a food reward (Karplus et al. 2007; Zion et al. 2007; Bajer et al. 2010), which might allow for innovative strategies to apply the toxicant by exploiting cognitive aspects of carp's foraging behavior. For example, in a small lake in Midwestern U.S., Bajer et al. (2010) showed that carp (75% of the population) were attracted to plant-based bait (corn) within 6 days, whereas native fishes were not. Overall, it seems plausible that ANT-A could be delivered to carp as an oral toxicant in a corn-based bait by first training carp to consume corn at selected times and locations, after which time the bait would be replaced (for brief periods of time) with one that contains lethal doses of ANT-A. This strategy might result in relatively high mortality of carp with minimal impact on native biota. However, no proof-of-concept experiment has examined if a corn-based bait containing ANT-A could selectively target carp and not native species.

In this study, corn-based bait containing ANT-A was developed and experiments were conducted to (1) determine the lethal dose of ANT-A to carp, (2) quantify the leaching rate of ANT-A from the bait, (3) test species-specificity of the bait in mixed-species lab trials, and (4) test species-specificity in mixed-species pond trials. Our study has important implications for developing novel and practical management strategies for the common carp.

Methods

Four experiments were conducted to test if ANT-A could be incorporated into a corn-based bait to selectively kill carp. First, the lethal dose was examined in gavage trials. This information was then used to develop bait that would be lethal to carp after consuming a single pellet. A leaching trial was then conducted to examine how much ANT-A leached into the water from bait containing a lethal dose of ANT-A and whether leaching caused any fish mortality. This assay involved carp as well as bluegill (*Lepomis macrochirus*), which are particularly sensitive to ANT-A. Following the leaching experiment, we conducted a mixed-species laboratory species-specificity test, in which we provided toxic bait (the same amount as in the leaching trial) to carp and the following three native species from families commonly found in lakes where this type of control is likely to be applied: centrarchids [bluegill], percids [yellow perch (*Perca flavescens*)], and cyprinids [fathead minnow (*Pimephales promelas*)]. Finally, in a mixed-species pond species-specificity experiment, carp, bluegills, and perch were used to test if carp could be targeted in a selective manner in a larger, more natural environment. Fathead minnows were not used in the pond trial because their small size would make it difficult to assess mortality.

Bait formulation

A batch of ANT-A was fermented and extracted by the University of Minnesota Biotechnology Resource Center (St. Paul, MN) contracted through Aquabiotics, Inc. (Bainbridge Island, WA). Produced ANT-A powder was determined to contain less than 10% impurities that were not characterized but likely consisted of residual fermentation media. ANT-A powder was then encapsulated into a microparticle developed at the U.S. Geological Survey Upper Midwest Environmental Sciences Center (La Crosse, WI; UMESC) prior to incorporation into a corn-based bait. Microparticles were produced similarly to the methods described in Hawkyard et al. (2011) and Langdon et al. (2008). This microparticle was a spray-atomized product of a core with ANT-A, refined beeswax (Sigma-Aldrich, St. Louis, MO, USA), and sorbitan monopalmitate (Sigma-Aldrich, St. Louis, MO, USA). Microparticles had a diameter of ~ 0.35 µm and a nominal ANT-A concentration of 20% weight by weight (w/w). Microparticles were stored at − 20 °C in plastic containers until use. Specific concentrations of ANT-A in microparticle, or later in the bait (see below) were not measured beyond this point, thus all concentrations reported below were nominal. However, manufacturer's specifications (storage at − 20 °C) were followed to minimize the potential breakdown of ANT-A in the microparticle or bait until it was applied. Our process of microparticle formulation required ANT-A in a dry powder form; therefore we decided not to use the commercially available aqueous ANT-A formulation (Fintrol™) registered by the U.S. Environmental Protection Agency (EPA).

The bait was made using corn meal (Quaker Oats Company, Chicago, IL; 80% by weight), gelatin (Knox Gelatine, Kraft Foods Group Inc., Northfield, IL; 10% by weight), and microparticle (10% by weight). Thus, the bait contained a nominal concentration of 20 mg ANT-A/g. The corn meal and microparticle were mixed by hand using a plastic spatula. The gelatin was prepared according to manufacturer's instructions, cooled to room temperature, poured into the corn meal-microparticle mixture and mixed by hand using plastic spatula to produce a slurry that was then placed into plastic bags and chilled to 4 °C, until the mixture became similar to the consistency of cold putty. The mixture was then extruded from a small opening in a plastic bag to form long lines on a glass plate. The lines were allowed to fully harden at 4 °C until they could be cut with a razor blade to a size that was sufficient to pass the gape of fish used in the trials: a diameter of approximately 4 mm and a length of 8 mm for the carp $<$ 200 mm, and a diameter of approximately 10 mm and a length of 20 mm for the carp $>$ 200 mm. Any fish whose gape was too small to consume the entire pellets could have still fed on the bait because it was friable in the water. Bait was stored at − 20 °C in plastic containers until use. Nontoxic (blank) bait, which was used in control treatments and during acclimation phases of the experiments (see below), was prepared in the same way, except that the microparticle used to make it contained no ANT-A.

Test animals

Fathead minnows, bluegill, and yellow perch were reared from eggs at the Upper Midwest Environmental Sciences Center (UMESC). Animal husbandry procedures followed UMESC Standard Operating Procedures for fish care and maintenance. Methods used to conduct research for this research protocol (AEH-16-

CCT-01) were approved by the UMESC Animal Care and Use Committee. The juvenile carp used in all trials were obtained from Osage Catfisheries, Inc. (Osage Beach, MO). Adult carp used in the pond species-specificity trial were collected from a lake in Minnesota (Long Lake, Ramsey County; University of Minnesota Animal Care Protocol 1601-33424A). All fish used in the experiments were capable of ingesting the bait pellets, either by swallowing them whole, or by ingesting portions of pellets.

Gavage trial

Common carp (94–146 mm in total length [TL]; 38–128 g) were acclimated for 5 d to fiberglass, round, flat-bottom, 227-L tanks containing 150 L heated (~ 24 °C) well water with a pH of approximately 7.9 and continuous water flow (minimum of 1 tank-volume exchange/h). During acclimation, carp were offered daily a diet of bloodworms and the non-toxic bait each at 1% body weight (BW). The bloodworms were used for nutritional reasons because they often dominate carp's diet in natural systems and are highly palatable (Garcia and Adelmen 1985; Kasumyan 1997); in other trials (see below) bloodworms were used to mimic food sources found in natural systems. During the trial, seven tanks were used, each containing five carp. Two tanks were randomly assigned to each of three ANT-A dose-level treatments (n = 10 carp per treatment), while the remaining tank was used as a control (N = 5 carp). The three different ANT-A dose levels were: 4.0, 8.0, 16.0 mg ANT-A/kg BW, equivalent to ingesting the toxic bait at 0.02, 0.04, or 0.08% BW, respectively. Percent BW calculations were based on the mean weight of fish in each tank, weighed before being placed in the tanks. Total fish BW varied from 64–74 g in all tanks. In the control treatment, non-toxic bait was administered by gavage at 0.08% BW, equivalent to the amount of bait administered at the highest ANT-A dose. To administer a dose, carp were removed from tank and anesthetized to surgical plane (50 mg tricaine methanesulfonate [TMS]/L; Tricaine-S™, Western Chemical Inc., Ferndale, WA). A 5-mL plastic syringe with the tip removed was filled with appropriate amount of bait and inserted into the mouth of the anesthetized fish past the pharyngeal teeth. The plunger was then depressed to deliver the bait. Fish were immediately placed back into their respective tank where mortality was recorded 1, 3, and 24 h post-gavage. Fish surviving at the end the trial were euthanized by TMS-overdose (200 mg TMS/L). All fish were measured for total length (nearest mm), and wet weight (nearest 0.1 g) at the conclusion of the trial. Water quality parameters (dissolved oxygen [DO], temperature, pH) were measured at 1 and 24 h with a YSI Handheld Dissolved Oxygen Meter (Yellow Springs, OH), and a Beckman-Coulter pH Meter Φ410 (Brea, CA) (Online Resource 1).

Leaching trial

The trial was conducted in fiberglass tanks (n = 5) using conditions described in the gavage trial except that the water temperature was 20 °C. Carp (n = 6; 75–179 mm TL; 7–72 g) and bluegill (n = 6, 86–152 mm TL; 12–70 g) were stocked in each tank. Fish were acclimated to the tank conditions for at least 5 d during which they were offered a mixture of bloodworms and non-toxic bait each at 1% BW.

During the trial, 1 g of the 4-mm ANT-A bait was placed at the bottom of each tank. Instantaneous leaching of all ANT-A present in this amount of bait would have resulted in a water concentration of 0.13 mg ANT-A/L, approximately 300 times higher than the LC_{50} for common carp (0.35 ug/L/96 h; Marking 1992). The bait was placed inside an enclosure that allowed water to circulate around the bait while preventing fish from ingesting or disturbing it. The bait was placed inside a polyvinyl chloride (PVC) pipe (0.6 cm diameter, 10 cm long) with 35 mm mesh on both ends, that was then placed inside a plastic container (47 cm × 23 cm × 17 cm; Rubbermaid™) with > 20 holes (diameter = 3.2 mm) drilled in each side. An airstone was placed near the container to ensure there was water movement near the enclosure. Water flow to the tank was stopped concurrent with placing the bait in the tank.

Water samples (25 mL) were taken by submerging a 50-mL centrifuge tube (VWR, Radnor, PA) ~ 1 cm below the surface of the water immediately before the addition of bait and at 1, 4, 8, 24, 48, and 72 h after. These time points were selected to examine ANT-A concentration at frequent intervals immediately after the bait was placed in the water when we thought most of the leaching would occur (Table 1). Water samples were processed using solid phase extraction (SPE) to

Table 1 Antimycin-A concentration (μg/L) in the water during leaching trials

Tank	Time (h)					
	1 h	4 h	8 h	24 h	48 h	72 h
1	N.D.	N.D.	0.013	N.D.	N.D.	N.D.
2	N.D.	N.D.	0.030	N.D.	0.009	N.D.
3	N.D.	N.D.	0.012	N.D.	N.D.	N.D.
4	N.D.	N.D.	0.018	0.020	7.48[a]	N.D.
5	N.D.	N.D.	0.019	N.D.	N.D.	N.D.

N.D. Below the threshold of detection of 8 ng/L

[a]Water drained nearly completely from the tank between 24 and 48 h and was re-filled. Water sample at 48 h for tank 4 was taken before tank was refilled

concentrate ANT-A 25 fold as described in Bernardy et al. (2013). ANT-A concentration was then quantified using an Agilent 6530 Accurate-Mass Quantitative Time of Flight Liquid Chromatography Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA), with a detection limit of 8 ng/L and a quantification limit of 0.32 μg/L. Fish mortality was recorded at each water-sampling period. Water quality parameters (DO, temperature, pH) were measured 1, 24, 48, and 72 h after placing the bait in the tank (Online Resource 2). At the end of the trial, all fish were euthanized, measured and weighed.

Laboratory species-specificity trials

The trial was conducted in fiberglass tanks (n = 6) using conditions described in the gavage trial. Each tank contained six common carp (54–80 mm TL; 5–16 g), five fathead minnows (45–72 mm TL; 1–9 g), six yellow perch (47–61 mm TL; 1–4 g), and six bluegills (82–123 mm TL; 16–66 g). Fish were acclimated to test conditions for 7 d during which they were offered the non-toxic bait and bloodworms each at 1% BW. Three tanks were then randomly selected as treatment tanks and three as control tanks. Fish in the treatment tanks were offered 1 g of toxic bait ($\sim$ 0.30% body weight; 59 mg ANT-A/kg BW). The control tanks were offered 1 g of non-toxic bait. We chose to offer 1 g of bait to be consistent with the leaching trial. Fish mortality was monitored every hour for the first 6 h, and then at 24 h, at which time water quality parameters (DO, temperature, pH) were measured. Dead fish were removed from the tank during each monitoring point and weighed and measured. Fish that survived in the treatment tanks were euthanized by overdose of TMS and measured and weighed.

Fish in the three control tanks were then offered the acclimation diet (bloodworms and non-toxic bait at 1% BW each) for 3 d. Two of the 3 tanks were then randomly selected as treatment tanks and the test with toxic bait was repeated while the remaining single tank was used as a control. This design resulted in five replicates of the toxic bait treatment and four replicates of the control treatment with all tanks but one being eventually exposed to the toxic bait treatment. Some fish died between the end of the first trial and the initiation of the second trial, thus the second trial contained fewer fish (Table 2). Water quality parameters were measured at 1 and 24 h post-exposure (Online Resource 3). All fish were measured for weight and length at the conclusion of the trial.

Table 2 Results of the laboratory species-specificity trial

Trial #	Bait type	Number of individuals in tank			
		Carp	Bluegill	Yellow perch	Fathead minnow
Trial 1	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Toxic	2 (6)	0 (6)	1 (6)	5 (5)
	Toxic	3 (6)	0 (6)	1 (6)	5 (5)
	Toxic	0 (6)	0 (6)	1 (6)	4 (5)
Trial 2	Blank	0 (6)	0 (6)	1 (3)	0 (2)
	Toxic	4 (6)	0 (6)	0 (6)	1 (6)
	Toxic	5 (6)	0 (6)	1 (2)	5 (5)

Shown is the number of fish that died in each tank over the course of the experiment. Numbers in parentheses show how many fish were placed in each tank at the beginning of the experiment

Pond species-specificity trials

Six concrete ponds (10.4 m long × 5.5 m wide × 0.75 m deep; no water flow; ~ 12 °C) were stocked with 10 adult common carp (265–483 mm TL; 570–3000 g), 9 juvenile common carp (98–179 mm TL; 34–130 g; fewer juvenile carp were available), 20 yellow perch (46–136 mm TL; 4–33 g), and 20 bluegill (58–149 mm TL; 8–106 g). Fish were allowed to acclimate for 7 d, during which they were offered a mixture of bloodworms and the non-toxic bait (1 and 3% BW, respectively). Following the acclimation period, three ponds were randomly assigned to either the toxic bait treatment or the control treatment. Fish in three ponds assigned to the toxic bait treatment were offered the toxic bait at an overall dosage of 1% BW per day, equivalent to an ANT-A dose of 28 mg ANT-A/kg BW/d. Bloodworms (1% BW/d) and cracked field corn (~ 100 g/d) were offered concurrent with the toxic bait. We chose to continue offering bloodworms and to add cracked corn to simulate field conditions in which carp would have access to other foodstuffs in the environment and where toxic bait might be mixed with a non-toxic food reward (e.g. cracked corn) to attract more carp and avoid scenarios in which a single carp might consume large amounts of toxic pellets, reducing cost-efficiency. Fish in the control ponds were offered the same foodstuffs except that the non-toxic bait was offered in lieu of the toxic bait. Fish in all ponds were fed in the evenings and remaining food was removed in the morning with a net. The experimental period during which fish were offered the aforementioned diet combinations lasted for 6 days. Mortality was monitored twice daily. All dead fish were removed from the pond and total length and weight were recorded. Water quality parameters (DO, temperature, and pH) were measured daily throughout the experiment (Online Resource 4).

Statistical analysis

We elected to use the minimum number of tanks or ponds and the minimum number of animals per treatment to convincingly demonstrate that the toxic bait had the capacity to eliminate a biologically meaningful number of carp in our experiments (> 30%). We did this to avoid unnecessarily exposing large numbers of animals to the toxin. This pertains especially to the species-specificity experiments in the laboratory and in the ponds. Given the nature of the experiments (application of a toxin over a short period of time), we assumed that mortality in treatment tanks would be high (> 30% and consistent), while mortality in control tanks would be nil. We also assumed that we would be using a t test to analyze the results of our experiments. Power analysis using such assumptions (power = 0.8, $\alpha = 0.05$, mean difference > 0.3, standard deviation in treatment and controls ~ 0.1) suggested that three replicates or more would be sufficient for treatment and control experimental units (lab tanks or ponds). Thus, we used three replicates for the pond experiment (where space was more limited) and five replicates of the treatment group in the lab experiment where tanks more easily available. Similar approach was employed by Rach et al. (1994) where three ponds were used to conduct early tests of ANT-A as a toxin for common carp.

For the gavage and leaching trials, fish mortality was recorded at each treatment level. For the laboratory species-specificity trials, a one-sided Wilcoxon Rank Sum Test ($P = 0.05$) was used to test the hypothesis that mortality in treatment tanks was greater than mortality in control tanks for each species. Similarly, for the pond species-specificity trial, a one-sided Wilcoxon Rank Sum Test ($P = 0.05$) was used to test the hypothesis that mortality in treatment ponds was greater than mortality in control ponds for each species.

Results

Gavage trials

No carp died in the control tanks. Five of the 10 carp died after gavage of 4 mg ANT-A/kg BW; suggesting that the LD_{50} for carp in our experiments was approximately 4.0 mg ANT-A/kg BW. All carp died after gavage of 8.0 mg ANT-A/kg BW. Nine out of 10 carp died after gavage at 16.0 mg ANT-A/kg BW; the reason for the incomplete mortality in the highest dose treatment was unknown but it might have been caused by regurgitation (i.e. the bait not being inserted deep enough past pharyngeal teeth).

Table 3 Results of the pond species-specificity trial

	Control 1		Control 2		Control 3		Treatment 1		Treatment 2		Treatment 3	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Carp adult	9	1[a]	10	0	10	0	6	4	6	4	7	3
Carp juvenile	8	1	9	0	9	0	9	0	9	0	9	0
Bluegill	16	0	15	4	17	2	18	0	17	0	18	0
Perch	20	0	20	0	20	0	17	0	19	0	20	0

Shown are the numbers of fish that survived or died in each control or treatment pond. Fish in treatment ponds were offered toxic bait containing antimycin-a whereas fish in control ponds were offered non-toxic bait without antimycin-a

[a]Fish jumped out of the pond

Leaching trials

No fish died in any of the tanks during the leaching trial. ANT-A was not detected in the water at either the 1 or 4 h time intervals (Table 1). ANT-A was detected in all tanks at 8 h at less than 0.03 μg/L, equivalent to leaching of less than 0.1% of the initial mass of ANT-A present in the bait at the start of the trial (Table 1). This suggests that only minor leaching occurred within first 8 h. ANT-A was generally not detected at 24 h and beyond (Table 1), possibly due to degradation of ANT-A in water (the half-life is 12 h at 25 °C; EPA 2007). Accidentally, the water drained almost completely from one of the tanks between the 24 and 48 h and ANT-A concentration reached 7.48 mg/L (Table 1), however, no fish mortality occurred because of short exposure time. Detailed estimates of the amount of ANT-A that leached out of the pellets are not provided here because they are complicated by natural degradation in the water (EPA 2007), and in the bait, which is unknown.

Laboratory species-specificity trial

Fourteen of 30 ($\sim$ 47%) common carp died in treatment tanks whereas none died in control tanks (Table 2; $P = 0.02$; df = 3; W = 2). Twenty of 26 ($\sim$ 77%) fathead minnows died in treatment tanks whereas none died in control tanks; (Table 2; $P = 0.007$; df = 3; W = 20). Four of 26 ($\sim$ 15%) yellow perch died in treatment tanks, whereas one of 21 ($\sim$ 5%) died in control tanks (Table 2; $P = 0.15$; df = 3; W = 5.5). No bluegills died in either treatment or control tanks (Table 2).

Pond species-specificity trial

Eleven of 30 adult carp (37%) died in treatment ponds, while only one of 30 (this fish jumped out of the pond) died in control ponds (Table 3; $P = 0.03$; df = 2; W = 9). No juvenile carp died in treatment ponds and one juvenile carp died in the control ponds (Table 3; $P = 0.91$; df = 2; W = 6). No bluegill died in treatment ponds and 6 of 48 (13%) died in control ponds (Table 3; $P = 0.96$; df = 2; W = 7.5). No yellow perch died in either treatment or control ponds (Table 3).

Discussion

This study is the first to indicate that ANT-A incorporated into a corn-based bait might be used to selectively control populations of carp. The efficacy and selectivity observed in our study indicates that such a strategy might be most effective in lakes where the fish community is dominated by centrarchids and percids. While we did observe some mortality of perch in our laboratory trial, it occurred both in control and treatment tanks, was not significant, and most likely was related to disease or stress. No mortality of perch occurred in the pond trial, which lasted longer than the laboratory trial, included repeated exposure to ANT-A pellets, and more closely resembled natural conditions. No mortality of bluegills occurred in either laboratory or pond trials. The laboratory specificity experiment did also show that corn-based bait could impact native cyprinids. These concerns need to be carefully examined. Non-target mortality of native cyprinids may not be a major concern in many lakes in

North America where carp populations are especially problematic, including the shallow lakes of the Great Plains ecoregion. For example, 15 species of cyprinids occur in Great Plains lakes of south-central Minnesota (Drake and Pereira 2002), but only four of those are omnivorous and might overlap in diet with the carp (Drake and Pereira 2002). Additionally, these native cyprinid species are small, thus, to exclude them, large, hard pellets could be used, which only adult carp could ingest and crush with their pharyngeal teeth. Non-specific mortality could be further reduced by applying the bait at times and within sites where carp, and not native fish, are most likely to consume it. For example, applying the bait at night, when carp forage most actively, and in deeper areas might exclude native cyprinids with diurnal feeding patterns. Cognitive aspects of carp foraging behavior should also be exploited to behaviorally condition those fish before the bait is applied (Bajer et al. 2010). Carp's gustatory preferences could additionally be exploited by, for example, adding amino acids like cysteine to the bait, which carp have been shown to be attracted to (Kasumyan and Morsi 1996). We chose corn because carp readily ingest it and can be conditioned to aggregate in sites baited with it (Bajer et al. 2010). Aquaculture literature also indicates that corn was a reasonable choice because its main amino acids, glutamic acid and proline (http://www.fao.org/docrep/t0395e/t0395e03.html) are highly palatable to carp (Kasumyan and Morsi 1996). Carp also have relatively high amylase activity that allows them to digest complex carbohydrates, such as starch, which constitutes approximately 70% of corn (Takeuchi et al. 2002; Li et al. 2016). Nevertheless, the potency and specificity of the bait could undoubtedly be improved.

Catostomids are another group of native fish that could be impacted in lakes of North America, because, like carp, they also often feed on plant material (Cooke et al. 2005). However, in lakes invaded by carp, catostomids are represented primarily by bigmouth buffalo (*Ictiobus cyprinellus*) and white sucker (*Catostomus commersonii*). Bigmouth buffalo is planktivorous and not likely to be attracted to benthic bait, and the white sucker feeds predominately on zooplankton and zoobenthos (Saint-Jacques et al. 2000). Though the attraction of native fishes to corn-based bait is poorly documented, Bajer et al. (2010) used telemetry and cameras to show that in a natural lake in Minnesota, approximately two-thirds of the carp population learned to visit a site baited with corn in less than a week, whereas no native cyprinids or catostomids were attracted to corn, even though white suckers were common in the lake (http://www.dnr.state.mn.us/lakefind/showreport.html?downum=10001300). Further, corn-baited traps have been used to lure and remove carp from at least six lakes in south-central Minnesota showing nearly 100% selectivity for carp (P. G. Bajer, unpublished data, University of Minnesota 2010–2017). Catfishes, including the black bullhead (*Ameiurus melas*), are also commonly found in lakes with high carp abundance in North America. However, they have much higher tolerance levels to ANT-A (LC_{50} = 25–200 ug/L/96 h; Finlayson et al. 2002) and would most likely not be impacted; ANT-A is commonly used in catfish farms to eliminate other fish while maintaining catfish monoculture. Although more studies are needed in natural systems, corn-based bait could offer high selectivity as a carrier for oral toxicants for the carp in many areas of North America. Where little site-specific information exists, we recommend that underwater cameras or traps are used prior to toxin application to assess potential non-target impacts.

It is not well known what mortality levels are needed to control populations of invasive fish using oral toxicants, but Lechelt and Bajer (2016) suggested that 30– 50% annual removal rates might be sufficient to control carp populations in systems with abundant predators, like bluegill, who consume carp eggs and larvae, and by doing so limit carp's reproductive success (Bajer and Sorensen 2010; Silbernagel and Sorensen 2013). Weber et al. (2016) suggested that carp removal in large, inter-connected systems with relatively low abundance of egg and larval predators, might be less effective, and exploitation rates of 50% may be needed to control carp abundance. In our experiments, approximately 40% of the carp died after being offered the toxic bait over only short periods of time. We suspect that our experiments provided conservative estimates of carp mortality. In the laboratory experiment, only 1 g of bait was provided to fish to keep the amount of bait consistent with the leaching trial, and bait was only provided once (single feeding). Larger amounts of bait and numerous exposures would likely result in higher carp mortality. The mortality of carp would also likely have been higher in the pond experiment if these tests were conducted earlier in the season. Pond experiments were conducted in November when water temperatures were below 12 °C, at which point carp consumption rates are known to diminish (Goolish and Adelman 1984). Late summer through early fall is probably the best time period to apply oral toxicants to carp, because these fish are highly attracted to corn at that time (Bajer et al. 2010).

ANT-A is currently registered as a restricted use pesticide that can be applied directly to water (Fintrol^{TM}) to control nuisance fish populations. Use of ANT-A in an oral delivery formulation for fish in the United States would require an additional approval process. While the fate of ANT-A in aqueous solution (Fintrol^{TM}) including the rate and products of breakdown is relatively well documented (EPA 2007), the fate of ANT-A as an ingredient of carp bait is not known. For example, it is not known if ANT-A that is incorporated into the microparticle and then into the bait might degrade slower that ANT-A applied directly into water where it can be hydrolysed more rapidly. Products of ANT-A metabolism once it passes through fish digestive system are also unknown. Non-target, chronic and sub-lethal effects on humans and biota would also need to be carefully examined. Available information suggests that the risks associated with oral application of ANT-A to control carp populations might be acceptable, but potential issues would need to be addressed. ANT-A delivered through oral exposure routes (i.e. toxic bait) is lethal to fishes in concentrations considerably less than for higher vertebrates (Lennon and Berger 1970; Finlayson et al. 2002). The acute (48 h) LD_{50} for rats (*Rattus* sp.) was nearly 100 times higher than that for fish (EPA 2007) and there was no mortality in rats offered ANT-A in the diet (dose = 5 mg/kg BW/d for 4 weeks, and 10 mg/kg/d for an additional 4 weeks; Herr et al. 1967). ANT-A is highly toxic to some water birds, such as the Mallard (*Anas platyrhynchos*, LD_{50} = 2.9 mg/kg; EPA 2007), thus care would need to be taken to prevent aquatic birds from feeding on the pellets. This could be accomplished by designing feeders from which only the carp could consume the pellets. For example, as a rudimentary solution, we commonly use soft mesh bags for that purpose, where carp can eat the pellets through the mesh, but pellets remain in the bags if uneaten and can later be removed. The pellets could be applied at night, when carp forage most actively, and then be retrieved in the morning. Consuming dead carp by predatory birds or mammals should not pose a significant risk because these organisms have an LD_{50} greater than that of carp, suggesting that that large quantities of carp would need to be consumed by these animals to affect mortality. For example, LD_{50} values reported for mammals (rats) suggest that a predatory mammal would need to consume an infeasible amount of carp tissue to affect mortality (> 10 kg of carp tissue per one kg of the predators' BW). Further, given ANT-A's short half-life and breakdown into non-toxic metabolites when delivered to water (at least in the case of Fintrol^{TM}, it seems likely the toxicant will decay quickly within the body of the carp (EPA 2007) further reducing the risk of non-target impact, though studies need to address this. Carp carcasses could be collected in the morning following an overnight application to mitigate that risk. Some predatory fishes might be impacted, but carp are often large enough to have few predators except during early development. Invertebrate communities are also likely to be impacted within application sites, but broader effects are unlikely (Dinger and Marks 2007). Evidence from streams where Fintrol^{TM} was applied show that invertebrate communities rebound quickly after the application of ANT-A (Dinger and Marks 2007). Human health concerns would also need to be carefully examined and addressed. For Fintrol^{TM} applications, the EPA rules that fish cannot be harvested for 12 months after treatment, drinking water intakes in treatment area are closed until ANT-A levels decline below 0.015 μg/L, and treated areas are restricted from access by the public during treatment and 7 days following. Outflows from systems treated with Fintrol^{TM} are also treated with potassium permanganate to minimize downstream exposure.

The use of toxic bait could help managers control carp populations in systems where conventional management schemes using simple removal techniques are unlikely to be sustainable. First, the toxic bait could target both juvenile and adult carp, since both life stages share a similar diet (Yilmaz et al. 2003). Targeting multiple life stages may be necessary to reach carp management goals in areas where carp recruitment is frequent (Lechelt and Bajer 2016). Since ANT-A appears to be undetectable to fish (Marking 1992), carp are not likely to avoid the bait, and treatment efficiency might be relatively consistent with each application. This is of high practical importance because conventional control schemes, such as removal with nets, often result in reduced efficiency over time due to strong avoidance behaviors (Hunter and Wisby 1964). Nevertheless, future studies should determine the possibility of developing avoidance behaviors due to sub-lethal exposure, which is an important unknown. Biological realism of tests used to assess the efficacy and specificity of toxic baits that incorporate ANT-A also needs to increase. Future experiments should be conducted in larger, more natural systems and need to incorporate a larger diversity of native fishes. Economic factors also need to be examined in comparison to traditional control methods. Currently, the cost of ANT-A is high (approximately $15 per one adult carp) due to limited availability and limited demand, but it is likely to decrease rapidly if this control strategy was popularized. Other aspects, such as the production of pellets, appear to be relatively simple and could be easily scaled-up. While the use of toxic pellets might have its limitations in large and open ecosystems (e.g. the Murray-Darling in Australia or the Mississippi in North America), we believe that this approach could offer new and practical management solutions in smaller and more isolated ecosystems, such as lakes and reservoirs.

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M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 4.2: Common carp management using biocontrol and toxins: Phase II
SUBPROJECT MANAGER: Przemek Bajer
AFFILIATION: University of Minnesota
MAILING ADDRESS: 135 Skok Hall, 2003 Upper Buford Circle
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WEBSITE: https://fwcb.cfans.umn.edu/personnel/przemek-bajer
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $406,000
AMOUNT SPENT: $348,913
AMOUNT REMAINING: $57,087

Sound bite of Project Outcomes and Results

This project found that bluegill sunfish can reduce production of carp fry by 8-fold in shallow lakes. It also found that corn-based food pellets that contain a toxin might be used to selectively target carp with little risk to native fish. Both of these are promising strategies for carp control.

Overall Subproject Outcome and Results

This project aimed to test new management tools for the common carp, Minnesota's most abundant invasive fish. We used a whole lake experiment to test if bluegill sunfish can reduce production of carp fry in shallow lakes (Activity 1). We also used a series of lab, pond and lake experiments to test if corn-based food pellets that contain a toxin can be used to selectively target carp without harming native fish (Activities 2, 3, 4). Activity 1 (bluegill experiment in 6 small lakes) showed that bluegills can suppress the production of carp fry in shallow lakes by 8-fold. Thus, maintaining healthy bluegill populations in lakes would serve as an important biocontrol strategy for carp in Minnesota.

Activities 2, 3, and 4 showed that common carp readily consume corn pellets that contain a toxin (Antimycin-A, ANTA) and cannot distinguish between pellets with or without the toxin. Further, in a pond experiment with carp and three native species (white sucker, bluegill, yellow perch), only carp ate the toxic pellets and perished. Finally, in a natural lake experiment where we tagged nearly 500 carp and 900 native fish, only carp were attracted to corn-based pellets (we did not use toxin in the lake experiment). This was further verified using underwater cameras. Overall, corn-based food pellets appear to be very powerful and relatively species-specific attractant for carp. Toxins, such as ANTA, could be incorporated into such pellets to target carp. Our work also showed that corn (without toxin) can be used as bait to train carp to form large feeding aggregations that could be targeted using simpler and safer means than toxins, such as nets.

Future directions might include: 1) Focusing on risks and costs associated with using corn-based pellets that contain ANTA or other toxins to control common carp, 2) Focusing on how baiting with corn can be used to induce large feeding aggregations of carp than could be removed with nets. This is being addressed in Phase III.

Subproject Results Use and Dissemination

Two manuscripts have been published:

Poole, J. R., Sauey, B. W., Amberg, J. J., & Bajer, P. G. (2018). Assessing the efficacy of corn-based bait containing antimycin-a to control common carp populations using laboratory and pond experiments. *Biological Invasions*, *20*(7), 1809-1820.

Poole, J. R., & Bajer, P. G. (2019). A small native predator reduces reproductive success of a large invasive fish as revealed by whole-lake experiments. *PloS one*, *14*(4), e0214009.

One manuscript has been submitted for publication:

Hundt, P. J., Amberg, J. J., Sauey, B. W., & Bajer, P. G. 2019. Toward a new Common Carp (*Cyprinus carpio*) management tool: Laboratory and mesocosm experiments testing a species-specific corn-based bait containing a toxin. Submitted to Management of Biological Invasions

One manuscript is in preparation:

Hundt, P.J, Bajer, P. G. Can corn-based food pellets be used to selectively induce feeding aggregation of invasive fish, Common Carp (*Cyprinus carpio*), in a natural lake? To be submitted for Fisheries Management and Ecology

Presentations:

Poole, J.R., B.W. Sauey, J.J. Amberg, and P.G. Bajer. (2017). Controlling common carp through biocontrol and species-specific toxin delivery. Contributed paper presented at annual meeting of the Minnesota Chapter of the American Fisheries Society. Saint Cloud, MN. February 22, 2017.

Poole, J.R., B.W. Sauey, J.J. Amberg, and P.G. Bajer. (2017). Exploiting Dietary Differences to Develop Species-Specific Control of Common Carp Using Toxic Food Pellets. Contributed paper presented at annual National meeting of the American Fisheries Society. Tampa, FL. August 22, 2017.

Poole, J.R., B.W. Sauey, J.J. Amberg, and P.G. Bajer. (2017). Exploiting Dietary Differences to Develop Species-Specific Control of Common Carp Using Toxic Food Pellets. Contributed paper to be presented at annual International Conference for Aquatic Invasive Species. Fort Lauderdale, FL. October 23, 2017.

Poole, J.R., B.W. Sauey, J.J. Amberg, and P.G. Bajer. (2017). Control of common carp through species-specific toxin delivery. Poster presented at the Minnesota Aquatic Invasive Species Research Center Showcase. Saint Paul, MN. September 13, 2017.

Poole, J.R. and P.G. Bajer. (2017) Control of common carp through biocontrol and species-specific toxin delivery. Friday Noon Seminar Presentation at the University of Minnesota, Twin Cities. Saint Paul, MN. November 11, 2017.

Hundt PJ and Bajer PG. Toward a new common carp management tool: Testing species-specific corn-based toxic bait. UMISC - NAISMA Joint Conference, October 2018, Rochester, Minnesota

Hundt PJ and Bajer PG. New common carp management techniques: Selective toxins and Whooshh. 2018 MAISRC showcase. St. Paul, MN. PowerPoint available:
https://www.maisrc.umn.edu/files/maisrcshowcasesept2018publicpptx

2013 Project Abstract
For the Period Ending December 31, 2016

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project 5: Developing and evaluating new techniques to selectively control invasive plants phase I A: manipulating sunfish to enhance milfoil weevils

PROJECT MANAGER: Raymond M Newman

AFFILIATION: University of Minnesota – Minnesota Aquatic Invasive Species Research Center

MAILING ADDRESS: Fisheries, Wildlife and Conservation Biology, University of Minnesota, 2003 Upper Buford Circle

CITY/STATE/ZIP: St. Paul, MN 55108

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $194,415
Amount for this Activity: $167,080

Overall Project Outcomes and Results

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most widespread and problematic invasive aquatic plants in Minnesota. Approaches to improve its management are needed to reduce economic and ecological costs of invasive control. We focused on assessing factors that limit biological control of Eurasian watermilfoil by the native milfoil weevil and other herbivores.

Enclosure experiments to assess the effect of sunfish predation on herbivore and milfoil abundance were largely unsuccessful. Weevil populations developed in the enclosures but there were no differences in weevil or milfoil abundance due to fish stocking. We failed to recover stocked fish from the enclosures and suspect that predation by herons removed the fish. Realistic enclosure experiments in natural lakes may not be feasible and experimental manipulations might be better conducted in small natural or artificial ponds or in large tanks.

We assessed herbivore abundance in metro lakes and found milfoil weevils in 12 of the 19 lakes surveyed. Herbivore abundance was higher in 2015 than 2016, but abundance during both years was lower than some prior years. Only 1 weevil was found in over 450 sunfish stomachs examined, in part due to low milfoil weevil density in many lakes. Milfoil weevil abundance was negatively correlated (r=-0.44) with sunfish abundance; lakes with high sunfish populations (> 50 sunfish/trapnet) will likely not support sufficient herbivore populations and biological control should not be considered in these lakes until sunfish are reduced.

However, some lakes with low sunfish populations also have low herbivore densities and factors other than sunfish are apparently limiting herbivores and biocontrol in these lakes. Possible limiting factors include lack of access to shoreline overwinter habitat, extensive mechanical harvesting or herbicidal control, and poor water or plant quality. Further work that also accounts for environmental variability is needed to identify factors limiting milfoil herbivores and biocontrol.

Project Results Use and Dissemination

Information on milfoil ecology and biological control has been provided on the MAISRC website and twice at the MAISRC showcase. A summary of the project was presented at the Upper Midwest Invasive Species Conference in La Crosse, WI. We provided overviews of our work to Ramsey-Washington Lake Association and the Minnesota Invasive Species Advisory Council.

Assessment of factors affecting the biological control of Eurasian watermilfoil

Final Report to the Minnesota Aquatic Invasive Species Research Center
ENRTF Phase I Project: Developing and evaluating new techniques to selectively control invasive plants: Activity 2 manipulating sunfish to enhance milfoil weevils

Raymond M. Newman
With assistance and input from Adam R. Kautza and Thomas J. Ostendorf
Department of Fisheries, Wildlife and Conservation Biology
University of Minnesota
St. Paul, MN 55108

Abstract:
Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most widespread and problematic invasive aquatic plants in Minnesota. Approaches to improve its management are needed to reduce economic and ecological costs of invasive control. We focused on assessing factors that limit biological control of Eurasian watermilfoil by the native milfoil weevil and other herbivores.

Enclosure experiments to assess the effect of sunfish predation on herbivore and milfoil abundance were largely unsuccessful. Weevil populations developed in the enclosures but there were no differences in weevil or milfoil abundance due to fish stocking. We failed to recover stocked fish from the enclosures and suspect that predation by herons removed the fish. Realistic enclosure experiments in natural lakes may not be feasible and experimental manipulations might be better conducted in small natural or artificial ponds or in large tanks.

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However, some lakes with low sunfish populations also have low herbivore densities and factors other than sunfish are apparently limiting herbivores and biocontrol in these lakes. Possible limiting factors include lack of access to shoreline overwinter habitat, extensive mechanical harvesting or herbicidal control, and poor water or plant quality. Further work that also accounts for environmental variability is needed to identify factors limiting milfoil herbivores and biocontrol.

Introduction:

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most troublesome aquatic weeds in North America (Smith and Barko 1990). Chemical control of Eurasian watermilfoil with 2,4-D or triclopyr (Cason and Roost 2011, Netherland and Jones 2015) and fluridone (Wagner et al. 2007) can be effective at controlling the plant for several years, often with few negative effects on native plants. However, herbicide treatments are expensive, often need to be repeated every several years and can cause significant negative effects on native plant communities and systems (Wagner et al. 2007, Valley et al. 2006, Cason and Roost 2017. Furthermore, some stakeholders object to chemical treatments and desire different approaches. This led to an interest in biological control with herbivorous insects (Creed and Sheldon 1995, Sheldon and Creed 1995) and the most promising agent is the native milfoil weevil *Euhrychiopsis lecontei* (Newman 2004).

The milfoil weevil is native to North America (Creed 1998); its natural host plants were likely the native northern watermilfoil (*M. sibiricum*) and other native watermilfoils such as *M. verticillatum* (Solarz and Newman 2001). The milfoil weevil captured Eurasian watermilfoil as a new and preferred host when it was introduced to North America. Extensive host range testing indicated that the milfoil weevil is specialist on plants within the watermilfoil (*Myriophyllum*) genus (Solarz and Newman 2001, Sheldon and Creed 2003, Newman 2004) but that the insect performs best on the exotic Eurasian watermilfoil and poorest on the native northern watermilfoil; performance on a hybrid of the two species is better than on the native and may better (Borrowman et al. 2015) or worse than on the Eurasian variety (Roley and Newman 2006). The milfoil weevil spends the summer submersed on milfoil plants, completing all 4 life stages (egg, larva, pupa and adult) underwater and producing 3 to 4 generations before the adults move to shore to overwinter in leaf litter (Newman et al. 2001). In the spring, adults return to the lake and begin to lay eggs. Suitable overwinter habitat (dry sites with duff near shore) is required to sustain weevil populations (Thorstenson et al. 2013). In-lake densities have been related to amount of natural shoreline (Jester et al. 2000), but summer in-lake factors appear more important to weevil populations when shoreline habitat is available (Newman et al. 2001). The native milfoil weevil is widespread in Minnesota and North America (Creed 1998, Tamayo et al. 1999) and likely occurs naturally in most lakes that have Eurasian or northern watermilfoil (Borrowman et al. 2014).

The milfoil weevil has caused declines of Eurasian watermilfoil under controlled conditions (Creed and Sheldon 1995, Sheldon and Creed 1995, Newman et al. 1996) and in a number of lakes (Sheldon and Creed 1995, Newman and Biesboer 2000, Newman 2004), although there is considerable variability in effects across lakes (Reeves et al. 2008, Reeves and Lorch 2012). Summer-long densities of 0.25 to 0.5 weevils per stem may be sufficient to control the plant and densities > 1/stem have resulted in control (Newman 2004). In many lakes weevil populations do not reach sufficient density to control the plant (reviewed in Newman 2004). Identification and amelioration of factors limiting populations would enhance chances for successful control.

Work in Minnesota and elsewhere suggested that predation by sunfish (*Lepomis spp.* but, primarily bluegill, *L. macrochirus*, and its hybrids) can limit herbivore and milfoil weevil populations and thus its control of Eurasian watermilfoil. In experimental manipulations, weevil and other herbivore densities were reduced in the presence of sunfish (Ward and Newman 2006) and in a comparison across 11 lakes, milfoil weevil densities were negatively related to sunfish relative abundance (Ward and Newman 2006). Sunfish densities > 25-30 per trapnet can limit herbivore abundance and sunfish densities > 50 per trapnet allow few herbivores. In Minnesota and elsewhere, sunfish densities appear lower in lakes where herbivorous insects are controlling Eurasian watermilfoil (Newman 2004, Parsons et al. 2011, Parsons 2012). EnviroScience has stocked over 200 lakes in the US and Canada. Although they purport good success from stocking, the published evidence is equivocal (Reeves et al. 2008) and effective methods to reduce predation by fish would enhance the success of both natural and stocked (augmented) populations of milfoil weevils and other herbivores. If biological control with insects is to be operationally successful, management to reduce overabundant or stunted sunfish populations may be needed.

Overabundant and stunted sunfish are a major problem in Minnesota lakes (Drake et al. 1997, Shroyer et al. 2003, Jacobson 2005) and reducing sunfish density is not a trivial task. It is likely that a combination of predator enhancement and regulations to reduce harvest of large sunfish is required (Beard and Essington 2000, Aday et al. 2006), perhaps along with direct reduction by trapnetting or tournaments. However, if sunfish densities can be reduced and sunfish size-structure enhanced, this could create a quality sunfish fishery while also enhancing biological control of Eurasian watermilfoil.

To assess the potential to enhance biological control of Eurasian watermilfoil, enclosure experiments and field surveys were conducted. Enclosure experiments were conducted to determine if sunfish limit herbivore abundance and control of milfoil. To determine factors limiting herbivore abundance in lakes and the extent of sunfish consumption of herbivores, twenty lakes were surveyed for milfoil, herbivores and sunfish, most in both years. Sunfish stomach contents were assessed in ten of these lakes. These results were used to propose further study.

Methods

Enclosure experiments

Enclosure experiments were conducted in summer 2015 and 2016 in Cedar Lake (DOW 270039) and Peltier Lake (020004). Sites with Eurasian watermilfoil beds that also had some native plants in water depths between 1 and 2m were located in each lake.

In July 2015 three enclosures were installed in each lake. The vinyl impermeable enclosures (2.4m deep x 38m circumference) enclosed an area of approximately 100m^2 with sides embedded in sediment with rebar "staples" and a lead line and held upright by floats along the top surface. The enclosures were allowed to equilibrate for a week before pre-treatment plant and water quality data were collected (see below). At the same time, two adjacent and similar areas were selected and marked to be used as open controls. Each of the three enclosures in a lake was randomly assigned a fish treatment level and then 0, 5 (0.05/m^2) or 20 (0.2/m^2) bluegill sunfish (collected from same lake) were stocked into the enclosures. Fish in Cedar Lake ranged from 100 to 160mm in length (22-80g) and in Peltier from 110 to 180 mm (30-150g) and each fish had a PIT tag implanted before stocking.

Prior to fish stocking weevil surveys were conducted and plant biomass and water quality measures were assessed within the enclosures and the control plots. Weevil surveys were conducted by collecting 8 milfoil stems (top 50 cm) from each of 6 locations (samples) within an enclosure or control area. Each sample of 8 stems was kept in a Ziploc bag until processing in the laboratory. Plant biomass was collected from 5 sites within each enclosure with the rotating rake method (Johnson and Newman 2011). Samples were kept in sealable bags in a cooler until they could be processed in the lab. Within each enclosure Secchi depth was measured as was transparency in a Secchi tube. Dissolved oxygen, temperature and light (PAR) profiles were measured with readings at the surface, 0.5m and 1m.

Weevils survey samples were counted for stems and meristerms and examined under 3x magnification for eggs, larvae, pupae and adult weevils, which were enumerated and preserved. Other herbivores such as the lepidopterans *Acentria* and *Parapoynx* were also enumerated. Results for each sample were expressed as numbers per stem and samples were averaged within an enclosure. Plant samples were kept in a cooler at 4 ˚C until processed, when they were sorted, identified to species, weighed, dried (65 ˚C for 2 days) and reweighed. Biomass (g dry/m^2) was determined for each species and for Eurasian watermilfoil and all native taxa combined.

Biomass samples and water quality data were collected at the beginning, middle and end of the experiment and weevil surveys were conducted once per month. We attempted to retrieve fish at week three and thereafter using a combination of angling and trot lines as well as visual observation. The experiment ended in early October 2015.

We repeated these experiments in 2016 with an earlier start. Enclosures were installed in both lakes in June and randomly stocked the following week with 0, 5 or 20 sunfish. Fish were slightly bigger in 2016 with a range of 120-160 mm in Cedar and 120 to 200mm in Peltier. We spent more time securing the enclosures, using larger pins and a diver to check the seal. We also staked some of the enclosure to reduce escape of fish. Using the methods of 2015, we took plant biomass samples (5 per enclosure or control) at the beginning, middle and end of the experiment, measured water quality 4 times during the experiment and conducted weevil surveys once per month (6 samples per enclosure).

Field surveys

To further define the relationship between sunfish and herbivores, surveys of lakes for milfoil weevils and other herbivores were conducted and results compared to estimates of sunfish density. Point intercept surveys of aquatic macrophytes were conducted on a subset of lakes to quantify milfoil and native plant occurrence. Lakes were selected that had recent or planned fisheries surveys to get estimates of sunfish abundance and lakes that were known or recommended by contacts to have had abundance milfoil populations in the past.

In 2015 fourteen lakes were surveyed and in 2016 eighteen lakes were surveyed (Table 1). Over half the lakes were sampled two or more times each year. For each survey, approximately 30 sample stations were located at each lake, and stations were typically distributed around the lake on 10 transects with stations near shore (shallow, ≤1m), midway to edge of bed (1.5-2.0 m) and the outer edge of the bed (ca. 3m). At each station, 8 milfoil stems (top 50 cm of plant) were collected and placed into a sealable plastic bag. Samples were returned to the laboratory and kept refrigerated until they were processed (usually within 24h and always within 48h). For each sample, stems and meristems were counted as were eggs, larvae, pupae and adult weevils and lepidopteran larvae, which were preserved in 80% ETOH. Plants were examined under 3x magnification and if needed under a dissecting scope to verify eggs and larvae. Herbivore abundance is expressed as number per stem averaged over the number of samples collected.

Fish were collected for stomach samples from 6 lakes in 2015 and 10 lakes in 2016. In 2015 most fish were collected by electrofishing, whereas in 2016 fish were also collected by trapnet and angling. Stomach contents of each captured fish were obtained via gastric lavage and the contents were preserved in 80% ETOH. Stomach contents were later examined under a dissecting microscope (4-25X) and herbivores enumerated along with general groups of taxa (e.g., zooplankton, snails, chironomids, amphipods, etc.).

Plant communities were surveyed with point intercept sampling on 7 lakes to provide background for future study but those results are not presented here.

Results and Discussion

Enclosures

The enclosures stayed in place in all lakes but may have shifted slightly in 2015 after a large storm; the extra measures in 2016 appeared to eliminate any movement. Water clarity declined in both lakes throughout the summer in both 2015 and 2016 to 0.3-0.8m in July and August in Peltier and 1m in Cedar. Clarity was somewhat variable among enclosures in 2015 but in 2016 was very similar to in-lake clarity. Temperatures within the enclosures were slightly higher than outside on occasion but never exceeded 29 ˚C and dissolved oxygen was generally above 8 mg/L, although it was occasionally <4mg/L at the bottom of the Peltier enclosures. Environmental conditions did not appear limiting.

Plant biomass was variable among enclosures, lakes, and years even though we attempted to place the enclosures and controls in similar density beds each year (Table 2). Biomass of native plants and milfoil was generally higher in 2016 than 2015 and Cedar milfoil biomass was generally higher than Peltier in both years. In Cedar the native biomass was dominated by coontail. In Peltier, coontail was the most common native but Elodea was often nearly as abundant. Other taxa were present at low abundance and often sporadic but Peltier had greater diversity than Cedar.

There was no apparent effect of enclosure or fish treatment on milfoil or native plant biomass in either lake or either year (Table 2). Milfoil biomass generally declined over the season in all treatments, possibly along with decreases in clarity but there was no pattern or effect of treatment on the changes. Weevil densities were also highly variable although densities in Cedar in 2016 were extremely low in the lake and enclosures (only 1 weevil was found). In 2015 weevil densities increased in Cedar plots from <0.05 in July to > 0.27 in August and densities were highest in the no and low fish treatments and lowest in the high fish treatment and controls (Table 3). Density remained high in the low fish treatment but not in the no fish treatment. In contrast, weevil densities in Peltier decreased from a high of 0.2-0.6 in July to few in August and September. Similarly in 2016 densities in Peltier were highest in June and July with few weevils in August. There was no clear relationship to fish stocking density.

Table 1. Lakes surveyed for herbivores in 2015 and 2016 with lake Division of Waters ID number, area (ha), year of most recent DNR fisheries survey, mean number of sunfish (all *Lepomis spp.*) per trapnet found in the survey and years of weevil surveys.

Lake	DOW ID	Area (ha)	Fish Survey	Sunfish/net	Weevils Sampled
Auburn	10004400	114	2012	78	2015-2016
Cedar	27003900	66	2009	58	2015-2016
Cenaiko	02065400	12	2009	16	2015-2016
Centerville	02000600	192	2013	40	2015-2016
Christmas	27013700	108	2013	34	2015-2016
Firemen's	10022600	3	2010	38	2016
Minnetonka	27013300	5751			
Smiths Bay					2015-2016
Veterans Bay					2015-2016
Mitchell	27007000	46	2015	71	2015-2016
Otter	02000400	122	2013	26	2015-2016
Peltier	02000300	123	2013	5	2015-2016
Pierson	10005300	120	2013	23	2016
Rebecca	27019200	106	2011	271	2015
Riley	10000200	120	2015	12	2015-2016
Round	27007100	12	2015	17	2016
Schmidt	27010200	15	1990	22	2016
Steiger	10004500	67	2014	86	2015-2016
Susan	10001300	35	2014	19	2015-2016
Zumbra	10004100	94	2015	31	2016

Table 2. Plant biomass (g dry/m^2) of Eurasian watermilfoil (MSPI), native plants and all plants and number of taxa in enclosures by lake, date and fish treatment (C= open control).

Peltier	Treat	MSPI	Native	Total Biomass	N/sample
7/23/15	No Fish	9.7	333.7	343.4	3
9/2/15	No Fish	.	.	.	.
10/2/15	No Fish	26.7	74.9	101.7	3
7/23/15	Low Fish	21.3	598.5	619.9	3
9/2/15	Low Fish	21.1	180.7	201.8	3
10/2/15	Low Fish	12.6	307.7	320.3	3
7/23/15	High Fish	41.1	472.0	513.1	3
9/2/15	High Fish	22.0	624.1	646.1	3
10/2/15	High Fish	33.6	282.4	316.1	3
7/23/15	C1	71.2	598.5	669.7	3
9/2/15	C1	30.9	180.7	211.6	3
10/2/15	C1	36.6	307.7	344.3	3
7/23/15	C2	33.2	598.5	631.7	3
9/2/15	C2	3.5	180.7	184.2	3
10/2/15	C2	16.2	307.7	323.9	3

Cedar	Treat	MSPI	Native	Total Biomass	Taxa
7/30/15	No fish	1132.4	507.8	1640.2	3
9/4/15	No fish	141.4	125.4	266.8	3
10/2/15	No fish	161.9	106.1	267.9	2
7/30/15	Low Fish	989.3	582.7	1572.0	2
9/4/15	Low Fish	217.4	148.7	366.1	2
10/2/15	Low Fish	111.9	110.4	222.3	3
7/30/15	High Fish	695.3	632.6	1327.9	2
9/4/15	High Fish	90.7	280.4	371.1	3
10/2/15	High Fish	200.4	233.4	433.8	2
7/30/15	Control 1	1765.7	3580.9	5346.6	4.0
9/4/15	Control 1	190.8	486.8	677.6	3
10/2/15	Control 1	143.3	174.8	318.1	3
7/30/15	Control 2	928.2	643.8	1572.0	3
9/4/15	Control 2	372.4	216.8	589.3	3
10/2/15	Control 2	251.0	255.2	506.2	3

Table 2. continued.

Peltier	Treatment	MSPI	NATIVE	TOTAL BIOMASS	TAXA/SAMPLE
6/29/16	No Fish	13.6	16.2	37.8	4
7/27/16	No Fish	2.1	1288.7	1327.5	4.8
8/24/16	No Fish	10.4	561.9	589.0	4.8
6/29/16	Low Fish	47.5	259.2	336.9	5.2
7/27/16	Low Fish	45.8	395.5	456.7	5.6
8/24/16	Low Fish	58.2	306.8	366.3	4.8
6/29/16	High Fish	2.8	220.3	244.9	5.4
7/27/16	High Fish	8.2	490.2	523.5	4.8
8/24/16	High Fish	3.0	273.2	279.5	3.2
6/29/16	Control 1	40.2	75.9	120.5	5
7/27/16	Control 1	2.4	205.5	211.5	3.2
8/24/16	Control 1	13.1	505.8	420.2	4.25
6/29/16	Control 2	0.0	75.9	94.4	4.2
7/27/16	Control 2	0.0	203.9	209.3	3.2
8/24/16	Control2	0.0	185.5	188.0	1.8

Cedar	Treatment	MSPI	Natives	Total Biomass	Taxa/Sample
6/30/16	No Fish	120.4	299.0	419.9	2.6
7/26/16	No Fish	42.9	196.9	240.1	2.8
8/26/16	No Fish	53.9	652.3	706.2	3
6/30/16	Low Fish	411.7	281.7	698.2	4.4
7/26/16	Low Fish	258.8	220.0	480.0	4
8/26/16	Low Fish	289.3	363.0	652.3	3.8
6/30/16	High Fish	214.0	235.0	453.6	2.8
7/26/16	High Fish	5.9	182.3	188.9	1.8
8/26/16	High Fish	11.3	471.0	482.4	2.6
6/30/16	Control 1	132.4	139.1	272.8	4
7/26/16	Control 1	34.5	66.9	101.9	2.4
8/26/16	Control 1	207.3	429.4	637.1	2.8
6/30/16	Control2	585.2	323.6	917.9	3.4
7/26/16	Control2	53.4	111.7	165.7	1.6
8/26/16	Control2	88.1	436.4	524.7	2.4

Table 3. Milfoil weevil densities (total of all life stages/stem) in enclosures (fish density, none, low or high) and control plots (C1 and C2) in 2015 and 2016 at Peltier and Cedar Lakes.

	Weevils (total/stem)			
Peltier	7/17/15	8/24/15	9/14/15	10/3/15
None	0.606	0.043	0.068	0.063
Low	0.194	0.048	0.043	0.000
High	0.533	0.086	0.000	0.000
C1	0.421	0.083	0.000	0.000
C2	0.265	0.042	0.109	0.000
Cedar	7/30/15	8/26/15	9/15/15	10/3/15
None	0.091	0.596	0.000	0.000
Low	0.000	0.674	0.426	0.103
High	0.000	0.271	0.128	0.143
C1	0.043	0.022	0.022	0.000
C2	0.040	0.167	0.000	0.024
Peltier	6/29/16	7/20/16	8/24/16	
None	0.146	0.417	0.000	
Low	0.208	1.039	0.339	
High	0.033	0.224	0.000	
C1	0.361	0.707	0.000	
C2	0.049	.	0.000	
Cedar	6/30/16	7/19/16	8/26/16	
None	0	0	0	
Low	0	0	0.021	
High	0	0	0	
C1	0	0	0	
C2	0	0	0	

Despite multiple efforts with traps, angling and trot lines, starting at the midpoint of each experiment as well as the end, we were not able to retrieve any of the stocked fish from the enclosures. Snorkeling observations (though limited by the poor clarity) also failed to reveal fish large enough to have been stocked. Observations in 2016 lead us to suspect that herons, which would perch on the floating rims of the enclosures, consumed many if not all of the stocked fish. Thus it is likely that we did not sustain a differential fish density and predation pressure which would also explain the lack of differences in weevil density or milfoil or plant biomass. The declining and low milfoil biomass in Peltier enclosures in 2016 could be due to the high abundance of weevils in July but the disappearance of weevils in August is puzzling. Similarly, the general decline of milfoil in Cedar enclosures in 2015 could be related to the high density of weevils found at mid-experiment, but differences among enclosures do not appear related to weevil density.

Conducting good enclosure experiments is a challenge; it is difficult to find sites with high milfoil biomass that include native plants and that are similar across locations. For example, in Peltier the sites we used in 2015 had almost no milfoil in 2016 so sites on the other side of the lake needed to be used. Year to year differences in water clarity and changes in clarity can also be important and the poor clarity in Peltier and in 2016 in Cedar likely affected plants as well as inhibited our ability to monitor the fish populations. If heron predation is a factor, ways to prevent predation need to be devised. Mesh covers pose their own problems. For future experiments, sites in lakes with better clarity may be more suitable and an even earlier start of the experiment may be good. Alternatively, it may be more effective to conduct these experiments in artificial or natural ponds or in very large ($>25m^2$) deep ($\geq 1.5m$) tanks.

Field Surveys

Milfoil weevils were found in 12 of the 19 lakes surveyed (Tables 4 and 5). Aquatic lepidopterans were found in 8 lakes though never as abundant as milfoil weevils. As is typical, weevil eggs were most common, followed by larvae and adults. Weevil abundance was generally higher in 2016 than 2015 and weevils were not found in several lakes in 2016 where they had been present in 2015. Highest densities (0.3-0.8/stem) were found in Centerville, Peltier, and the bays of Lake Minnetonka. Weevils were relatively abundant in Auburn and Susan in early 2015 but were not found in surveys in later 2016. Densities both years, but particularly in 2016, were lower than in years past and many previous studies (Newman 2004) and no lakes attained a density of 0.5/stem or sustained a density ≥ 0.25/stem throughout the summer.

Total weevil density was negatively related to sunfish density (sunfish per trapnet set; Fig. 1) with a correlation of -0.44, a marginally significant correlation ($p = 0.066$ for 1 tailed test). It is clear that few weevils are found in lakes with sunfish densities greater than 70 sunfish per trapnet but there are also lakes with no or few weevils despite a low sunfish catch per trapnet (<20/net). At high sunfish densities, weevils may be limited by sunfish predation if other factors are not limiting but other factors may be limiting weevils in some lakes that have low sunfish densities. Currently, it is not clear what those factors may be, but they could include overwinter habitat, water temperature, harvesting or herbicidal control. Both mechanical harvesting (Newman and Inglis 2009) and herbicidal control (Knight and Havel 2016) have been shown to limit weevil populations.

To determine the degree of predation on milfoil weevils by sunfish we examined the stomachs of over 450 sunfish from 10 lakes (Table 6). We found 1 adult milfoil weevil in these samples (Peltier 2016). Although some samples were from open water and contained primarily zooplankton (Table 6) many stomachs contained snails, amphipods and chironomids that are typically associated with plants. This is a much lower occurrence of milfoil weevils than found by Sutter and Newman (1997), but may in part be explained by the relatively low densities of weevils we encountered during our weevil surveys. If weevils are rare they will not likely be found in the diet. It is possible that sampling earlier in the season would reveal more predation but Sutter and Newman found equally high rates in August compared to June and July.

Table 4. Weevil and lepidopteran density (N/stem and 2SE) of all life stages in surveys in 2015. Number of samples is given beneath the lake name.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Lepidopt
Auburn	6/2/15	0.048	0.012	0	0.011	0.071	0
27	2SE	0.040	0.013	0	0.013	0.055	0
Auburn	8/31/15	0	0	0	0	0	0
27	2SE	0	0	0	0	0	0
Cedar	6/11/15	0	0	0	0	0	0
30	2SE	0	0	0	0	0	0
Cenaiko	6/25/15	0	0	0	0	0	0
26	2SE	0	0	0	0	0	0
Cenaiko	8/20/15	0	0	0	0	0	0
26	2SE	0	0	0	0	0	0
Centerville	7/15/15	0.150	0.030	0.008	0.119	0.307	0
24	2SE	0.213	0.029	0.017	0.071	0.225	0
Christmas	6/15/15	0.015	0.008	0	0.006	0.029	0
46	2SE	0.017	0.009	0	0.009	0.020	0
Christmas	8/11/15	0.024	0	0.003	0.050	0.076	0.003
50	2SE	0.022	0	0.005	0.031	0.041	0.005
Mitchell	6/8/15	0	0	0	0	0	0
31	2SE	0	0	0	0	0	0
Mitchell	7/20/15	0	0	0	0	0	0
28	2SE	0	0	0	0	0	0
Mitchell	8/21/15	0	0	0	0	0	0
28	2SE	0	0	0	0	0	0
Otter	7/20/15	0.179	0.004	0	0.031	0.213	0.016
27	2SE	0.123	0.007	0	0.033	0.135	0.015
Peltier	6/23/15	0.060	0.004	0	0.087	0.151	0.004
30	2SE	0.064	0.008	0	0.058	0.078	0.008
Rebecca	6/19/15	0	0	0	0	0	0
30	2SE	0	0	0	0	0	0
Riley	6/1/15	0.061	0.018	0	0.009	0.088	0.076
36	2SE	0.055	0.017	0	0.012	0.062	0.146
Riley	7/29/15	0.079	0.004	0	0.031	0.115	0
28	2SE	0.074	0.009	0	0.024	0.094	0
Riley	8/31/15	0.149	0.093	0.005	0.026	0.273	0.003
30	2SE	0.148	0.069	0.012	0.031	0.222	0.007
Smith's Bay	6/29/15	0	0.011	0	0.011	0.022	0
39	2SE	0	0.013	0	0.018	0.024	0
Smith's Bay	8/17/15	0.025	0.004	0.009	0.034	0.071	0
32	2SE	0.025	0.008	0.013	0.021	0.047	0
Steiger	6/9/15	0	0	0	0	0	0
27	2SE	0	0	0	0	0	0
Susan	6/3/15	0.003	0.004	0	0	0.007	0
27	2SE	0.005	0.008	0	0	0.010	0
Susan	7/30/15	0.102	0	0	0.004	0.106	0
29	2SE	0.091	0	0	0.009	0.091	0
Susan	9/2/15	0	0.005	0	0.010	0.010	0
26	2SE	0	0.010	0	0.013	0.019	0
Vet's Bay	7/21/15	0.154	0.033	0.006	0.032	0.224	0
35	2SE	0.098	0.035	0.011	0.022	0.116	0
Vet's Bay	8/25/15	0.058	0	0	0.033	0.091	0
35	2SE	0.061	0	0	0.028	0.073	0

Table 5. Weevil and lepidopteran density (N/stem and 2SE) of all life stages in surveys in 2016. Number of samples is given beneath the lake name.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Lepidopt
Auburn	6/7/16	0	0	0	0	0	0
30	2 SE	0	0	0	0	0	0
Auburn	7/18/16	0	0	0	0	0	0
33	2 SE	0	0	0	0	0	0
Cedar	6/1/16	0	0	0	0	0	0
32	2 SE	0	0	0	0	0	0
Cedar	8/16/16	0	0	0	0	0	0
31	2 SE	0	0	0	0	0	0
Cenaiko	6/7/16	0	0	0	0	0	0
26	2 SE	0	0	0	0	0	0
Cenaiko	7/25/16	0	0	0	0	0	0
26	2 SE	0	0	0	0	0	0
Centerville	6/8/16	0.006	0.005	0	0	0.011	0
25	2 SE	0.011	0.010	0	0	0.015	0
Centerville	7/21/16	0.074	0	0	0.004	0.078	0.010
25	2 SE	0.082	0	0	0.008	0.083	0.014
Christmas	7/6/16	0.003	0.016	0.006	0.013	0.038	0
47	2 SE	0.006	0.014	0.008	0.011	0.023	0
Christmas	7/28/16	0.024	0	0	0.003	0.027	0
53	2 SE	0.025	0	0	0.005	0.027	0
Christmas	8/22/16	0.020	0	0	0.035	0.055	0
48	2 SE	0.022	0	0	0.045	0.055	0
Firemen's	8/24/16	0	0	0	0	0	0
28	2 SE	0	0	0	0	0	0
Mitchell	6/14/16	0	0	0	0	0	0
21	2 SE	0	0	0	0	0	0
Mitchell	7/13/16	0	0	0	0	0	0
22	2 SE	0	0	0	0	0	0
Mitchell	8/17/16	0	0	0	0	0	0
8	2 SE	0	0	0	0	0	0
Otter	6/2/16	0.024	0.004	0.004	0	0.032	0
33	2 SE	0.021	0.008	0.009	0	0.023	0
Otter	7/12/16	0.008	0.013	0	0	0.021	0
32	2 SE	0.016	0.015	0	0	0.021	0
Otter	8/15/16	0.004	0.022	0	0.005	0.031	0
31	2 SE	0.008	0.037	0	0.009	0.039	0

Table 5
Continued

Peltier	5/26/16	0.101	0.150	0.021	0	0.273	0
30	2 SE	0.076	0.074	0.024	0	0.105	0
Peltier	6/27/16	0.042	0.031	0.013	0.043	0.128	0
30	2 SE	0.083	0.036	0.018	0.038	0.123	0
Peltier	8/18/16	0.099	0	0	0.004	0.104	0
28	2 SE	0.122	0	0	0.009	0.124	0
Piersons	8/2/16	0.025	0	0	0	0.025	0
32	2 SE	0.025	0	0	0	0.025	0
Riley	6/1/16	0.051	0	0	0	0.051	0
36	2 SE	0.102	0	0	0	0.102	0
Riley	7/26/16	0.063	0.034	0	0.011	0.107	0
30	2 SE	0.058	0.027	0	0.015	0.069	0
Riley	8/22/16	0.020	0	0	0	0.020	0
25	2 SE	0.024	0	0	0	0.024	0
Round	7/28/16	0.051	0.005	0	0.017	0.073	0.004
31	2 SE	0.056	0.011	0	0.020	0.061	0.008
Schmidt	8/15/16	0	0	0	0	0	0
30	2 SE	0	0	0	0	0	0
Smith Bay	7/14/16	0.102	0.006	0	0.035	0.143	0
44	2 SE	0.096	0.008	0	0.046	0.108	0
Steiger	7/25/16	0	0	0	0	0	0.005
27	2 SE	0	0	0	0	0	0.009
Susan	6/1/16	0.003	0.005	0	0	0.008	0
23	2 SE	0.006	0.010	0	0	0.011	0
Vet's Bay	7/21/16	0.185	0.012	0.002	0.009	0.209	0.003
42	2 SE	0.099	0.019	0.005	0.010	0.103	0.006
Zumbra	8/4/16	0	0	0	0	0	0
32	2 SE	0	0	0	0	0	0

Table 6. Fish sampled for stomach contents in 2015 and 2016 and dominant prey taxa for each sampling session. Only 1 milfoil weevil was found; an adult weevil in Lake Peltier in 2016.

Lake	Date	Bluegill	Pumpkinseed	DominantTaxa
Auburn	8/31/15	25	0	Zooplankton
	9/15/15	19	0	Zooplankton
	8/4/16	50	0	Amphipods
Cedar	8/4/15	29	0	Aquatic Diptera
	8/10/15	26	0	Aquatic Diptera
	7/11/16	2	3	Snails
	7/12/16	25	0	Snails
Centerville	8/11/15	26	1	Aquatic Diptera
	9/1/15	13	12	Aquatic Diptera
	8/1/16	9	4	Chironomids
Christmas	8/23/15	3	9	Snails and insects
	9/14/15	7	5	Snails and insects
	8/18/16	7	4	Chironomids
Otter	8/12/15	2	25	Snails and insects
	9/3/15	0	27	Snails and insects
	8/9/16	0	4	Chironomids
	8/17/16	5	7	Chironomids
Peltier	8/3/15	23	3	Zooplankton
	8/5/15	27	3	Zooplankton
	7/5/16	1	4	Chironomids
	7/8/16	24	0	Chironomids
Piersons	8/2/16	45	4	Amphipods
Round	8/9/16	20	0	Zooplankton
Steiger	8/3/16	49	1	Chironomids
Zumbra	8/3/16	44	6	Amphipods

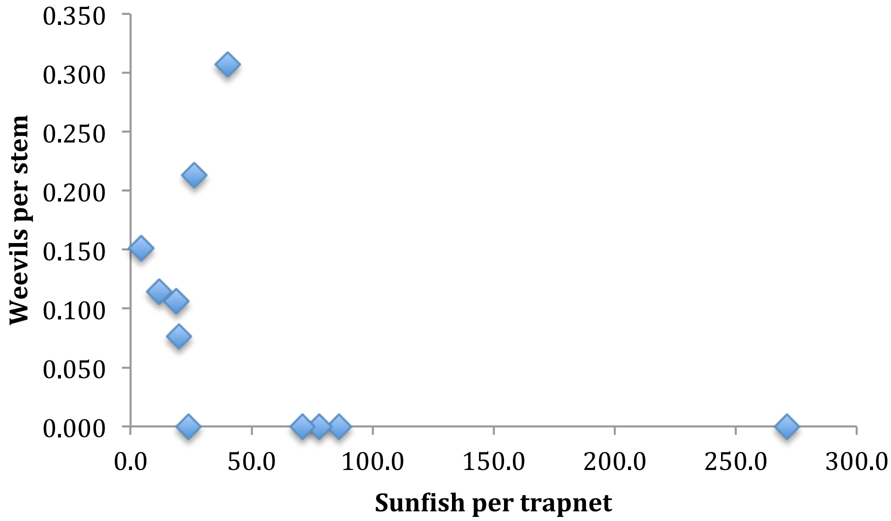

Figure 1. Relationship between number of weevils per stem (total of all life stages) and sunfish catch in survey lakes. R = -0.44

Conclusions

Lakes with high sunfish populations will likely not support sufficient herbivore populations to control milfoil and biological control should not be promoted in these lakes until sunfish are reduced. However, some lakes with low sunfish populations also have low herbivore densities and factors other than sunfish are apparently limiting herbivores and biocontrol in these lakes. Possible limiting factors include lack of access to shoreline overwinter habitat (Jester et al. 2000, Thorstenson et al. 2013), extensive mechanical harvesting (Newman and Inglis 2009) or herbicidal control (Havel et al. 2017, *in review*), and poor water or plant quality (Miller et al. 2011, Marko and Newman *in press*). These results indicate that more work is needed to assess factors limiting milfoil weevil populations. The relative importance of these factors is unknown and work that also accounts for year to environmental variability is needed to determine the importance of factors limiting milfoil herbivores and biocontrol.

Longer term data sets will be needed to help identify these factors. We will conduct a broader analysis of the data from this project in combination with previous data from 2011-2014 and a series from 1994-2004 to see if we can detect a climate or environmental signal or identify other factors that might explain variation in milfoil weevil abundance.

Acknowledgements:

Many people in addition to Dr. Adam R. Kautza and T. J. Ostendorf assisted with data collection and execution of this project: Melaney Dunne, Chloe Fouilloux, Matt Gilkay, Madison Jaske, Aislyn Keyes, Todd Meilzarek, Leo Rubenstien, and Carleigh Windhorst. Their assistance and input is greatly appreciated. The input and cooperation of the Minnesota DNR (especially Daryl Ellison, TJ Debates, and Charles Anderson) was also appreciated as was the cooperation and access provide by the Minneapolis Park and

Recreation Board (Rachel Crabb) the Rice Creek Watershed District (Matt Kocian), Eden Prairie (Leslie Stovring), Chaska and Carver County (Charlie Sawdey and Bill Monk), Three River Park District (Rich Brasch) and Anoka Parks (Jeff Perry). Steve McComas provided helpful input. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

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2013 Project Abstract
For the Period Ending December 31, 2016

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project 5: Developing and evaluating new techniques to selectively control invasive plants phase I B: factors influencing selective herbicide control of curlyleaf pondweed

PROJECT MANAGER: Raymond M Newman

AFFILIATION: University of Minnesota – Minnesota Aquatic Invasive Species Research Center

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $194,415
Amount for this Activity: $27,335

Overall Project Outcomes and Results

Curlyleaf pondweed (*Potamogeton crispus*) is one of the most widespread and problematic invasive aquatic plants in Minnesota. It sprouts from turions (winter buds) in the fall and winter and grows rapidly to the surface in the spring before senescing in early summer. Selective control can be attained with early-season herbicide treatments.

To provide an analysis of factors affecting curlyleaf abundance in untreated and herbicide-treated lakes, we collated pre-existing data from a variety of agencies and researchers; we analyzed data on curlyleaf pondweed frequency of occurrence and relative density from 60 lakes across Minnesota. The lakes had surveys conducted in May (pretreatment timing) or June (peak curlyleaf coverage) between 2006-2015; several lakes had data for all ten years. Forty-nine lakes had data for years not treated with herbicide, with one to eight years of data from each (mean of three years). Twenty-two lakes had data associated with curlyleaf pondweed herbicide treatments (one to nine years of treatment; mean of 3.8 years).

For the untreated lakes, productivity (as indicated by prior summer Secchi depth) and over-winter conditions (snow cover or ice duration) were important predictors of curlyleaf with greater curlyleaf abundance in lakes with higher productivity and milder overwinter conditions (shorter duration of ice cover and lesser snow depth). For herbicide treated lakes, consecutive years of treatment was also important; early season abundance decreased with more years of prior treatment. There were diminishing returns from repeated treatment and curlyleaf abundance can rebound quickly once treatment stops. June density and frequency appeared less affected by overwinter conditions and more by spring growing conditions and the effect of treatment that year. Mild winters will likely result in more abundant populations that spring, and managers should plan for more extensive treatments following mild winters. Repeated treatments will decrease curlyleaf frequency and abundance, but must be sustained.

Project Results Use and Dissemination

Information on curlyleaf pondweed ecology and control has been provided on the MAISRC website and at the MAISRC showcase. The results of the curlyleaf pondweed analysis were presented at the 56th Annual meeting of the Aquatic Plant Management Society in Grand Rapids, MI and a summary of the analysis was presented at the Upper Midwest Invasive Species Conference in La Crosse, WI. We provided overviews of our work to Ramsey-Washington Lake Association and the State of Waters Conference. We plan to develop and submit a manuscript on the curlyleaf pondweed responses to a peer-reviewed journal by July 2017. The data set assembled and organized will also be used by a graduate student to further assess the response of native plants to curlyleaf pondweed abundance and control.

Factors affecting the abundance and control of curlyleaf pondweed in managed and unmanaged systems: analysis of results from 60 lakes

Final Report to the Minnesota Aquatic Invasive Species Research Center
ENRTF Phase I Project: Developing and evaluating new techniques to selectively control invasive plants: Activity I factors influencing selective herbicide control of curlyleaf pondweed

Raymond M. Newman
With assistance and input from Adam R. Kautza and Thomas J. Ostendorf
Department of Fisheries, Wildlife and Conservation Biology
University of Minnesota
St. Paul, MN 55108

Abstract:

Curlyleaf pondweed (*Potamogeton crispus*) is one of the most widespread and problematic invasive aquatic plants in Minnesota. It sprouts from turions (winter buds) in the fall and winter and grows rapidly to the surface in the spring before senescing in early summer. Selective control can be attained with early-season herbicide treatments.

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Background:

Curlyleaf pondweed (*Potamogeton crispus)* is a major nuisance in Minnesota and North America and has been widespread since the early 1900s (Bolduan et al. 1994, ISP 2013). It occurs in over 750 waterbodies in Minnesota (ISP 2013). Its life history makes the plant particularly problematic (Woolf 2009). In many lakes it sprouts from turions in late summer or fall, grows until temperatures decline below 5 ˚C, and overwinters under the ice (Bolduan et al.

1994). When water temperatures warm above 10 ˚C in the spring the plant starts growing rapidly and can outcompete native plants. Surface mats are often produced along with the vegetative turions at temperatures around 25 ˚C and the plant will then senesce and decay. Poor water clarity after senescence often further inhibits native plant communities. The dormant turions persist in the sediment through summer to sprout in the fall when temperatures decline and clarity improves (Bolduan et al. 1994). Curlyleaf pondweed can be controlled with physical and mechanical methods, but regrowth is an issue (McComas and Stuckert 2000, Woolf 2009) and no selective biological controls are available (Woolf 2009).

Methods to selectively control curlyleaf pondweed with low-dose, early-season, lake-wide treatments with endothall were developed by the Army Corps (Poovey et al. 2002, Skogerboe et al. 2008). These treatments are usually conducted in late May or early June prior to peak curlyleaf growth when water temperatures are between 10 and 15 ˚C to minimize effects on native plants. Recent assessments indicate that these treatments can reduce curlyleaf abundance and turion production in the year of treatment (Johnson et al. 2012) with relatively little harm to native plants (Jones et al. 2012). However, substantial stocks of viable turions remain even after three or more years of treatment and it is not clear how quickly curlyleaf will return to nuisance levels after treatment stops (Johnson et al. 2012). After 3 years of whole lake treatment (entire littoral) with endothall McCommas et al. (2015) were able to reduce effort to spot treatments (4 to 32% of littoral), but treatment was required each of the subsequent 4 years. There are both financial and environmental concerns if treatment must continue every year to maintain control.

In addition to assessing the effects of herbicidal treatments on curlyleaf, a better understanding of the factors that affect curly occurrence and abundance in lakes would be useful to further guide management. Valley and Heiskary (2012; see also Heiskary and Valley 2012) presented evidence that winter conditions (cumulative snow depth) could affect curlyleaf frequency of occurrence with reduced frequency following winters with heavy snow cover. Winter conditions could therefore influence the need for or extent of management in the following spring.

These previous studies focused on a limited set of lakes and the aim of this project was to obtain results from a broader set of lakes across Minnesota to see if the results hold over a broader range of locations and longer time period and to determine if there are other factors that affect curlyleaf abundance or effectiveness of control. An analysis of existing data collected by the DNR, watershed and park districts and consultants may be able to address these issues in lieu of a complete new multi-year study. Plant surveys from these lakes, which are distributed across the state and express a range of water quality, will also be useful to help factor out climatic and annual variability in plant abundance.

Methods:

We contacted over 15 consultants, agency personnel and researchers identified by us and the DNR who were known to have conducted plant surveys that would include curlyleaf pondweed. We requested data sets that included point-intercept survey data with at least one survey in spring or early summer to capture peak curlyleaf growth. We combined these surveys with data we obtained on a previously published project (11 lakes, Johnson et al. 2012, Jones et al. 2012), ongoing data from 5 lakes in the Purgatory Bluff Creek Watershed District and 13 lakes from the Minnesota DNR Sentinel Lakes program (D.L. Dustin). In total, we obtained data for 67 lakes; data from 60 of these lakes (Fig. 1) were suitable for our analysis with point intercept surveys conducted in May (pretreatment timing) or June (peak curlyleaf coverage). These sixty lakes cover the period of 2006-2015; several lakes had data for all ten years. Data for years not treated were available from forty-nine lakes with one to eight years of data for each (mean of three years). Twenty-two lakes had data associated with curlyleaf pondweed herbicide treatments (one to nine years of treatment; mean of 3.8 years).

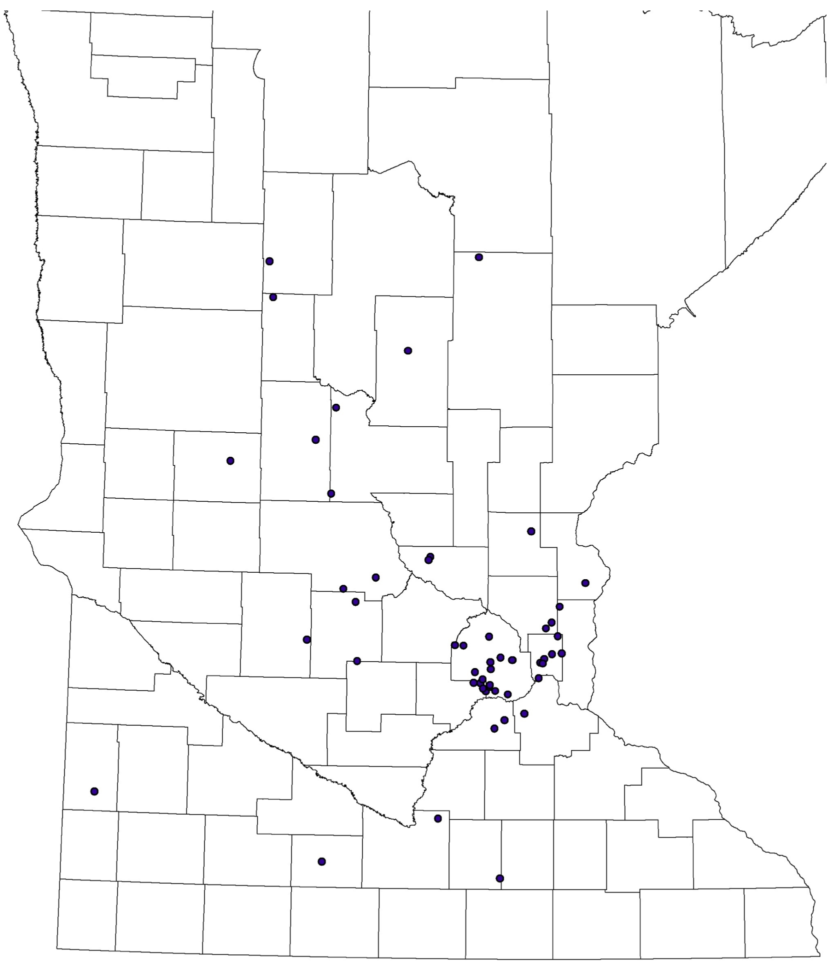

Fig. 1. Distribution of curlyleaf pondweed lakes used in the analysis.

For this analysis we focused on curlyleaf pondweed response and thus on the early season May and June curlyleaf data. We collated and organized the native plant data from mid-summer surveys for future analysis but did not analyze those results, which will require more sophisticated analyses. For the curlyleaf data sets, we used frequency of occurrence and relative density (relative rake rating) as the response. All data sets had frequency of occurrence responses and to standardize the maximum depth considered, we restricted the analysis to depths ≤ 3.7m (i.e. frequency of occurrence in depths ≤ 3.7m). We also computed and analyzed for mean relative rake density for the 30 lakes that had relative density ratings (1 to 4, with 1 being low density – one or few stems and 4 being high density, filling the rake). We computed the mean rating for only sites with curlyleaf (e.g., no ratings of zero). This provides an estimate of relative abundance or density when the plant is present. Each lake was classified each year as treated (permitted and generally delineated) or not treated (may include local homeowner shoreline treatments, but not large scale or offshore treatments) and contiguous years of treatment was used as an indication of duration of treatment.

We obtained water quality data from the Minnesota Pollution Control Agency (https://cf.pca.state.mn.us//water/watershedweb/wdip/) and snow depth and duration of ice cover data from the Minnesota DNR and State Climatology Office (http://www.dnr.state.mn.us/climate/historical/index.html). We used the previous year August Secchi depth as an index of lake productivity (data for TSI and P concentration were sparser) and decimal latitude as an index of growing conditions. We then used mixed effects linear models (e.g. Valley and Heiskary 2012) with lakes as random effects, and treatment, year, years of treatment and other climatic and environmental factors as fixed effects to assess factors that affect curlyleaf frequency of occurrence or relative density separately in treated and untreated lakes and separately for pretreatment surveys (May) and June (post treatment or time of peak curlyleaf in untreated lakes) surveys. Models were selected based on the lowest AIC and also significance of variables within the model.

Results and discussion:

Treated lakes had lower frequencies of occurrence and relative density than untreated lakes in both May and June (Fig. 2, Table 1). Although May frequency was not significantly lower in treated lakes, relative density was, suggesting that the prior years of treatment reduced density in the following May. As expected, June frequency was significantly reduced by treatment and there was not a significant change in frequency in untreated lakes. Relative density in treated lakes was significantly lower than untreated lakes in both May and June (Table 1).

Table 1. Mean (and 2 SE) early season (May; pretreatment) and June frequency of occurrence (Freq) and relative density (Rel Dens; 1-4) at sites where plants were found.

Lake	May Freq	Jun Freq	May Rel Dens	Jun Rel Dens
Treated	0.37	0.13	1.31	1.20
2 SE	0.05	0.03	0.10	0.14
Untreated	0.41	0.36	1.96	2.07
2 SE	0.08	0.05	0.32	0.18

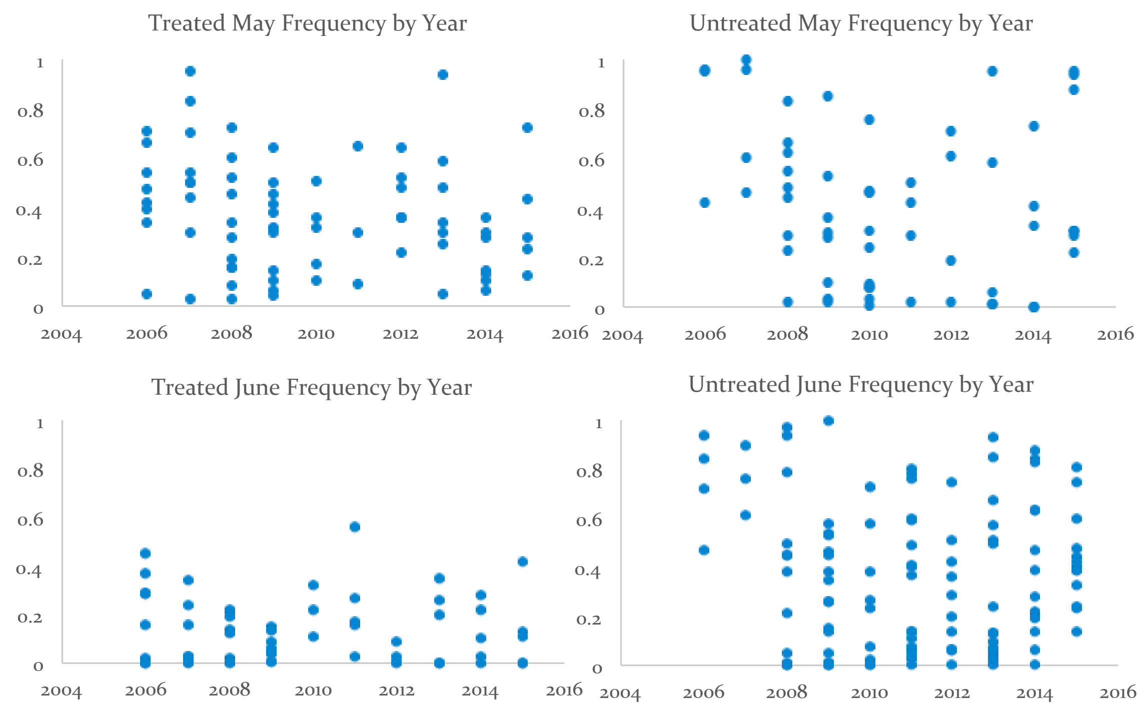

Fig. 2. May and June curlyleaf frequency of occurrence by year in treated and untreated lakes.

The mixed effects models revealed that for lakes treated with herbicides to control curlyleaf, the number of years treated was a significant predictor of early-season, pre-treatment curlyleaf frequency and relative density (Table 2), suggesting that repeated treatment with herbicides restricts curlyleaf distribution and abundance in the spring. Early season frequency in treated lakes was also influenced by the previous summer August Secchi depth (an overall indication of clarity and productivity) and winter conditions (ice duration or snow depth), but relative density (where plants occurred) appeared less affected by winter conditions. In untreated lakes, spring early season curlyleaf frequency and relative density were best predicted by a combination of environmental factors including mean snow depth, duration of ice cover, and previous summer Secchi depth (Table 2). The negative relationships with Secchi indicate curlyleaf is more frequent and dense in more eutrophic lakes, and negative relationships with snow and ice cover indicate the overwinter effects of reduced light on curlyleaf frequency and relative abundance.

These results suggest that more severe winter conditions and repeated herbicide treatment create conditions less favorable for curlyleaf pondweed distribution and growth the following spring. For June peak curlyleaf relative density, years treated was less important (only the current year of treatment has an effect) and although winter environmental conditions appeared in some models they were generally not significant and not always negative. This suggests that aside from the immediate treatment effects, peak curlyleaf density is more influenced by spring growing conditions than prior year management or winter conditions.

Table 2. Results of best fit mixed effects models (lowest AIC with significant effects) for Early Season (May or April) curlyleaf frequency of occurrence (depth ≤3.7m) and relative density (1-4 for sites with plants) and June relative density.

Early Season Frequency best models

Treated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	6.703	2.998	2.236	0.025*
No. years treated	-0.546	0.239	-2.284	0.022*
Days ice cover	-0.042	0.021	-2.026	0.043*
Previous year Aug. Secchi	-1.019	0.610	-1.670	0.095

Untreated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	2.234	1.132	1.974	0.048*
Mean depth snow	-0.110	0.055	-2.004	0.045*
Previous year Aug. Secchi	-1.718	0.843	-2.309	0.042*

Early Season Relative Density best models

Treated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	0.432	0.131	3.303	0.001*
No. years treated	-0.060	0.018	-3.367	0.001*
Previous year Aug. Secchi	-0.012	0.072	-0.164	0.870

Untreated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	2.076	0.708	2.931	0.003*
Days ice cover	-0.011	0.005	-2.124	0.034*
Previous year Aug. Secchi	-0.044	0.139	-0.315	0.753

June Peak Relative Density best models

Treated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	0.225	0.181	1.248	0.212
Previous year Aug. Secchi	-0.087	0.149	-0.587	0.557

Untreated lakes

Fixed effects	Estimate	SE	z	*p*
Intercept	0.764	0.103	7.401	<0.001*
Previous year Aug. Secchi	-0.063	0.057	-1.092	0.275

Previous work (Johnson et al. 2012) had also suggested that repeated treatments could decrease curlyleaf frequency and biomass the following spring, and this larger data set suggests the reductions are consistent but not large (Table 3), with frequency declining from 48% occurrence to 35% after three years and 31% after 5 years of treatment. The post treatment reduction (from May to June) was much larger and after two or more years of treatment June frequency was around 10%. Thus repeating treatment may result in somewhat better control and lower post treatment occurrence, but effects on frequency in the following spring diminish.

Table 3. Curlyleaf pondweed frequency of occurrence in May (before treatment) and June (after treatment) in treated lake by years of consecutive treatment (±2SE).

YrsTrt	May	June
1	0.48 ±0.10	0.21 ±0.07
2	0.42 ±0.11	0.12 ±0.07
3	0.35 ±0.13	0.10 ±0.05
4	0.32 ±0.13	0.05 ±0.04
5	0.31 ±0.13	0.14 ±0.08

An unresolved question is how rapidly curlyleaf will return if treatments are stopped. Unfortunately, monitoring is often stopped when treatments are stopped. In the present data set there are 7 instances from 6 lakes where treatment was stopped and frequency was monitored in the untreated year. It does not appear that there is any noticeable effect on May frequency. However, there was always an increase June in the untreated years compared to treated years (mean of 0.23) and in several lakes the increase was substantial (from 0.09 to 0.73 and 0.22 to 0.56). Thus even stopping treatment for 1 year can result in substantial rebounds that would call for treatment again in the following year.

Our results provide additional support for Valley and Heiskary's (2012) finding that winter conditions, particularly winter snow depth, can affect curlyleaf, with decreasing curlyleaf frequency in years with deeper snow cover. Our results indicated that both snow cover and ice duration are associated with decreases in curlyleaf frequency and abundance in May. Managers can thus expect the need for more treatment over larger areas following shorter or milder winters with less snow cover. Our results also show that May pretreatment curlyleaf frequency and relative density decrease with repeated years of treatment, but the decreases are not large and substantial populations remain even after 5 years of treatment. In many instances the curlyleaf will quickly rebound if treatments cease.

Acknowledgements:

We thank Chip Welling of the Minnesota DNR who suggested this project and provided significant input and assistance with obtaining data sets. Data for 12 lakes were collected by graduate students James Johnson and Ajay Jones as part of a curlyleaf whole lake treatment study funded by the Minnesota DNR (Wendy Crowell was key to that project). Data for 5 lakes was collected by graduate students Josh Knopik, John JaKa and Melaney Dunne with funding from the Riley Purgatory Bluff Creek Watershed District. Additional data sets were provided by the Minnesota DNR SLICE program (Donna Dustin), Minnesota DNR Invasive Species Program (Allison Gamble and Keegan Lund), Capitol Region Watershed District (Britta Suppes), Ramsey Washington Watershed District (Simba Blood), Three Rivers Park District (Rich Brasch), Minnehaha Creek Watershed District (Eric Fieldseth), Rice Creek Watershed District (Matt Kocian), and consulting firms Barr Engineering (Meg Rattei), Bluewater Science (Steve McComas) and Freshwater Scientific Services (James Johnson). Their cooperation was key to this project and greatly appreciated. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

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M.L. 2013 Project Abstract
For the Period Ending September 30, 2017

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project 6: Determining Heterosporosis Threats to Inform Prevention, Management, and Control
PROJECT MANAGER: Paul Venturelli
AFFILIATION: University of Minnesota
MAILING ADDRESS: 135 Skok Hall, 2003 Upper Buford Circle
CITY/STATE/ZIP: St Paul/MN/55108
PHONE: 612-624-4228
E-MAIL: pventure@umn.edu
WEBSITE: http://fwcb.cfans.umn.edu/Faculty/Venturelli/
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $111,889
AMOUNT SPENT: $111,889
AMOUNT REMAINING: $0

Overall Project Outcomes and Results

Heterosporosis is an emerging disease of concern in Minnesota that is caused by the parasite *Heterosporis sutherlandae*. It damages fish muscle and renders them inedible. Heterosporosis was discovered in Leech Lake in 1990 and has since been detected in ~30 waterbodies and in over a dozen species. Heterosporosis was identified as a high research priority by the 2014 MAISRC Research Needs Assessment because it can infect up to 40% of fish and we knew little about the disease or its population-level effects. Our objectives were to collect data to better understand this disease, and to estimate the threat that heterosporosis poses to perch harvest in a typical Minnesota lake.

We collected perch and other fishes from Leech Lake seasonally from fall 2015 to winter 2017, and from Cass and Winnibigoshish lakes in fall 2015 and 2016. Heterosporosis was rare among all species, seasons, and lakes. We detected the disease in only 9% of perch, and 20-30% of these fish had visible muscle damage. Heterosporosis did vary seasonally, and infected perch were not more susceptible to angling. In the lab, we found a 32-34% infection rate when fish were fed infected tissue and a 2-17% infection rate with passive transmission from cohabitating healthy and infected fish. We found no evidence of a relationship between growth or survival and infection.

We used this and other information to develop a population model that suggested that heterosporosis can have short-term impacts on yellow perch harvest (e.g., in a naïve population or after a bad year), but that long-term impacts are unlikely. Sensitivity analysis indicated that disease associated parameters had little effect on overall harvest. Based on the results of this project, we do not consider heterosporosis to be a significant threat to Minnesota fish, but recommend further research to improve the model, because threats to aquaculture or laboratory fish may be higher.

Project Results Use and Dissemination

We generated a heterosporosis fact sheet that is available on the MAISRC website (http://www.maisrc.umn.edu/fishdisease/) and was distributed to participating resorts and an interested fishing guide. We have maintained contact with two resorts (one on Leech Lake and one on Cass Lake), both of which contributed angler log book data that we used to estimate heterosporosis prevalence. We also had many positive conversations with individuals who approached us during field work. We have given numerous presentations of this work to a combined audience of over 300 researchers, managers, policymakers, and stakeholders. These include three presentations at MAISRC Showcase events, a presentation at the MN DNR's summer 2017 Fisheries Research Meeting, presentations at four academic conferences, and internally at the University of Minnesota. Our research has been highlighted in local and national media outlets, and our first paper is currently in review with the *Journal of Aquatic Animal Health.* Masters student Megan Tomamichel was recently awarded a competitive, $2,500 Judd Fellowship through the University of Minnesota to travel to Chile and adapt her model to sea lice infestations in salmon farms.

2013 Project Abstract
For the Period Ending June 30, 2016

PROJECT TITLE: Developing eradication tools for invasive carp species. Phase I: Understanding the virome of carp species in the Upper Midwest
PROJECT MANAGER: Dr. Nicholas Phelps
AFFILIATION: Minnesota Aquatic Invasive Species Research Center
MAILING ADDRESS: 2003 Upper Bufford Circle, 135 Skok Hall
CITY/STATE/ZIP: St. Paul, MN 55108
PHONE: 612-624-7450
E-MAIL: phelp083@umn.edu
WEBSITE: www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $206,754

Overall Project Outcome and Results

Although ambitious, eradication of aquatic invasive species is an ultimate goal of the MAISRC. One possible method would be through the introduction or promotion of species-specific pathogens. This high-risk, high-reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. As a first step in Phase I of a multi-phase project, invasive carp species were surveyed to identify viruses circulating in these populations. Nearly 700 common carp were collected from Minnesota lakes, 120 silver carp from the Fox and Illinois Rivers, and a variety of carp species from eight mortality events. All fish were negative for cyprinid herpes viruses 1, 2, and 3, carp edema virus, and spring viremia of carp virus. However, advanced molecular approaches and virus isolation detected several known and unknown viruses of significance. This included novel viruses from at least seven RNA virus families: picornavirus, reovirus, hepatovirus, astrovirus, hepatitis virus, betanodavirus, and paramyxovirus. The novel carp paramyxovirus was associated with a mortality event and shows particular promise for further evaluation as a biocontrol agent. The standard operating procedures developed during Phase I will be essential to advance future work on this and related pathogen discovery research. Unfortunately, Phase I was met with several unforeseen challenges that hindered completion of all proposed activities, including laboratory renovation progress, service provider availability and delays, and access to mortality events. In spite of these setbacks, this project has significantly advanced our understanding of invasive carp viruses and positioned us well to for future research efforts. Phase I of this project provided researchers and managers with baseline data on viruses circulating in invasive carp populations in the region. These data have been broadly disseminated at scientific conferences, peer-reviewed and lay publications, and through MAISRC communications. Continued efforts to build upon this line of research will commence in Phase II of this long-term effort.

Project Results Use and Dissemination

The data generated from this study was presented five times in different scientific and stakeholder conferences. The research data from this study will generate three or more publications, which are currently in preparation. These are tentatively titled (i) Prevalence of RNA viruses in invasive carp populations in Minnesota; (ii) Genomic-based characterization of novel RNA viruses present in invasive carp population in Minnesota; (iii) Molecular characterization of novel RNA viruses associated with fish mortality events in different lakes in

Minnesota; (iv) Next generation sequencing as a tool for diagnosis and discovery of novel pathogens.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 7.2: Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents
SUBPROJECT MANAGER: Dr. Nicholas Phelps
AFFILIATION: University of Minnesota Department of Fisheries, Wildlife and Conservation Biology
MAILING ADDRESS: 2003 Upper Bufford Circle
CITY/STATE/ZIP: St. Paul, MN 55108
PHONE: 612-624-7450
E-MAIL: phelp083@umn.edu
WEBSITE: http://www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $445,210
AMOUNT SPENT: $422,667
AMOUNT REMAINING: $22,543

<u>**Sound bite of Subproject Outcomes and Results**</u>

Researchers identified many new and important viruses in Minnesota fish populations, including Koi Herpes Virus, which caused high mortality in common carp and was not detected in native fish species. This virus will be evaluated as a potential biocontrol agent for common carp in the next phase of the project.

<u>**Overall Subproject Outcome and Results**</u>

One possible component to an effective integrated pest management plan for aquatic invasive species would be through the introduction or promotion of species-specific pathogens. This high-risk, high-reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. In Phase II of this long-term effort, we characterized the virome invasive and native fish species and zebra mussels. *We achieved our ultimate goal of this project and identified a candidate virus (koi herpes virus) that caused high mortality in common carp and was not detected in native fish species – this virus will be the focus of Phase III.* We also identified many other novel and undescribed viruses in health and dead fish, however the implications of these results are unknown and warrant additional research to better understand the threat to native species and/or potential as biocontrol agents. The virome of zebra mussels was also interesting with lower viral diversity than the fish species investigated; however, no viruses emerged as potential zebra mussel biocontrol candidates from field samples or laboratory trials.

This study emphasized the value of advanced molecular approaches to unbiased viral discovery and diagnostics. The methods we developed and optimized for sample collection, processing, and sequence analysis (all together called a ‘pipeline’), have informed testing protocols at the Minnesota Veterinary Diagnostic Laboratory. We have also elevated awareness among managers that viral diversity is much higher than currently known and deserves more attention as early indicators of potential threats.

The project team spent considerable time during Phase II engaging with managers, scientists, and the public in multiple formats. It is important that this type of research is transparent and understandable to all stakeholders. To that end, we held formal in person meetings, attended local-national-international scientific conferences, published a peer-review manuscript, networked with internationally-renowned experts, produced two videos, and provided interviews for print, radio and TV media.

<u>**Subproject Results Use and Dissemination**</u>

We had learned during Phase 1 of this project (MAISRC Sub Project 7.1) that communication, outreach and transparency were very important for this type of project. To that end, the project team has spent considerable time engaging with managers, scientists, and the public in multiple formats. This has included formal in person meetings, local-national-international scientific conferences, peer-review publication, networking with internationally-renowned experts, video production, and print, radio and TV media. A summary of this is listed below:

Formal in-person meetings: Great Lakes Fish Health Committee, MN DNR Koi Herpes Virus Working Group.

Scientific conferences: American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop, MAISRC showcase (x3), International Conference on Aquatic Invasive Species, Minnesota Veterinary Diagnostic Laboratory, Aquatic Invaders Summit III, Freshwater Mollusk Conservation Society, International Symposium on Aquatic Animal Health. NOTE: Most of these conferences were supported by non-LCCMR funding.

Peer-review publication: Padhi, S. K., I. E. Tolo, M. McEachran, A. Primus, S. K. Mor, N. B. D. Phelps. In press. Koi herpesvirus and carp edema virus: Infections and coinfections during mortality events of wild common carp in the United States. Journal of Fish Disease. Several other publications are in progress.

Networking with experts: Dr. Ken McColl, Dr. Tom Waltzek, Dr. Mikolaj Ademek, and others.

Video production: Video 1 (viewed 822 times as of 8/8/19), Video 2 (viewed 96 times as of 8/8/19).

Media: New York Times, KSTP 5, KARE 11, Star Tribune, Minnesota Daily, MN DNR Press release, MAISRC newsletters.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 8: Risk assessment, control, and restoration research on aquatic invasive plant species
SUBPROJECT MANAGER: Daniel Larkin
AFFILIATION: University of Minnesota
MAILING ADDRESS: 135 Skok Hall, 2003 Upper Buford Circle
CITY/STATE/ZIP: St. Paul, MN 55108
PHONE: 612-625-6350
E-MAIL: djlarkin@umn.edu
WEBSITE: http://larkinlab.cfans.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $822,000
AMOUNT SPENT: $820,251
AMOUNT REMAINING: $1,748

Sound bite of Subproject Outcomes and Results
This project predicted invasion risk, assessed ecological impacts, evaluated control efficacy, and investigated factors limiting post-control recovery of native aquatic plants. This was applied to starry stonewort, Eurasian watermilfoil, and curlyleaf pondweed. This will refine approaches for invasion prevention, reduce populations of established AIS, and restore native species.

Overall Subproject Outcome and Results
Aquatic invasive plants can lower native plant diversity, reduce habitat quality for fish and other animals, and interfere with recreation. To protect Minnesota's water resources, steps need to be taken to prevent new invasions, control existing populations, and support recovery of native biodiversity. These efforts require sound, science-based guidance. To provide such support, we conducted research to predict invasion risk, assess ecological impacts, evaluate control efficacy, and investigate factors limiting post-control recovery of native aquatic plants. This work was applied to three target species at different stages of invasion: (1) *Nitellopsis obtusa* (starry stonewort), first found in Minnesota in 2015 and now known in 14 lakes; (2) *Myriophyllum spicatum* (Eurasian watermilfoil), found in 1987 and established in >300 lakes; and (3) *Potamogeton crispus* (curly-leaf pondweed), here for >100 years and in >750 lakes. For starry stonewort, we developed models to predict risk of further spread and prioritize search locations for statewide volunteer search efforts, experiments to determine how long starry stonewort remains can survive out of water (i.e., remain transportable by boaters), and field and lab-based control experiments to guide management. For Eurasian watermilfoil and curly-leaf pondweed, we investigated relationships with native plant biodiversity, finding that they displace native species, an effect compounded by lower water clarity, and contribute to "biotic homogenization"—loss of ecological distinctiveness. We are investigating how to better control these invasive species and foster recovery of native vegetation by synthesizing thousands of aquatic plant surveys and management records collected in Minnesota and by conducting in-lake removal and restoration experiments. This work will continue under a follow-up project (MAISRC Subproject 8.2: Impacts of invader removal on native vegetation recovery). Our findings help Minnesotans by highlighting practices needed to protect lake ecosystems and refining approaches for preventing invasions, reducing populations of established AIS, and restoring native species.

Subproject Results Use and Dissemination
Information from this project has been disseminated through 10 peer-reviewed journal articles, 30 invited talks, 20 contributed presentations, 45 media stories, and resources published on the MAISRC website. Fully published

articles (7 of the 10) are included as attachments. Project findings are being used to guide AIS spread prevention and management efforts involving the Minnesota Department of Natural Resources, lake associations, and other stakeholders. This project has also contributed significantly to MAISRC Subproject 10 (“Citizen Science and Professional Training Programs to Support AIS Response”).

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Review

Biology, ecology, and management of starry stonewort (*Nitellopsis obtusa*; Characeae): A Red-listed Eurasian green alga invasive in North America

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ABSTRACT

Nitellopsis obtusa (starry stonewort) is a green macroalga (family Characeae) native to Europe and Asia that is of conservation concern in its native range but expanding in North America. We synthesize current science on *N. obtusa* and identify key knowledge gaps. *Nitellopsis obtusa* is able to reproduce sexually or asexually via fragments and bulbils. Native populations reproduce primarily asexually; sexual fertility increases with longer growing seasons and in shallower waters. In North America, only males have been observed. *Nitellopsis obtusa* has been known from North America for four decades and confirmed in seven U.S. states and two Canadian provinces. It is typically associated with low-flow areas of lakes with alkaline to neutral pH and elevated conductivity. *Nitellopsis obtusa* has ecological benefits in its native range, contributing to food webs and water clarity. In its invaded range, *N. obtusa* could negatively influence native macrophytes and habitat quality, but there has been little research on impacts. There have been many efforts to control *N. obtusa* through physical removal or chemical treatments, but little systematic evaluation of outcomes. Substantial areas of uncertainty regarding *N. obtusa* include controls on reproduction, full distribution in North America, ecological impacts, and control strategies.

1. Introduction

Nitellopsis obtusa (Desv. in Loisel.) J. Groves (common name: starry stonewort) is a freshwater green macroalga of the family Characeae that is native to Europe and Asia. It is the only extant member of the genus *Nitellopsis* (Soulié-Märsche et al., 2002) and is of conservation concern in much of its native range (Stewart and Church, 1992; Blaženčić et al., 2006; Caisová and Gąbka, 2009; Korsch et al., 2012; Westling, 2015).

Despite threats to *N. obtusa* in its native range, it is of increasing concern as an invasive species in North America, where it has been recorded for four decades (Geis et al., 1981; Karol and Sleith, 2017). This phenomenon—of a species being rare or declining in its native range while finding new success as an invader—has been observed in other invasive plant and animal taxa (see examples in Callaway and Ridenour, 2004; Escobar et al., 2016). This makes the biogeography and ecology of *N. obtusa* of interest from both a species management perspective and as an example of a broader phenomenon in biological invasions. Furthermore, we know of no other characeans that are classified as invasive—though some may be considered a nuisance in highly managed systems like rice fields or canals of the western United States (DiTomaso et al., 2013).

Unfortunately, there has been little applied research on *N. obtusa*. For example, a search in early 2018 yielded 212 peer-reviewed articles containing the keywords *Nitellopsis obtusa* (Thomson Reuters, 2018), but most of those involved its use as a model species for cell biology research; only 12 papers addressed *N. obtusa* as a non-native species in North America (Geis et al., 1981; Schloesser et al., 1986; Nichols et al., 1988; Griffiths et al., 1991; Sleith et al., 2015; Escobar et al., 2016; Midwood et al., 2016; Alix et al., 2017; Brainard and Schulz, 2017; Karol and Sleith, 2017; Romero-Alvarez et al., 2017). Similarly, though *N. obtusa* occurs on many national and regional conservation Red Lists, there has been relatively little published research on *N. obtusa*

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conservation in its native range (but see Rey-Boissezon and Auderset Joye, 2012; Kato et al., 2014; Auderset Joye and Rey-Boissezon et al., 2015; Boissezon et al., 2017).

The goals of this paper are to synthesize current knowledge of *N. obtusa*, drawing upon research from both its native and invasive ranges, and identify information gaps to inform future research efforts. The global distribution of *N. obtusa* is highly dynamic, and key questions pertaining to its reproduction, genetics, ecological roles, and management remain unanswered.

2. Species description

2.1. Classification

The taxonomic history of *Nitellopsis obtusa* has been complex and confusing. The species was first described as a member of the genus *Chara* (*C. obtusa* Desv. in Loisel.) in 1810, but has been classified as a member of four different genera during the next 110 years: *Lychnothamnus*, *Nitella*, *Nitellopsis*, and *Tolypellopsis*. The tribal placement of *Nitellopsis* has also varied. Though accepted as a member of tribe *Chareae* (with *Chara*, *Lamprothamnium*, and *Lychnothamnus*), its classification relative to these three genera has been inconsistent. Wood (1962) proposed subtribe *Nitellopsinae* to include only *Nitellopsis*, uniting the remaining three genera in subtribe *Charineae*. In contrast, molecular phylogenetic work supported *Nitellopsis* as more closely related to *Lychnothamnus* than to *Chara* or *Lamprothamnium* (McCourt et al., 1996), which suggests that *Charineae* is paraphyletic.

2.2. Morphology

Nitellopsis obtusa is a dioecious species reaching heights of 30 to 120 cm in the water column. The alga is bright green to dark green to brown depending on phenology and growing conditions. The main axis is slender to robust, 0.7–2 mm in diameter (Fig. 1). White, conspicuous, star-shaped bulbils, which function as asexual reproductive structures and organs for hibernation (Bharathan, 1987), arise from rhizoid nodes and green bulbils arise from main axes and branchlet nodes. Branchlets are 5–8 per whorl, up to 9 cm in length, and composed of 2 to 3 segments. Gametangia are formed on all branchlet nodes, solitary or in pairs. Mature antheridia are orange to bright red, 800–1500 μm in diameter. Oogonia (not yet observed in North America) are nearly spherical, bright red to light green, and have a very small five-celled coronula (Fig. 1). Oospores are ellipsoidal with truncated bases; calcified oospores (gyrogonites) are inverted-pear shaped to sub-cylindrical (Groves, 1919; Corillion, 1957; Krause, 1997; Bailly and Schaefer, 2010; Mouronval et al., 2015; Kabus, 2016; Boissezon et al., 2017).

2.3. Origins

Nitellopsis obtusa is the only surviving member of an evolutionary lineage that arose during the Cretaceous-Tertiary boundary (Soulié-Märsche, 1979). Reconstruction of the historical biogeography of the lineage (Sanjuan and Martin-Closas, 2015) showed that it was initially restricted to Europe (for ca. 10 MY) before expanding eastward. Fossil remains of *N. obtusa* from the Early Quaternary to present represent the most recent phase of the lineage's biogeographic history (excluding contemporary human-assisted relocation) and indicate a generally northern, Eurasian distribution, ranging from Spain to Japan (Corillion, 1975). While fossil gyrogonites of *N. obtusa* have been found within Early Holocene deposits from the Sahara (Soulié-Märsche et al., 2002), these correspond to the last humid period in North Africa and the species has not been found in deposits younger than 4500 YBP.

2.4. Native distribution and conservation status

Known populations of *N. obtusa* have a disjointed distribution through Occidental and Central Europe and Asia and are absent from Africa. There is some evidence of recent changes in the native range of the species during the last three decades, concurrent with accelerated climate warming. Krause (1985) reported that *N. obtusa* was expanding in Europe. In France, its range has shifted from west to east (Bailly and Schaefer, 2010) and it has been discovered in southern France in seven new localities since 2012 (Mouronval et al., 2015). New localities have also been recorded since 2006 in the Wielkopolska region of Poland (Gąbka, 2009) and in newly dug ponds in floodplains in Germany (Korsch et al., 2008). In Switzerland, *N. obtusa* has expanded into large,

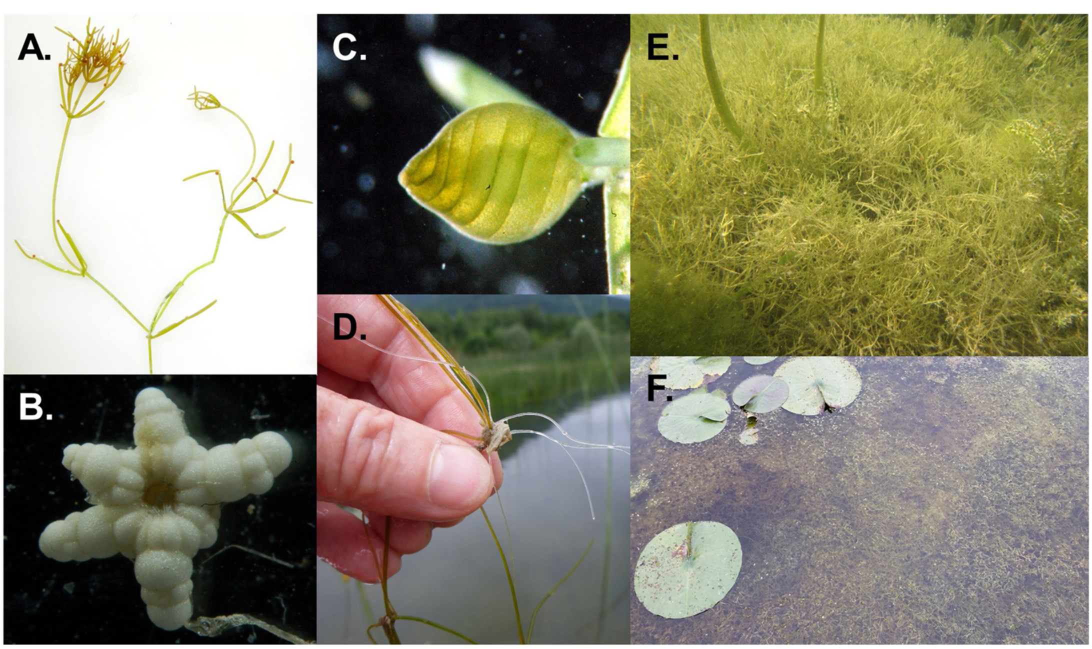

Fig. 1. Photos showing morphological characteristics and growth of *N. obtusa*: A. a male individual exhibiting red antheridia, B. a star-shaped bulbil, C. an oogonium, D. clear filamentous rhizoids, E. underwater image (New York, U.S.A.), F. mixed vegetation dominated by *N. obtusa* reaching surface at shallow water depth (Minnesota, U.S.A.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

moderately eutrophic lowland lakes (Dienst et al., 2012; Auderset Joye and Rey-Boissezon et al., 2015; Rey-Boissezon and Auderset Joye, 2015). It has also recently colonized two lakes in the Swiss and French Jura Mountains at elevations of 850 and 1004 m, respectively (Bailly et al., 2007).

The Red List status of *N. obtusa* varies among regions: it is considered near threatened in Switzerland (Auderset Joye and Schwarzer et al., 2012), vulnerable to critically endangered in Germany (Hamann and Garniel, 2002; Kabus and Mauersberger, 2011; Korsch et al., 2012), vulnerable or regionally extinct in eastern Europe (Blaženčić et al., 2006; Caisová and Gąbka, 2009), and vulnerable in Nordic countries (Johansson et al., 2010; Koistinen, 2010).

Increased occurrences of *N. obtusa* in parts of its native range have led to recent reclassifications of the species' conservation status. In Sweden, its status was lowered from endangered to vulnerable between 2005 and 2010 (http://artfakta.artdatabanken.se/taxon/1093). In Germany, *N. obtusa* is no longer considered threatened (Korsch et al., 2008; Auderset Joye and Schwarzer et al., 2012). In Asia, *N. obtusa* is present in China and was recently rediscovered in Japan, where it had been thought to be extinct (Kato et al., 2014). In the Netherlands, variation in *N. obtusa* abundance associated with changes in trophic state is synchronous with variation in breeding populations of red-crested pochard (*Netta rufina*) (van Turnhout et al., 2010). Hence, conservation of *N. obtusa* is a priority for lake restoration plans in several European regions (van den Berg et al., 1998).

2.5. Reproductive biology and dispersal

Characeae are able to reproduce both sexually and vegetatively. Extant populations of *N. obtusa* in its native range reproduce primarily through vegetative propagules (fragments and bulbils) and low sexual fertility was reported as early as the late 1800s (Migula, 1897). However, with colonization of shallower waters, there appears to be a shift toward increased sexual fertility (Krause, 1985). The influence of water temperature on growth and fertility of *N. obtusa* was studied by Willén (1960) and Boissezon et al. (2017); both found that development of gametangia could be triggered by a warm, sunny growing season.

Bulbils serve as organs for hibernation and clonal multiplication in permanent habitats (Bociąg and Rekowska, 2012). They are consistently produced on *N. obtusa* rhizoids and thalli (main axes). But clonality may be a less effective reproductive strategy in shallow habitats where viability of fragments and bulbils is limited by winter freezing or summer drying. Allocation of resources to sexual reproduction may be a strategy to ensure that long-lived, resistant propagules are produced (Boissezon, 2014). Oospores within sediments, particularly gyrogonites, can persist for long periods in a dormant state in sediment and be transported by waterfowl to distant waterbodies (endozoochory). In contrast, bulbils are short-lived and can only be transported over short distances (van den Berg et al., 2001; Bonis and Grillas, 2002; Boedeltje et al., 2003).

To date, only sterile or male plants have been observed in North America (Mann et al., 1999; Sleith et al., 2015). Prior reports of orange "oocysts", "oocytes," or "oospores" on North America specimens have been reexamined and shown to only depict male antheridia, not oogonia or zygotes (Sleith et al., 2015). In native habitats where both males and females occur, *N. obtusa* exhibits protandry: male organs develop throughout the growing season and prior to emergence of female organs, which emerge late in the growing season (Boissezon et al., 2017). Sub-optimal environmental conditions, such as deep habitats, high latitudes, or cold climates, may prevent the development of female organs by truncating the growing season, thereby leading to only sterile or male individuals being observed. Protandry or environmental conditions might explain the apparent absence of female individuals in North America. Alternatively, it is possible that only male individuals have survived introduction and have spread clonally in North America. It is also possible that distinct ecotypes are playing a role in manifestation or suppression of sexual reproductive structures. Genetic analyses are needed to clarify these mechanisms.

3. Invasion history in North America

The historical pattern of *N. obtusa* records for North America is consistent with initial invasion into large water bodies (Lake Ontario, Lake St. Clair) followed by secondary spread into smaller, inland water bodies. An important consideration in reconstructing the spread of any invading species is that observations may include inaccuracies, spatial sampling biases, or other artifacts (Aikio et al., 2010). Thus, the spread history of *N. obtusa* described below should be considered an approximation of its true introduction and spread.

The oldest published record of *N. obtusa* in North America was in the St. Lawrence River in New York's Jefferson and St. Lawrence counties in 1978 (Geis et al., 1981). However, while the Characeae collection at the New York Botanical Garden (NY) was being inventoried, a specimen dated from 1974 that was identified as "?*Nitellopsis* sp." from the St. Lawrence River was found (Karol and Sleith, 2017). The collection is undoubtedly *N. obtusa*, indicating that the alga was established in the Montreal, Québec portion of the St. Lawrence River at least four years prior to the 1978 finding by Geis et al. (1981).

In 1983, *N. obtusa* was recorded in the St. Clair-Detroit River system in Michigan (Schloesser et al., 1986; Griffiths et al., 1991). And in 2005, it was reported from Upper Little York Lake in interior New York (Sleith et al., 2015). By 2012, reports began to rapidly increase and expand to Pennsylvania, Indiana, and interior Michigan (Fig. 2). *Nitellopsis obtusa* was confirmed in Wisconsin in 2014. In 2015, there were first records for Minnesota and Vermont. There have been few official reports from Canada but Midwood et al. (2016) recently reported *N. obtusa* from Presqu'ile Bay, Lake Ontario. There have also been unpublished reports from Lake Scugog in interior Ontario (https://scugoglakestewards.com/monitoring-in-lake-scugog-in-2015/). The current known extent of *N. obtusa* in North America encompasses two Canadian provinces and seven U.S. states (Fig. 3).

Total numbers of unvouchered or unconfirmed reports in North America should be interpreted with caution as they could lead to overestimation (Figs. 2, 3); indeed, in preparing this manuscript we identified several inaccurate reports. In addition, there has been little awareness of *N. obtusa* or systematic search effort in regions where it has only recently been identified. With more comprehensive sampling effort, we anticipate detection of additional populations. All confirmed occurrence data indicate *N. obtusa* is at a relatively early stage of invasion in North America, and may be undergoing increase following a multi-decade lag phase (Fig. 2), as has frequently been observed in plant invasions (Aikio et al., 2010; Larkin, 2012). Alternatively, this pattern could be an artifact of increased awareness and search effort. Regardless, it is unlikely that *N. obtusa* has reached the full extent of its potential range in North America. For example, using climate-based ecological niche modeling, Escobar et al. (2016) predicted that large portions of North America where *N. obtusa* has not been found to date (including the Mid-Atlantic, Intermountain West, and Great Plains ecoregions), could be susceptible to *N. obtusa* invasion should it be introduced into suitable water bodies. Likewise, using water-chemistry based modeling, Sleith et al. (2018) identified areas of the Northeast U.S.A. (including eastern New York and western Vermont) with suitable habitat that have yet to be invaded (Fig. 3).

Overland dispersal on boats or boating equipment is implicated in *N. obtusa* spread. For example, in 2014, Sleith et al. (2015) surveyed 20 lakes lacking boat launches within the most heavily *N. obtusa*-invaded region of New York and *N. obtusa* was not detected. It is true that endozoochory by water birds is a known dispersal mechanism for Characeae (Proctor, 1962). However, only male *N. obtusa* has been documented in North America to date (Mann et al., 1999; Sleith et al., 2015); development and animal consumption and deposition of viable oogonia is impossible in the absence of females.

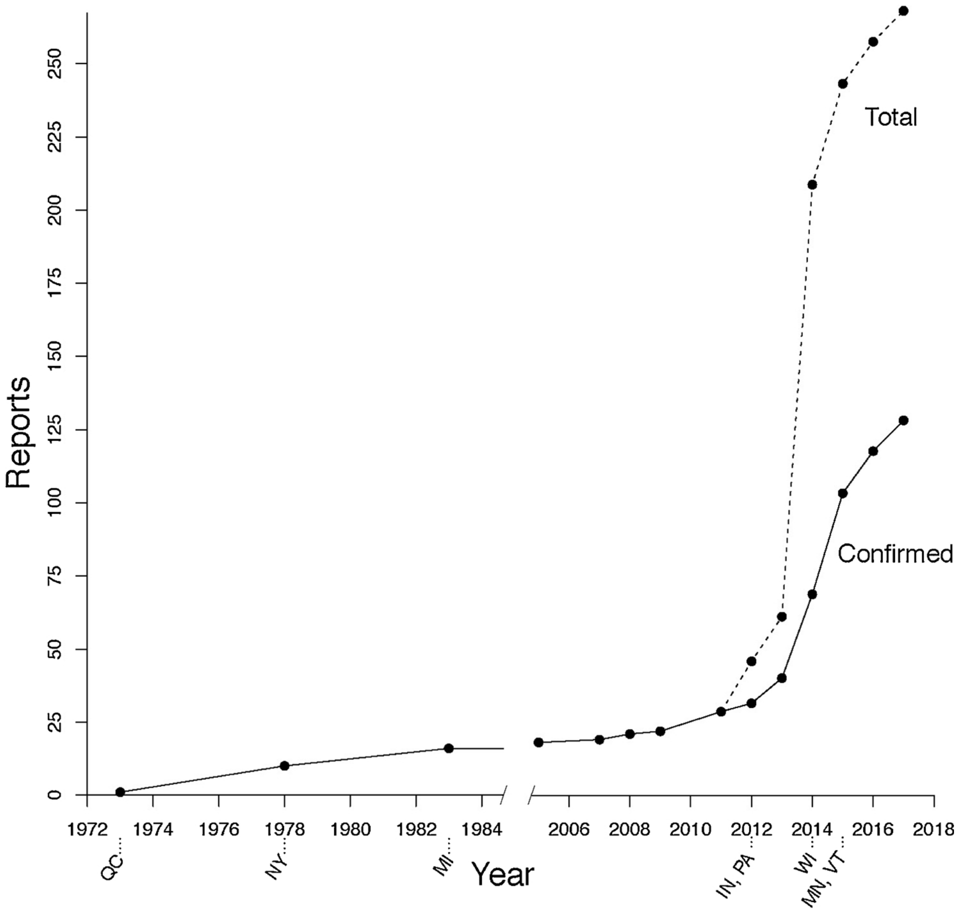

Fig. 2. Accumulation of *N. obtusa* occurrences (unique waterbodies) in North America over time, with differentiation of reports that have (Confirmed) and have not (Total) been confirmed through examination of voucher specimens by experts. Abbreviations on the x-axis indicate years when first records were confirmed for Québec, Canada (QC) and the U.S. states of New York (NY), Michigan (MI), Indiana (IN), Pennsylvania (PA), Wisconsin (WI), Minnesota (MN), and Vermont (VT) in the United States.

4. Habitat associations

4.1. Environment

In its native range, *N. obtusa* has been recorded in deep and shallow lakes, abandoned gravel pits, rivers, oxbows, and secondary channels at water depths of 0.5 to > 14 m (Korsch et al., 2008; Janauer et al., 2010). It preferentially colonizes calcareous, neutral to alkaline, mesotrophic to eutrophic waters (Bailly et al., 2007; Hutorowicz and Dziedzic, 2008), generally on sediments that are calcareous and rich in nutrients and clay (Table 1). *Nitellopsis obtusa* has also been found in brackish waters near the Baltic Sea (Langangen et al., 2002). Formation of large, dense mats has typically been observed under still conditions in lowland freshwater lakes (Corillion, 1975; Stewart and Church, 1992; Rey-Boissezon and Auderset Joye, 2015). Such mats can be monospecific or contain only a few individuals of other Characeae or vascular plant species. Frequently co-occurring species include *Stuckenia pectinata* (*Potamogeton pectinatus*), *Myriophyllum spicatum, Najas marina, Chara contraria, C. globularis,* and *C. tomentosa* (Pełechaty, 2005; Sanda et al., 2008; Rey-Boissezon and Auderset Joye, 2012).

In its introduced range, *N. obtusa* can be found in a variety of habitats, from bays of the Great Lakes to small inland ponds (Sleith et al., 2015). As in its native range, *N. obtusa* occurs in calcareous, neutral to alkaline, mesotrophic to eutrophic waters (Table 1). It has been found on a variety of substrates, from rocky, sandy bottoms of the St. Lawrence River to organic-rich, mucky sediments of inland lakes (e.g., Upper Little York Lake in Cortland Co., NY). *Nitellopsis obtusa* has been reported from depths of 0.5–7 m (Geis et al., 1981; Sleith et al., 2015). It can form large, dense, nearly monotypic mats or occur intermixed with native macrophytes. Composition of co-occurring macrophytes has not been systematically sampled across the invaded range, but taxa observed to co-occur with *N. obtusa* in Michigan, Minnesota, New York, and Vermont include *Ceratophyllum* spp., *Myriophyllum* spp., *Chara braunii, C. contraria, C. vulgaris, C. globularis, Najas flexilis, N. guadalupensis, Nitella flexilis, N.* aff. *montana, Nuphar variegata, Potamogeton crispus, P. friesii, P. richardsonii, P. zosteriformis, Stuckenia pectinata, Tolypella intricata, Tolypella glomerata, Utricularia macrorhiza*, and *Vallisneria americana* (A. K. Monfils, CMU, unpub. data; R. Sleith, NYBG, unpub. data; M. Verhoeven, UMN, unpub. data).

4.2. Disturbance

Species of Characeae have been found to be fast-growing, pioneer species that can outcompete vascular aquatic plants in ecosystems disturbed by flooding or drought or that are nutrient-limited (Forsberg, 1964; Littlefield and Forsberg, 1965; Bonis and Grillas, 2002; Lambert-Servien et al., 2006). Disturbances like drought act as abiotic filters in aquatic communities that shape species diversity and composition by eliminating standing competitors, thereby creating gap opportunities for recruitment of pioneer species (Connell and Slatyer, 1977). However, counter to the disturbance tolerance observed in other characeans, Boissezon et al. (2017) found that *N. obtusa* abundance in a semi-permanent shallow lake decreased rapidly following drawdowns, limiting the species to deep areas that were continuously inundated. Concurrently, richness and heterogeneity of pioneer aquatic plant species increased with these drought events. This sensitivity of *N. obtusa* to drought may explain why it is mainly observed in quiet, permanent waters.

Eutrophication is another disturbance to which *N. obtusa* has shown sensitivity (Auderset Joye and Schwarzer, 2012; Kabus, 2016). Elevated nutrient concentrations and decreased water clarity have been implicated in reduced *N. obtusa* abundance in Scanian lakes of southern

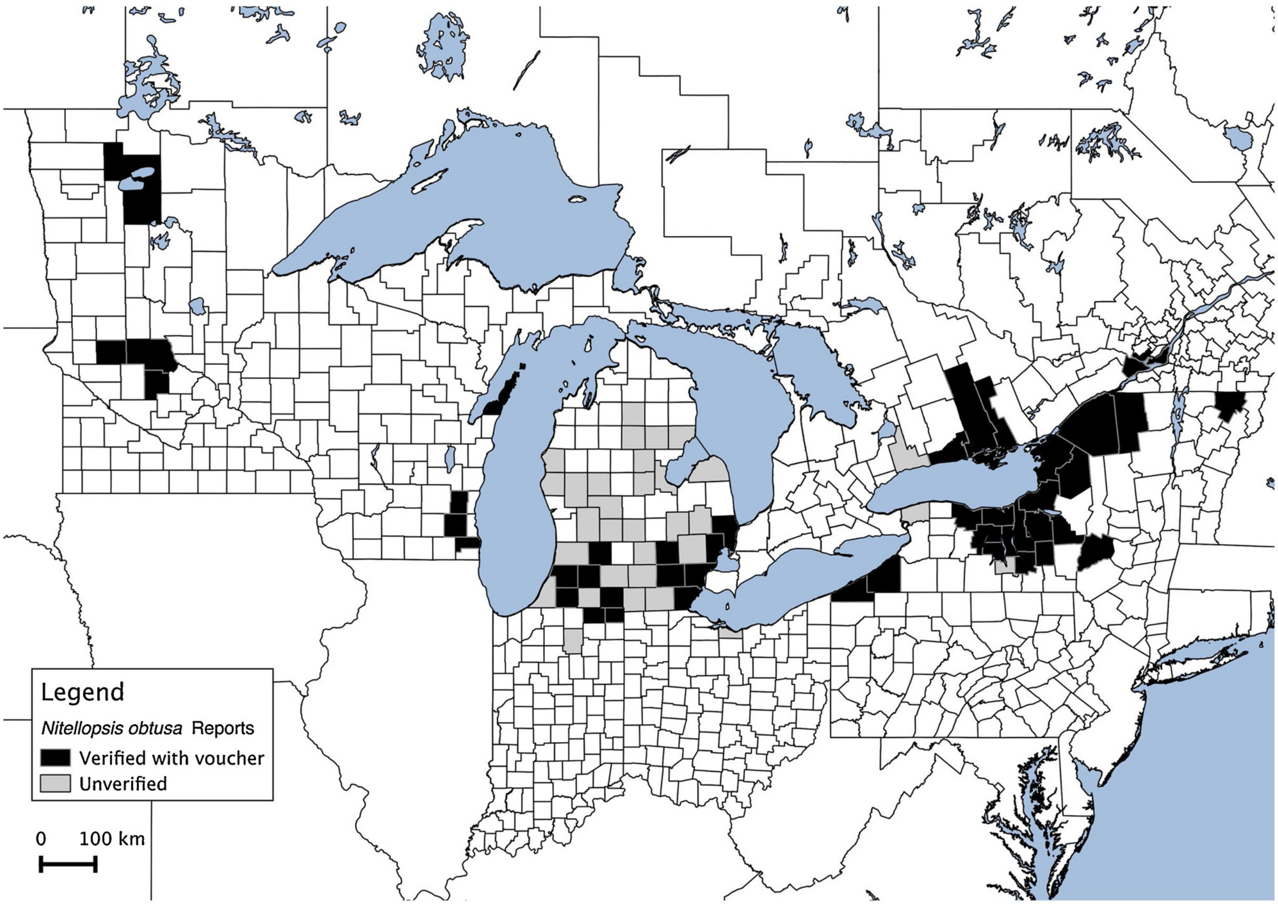

Fig. 3. Map of the Great Lakes region of North America showing reported distribution of *N. obtusa* at the county level, including both counties with and without expert-verified voucher specimens (black and grey shading, respectively).

Table 1
Published environmental data associated with occurrences of *Nitellopsis obtusa* in its native and introduced ranges. Native range values from France (Otto-Bruc, 2001; Bailly et al., 2007; Rey-Boissezon and Auderset Joye, 2012; Coppin, 2013); Germany (Doege et al., 2016); Poland (Królikowska, 1997; Pełechaty, 2005; Hutorowicz and Dziedzic, 2008; Chmara et al., 2014; Pełechaty et al., 2014); and Switzerland (Auderset Joye and Schwarzer et al., 2012; Auderset Joye and Rey-Boissezon et al., 2015; Rey-Boissezon and Auderset Joye, 2015). Introduced range values from New York, U.S.A. (Sleith et al., 2015).

	Native range			Introduced range		
Parameter	Min.	Max.	Mean	Min.	Max.	Mean
Depth (m)	0.4	31	3.9	—	—	—
Summer temperature (C)	14.0	28	16.1	18.2	25.4	23.0
Dissolved O_2 (mg/L)	—	—	—	3.4	13.5	9.3
Oxidation reduction potential (mV)	—	—	—	46.3	277.1	98.4
pH	3.8	9.8	8.0	7.3	9.2	8.5
Conductivity (μS/cm)	32	2,880	228.3	160.7	499.2	301.3
N-NH_4 (μg/L)	0	494	218.0	9.7	171.6	56.0
N-NO_3 (μg/L)	0	660	177.7	2.4	1,732	230.9
Total N (μg/L)	0	7,800	873.9	—	—	—
Soluble reactive PO_4 (μg/L)	0	1,015	12.0	0.6	110.7	11.9
Total dissolved P (μg/L)	2	430	50.2	6.6	172.2	24.6
Dissolved organic C (mg/L)	—	—	—	3.6	50.2	10.3
Ca (mg/L)	5.2	172	92.5	28.8	107.1	50.8
Mg (mg/L)	3.4	17.5	10.7	1.2	20	9

Sweden (Lundh, 1951; Blindow, 1992a). In Europe's second largest lake (Lake Constance; Germany, Switzerland, and Austria), strong recovery of *N. obtusa* over a nearly 50-year period was associated with a return to mesotrophic conditions and concurrent reductions in shading by *Cladophora* spp. (Murphy et al., 2018).

Use of herbicides to control vascular macrophytes is another disturbance that may influence *N. obtusa*—possibly increasing its abundance as has been found with other Characeae species in the U.S.A. In a Minnesota lake, application of multiple fluridone treatments to control *Myriophyllum spicatum* (Eurasian watermilfoil) was followed by increased frequency of *Chara* spp. from 33% to 100% of sampled points (Crowell et al., 2006). Similarly, Wagner et al. (2007) reported increases in *Chara* frequency in two out of four Wisconsin lakes treated with fluridone; Netherland and Jones (2015) observed increased frequency of *Chara* spp. in one out of two study bays following treatment of *M. spicatum* with triclopyr; Parsons et al. (2007) found increases in *Nitella* spp. following application of diquat for *Egeria densa* (Brazilian elodea) management in a lake in Washington; and Kelly et al. (2012) found minimal impacts of diquat, endothall, and fluridone on several New Zealand Characeae species. How treatments targeting vascular macrophytes influence *N. obtusa* occurrence and density merits investigation.

5. Ecological impacts

5.1. Ecological value in the native range

In general, Characeae are key contributors to ecological and environmental functions in shallow water bodies (Kufel and Ozimek, 1994; van den Berg et al., 1998; Christensen et al., 2013). As primary benthic producers, they provide habitat, food, and refugia for periphyton, invertebrates, fish, amphibians, and birds (Noordhuis et al., 2002; van Nes et al., 2003). In the case of *N. obtusa* specifically, it is grazed preferentially by the red-crested pochard, a large diving duck (Ruiters et al., 1994).

Characeans also help maintain clear water states in shallow waterbodies through contributions to biogeochemical cycles (e.g., organic carbon production, phosphorus immobilization, and allelopathy) and sediment stabilization (van Donk and van de Bund, 2002; Berger and Schagerl, 2004; Hilt et al., 2006). There is evidence that *N. obtusa* in particular can increase water quality. Blindow (1992b) reported that dense beds of *N. obtusa* in two Swedish lakes functioned as phosphorus sinks—and likely slowed water movement and reduced sediment suspension—thereby improving water quality. Hilt et al. (2010) related the return of dense mats of *N. obtusa* in Lake Scharmützelsee in Germany to the stabilization of a clear-water state. And in an analysis of water quality and submersed macrophyte communities in 49 temperate shallow lakes that had turned turbid and were subsequently restored, Hilt et al. (2018) found that recovery of dense mats of charophytes, including *N. obtusa*, was critical for maintaining clear-water states.

5.2. *Ecological effects in the invaded range*

Numerous non-native, aquatic macrophytes have been transported to North America through ballast water from trans-oceanic shipping, the ornamental gardening trade, and other vectors (Kay and Hoyle, 2001; Padilla and Williams, 2004). Once they become established, it is rare that invasive macrophytes can be eradicated, though their abundance can be reduced through mechanical, biological, or chemical control methods (Hussner et al., 2017). *Hydrilla verticillata* (hydrilla), *Myriophyllum spicatum*, *Eichhornia crassipes* (water hyacinth), and other invasive plants are known for their ability to form large, monospecific stands that impede recreation and can cause ecological harm, including reductions in native plant diversity and degradation of habitat quality for fish and other animals (Mitchell, 1976; Aiken et al., 1979; Colle and Shireman, 1980).

Nitellopsis obtusa could have similar impacts as other invasive macrophytes; this warrants further study (Pullman and Crawford, 2010; Hackett et al., 2014; Brainard and Schulz, 2017). Its ability to form large, dense mats suggests that its expansion within a lake could lead to displacement of native vascular plants or algae. *Nitellopsis obtusa* is also taller than most native Characeae and can fill the water column at shallow depths; this could cause native species to become light-limited. In addition, characeans can act as ecosystem engineers, altering water chemistry and nutrient cycling through high rates of productivity and nutrient uptake and low rates of decomposition (Kufel and Ozimek, 1994; Kufel and Kufel, 2002). It is possible that large beds of *N. obtusa* might restrict nutrients available to native plants through such mechanisms, as has been shown in other invasive macrophytes (Larkin et al., 2012). Potential ecological impacts of *N. obtusa* are largely unknown due to a lack of peer-reviewed literature. However, Brainard and Schulz (2017) documented decreased native plant species richness and biomass associated with increasing *N. obtusa* abundance in four lakes in New York, U.S.A.

Potential impacts to fish or other aquatic animals are uncertain. Relationships between fish and macrophyte communities are complicated, difficult to study, and not well-resolved even under undisturbed, reference conditions (Valley et al., 2004) or in the context of long-established, well-studied invasive plant species (Kovalenko et al., 2010). Throughout the invaded range of *N. obtusa*, submersed vegetation is an important resource for game and non-game fish, and the extent of macrophyte cover can be a limiting factor for fish populations (Randall et al., 1996). Conditions for fish may be undermined when either too little or too much of a basin has submersed vegetation—it is the latter possibility that motivates concern about *N. obtusa*. However, fish are mobile and flexible in their use of different microhabitats, which could mitigate impacts except, perhaps, in extreme cases of *N. obtusa* dominance.

Nitellopsis obtusa could also interact with crayfish, which can substantially reduce density, survival, and biomass of submersed macrophytes via direct feeding and fragmentation (Lodge et al., 1994; van der Wal et al., 2013). For example, the globally widespread species *Procambarus clarkia* (red swamp crayfish) has been shown to preferentially feed on finely branched macrophytes in general and on characeans specifically (Cronin et al., 2002; Cirujano et al., 2004). It is possible that resident populations of crayfish could limit establishment of *N. obtusa*; this merits further investigation as a potential source of invasion resistance.

Despite the potential for *N. obtusa* to have negative ecological effects, we could find almost no quantification of such effects in our review of published research and publically available grey literature (but see Brainard and Schulz, 2017). Despite this, anecdotal claims of harm have been widely circulated. Given the recent rapid spread of *N. obtusa* in North America and its ability to form large, nearly monotypic stands resistant to control, concern is warranted. However, improved understanding of potential threats based on sound empirical evidence is needed to guide effective management responses.

6. Management of invasive populations

6.1. *Chemical treatment*

Nitellopsis obtusa has typically been treated with various formulations of copper-based algaecides (copper sulfate and chelated copper compounds). Copper-based algaecides have been shown to be effective for short-term control of microscopic and filamentous algae (Murray-Gulde et al., 2002; de Olivira-Filho et al., 2004). However, published data demonstrating the effectiveness of copper-based algaecides for Characeae control in general, and *N. obtusa* in particular, are lacking (Fernández et al., 1987; Guha, 1991; Kelly et al., 2012).

When copper compounds are used for *N. obtusa* management, they are often applied multiple times in a single growing season or over multiple years. Glisson et al. (2018) evaluated the effects of two chelated copper treatments applied to a Minnesota lake in a single growing season. The first application significantly reduced *N. obtusa* biomass compared to an untreated reference area, but a second application did not further reduce biomass, and bulbil viability and abundance were not reduced by treatment, suggesting high capacity for regeneration. Following multiple chelated copper applications in a Michigan lake, there were no significant differences in *N. obtusa* biomass or height between treated and untreated sites at two or four weeks following the first and second treatment applications (A. K. Monfils et al., CMU, unpub. data). Use of copper-based compounds can lead to accumulation of copper in sediments (Prepas and Murphy, 1988; Van Hullebusch et al., 2003; Liu et al., 2006) and have negative effects on aquatic biota (Hanson and Stefan, 1984; Huggett et al., 1999; Mal et al., 2002; de Olivira-Filho et al., 2004). Recurring copper treatments can also give rise to copper-resistant populations of undesirable species (Izaguirre, 1992). Thus the effectiveness of repeated treatments should be further evaluated and considered in light of possible negative consequences.

Use of copper-based algaecides in combination with non-copper herbicides has been employed as a treatment strategy for *N. obtusa*. Flumioxazin and endothall are the herbicides most commonly used for these combination treatments. Tests of the effectiveness of endothall at suppressing Characeae growth have produced mixed results (Steward, 1980; Netherland and Turner, 1995; Hofstra and Clayton, 2001; Parsons et al., 2004) and this has not been directly tested on *N. obtusa* to our knowledge. Endothall is a broad-spectrum herbicide that can have negative effects on native plant communities under elevated treatment concentrations or exposure times (Skogerboe and Getsinger, 2001, 2002). Flumioxazin, which has been found to be effective on several macrophyte and algae species (Umphres et al., 2012; Glomski and Netherland, 2013), has been used in conjunction with copper algaecides on early infestations of *N. obtusa*. However, no empirical data support the efficacy of flumioxazin for controlling *N. obtusa*, it can be harmful to non-target species (Glomski and Netherland, 2013), and its effectiveness is lower in lakes with harder water and higher pH (Mudge and Haller, 2010)—characteristics broadly associated with *N. obtusa* occurrence (see above).

6.2. Mechanical removal

Over small scales, hand pulling and diver-assisted suction harvesting (DASH) can reduce cover and biomass of invasive macrophytes (Eichler et al., 1993; Boylen et al., 1996; Madsen, 2000). These methods involve divers removing biomass by hand and, in DASH, feeding it into a vacuum hose for disposal. While these methods can be effective and have high specificity, they are expensive, labor-intensive strategies that require long-term commitment (Bailey and Calhoun, 2008; Kelting and Laxson, 2010). For manual or DASH removal to be effective, all biomass at or below the substrate must be removed to minimize regrowth (Bailey and Calhoun, 2008). High densities of *N. obtusa* rhizoids and bulbils within invaded sediments can make this difficult to achieve. These methods were recently used on newly detected North American populations of *N. obtusa* (Little Muskego Lake, Waukesha Co., WI; Grand Lake, Stearns Co., MN), providing opportunities to evaluate the effectiveness of this approach.

At larger spatial scales, mechanical harvesters can be used to reduce biomass of nuisance macrophytes. Reduction in biomass is immediate but short-lived, and continued harvesting is needed (Rawls, 1975; Crowell et al., 1994). This method has been used for management of *N. obtusa* but requires further investigation—both to evaluate efficacy and because mechanical harvesters have the potential to disperse fragments and bulbils throughout a water body, possibly accelerating spread. This phenomenon has been documented in other macrophytes able to reproduce via fragmentation (Smith and Barko, 1990; Nino et al., 2005). Other concerns with mechanical harvesting include its non-selectivity and potential impacts to fish and invertebrate communities (Engel, 1990; Madsen, 2000). In a Minnesota lake, mechanical harvesting in combination with chelated copper treatment was found to significantly reduce *N. obtusa* biomass relative to an untreated reference area; harvesting alone was associated with a substantial but non-significant reduction in biomass (Glisson et al., 2018). In an inland Michigan lake, mechanical harvesting was performed in late summer, a time that corresponds with natural senescence of *N. obtusa* in this region. Evaluation of this treatment indicated that there were no significant differences in *N. obtusa* biomass or mat height between untreated and mechanically harvested areas (A. K. Monfils et al., CMU, unpub. data).

6.3. Physical management

Benthic barriers can be deployed on lakebeds to suppress growth of aquatic invasive plants and algae. Removable benthic barriers temporarily suppressed *Myriophyllum spicatum*, but re-colonization was rapid following barrier removal (Eichler et al., 1995; Helsel et al., 1996; Laitala et al., 2012). Caffrey et al. (2010) showed reduced growth of *Lagarosiphon major* using biodegradable jute matting. Over time the matting decomposed and the lakebed was recolonized by native plant and algae species. In Michigan U.S.A., an experiment is underway to evaluate the use of biodegradable benthic barriers as a component of an *N. obtusa* integrated management plan (A. K. Monfils et al., CMU, unpub. data).

Lake drawdowns can suppress seasonal regrowth of invasive macrophytes by exposing the lakebed to freezing and drying, thereby reducing viability of overwintering fragments and reproductive structures (Menninger, 2011). Winter drawdown has proven to be an inexpensive method for control *of Myriophyllum spicatum* and other invasive macrophyte species (Tarver, 1980; Siver et al., 1986). Limitations of this management strategy include its restriction to lakes with water-level controls and the fact that it is non-selective, potentially harming native macrophytes and benthic macroinvertebrates (Madsen, 2000; Harman et al., 2005). Lake level drawdowns are a potential strategy for *N. obtusa* control. Bulbil viability following desiccation and freezing is an important knowledge gap that is currently being investigated (K. G. Karol et al., NYBG, unpub. data).

7. Research needs

Our review of the literature on *N. obtusa* identified gaps in key knowledge areas important for understanding the basic biology of this species and guiding management responses in North America. Specifically, important questions remain unanswered pertaining to *N. obtusa* reproduction, environmental and biotic relationships, distribution and spread in North America, ecological impacts as a non-native species, and management.

Work addressing how environmental and genetic factors influence *N. obtusa* reproductive modes is needed. Little is known about the environmental cues required for germination of *N. obtusa* oospores or the contributions of sexual reproduction and genetic diversity to population dynamics. In North America, only male plants have been found. Further investigation is needed to assess this finding and determine whether there is a true absence of females or if females are present but not producing reproductive structures due to climatic or other factors. Emergence of fertile populations in the invaded range would be a major development that could increase persistence in already invaded waterbodies and potential for further spread (e.g., via long-distance dispersal of oospores by water birds).

We also have an insufficient understanding of the ecological niche of *N. obtusa*—and whether its niche differs between its native and invaded ranges. Field data indicate water chemistry associations that may be important for *N. obtusa* distribution, but several parameters have notably broad ranges (Table 1). Climatic niches occupied by *N. obtusa* in North America vs. Europe and Asia appear to differ (Escobar et al., 2016). But it is unclear whether this reflects a niche shift or is an artifact of populations in the invaded range, and possibly those in the native range, not being at equilibrium (i.e., the geographic extent of *N. obtusa* being dynamic).

Distributions of species are governed not only by environmental factors but also by biotic relationships within and across trophic levels (Noordhuis et al., 2002; Richter and Gross, 2013). Expansion of *N. obtusa* within North America has given rise to novel macrophyte assemblages; such changes could potentially contribute to local declines of native species (Parmesan, 2006; Stendera et al., 2012). Elucidating biotic interactions and incorporating them into projections of *N. obtusa* range expansion would improve threat assessment and predictive power; this is a major challenge for invasion ecology in general (Guisan and Thuiller, 2005; Gioria and Osborne, 2014). Characeae are known to be able to outcompete vascular plants (van Nes et al., 2003; Richter and Gross, 2013), but competition dynamics are likely to vary along resource gradients, and global change may lead to shifts in outcomes of competitive interactions between introduced and native species (Gioria and Osborne, 2014).

The full extent of the distribution of *N. obtusa* in North American is poorly understood. There are regions with few reports where there may be additional populations. Conversely, the lack of historical vouchering may be associated with false occurrence records. The need for systematic and vouchered studies is great. Along with improved distribution data, genetic analyses are needed to clarify relationships among populations. Such data would enable inferences to be made about the numbers and locations of initial introductions into North America and pathways of subsequent spread.

Relatively little is known about how *N. obtusa* invasion impacts aquatic ecosystems. Risks posed by invasive species increase with invasive potential, geographic extent, management difficulty, and ecological impacts (Molnar et al., 2008). Our review indicates high invasive potential, expanding geographic extent, and substantial management difficulty. There has been little information available to evaluate ecological impacts on plant communities, but publications are emerging (see Brainard and Schulz, 2017). Less well characterized are potential effects on water chemistry, invertebrates, fish, or other attributes. These knowledge gaps are problematic given that existing treatment options may have low efficacy or selectivity, requiring careful consideration of their relative costs and benefits.

In general, we have limited knowledge of the efficacy of methods currently available for *N. obtusa* control. More controlled, published studies on effectiveness of chemical treatments are needed to inform management. The same is true for the various physical and mechanical methods that have been employed (e.g., mechanical harvesting, DASH, benthic barriers, water-level management).

To support effective management, we need scientifically sound, well-designed, and replicated studies addressing management efficacy. Great strides have been made in the management of other aquatic invasive plants through multi-scale research programs that have tested treatment options in laboratory, mesocosm, and field settings, e.g., for *Myriophyllum spicatum* (Netherland and Getsinger, 1995; Getsinger et al., 1997; Netherland et al., 1997). Similar efforts are needed for *N. obtusa*. In addition to planned experiments, rigorous monitoring of ongoing treatments through research-management partnerships could accelerate learning. Relatively simple monitoring protocols can be incorporated into in-lake treatments to enable "learning while doing" (Zedler, 2005). For example, pre- and post-treatment measures of abundance of *N. obtusa* and native macrophytes in treated areas and untreated reference locations could provide a robust framework for evaluating management effectiveness.

In general, several of the applied knowledge gaps highlighted in this review can best be addressed through coordinated efforts across institutional and geographic boundaries. Research-management partnerships, sharing and synthesis of monitoring data, long-term studies of invasion dynamics and treatment outcomes, and home-and-away studies of *N. obtusa* ecology are important avenues for advancing *N. obtusa* science and management.

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Environmental filtering and competitive exclusion drive biodiversity-invasibility relationships in shallow lake plant communities

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Abstract

1. Understanding the processes that influence the diversity of ecological communities and their susceptibility to invasion by exotic species remains a challenge in ecology. In many systems, a positive relationship between the richness of native species and exotic species has been observed at larger spatial (e.g., regional) scales, while a negative pattern has been observed at local (e.g., plot) scales. These patterns are widely attributed to (1) biotic interactions, particularly biotic resistance, limiting

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invasions in high-diversity locations, producing negative local-scale relationships, and (2) native and exotic richness covarying at larger spatial scales as a function of environmental conditions and heterogeneity, producing positive large-scale relationships. However, alternative processes can produce similar patterns and need to be critically evaluated to make sound inferences about underlying mechanisms.

2. We aggregated a large dataset of aquatic vegetation surveys from 1,102 Minnesota shallow lakes collected over 13 years to quantify spatial and temporal patterns of community composition. Using those data and additional information on environmental conditions we evaluated evidence for four distinct mechanisms that could drive patterns of native and exotic species richness: biotic resistance, competitive exclusion, environmental filtering, and environmental heterogeneity.
3. We found the classic pattern of a negative native-exotic richness relationship at local scales and a positive relationship at lake scales. However, we found no evidence for local-scale biotic resistance; instead, competitive exclusion by invasive species appeared to reduce native species richness after locations became invaded. Evaluating the influence of environmental filtering and heterogeneity, we found that native and exotic species occupied somewhat different niches. Invaders were less sensitive to environmental gradients and more tolerant of a wider range of conditions. This segregation of habitat preferences alone could produce a negative local native-exotic richness relationship and a positive regional pattern without the involvement of biotic interactions.
4. *Synthesis:* Our findings conflict with established expectations, which come from research predominantly conducted in terrestrial ecosystems. This illustrates the

importance of explicitly evaluating underlying mechanisms in diversity-invasibility research and for comparisons across different types of ecosystems. Identification of different drivers of diversity also has direct implications for decisions about management of freshwater plant communities.

Keywords: Invasion ecology, Biodiversity, native-exotic richness relationship, biotic resistance, competitive exclusion, environmental filtering, heterogeneity, aquatic plants

Introduction

The relationship between the diversity of ecological communities and their propensity to be invaded by exotic species has been heavily debated (Levine & D'Antonio 1999; Levine 2000; Wardle 2001; Kennedy *et al.* 2002; Fargione & Tilman 2005). Much research, particularly modeling and small-scale experiments, has supported a negative relationship between diversity and invasibility. However, at larger (e.g., regional) scales the opposite pattern is frequently observed, with more diverse communities having more exotic species (Levine & D'Antonio 1999; Stohlgren *et al.* 1999; Cleland *et al.* 2004). This scale-dependent shift in the native-exotic richness relationship (NERR) remains difficult to explain, with multiple processes potentially interacting to produce overall patterns. At the same time, invasive species are a global ecological threat (*MEA* 2005, Bellard et al. 2016); thus, improving understanding of biodiversity-invasibility relationships is important for supporting conservation and management.

A common explanation for scale-dependent NERR differences is that separate processes drive local and regional patterns (Levine & D'Antonio 1999; Stohlgren *et al.* 1999). It has been posited that at local scales high diversity confers biotic resistance to invasion (Kennedy *et al.*

2002; Fargione & Tilman 2005), but that at broader scales, incorporation of new habitats that are favorable for native and invasive species alike increases diversity of both in parallel (Levine & D'Antonio 1999; Naeem *et al.* 2000). However, further work has highlighted other processes that may influence NERRs (Fridley *et al.* 2007). Spatial heterogeneity in environmental conditions may support positive NERRs (Davies *et al.* 2005) by increasing avenues for coexistence (e.g., Chesson 2000; Tilman 2004). The strength or direction of a local-scale NERR can also shift as a function of productivity, disturbance, or environmental gradients (Davies *et al.* 2007; Belote *et al.* 2008). For example, invaders that have broader environmental tolerances or prefer less productive conditions may occur, on average, in less diverse localities (e.g., Paavola, Olenin & Leppäkoski 2005) because those conditions tend to correlate with lower diversity. In such cases, negative NERRs may arise through a sampling effect without the need for any particular biotic interaction to be involved.

The management implications of an NERR can differ depending on its underlying mechanism(s). For example, a negative NERR resulting from diversity-driven biotic resistance would argue for efforts to create or maintain diversity to pre-empt invasion. In contrast, if such patterns are a result of competitive exclusion by the invader, efforts to increase diversity may offer little protection against invasion. At the regional scale, if a positive NERR arises because invasive and native species share environmental preferences, then the most resource-rich environments may be at the greatest risk of invasion. Alternatively, if heterogeneity is the driving mechanism for an NERR, the most variable locales may be most vulnerable.

Even in a single system, NERRs are likely to arise from multiple processes, especially across local and regional scales (Fridley *et al.* 2007). However, there is a growing consensus that biotic interactions tend to be key drivers of community structure at local scales while environmental conditions become more influential as spatial scale increases (Fridley *et al.* 2007). Thus a more mechanistic perspective that evaluates multiple processes at multiple scales is needed. Such studies are logistically difficult to conduct as experiments, but large-scale, long-term monitoring datasets offer an alternative means to address these dynamics.

Here we focus on four mechanisms that could influence native or exotic species diversity, three of which we could evaluate at multiple spatial scales. *Biotic resistance* to invasion has long been considered a potential benefit of diverse communities (Elton 1958) and is well-supported by experimental work (Stachowicz, Whitlatch & Osman 1999; Levine 2000; Naeem *et al.* 2000; Kennedy *et al.* 2002; Fargione & Tilman 2005), though the universality of this mechanism has been questioned (Capers *et al.* 2007). Invasive species can also *competitively exclude* resident species after establishment (Casas, Scrosati & Luz Piriz 2004; Yurkonis, Meiners & Wachholder 2005), producing a pattern of native and invader richness similar to biotic resistance but with a different temporal signature, i.e., loss of native diversity following invasion rather than lower likelihood of subsequent invasion in diverse locales. Thirdly, *environmental filtering* influences species' abilities to establish and persist in particular localities. Alignment of preferences between natives and invaders could produce positive regional NERRs, while competitive interactions determine local-scale outcomes (Davies *et al.* 2005; Cavender-Bares *et al.* 2009). Alternatively, if invaders have wider environmental tolerances than natives (Richards *et al.* 2006; Vazquez 2006), a negative local NERR could be produced by invaders establishing in marginal habitat with few native species. Lastly, *environmental heterogeneity* in conditions or habitat types is a key mechanism supporting overall diversity that can increase both native and invader richness (Davies *et al.* 2005). This effect is likely to become more pronounced over larger spatial scales as greater variability is accrued (Huston 1999).

In this study, we used an exceptionally large data set of aquatic vegetation surveys from Minnesota shallow lakes to characterize NERRs at local and regional scales and examine evidence for alternative mechanisms. Using data from sites with repeated sampling over time we tested for (1) native species richness conferring *biotic resistance* to invasion and (2) invaders *competitively excluding* native species after establishment. We used environmental data to (3) correlate native and invasive species richness with abiotic conditions to evaluate if *environmental filtering* acted similarly on both groups and (4) evaluate how native and invasive species responded to *environmental heterogeneity* as a potential driver of regional scale diversity.

Methods

Survey data

Vegetation data for the study were aggregated from 1,662 grid-based, point-intercept surveys conducted by the Minnesota Department of Natural Resources in 1,102 shallow lakes from 2002–2014. The lakes represent a broad range of shallow lakes across the state with varying levels and types of nearby land use, human activity, and management. Surveys were conducted with a thrown rake that was pulled along the benthic surface to collect vegetation. All macrophytes (aquatic vascular plants and macroalgae) were identified to species or lowest feasible taxon. For simplicity we refer to all taxa as "species," i.e., including those identified only to genus (See Supplementary Table 1 for a full list of taxa). The number of survey points varied between lakes (61.7 ± 37.9; mean ± SD), scaling with lake size.

We used these data to calculate species richness at point and lake scales. We distinguished species considered invasive in Minnesota based on established lists (Milburn, Bourdaghs & Husveth 2007; USDA 2016), and six were present in our surveys: *Lythrum salicaria* (purple loosestrife), *Myriophyllum spicatum* (Eurasian watermilfoil), *Phalaris arundinacea* (reed canarygrass), *Potamogeton crispus* (curly-leaf pondweed), *Typha angustifolia* (narrow-leaf cattail), and *Typha* × *glauca* (hybrid cattail). In cases where identification was resolved to a taxonomic level encompassing both invasive and native species (e.g., *Typha* sp. is ambiguous with the native *Typha latifolia*) we conservatively assumed the native form. Similarly, while invasive European genotypes of *Phragmites australis* occur in Minnesota, lineages were not discriminated in our dataset. Thus we treated all *P. australis* as comprising the widespread native subspecies *P. australis* ssp. *americanus*. In a small number of lakes, invasive *Typha* was recorded both to species and to the grouped category "*T. angustifolia* or × *glauca*." We counted these as representing only a single invader species.

Using these data, we evaluated NERRs at local (individual sampling point) and regional (whole-lake) scales. All analyses were performed in R version 3.1.2 (R Core Team 2014). Using point-level data, we estimated the relationship between native and exotic species richness. To account for the integer nature of the response variable, we used a generalized linear model (GLM) with a Poisson error distribution (using the 'glm' function from the stats package) and evaluated significance using the 'summary.glm' function (this approach was used for all GLMs). We then calculated lake-level richness values and constructed a separate GLM for lake-level native and exotic species richness.

Biotic interaction mechanisms

To calculate the potential for native diversity to confer biotic resistance to invasion and for invasive species to competitively exclude native species, we analyzed temporal patterns in lakes that had been repeatedly sampled over multiple years. Because temporal analyses would be sensitive to changes in sampling effort or locations, we only included data from lakes where the same grids of sampling points were used among years; this comprised 179 lakes, each with 2-9 interannual surveys (mean = 3.22).

To quantify biotic resistance, we compared the relationship between native species richness and the probability of a sampling point becoming invaded at subsequent sampling times. Because invasive species themselves can potentially increase the likelihood of further invasions (via an invasional meltdown; Simberloff & Von Holle 1999) or increase resistance (Henriksson *et al.* 2016), we focused only on initial invasions, excluding all locations that were already invaded. While the potential effects of initial invaders on secondary invasions are of interest, the number of such records was insufficient to address this issue. Additionally, some locations may have been generally unsuitable for vegetation, producing zero values for richness that could artificially reduce estimates of species richness, thus we excluded from our analysis points lacking vegetation at any sampling time. We also excluded locations from lakes that did not contain any invasive species at the initial sampling point. Invasion in such cases would require colonization from another lake, a highly stochastic process that could bias estimates. We analyzed data from the remaining sites using a generalized linear mixed effects model (from the binomial family). Whether or not an uninvaded point was subsequently invaded was used as the response variable, native species richness was treated as a fixed effect, and lake identity was included as a random effect. The model was fit using the ‘glmer’ function from the lme4 package (Bates *et al.* 2015) and using the “bobyqa” optimizer (with the argument control=glmerControl(optimizer="bobyqa")); significance was evaluated using a parametric bootstrap. This approach first estimates the full mixed model with the variable of interest included, then a reduced model with the variable removed; change in fit between models was assessed using the ‘PBmodcomp’ function (from the pbkrtest package with 1000 simulations; Halekoh & Højsgaard 2014). We also evaluated biotic resistance at the lake scale, evaluating how whole-lake native species richness influences the probability of becoming invaded using a GLM from the binomial family.

To evaluate whether invaders competitively excluded native species, we estimated rate of change in native species richness for each sampling point by estimating a linear regression for native species richness with sampling year as the single independent variable. For each model, the coefficient for the time parameter provides an estimate of the average yearly change in species number, with negative values indicating species loss. Differences in average coefficient values were compared between sites that were invaded and those that remained uninvaded through all surveys, also using a linear model. We again excluded locations where no vegetation was recorded during any survey and used a linear mixed effect model (with the ‘lmer’ function from the lme4 package) to compare rates between invaded and uninvaded sites while accounting for lake as a random effect. Statistical significance was again evaluated using the same parametric bootstrap approach as above. Competitive exclusion was also evaluated at the lake scale using a standard linear model (with the “lm” and “summary.lm” functions) to compare rates of change in species richness between invaded and uninvaded lakes.

Environmental mechanisms of invasion

To investigate how environmental conditions influenced patterns of diversity, we collected data on a range of environmental parameters at both point and lake scales. During surveys, point-level measures of bottom depth and Secchi depth were recorded. We used GLMs to estimate influence of depth and Secchi depth on native and invasive species richness, assuming Poisson distributions for species richness. We calculated these relationships at point and whole-lake scales (using mean values across points). Because depth and Secchi depth were correlated, we used separate models to independently evaluate their relationships with richness rather than including both parameters in a single analysis. The total possible richness of invasive species was much lower than that of native species, thus we conducted analogous analyses using invader presence as a binomial response in GLMs to test for environmental preferences of invasive species in general. Additionally we calculated standard deviations (SD) of depth and Secchi depth for each lake as measures of within-lake heterogeneity and used these data to estimate GLMs testing relationships between lake heterogeneity and native and invasive species richness at the lake scale, again assuming Poisson distributions for species richness. We also conducted an additional analysis of invader response with invader presence as a binomial response in a GLM.

To estimate additional environmental parameters for lakes, we aggregated data from two publicly available sources. We collected measurements of lake area and long-term average Secchi depth (m) for ~11,000 lakes derived from remote sensing data by the University of Minnesota Remote Sensing and Geospatial Analysis Laboratory (Olmanson, Bauer & Brezonik 2008; Olmanson, Brezonik & Bauer 2014). In addition, the Minnesota Pollution Control Agency (MPCA) manages a large dataset of direct lake measurements (~6 million records) collected by state, local, and citizen-based organizations on a wide variety of environmental parameters. We focused on five parameters likely to influence macrophyte distribution that were sampled in large numbers of lakes: pH, conductance (μS), total Kjeldahl nitrogen (N; mg/L), total phosphorus (P; mg/L), and chlorophyll *a* concentration (μg/L). Data were heterogeneous in space and time and collected by groups with differing technical proficiency, thus we took several steps to assure data quality. We limited environmental measures to only those collected since the year 2000 and during the growing season (June–September). To remove data likely to be erroneous we calculated mean and SD for each variable across all lakes and excluded any samples with values >5 SD from the mean. Because SD was sometimes strongly influenced by extreme outliers, we then recalculated SD with outliers removed and repeated the process a second time. This left us with 139 lakes in the dataset with values for all parameters. For these lakes we aggregated all measurements of a given parameter into a single mean.

For surveyed lakes with data for all environmental parameters, we used GLMs with multiple fixed effects to identify environmental conditions associated with native or invasive species. GLMs included 6 environmental parameters as potential predictors (N, P, pH, conductance, chlorophyll *a*, and Secchi depth). Native and invasive species richness and invasion status were modeled as responses in separate analyses, using a Poisson error distribution for richness measures and invader presence/absence as a binomial response.

Results

Overall patterns

Vegetation data comprised 56,134 sampling points from 1,662 surveys in 1,102 lakes. Across surveys, 150,318 individual vegetation samples were identified to 172 taxa (generally species; Table S1). Invasive species were identified in nearly half of the lakes (546) and invaded lakes spanned the entire range of native species richness (Fig. 1). The average number of species at a sampling point was 2.69 ± 1.83 (mean ± SD) and within a lake was 10.13 ± 7.23. Consistent with the "invasion paradox" (Fridley *et al.* 2007), we observed a negative NERR at the point scale and a positive relationship at the whole-lake scale (Table 1, Fig. 2).

Biotic resistance

At the local scale, we observed no significant relationship between species richness and the probability that a location would become invaded in the subsequent survey (Table 1, Fig. 3a), i.e., no support for local-scale biotic resistance. Results showed high variability with many lakes showing positive relationships, while others displayed negative relationships, indicating very noisy data with little pattern rather than a consistent but small effect. At the lake scale, we also did not see a significant relationship between species richness and invasion, though the parameter estimate was positive (0.028; Table 1). Thus, while non-significant, the trend followed the opposite pattern, with higher species richness being associated with a greater propensity for invasion. However, this pattern may be largely noise.

Competitive exclusion

Our analyses did provide support for competitive exclusion of native species by invaders at the local scale (Table 1). Based on parameter estimates of the linear mixed effects model, species richness decreased at invaded sampling points by 0.02 species per year (after accounting for lake-to-lake differences; Fig. 3b), while at uninvaded points richness increased by 0.08 species per year. At the lake scale, there was no significant difference in rates of richness change between invaded and uninvaded lakes; richness tended to increase in both over time (Table 1).

Environmental filtering

At the local scale, both native and invasive species richness significantly varied with environmental conditions (Table 1; analyses using binomial GLMs based on invasive species presence generally show the same directionality and significance patterns as the analyses using invader richness, results can be seen in Table S2 and figures S1 and S2 in online Supporting Information). Native and invasive species had significant, but opposing, relationships with depth (Fig 4a-b); native richness decreased with greater depth, while invasive richness increased, though less strongly. Both native and invasive richness increased with water clarity (Fig 4c-d), but this relationship was much stronger for native ($z = 40.17$) than invasive species ($z = 3.897$), suggesting weaker light limitation in invaders. At the lake scale, native richness increased with mean lake depth and mean Secchi depth (Fig 4e,g). Invasive richness did not significantly differ with either parameter (Fig 4f,h), again suggesting broader tolerance.

Analyzing the larger set of environmental variables, we identified many significant relationships between lake-level environmental parameters and species richness (Table 1), but the significant variables differed between native and invasive species. All environmental conditions except N were significant predictors of native richness. In contrast, only pH and Secchi depth were significant predictors of invasive richness. Furthermore, directionality of some strong predictors of richness were reversed between native and invasive species. For example, native richness had a strong negative relationship with P, while the pattern was positive (though not significant) for invasive species. The opposite pattern was seen for Secchi depth; invasive richness decreased and native richness increased with greater clarity. Conductance and chlorophyll *a* were significant negative predictors of native richness and negatively correlated but not significant for invaders. The generally weaker responses of invasive richness to environmental conditions suggest that invaders had broader environmental tolerances. At the lake scale these patterns are potentially confounded by the general correlation of average depth and lake size. However, both lake size and average depth (as opposed to depth at a particular location), are likely proxies for overall habitat variability, which in turn drives increased native species richness rather than a direct influence of average depth or size. Thus the general pattern of stronger environmental constraints on native species than invaders exists independent of whether lake size and average depth are confounded.

Heterogeneity

Within-lake heterogeneity in depth and Secchi depth were significant positive predictors of native richness (Table 1, Fig 5a,c) but had no influence on invasive richness (Table 1, Fig 5b,d). This further supports the contention that invaders have lower sensitivity to environmental conditions.

Discussion

The aquatic plant communities we studied showed a strong negative relationship between native and invasive species richness at local (point) scales, but a positive relationship at regional (lake-wide) scales, matching patterns observed in numerous systems. However, when we evaluated mechanisms that could generate these patterns, we found varying levels of support, indicating that not all mechanisms were of equal importance. Similarly, no mechanism dominated and any given factor explained only a small amount of the patterns observed in native and invasive species richness. In contrast to many terrestrial systems (Naeem *et al.* 2000; Kennedy *et al.* 2002; Levine, Adler & Yelenik 2004), we found no evidence for local-scale biotic resistance. Our results also indicate a strong influence of environmental constraints on local-scale richness patterns, counter to general expectations that environmental filtering becomes more important at broader spatial scales (Fridley *et al.* 2007). Our findings illustrate that similar NERR patterns can be produced by different underlying mechanisms that can be difficult to discriminate. These alternative mechanisms may have very different implications for conservation and management of aquatic plant communities, underscoring the value of applying a mechanistic lens to evaluating patterns of community structure and diversity.

Strong, opposing roles of environmental drivers on native and invasive species

Contrasting patterns of negative NERRs at local scales and positive NERRs at regional scales have been seen in a variety of systems; we saw similar patterns in Minnesota aquatic plant communities. While it is recognized that multiple processes can influence NERRs, there is a general expectation that biotic interactions dominate at local scales but are supplanted by abiotic determinants at broader scales (Fridley *et al.* 2007). Our data do not support this prediction. Rather we found that environmental conditions were relatively important predictors of richness at regional *and* local scales while effects of biotic interactions were relatively weak. However, there was still substantial unexplained variance that may be influenced by environmental factors not considered as part of this study or by alternative ecological mechanisms.

Native and invasive species were sensitive to different environmental factors; in some cases even showing opposing responses to the same environmental gradients. For example, native richness was associated with lower water depth and P while invasive richness was associated with greater depth and P. For water clarity, native and invasive species both showed positive relationships at the local scale, but the relationship was weaker for invasive species.

These divergent preferences suggest that native and invasive species occupied somewhat different niches. Such niche segregation alone could produce a negative NERR without biotic interactions being involved.

The patterns we observed indicate that invasive species gained advantage over native species under more eutrophic conditions. This is presumably due to these species being better adapted to exploit higher resource availability and tolerate lower light levels (Nichols & Shaw 1986; Woo & Zedler 2002). Alternatively, it is also possible that poor water quality increased with greater human activity, and that human activity was the proximate cause of greater invasion rates via increased transmission opportunities.

Furthermore, while greater environmental heterogeneity was associated with increased richness of native species—consistent with a large body of ecological theory and literature (Pickett & Cadenasso 1995; Larkin, Bruland & Zedler 2016)—there was no such response by invasive species. This suggests that native species were more specialized to depth and light niches within lakes, while invasive species occupied broader niches and were thus able to exploit more marginal habitat. However, our analyses were limited to water depth and Secchi depth; it is possible that invaders may have exhibited greater responsiveness to heterogeneity in other environmental factors. Greater responsiveness to increased resource availability and broader environmental tolerances appear to be attributes of successful invasive plants in general (Davis, Grime & Thompson 2000; Zedler & Kercher 2004), and global drivers of change reinforce these advantages (Thompson & Davis 2011). In northern shallow lakes, recent findings point to persistent, anthropogenic shifts to more nutrient-rich, turbid alternative states (Ramstack Hobbs *et al.* 2016). Our findings suggest these changes will exacerbate aquatic plant invasions.

Invasive species win biotic interactions—competitive exclusion but not biotic resistance

How new invasions affect native plant communities depends on biotic interactions between resident native vegetation and invading species. We analyzed repeated surveys in the same locations to investigate biotic interactions and found mixed support for their importance. After sampling locations were invaded, native species richness tended to decrease over time, while uninvaded locations gained species. This supports the competitive exclusion hypothesis, i.e., that invaders reduce local-scale diversity by displacing native species. In contrast, when we evaluated biotic resistance, we found no evidence that more-diverse sites were less likely to be subsequently invaded. A caveat is that invasions are highly stochastic processes punctuated by relatively few invasion events (Mack *et al.* 2000; Simberloff 2009). Furthermore, it is difficult to resurvey precise locations over multiple years and imperfect detection may confound species' presence/absence records (Chen *et al.* 2013). These factors can result in noisy datasets and as such, the likelihood of type II errors (false negatives) may be particularly high and the ability to detect a signal low. Yet our ability to still identify competitive exclusion despite such noise suggests that our general approach is valid and that biotic resistance is likely weaker or potentially absent in this system, though it is difficult to make a direct comparison of process strengths. Thus while we found evidence of influential biotic interactions at the local scale, as expected (Fridley *et al.* 2007), we did not observe biotic resistance, which is often considered to be the key driver for a negative NERR (Levine *et al.* 2004; Fargione & Tilman 2005). Instead we found statistically significant evidence for competitive exclusion, which is less often cited as a driver of negative NERRs. Though the effects we observed were relatively modest, on the order of one more species being lost per decade in invaded sites relative to uninvaded sites, and there was high variability with many individual sites and lakes exhibiting the opposite pattern.

Over time, biotic resistance and competitive exclusion can produce similar negative NERR patterns. Studies in which richness is examined at only a single time point are inherently unable to discriminate these two processes. Yet the two mechanisms have different implications for conservation and management. A system with strong biotic resistance will be resilient to invasions (Naeem *et al.* 2000; Fargione & Tilman 2005) and managing for diversity can minimize risk. But where biotic resistance is weak and competitive exclusion likely, uninvaded communities are vulnerable and biodiversity will not reduce invasion risk.

The combination of broader environmental tolerance of invasive species and the potential for competitive displacement of native species may provide an important pathway for invasion. By taking advantage of marginal habitat for native species, invaders can establish in new areas without facing competition. Once established, propagule pressure can then promote spread into nearby habitat preferred by native species (Lockwood, Cassey & Blackburn 2005). Propagule pressure from nearby sources will far exceed that associated with rare long-distance dispersal events (Simberloff 2009) and could swamp effects of biotic resistance (Thomsen *et al.* 2006). This “leapfrogging” of invasive plants from marginal to preferred habitat has been demonstrated in invasion of European *Phragmites australis* in North America, which spreads across the landscape via highway corridors and anthropogenic habitat (Lelong *et al.* 2007; Taddeo & De Blois 2012), providing propagules that can then invade intact natural wetlands and displace native species (Price, Fant & Larkin 2014; Fant, Price & Larkin 2016). The importance of environmental conditions in determining native and invasive species richness suggest that management of those factors may be a key strategy for limiting invader establishment.

At the larger spatial scale of whole lakes, our findings more closely match expectations from other systems (Levine & D’Antonio 1999; Davies *et al.* 2005; Fridley *et al.* 2007). Our lake-scale analyses showed no evidence of biotic resistance or competitive exclusion, instead native and invasive species richness increased in concert. This is consistent with NERRs not being driven by biotic interactions at large spatial scales but instead broader environmental, historical, or biogeographic factors (Ricklefs 2004; Fridley *et al.* 2007; Cavender-Bares *et al.* 2009). Native species richness increased with environmental heterogeneity, which aligns with the expectation that the inclusion of broader environmental conditions drives regional-scale diversity patterns (Levine & D'Antonio 1999; Davies *et al.* 2005; Fridley *et al.* 2007), though we did not observe a similar pattern for invasive species with the environmental factors we evaluated. Nonetheless, even if invasive species have broad environmental tolerances and are not influenced by heterogeneity, stochastic processes could still lead to increased invader richness at larger spatial scales, resulting in a positive NERR (Fridley, Brown & Bruno 2004).

Is biotic resistance "all dry"?

While our results regarding the relative importance of biotic interactions vs. abiotic drivers run counter to previous findings—particularly with respect to the absence of biotic resistance—the cause of that inconsistency remains uncertain. It may be partly due to few studies simultaneously investigating multiple alternative mechanisms of NERRs (but see Fargione & Tilman 2005) or to patterns being attributed to mechanisms that are presumed to be common but have not been explicitly tested.

It is also possible that the preponderance of diversity-invasibility research that comes from terrestrial systems biases expectations. Strong (1992) asked whether trophic cascades were "all wet." Is biotic resistance "all dry?" Nearly all evidence for local-scale biotic resistance comes from grassland or other terrestrial systems (Naeem *et al.* 2000; Levine *et al.* 2004;

Fargione & Tilman 2005; Fridley *et al.* 2007; but see Stachowicz *et al.* 1999). Relatively little research has been conducted in aquatic plant communities and some past findings have run counter to terrestrial expectations. Capers et al. (2007) found no evidence of biotic resistance in lake plant communities in the northeastern U.S. In lakes across the U.S., Fleming et al. (2015) tested Darwin's naturalization hypothesis that niches being occupied by close relatives would repel invaders; they found no evidence of such resistance. Ström et al (2014) experimentally demonstrated a local-scale but *positive* NERR in boreal wetlands.

Why would diversity-invasibility relationships differ between land and water? There is some evidence that aquatic plant communities are more strongly structured by abiotic environmental constraints (Santamaría 2002; Heino *et al.* 2017). Difficult environmental conditions in aquatic communities, particularly at higher latitudes, may impose such a strong filter on the macrophyte habitat species pool that species interactions have limited influence on community assembly (Santamaría 2002). Similar patterns have been observed in aquatic invertebrate communities (Peckarsky, Horn & Statzner 1990; Milner *et al.* 2001), suggesting that this may be a common pattern for freshwater systems. If the relative importance of abiotic and biotic processes in NERRs systematically varies between terrestrial and aquatic systems, then the limited research performed in the latter could bias our general understanding of the ecological mechanisms contributing to these patterns.

Implications for biodiversity conservation and invasive species management

Invasive species are one of the most important drivers of global change and can drastically restructure ecosystems (Vitousek *et al.* 1997; W. H. Mason, Bastow Wilson & B. Steel 2007; Tylianakis *et al.* 2008). The relationship between diversity and composition of native communities and their invasibility has been a fundamental area of inquiry in ecology going back to Elton (1958) and even Darwin (Daehler 2001). Understanding the conditions that allow invasive species to establish and that mediate their impacts remain critical issues for conservation and management (Mack *et al.* 2000; Byers *et al.* 2002). Studying the relationship between diversity of native species and invasive species can offer important insights into these questions by helping to identify the factors that support or deter invasions. In particular, the idea of biotic resistance suggests a "virtuous cycle" wherein efforts to support biodiversity also help repel invasions. However, the patterns we observed suggest that watershed management to support water quality may be a more effective means of mitigating invasions and their impacts. Nonetheless, it is clear that diversity-invasibility patterns can be driven by multiple mechanisms and recognizing the context-specific importance of these different mechanisms can help refine management strategies.

Our analysis of alternative mechanisms underlying NERRs in shallow lakes reveals several concerning trends: (1) environmental conditions consistent with broad patterns of anthropogenic change benefit invasive species, (2) lakes with higher biodiversity value are more likely to become invaded, and (3) biotic interactions represented a "bad news-bad news" scenario wherein local-scale diversity does not confer resistance to invasion but invasion does reduce local-scale diversity via competitive exclusion. However, our results do support continued effort toward established strategies for invasive species management. Specifically, efforts to maintain or improve lake condition, reduce spread of invasive species, and restore diverse plant assemblages where they have been lost are needed to slow the erosion of native plant diversity in these important ecosystems.

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Author Contributions

NH-W managed data collection. RM and DJL designed the study and analyses. RM analyzed the data and wrote the initial draft of the manuscript. All authors participated in data interpretation and revising the manuscript.

Data Accessibility

Macrophyte community data, environmental data, and analysis scripts for this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.19cf1c2 (Muthukrishnan, 2018)

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Figure Captions

Figure 1. Frequency of lakes with different native species richness. Dark gray portions of bars indicate lakes that had no invasive species present and light gray portions indicate lakes with at least one invasive species.

Figure 2. Relationship between richness of native species and invasive species identified in individual samples (a) or aggregated to the lake level (b). Points are jittered along the y-axis to increase visibility of overlapping points. The solid red lines indicate the estimated value and dashed lines are the 95% confidence interval for the estimate.

Figure 3. Biotic resistance (a) is indicated by an estimate of the probability of invasion of individual sampling locations as a function of native species richness. Colored lines indicate the trends for individual lakes with purple lines indicating lakes where invasion risk decreases with greater native species richness (indicating biotic resistance) and green lines indicating higher risk of invasion. The dashed portions of lines indicate estimates calculated for native species richness beyond the range where actual data was observed. The solid black lines indicate the overall estimates after accounting for autocorrelation within lakes and the dashed lines indicate the 95% confidence interval for those estimates. Competitive exclusion (b) is evaluated by a comparison of the rate of change in native species richness between locations that are uninvaded across all sampling time points and those where an invader is present. Here green lines indicate lakes with higher values at invaded points while purple lines are lakes with lower values at invaded sites and the black lines again show the overall estimates with a 95% confidence interval.

Figure 4. Relationships between depth (a-b, e-f) or Secchi depth (c-d, g-h) and the richness of native species and invasive species at individual sampling locations (a-d) and aggregated across entire lakes (e-h). Points are jittered along the y-axis for clarity and red lines indicate the mean and 95% confidence interval of the estimated value.

Figure 5. Relationships between the heterogeneity of depth or Secchi depth in a lake and the richness of native species (a,c) and invasive species (b,d). Points are jittered along the y-axis for clarity and red lines indicate the mean and 95% confidence interval of the estimated value.

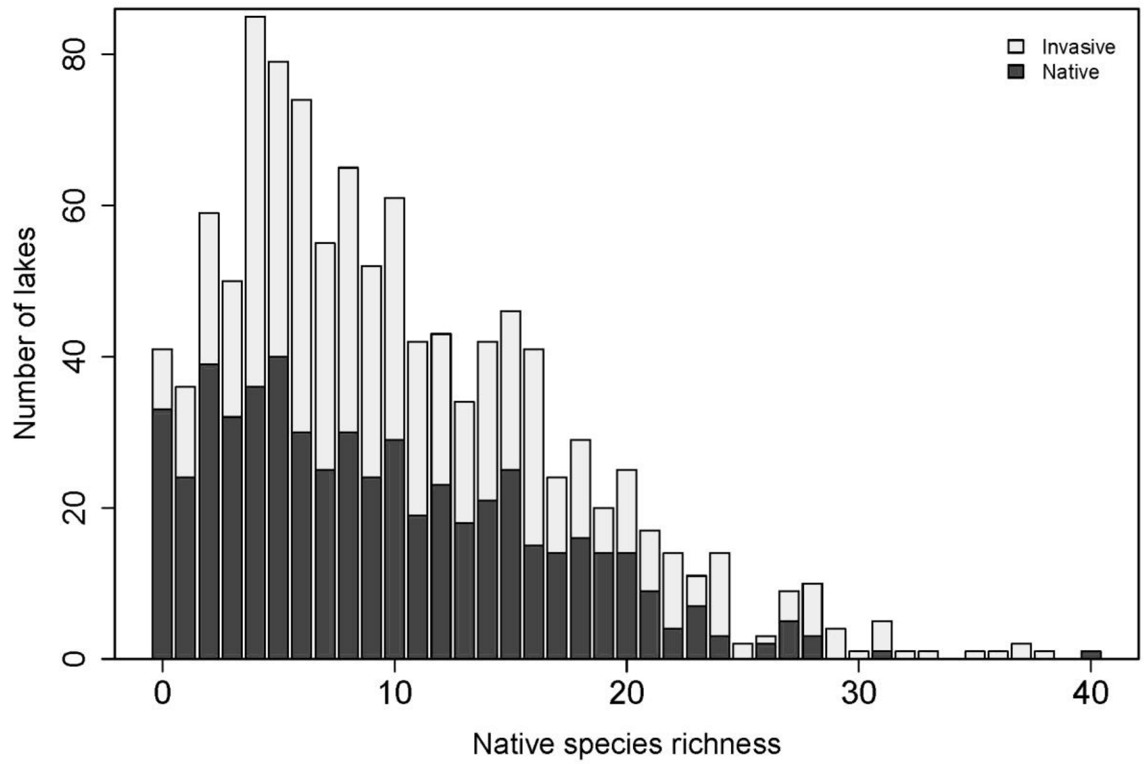
Tables

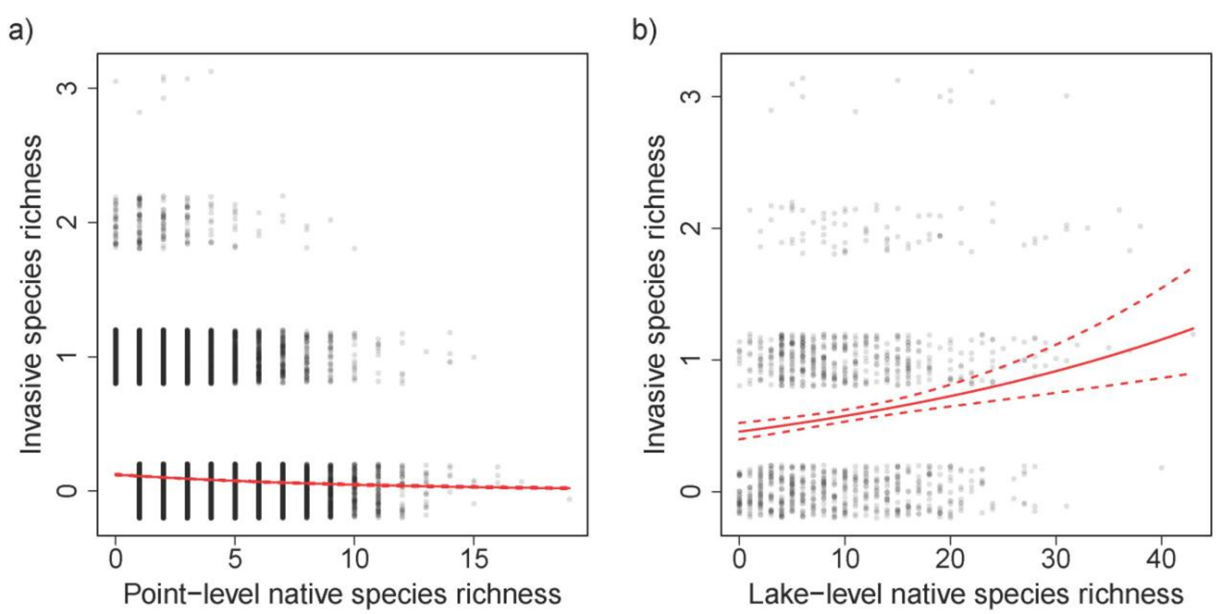
Table 1. Results of fixed effects from all statistical models. Generalized linear models were used in most analyses but mixed models were used for point scale analyses of biotic resistance and competitive exclusion to account for autocorrelation within lakes. Statistically significant results indicated with a “*”.

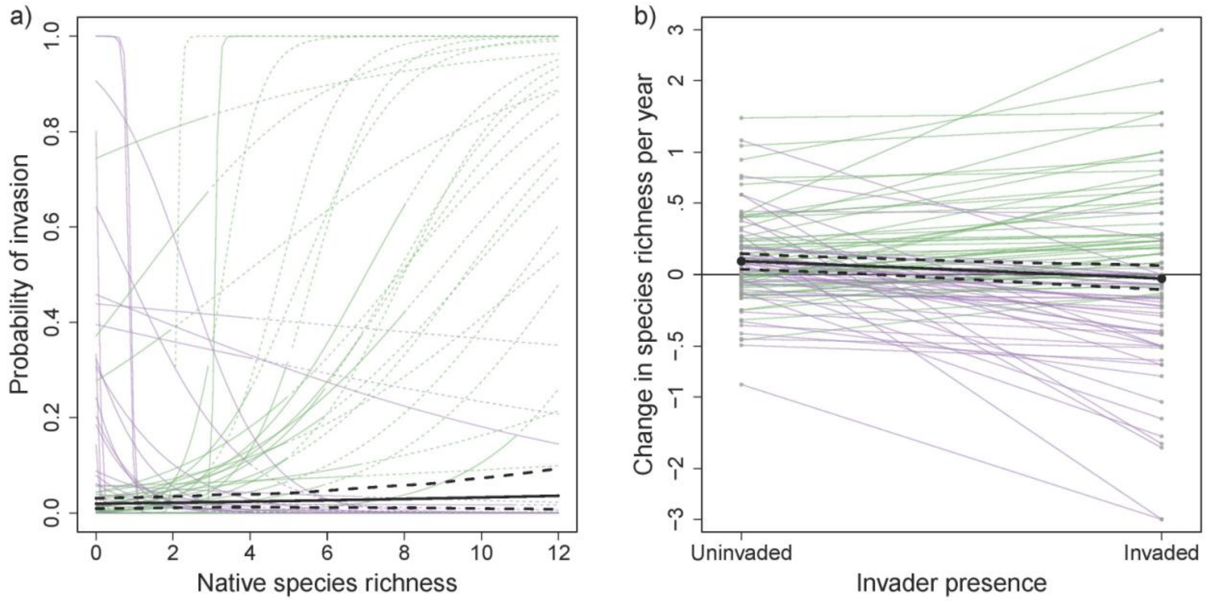
Analysis	Scale	Parameter	Estimate	Std. Error	Test statistic	p-value	Significant
Overall NERR	Point	Intercept	-2.103	0.024	-88.076	< 0.001	*
		Coefficient	-0.096	0.008	-11.308	< 0.001	*
	Lake	Intercept	-0.786	0.070	-11.277	< 0.001	*
		Coefficient	0.023	0.005	4.631	< 0.001	*
Biotic Interactions							
Biotic resistance	Point	Intercept	-3.921	0.286	-13.735		
		Coefficient	0.053	0.054	0.971	0.356	
	Lake	Intercept	-0.642	0.230	-2.798	0.005	*
		Coefficient	0.028	0.026	1.103	0.270	
Competitive exclusion	Point	Intercept	0.080	0.024	3.343		
		Coefficient	-0.101	0.025	-4.130	<0.001	*
	Lake	Intercept	0.021	0.182	0.114	0.910	
		Coefficient	0.239	0.221	1.080	0.282	
Environmental analyses							
Native richness ~ Depth	Point	Intercept	1.150	0.006	208.374	< 0.001	*
		Coefficient	-0.052	0.001	-40.172	< 0.001	*
	Lake	Intercept	2.268	0.020	114.602	< 0.001	*
		Coefficient	0.010	0.004	2.747	0.006	*

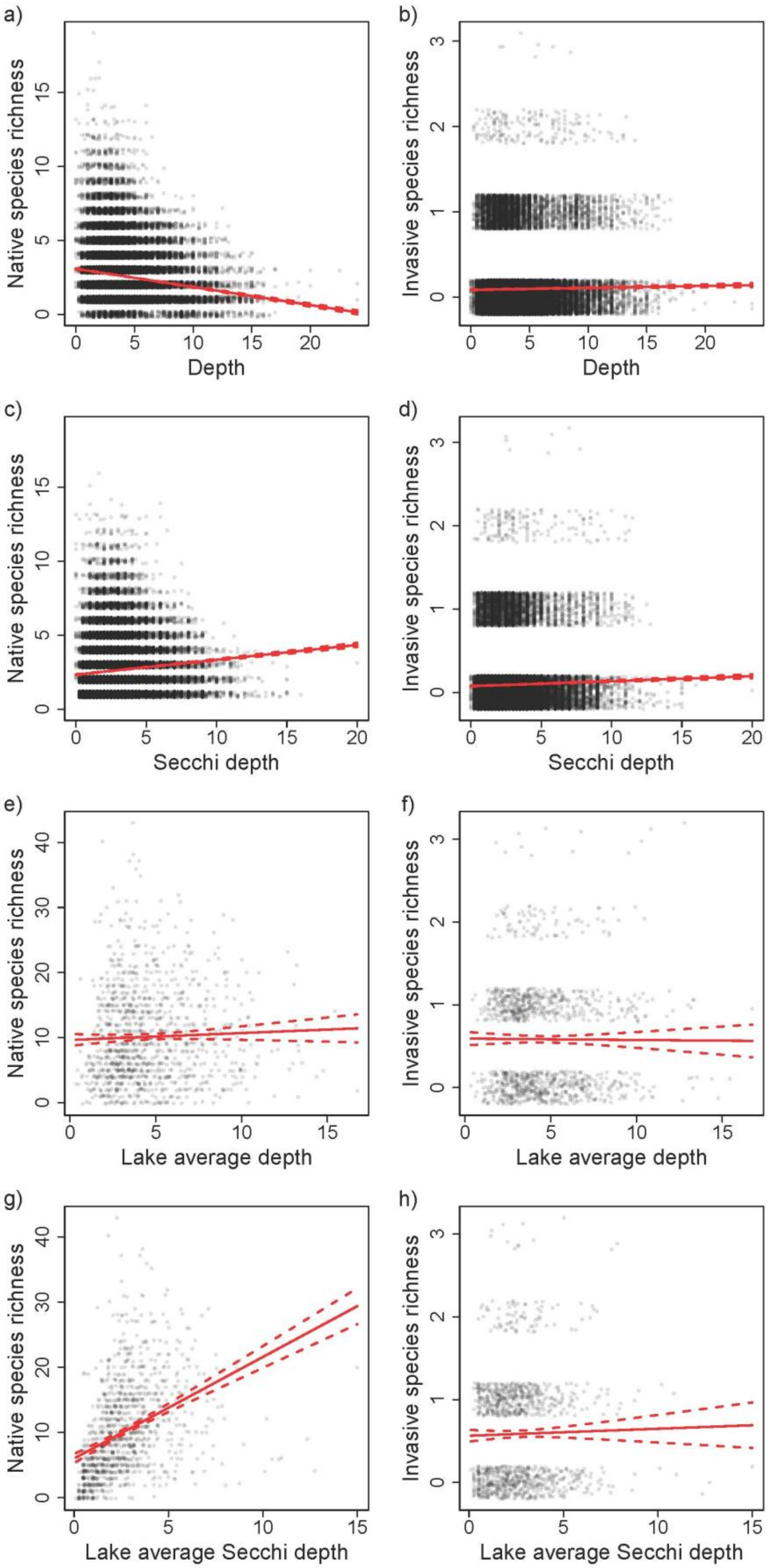
Invader richness ~Depth	Point	Intercept	-2.430	0.028	-87.339	< 0.001	*
		Coefficient	0.023	0.006	3.897	< 0.001	*
	Lake	Intercept	-0.524	0.083	-6.350	< 0.001	*
		Coefficient	-0.003	0.016	-0.178	0.859	
Native richness ~ Secchi depth	Point	Intercept	0.857	0.005	171.688	< 0.001	*
		Coefficient	0.037	0.001	25.767	< 0.001	*
	Lake	Intercept	1.957	0.016	119.019	< 0.001	*
		Coefficient	0.125	0.004	29.164	< 0.001	*
Invader richness ~Secchi depth	Point	Intercept	-2.545	0.026	-96.303	< 0.001	*
		Coefficient	0.059	0.007	8.107	< 0.001	*
	Lake	Intercept	-0.571	0.070	-8.144	< 0.001	*
		Coefficient	0.014	0.022	0.656	0.512	
Native richness ~ Environmental conditions	Lake	Intercept	2.283	0.357	6.388	< 0.001	*
		pH	0.087	0.043	2.006	0.045	*
		Conductance	-0.001	0.000	-6.518	< 0.001	*
		P	-1.359	0.380	-3.580	< 0.001	*
		N	0.028	0.051	0.550	0.582	
		Chlorophyll *a*	-0.006	0.001	-4.678	< 0.001	*
		Secchi depth	0.106	0.038	2.829	0.005	*
Invader richness ~ Environmental conditions	Lake	Intercept	-3.064	1.480	-2.070	0.038	*
		pH	0.412	0.175	2.355	0.019	*
		Conductance	0.000	0.000	-1.098	0.272	
		P	0.953	0.923	1.032	0.302	
		N	0.050	0.140	0.360	0.719	
		Chlorophyll *a*	-0.006	0.003	-1.866	0.062	
		Secchi depth	-0.316	0.157	-2.012	0.044	*
Heterogeneity analyses							

Native richness ~ Depth heterogeneity	Lake	Intercept	2.189	0.014	158.807	< 0.001	*
		Coefficient	0.079	0.006	13.223	< 0.001	*
Invader richness ~ Depth heterogeneity	Lake	Intercept	-0.510	0.058	-8.739	< 0.001	*
		Coefficient	-0.017	0.029	-0.576	0.565	
Native richness ~ Secchi depth heterogeneity	Lake	Intercept	2.089	0.013	155.818	< 0.001	*
		Coefficient	0.311	0.012	26.725	< 0.001	*
Invader richness ~ Secchi depth heterogeneity	Lake	Intercept	-0.561	0.055	-10.152	< 0.001	*
		Coefficient	0.050	0.058	0.856	0.392	

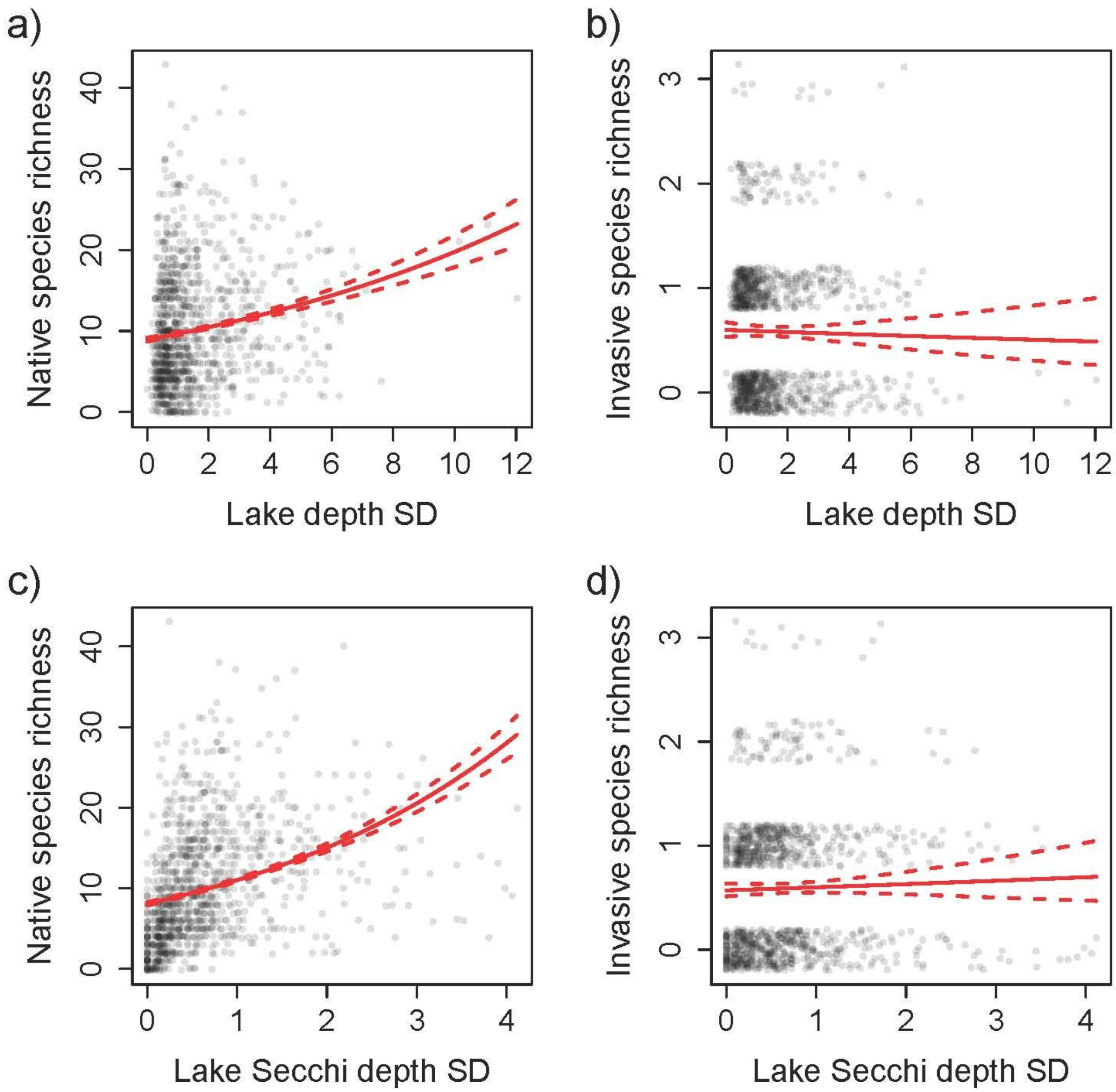

Invasive
Native
Number of lakes
0
20
40
60
80
0
10
20
30
40
Native species richness


a)
b)
Invasive species richness
Invasive species richness
0
1
2
3
0
5
10
15
Point−level native species richness
0
10
20
30
40
Lake−level native species richness


a)
Probability of invasion
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
12
Native species richness
b)
Change in species richness per year
3
2
1
.5
0
-.5
-1
-2
-3
Uninvaded
Invaded
Invader presence



a)
Native species richness
Depth
b)
Invasive species richness
Depth
c)
Native species richness
Secchi depth
d)
Invasive species richness
Secchi depth
e)
Native species richness
Lake average depth
f)
Invasive species richness
Lake average depth
g)
Native species richness
Lake average Secchi depth
h)
Invasive species richness
Lake average Secchi depth


a)
b)
c)
d)
Native species richness
Invasive species richness
Lake depth SD
Lake Secchi depth SD
0
10
20
30
40
0
1
2
3
0
2
4
6
8
10
12
0
1
2
3
4

RESEARCH ARTICLE

Forecasting distributions of an aquatic invasive species (*Nitellopsis obtusa*) under future climate scenarios

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Abstract

Starry stonewort (*Nitellopsis obtusa*) is an alga that has emerged as an aquatic invasive species of concern in the United States. Where established, starry stonewort can interfere with recreational uses of water bodies and potentially have ecological impacts. Incipient invasion of starry stonewort in Minnesota provides an opportunity to predict future expansion in order to target early detection and strategic management. We used ecological niche models to identify suitable areas for starry stonewort in Minnesota based on global occurrence records and present-day and future climate conditions. We assessed sensitivity of forecasts to different parameters, using four emission scenarios (i.e., RCP 2.6, RCP 4.5, RCP 6, and RCP 8.5) from five future climate models (i.e., CCSM, GISS, IPSL, MIROC, and MRI). From our niche model analyses, we found that (i) occurrences from the entire range, instead of occurrences restricted to the invaded range, provide more informed models; (ii) default settings in Maxent did not provide the best model; (iii) the model calibration area and its background samples impact model performance; (iv) model projections to future climate conditions should be restricted to analogous environments; and (v) forecasts in future climate conditions should include different future climate models and model calibration areas to better capture uncertainty in forecasts. Under present climate, the most suitable areas for starry stonewort are predicted to be found in central and southeastern Minnesota. In the future, suitable areas for starry stonewort are predicted to shift in geographic range under some future climate models and to shrink under others, with most permutations indicating a net decrease of the species' suitable range. Our suitability maps can serve to design short-term plans for surveillance and education, while future climate models suggest a plausible reduction of starry stonewort spread in the long-term if the trends in climate warming remain.

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Competing interests: The authors have declared that no competing interests exist.

Introduction

Starry stonewort (*Nitellopsis obtusa*, Characeae) is a species of concern for both its endangered status (in parts of its native range in Europe and Asia) and its invasive status (in North America). The 'starry' of its common name comes from its characteristic star-shaped bulbils, starchy reproductive structures that enable spread via asexual reproduction [1]. In North America, female individuals of this species have not been detected to date [2]. It has a higher ecological plasticity than other charophytes [1,3]. For example, starry stonewort can flourish in hardwater (i.e., water with high mineral content) and habitats of varying depth, light availability, and sediment characteristics [4]. In addition, starry stonewort can grow densely, which may lead to displacement of native aquatic plant species and could have consequences for habitat quality [2]. Dense growth may also impair recreational activities such as swimming, fishing, and boating [1,3]. Although populations of starry stonewort in their native distribution in Europe and Japan have been declining [5–7], the species has shown great capacity to spread as an aquatic invasive species in North America [3,8,9].

In 1978, starry stonewort was first recorded in North America in the St. Lawrence River, where it was likely introduced through ballast water discharge from trans-Atlantic shipping [10]. Marine currents could have played a role in starry stonewort's dispersion, but this has been not explored. Five years later, starry stonewort was reported for the first time in Michigan, United States [1,10]. To date, starry stonewort has been reported in Indiana, New York, Pennsylvania, Wisconsin, Vermont, Ontario, and, in August 2015, in Minnesota [3,8,11,12]. The introduction of starry stonewort to inland lakes has been speculated to be associated with recreational boat activities from the movement of bulbils and alga fragments between different lakes [1,3].

In light of limited knowledge about the potential spread and impacts of starry stonewort in the Americas, improved knowledge of the species' invasion ecology is a priority. Among other efforts, identifying areas on the leading edge of the invasion range (e.g., Minnesota) with suitable conditions for starry stonewort is a priority for targeting surveillance and control. Ecological niche modeling can support these efforts. Ecological niche models correlate environmental conditions with species' occurrence records to identify suitable habitats where a species can persist and increase in population size without the need of further immigration [13]. This methodology has been used successfully with different taxa, scales, and ecosystems [13–15]. Furthermore, ecological niche models can be applied to forecast probable distributions of species over longer time periods, e.g., under future climate scenarios [16–20]. Predicting areas where starry stonewort could establish could inform surveillance efforts for early detection, raise local awareness, and prioritize allocation of resources for control [21].

Local conditions can influence occurrence of starry stonewort in North America. For example, in Lake Ontario, starry stonewort's distribution is associated with high conductivity, short distances to marinas, and low fetch [3]. In New York, Sleith et al. [1] found high pH and conductivity to be associated with starry stonewort. However, invasive species' occurrences are defined not only by local-scale characteristics, but also by larger scales of environmental factors that promote or limit spread over space and time [22]. Invasion of starry stonewort in the Americas is likely an ongoing process that has not reached equilibrium, and more water bodies are likely to be affected [8].

Recent reports of starry stonewort in Minnesota provide an opportunity to explore climatic factors that may influence future expansion. Here, we have constructed a series of ecological niche models to answer three main questions: (i) Which areas are vulnerable to starry stonewort invasion in Minnesota under present-day climate conditions? (ii) Which areas in Minnesota have suitable conditions for starry stonewort under future climate scenarios?, and (iii)

How do decisions regarding the geographic region used in model calibration influence predictions? We propose a protocol (Fig 1) to improve the workflow of ecological niche models for forecasting species invasions.

Methods

The ecological niche modeling approach employed was based on the **BAM** framework [23], which summarizes three components to define a species' spatial range. The first component is **B**, the presence of other organisms that promote (e.g., prey, symbionts) or restrict (e.g., depredators, parasites) the distribution of the species in a region. The second component corresponds to the set of abiotic environmental conditions, **A**, e.g., temperature, that are suitable for a species to persist without need of immigration. The final component, **M**, corresponds to the ability of the species to colonize biotically (**B**) and abiotically (**A**) suitable regions. Thus, the spatial distribution of a species is defined as $\mathbf{B} \cap \mathbf{A} \cap \mathbf{M}$ [23]. We focused on a broad-scale exploration of **A** and **M**, as a preliminary assessment of the invasion potential of starry stonewort in terms of abiotic suitability and dispersal potential. We estimated **A** based on the association of starry stonewort occurrences with bioclimatic variables across its range, and estimated **M** based on using three regions for model calibration (Fig 1).

Occurrences

Occurrence records of starry stonewort were published in Escobar et al. [8], which used data from digital repositories including the Global Biodiversity Information Facility (GBIF) [24] and the Global Invasive Species Information Network [25] using the keywords "*Nitellopsis obtusa*," "*Nitellopsis obtusa* var. *ulvoides*," and "*Chara obtusa*". Occurrences from invaded areas in the US were also derived from additional reports and publications [1,4,9,26]. Minnesota records were updated based on 2016 reports of new localities from the Minnesota Department of Natural Resources (MDNR, http://www.dnr.state.mn.us/invasives/ais/infested.html).

Occurrences were individually inspected to assure credibility and geospatial accuracy. All Minnesota, Wisconsin, and New York records have been confirmed by a Characeae expert (Ken Karol, New York Botanical Garden). Michigan has the most records and not all have been verified by experts. It is possible that reports from Michigan (and GBIF or other databases) include false records. Unfortunately, this is the best information that is available at this time. We chose to include all records based on the expectation that the error rate is relatively low and that the invaded region most likely to include false records (Michigan) is in the center of the species' invaded range, such that false occurrences would be unlikely to have a strong influence on niche estimation.

Oversampled areas, as a form of sampling bias, can generate model overfit [27]. To prevent this, we calibrated present-day models using occurrences filtered to one-per-cell according to the spatial resolution of cells in our environmental layers [28]. All the remaining occurrences were used for modeling. From the initial pool of 2,260 occurrences, 84 single occurrences (i.e., occupied pixel cells) remained in the entire species' range: 29 in the native range (34.5%; 2 in Japan, 27 in Europe) and 55 in the invaded range in the US (65.5%; Fig 2).

Model calibration region M

The selection of **M**, the model calibration region, has a strong influence on ecological niche model predictions [29]. For instance, considering only invasive populations can result in incomplete information about the environmental preferences of the species [13], or be insufficient to characterize environmental tolerances [30]. Explicitly testing different extents of the calibration region facilitates comparison of models and informs interpretation of results [31].

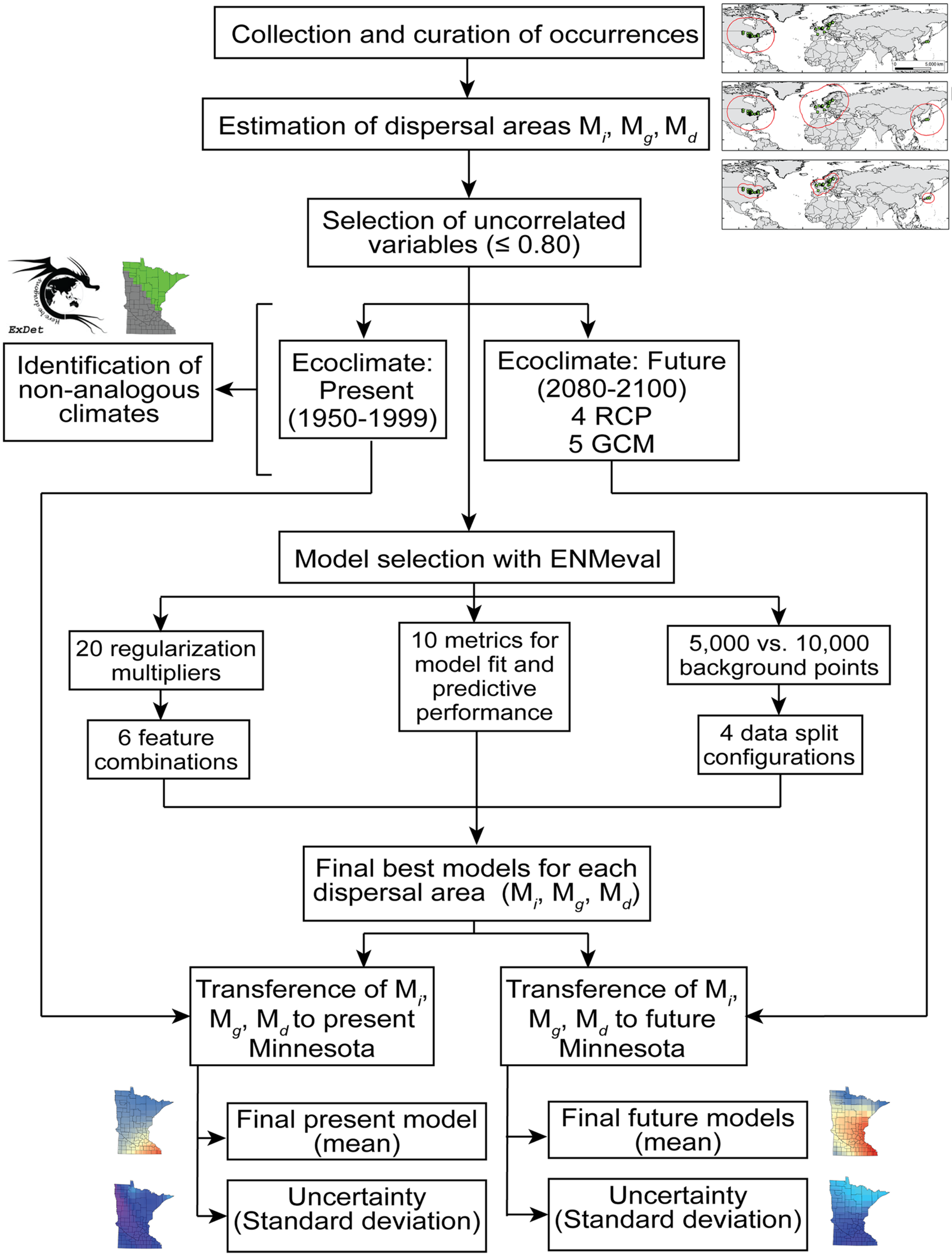

Fig 1. Workflow of the modeling process used in this study. Occurrences were collected, cleaned, and employed to estimate three model calibration regions (i.e., $\mathbf{M_i}$, $\mathbf{M_g}$, and $\mathbf{M_d}$). Present-day climatic variables were restricted to these model calibration regions and

compared to future climatic conditions in Minnesota. Models were parametrized using present-day climates in the three model calibration regions and the best models were projected to future climates in Minnesota using five climate models and four RCP scenarios.

https://doi.org/10.1371/journal.pone.0180930.g001

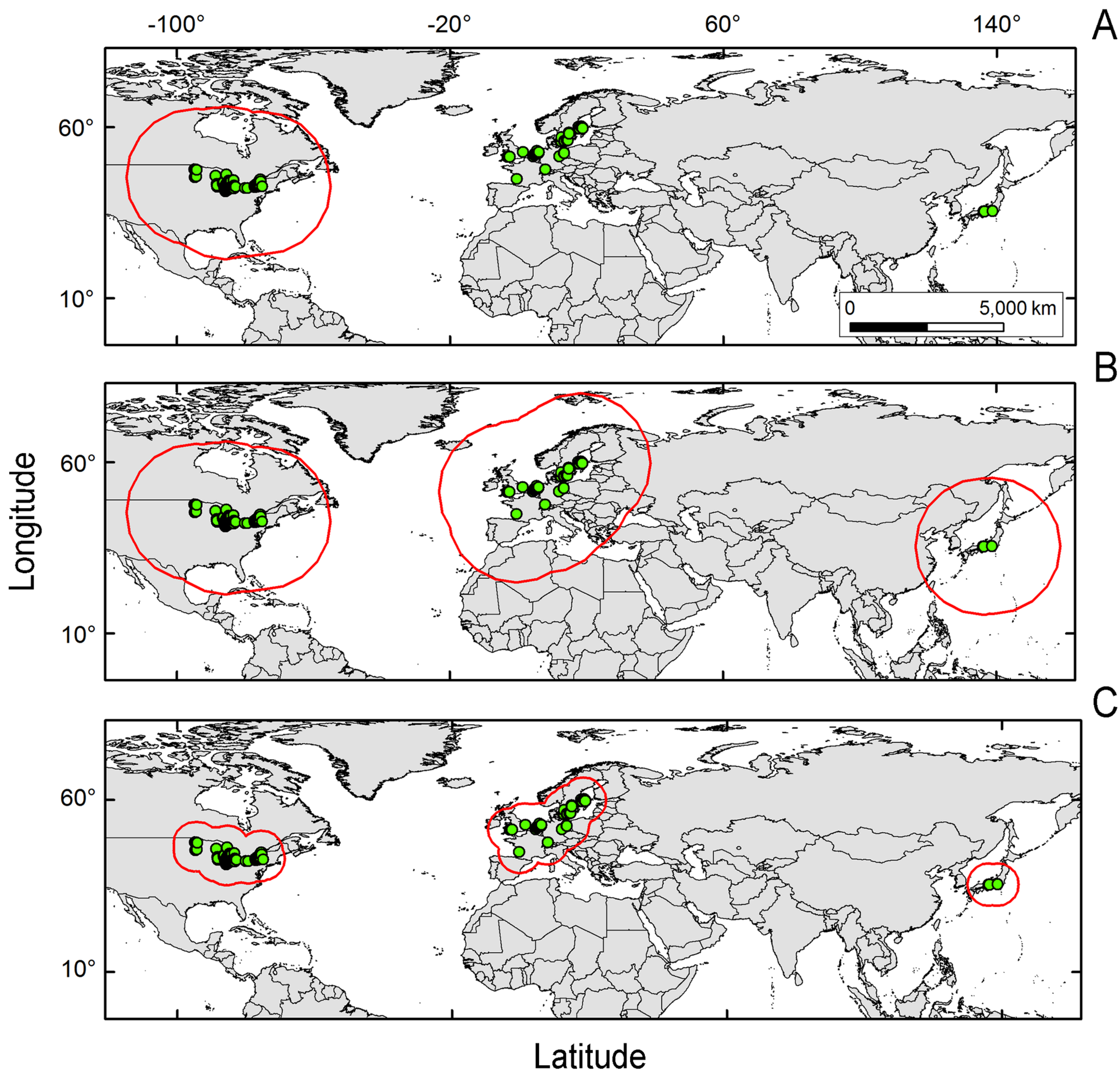

Fig 2. Model calibration region, M, explored in this study. Models were calibrated in three regions (red lines in A, B, and C) based on the distribution of starry stonewort populations (green points). **A.** Model calibration region based on an invasive population approach focused on starry stonewort populations in the invaded area of the United States and a high dispersal potential (i.e., 2,200 km), $\mathbf{M}_i$. **B.** Model calibration region considering the entire or global species' range in the United States, Europe, and Japan and a high dispersal potential (i.e., 2,200 km), $\mathbf{M}_g$. **C.** Model calibration region considering the entire or global species' range in the United States (left map), Europe (central map), and Japan (right map) and a reduced dispersal potential (i.e., 700 km), $\mathbf{M}_d$.

https://doi.org/10.1371/journal.pone.0180930.g002

Recent new records for starry stonewort in North America suggest that it may be expanding in North America from east to west and from south to north [8]. As a proxy of the dispersal potential of the species we used two distances for three **M** scenarios. First, we used the maximum distance between all known starry stonewort populations in the US (~2,200 km), as suggested by the data available (i.e., MDNR surveillance: http://www.dnr.state.mn.us/invasives/ais/infested.html) [23]. Considering that the species has been dispersing between distant lakes, we assumed that spatial barriers could be overcome in the model calibration regions. We used this distance as a buffer around starry stonewort occurrences to generate a model calibration region for the invaded range in the US ($\mathbf{M}_i$). This area corresponds to a model based on the invasive populations.

Furthermore, to account for starry stonewort environmental preferences across its entire range, we focused on two additional model calibration areas, including both native (Europe and Japan) and invasive populations (US). One of these calibration areas was based on the same maximum distance between all known starry stonewort populations in the US (~2,200 km; $\mathbf{M}_g$) and the other was a proxy of the maximum distance between closer neighbors populations in the US (~700 km; $\mathbf{M}_d$), which in our case corresponded to the distance between the last detection in Wisconsin and the first detection in Minnesota. We used these distances to generate a buffer around occurrences across the entire species' range (Fig 2). The $\mathbf{M}_i$ scenario encompasses inland and coastal regions of central and eastern Canada and all states in the continental US except those in the far west: California, Nevada, Oregon, Washington, and western portions of Arizona and Idaho. The $\mathbf{M}_g$ scenario encompasses all of those areas in addition to Europe, parts of northwestern Africa and Asia (Japan, North and South Korea, and parts of eastern China and Russia). The $\mathbf{M}_d$ scenario includes the Upper Midwest region in the US and southeastern Canada, portions of Southern, Northern, and Western Europe, and a small portion of Eastern Europe, and also Japan except by the Hokkaido island (Fig 2). All **M** scenarios included the area of interest for this study (Minnesota).

Environmental variables

As a proxy of **A**, we used the present-day Ecoclimate dataset (1950–1999) at 50-km spatial resolution [32]. Since starry stonewort occurs in both coastal and inland areas, we used climate variables covering both regions. This climate dataset is derived from the Coupled Model Intercomparison Project (CMIP5) and combines climatic patterns from multiple general circulation models from inland and marine ecosystems; thus, final climatic layers have global coverage. The role of oceanic dispersal in the invasion process of this species remains uncertain, however, we assumed that marine dispersal could play a role and include climate conditions in terrestrial and marine ecosytems in our model calibration regions. We used climatic variables likely to influence starry stonewort's macroscale distribution, selecting uncorrelated variables based on correlation coefficients $\leq$0.80 (Table A in S1 File). Specifically, we used annual mean temperature (˚C), mean diurnal temperature range (˚C), isothermality (%), temperature seasonality (˚C), maximum temperature of the warmest month (˚C), mean temperature of the wettest quarter (˚C), annual precipitation (mm/m^2), and precipitation seasonality (%) [32].

Climate models are considerably variable, thus, adding more scenarios of future climate would provide more information regarding the plausible variability in forecasts. Future climatic conditions for the end of the 21st century (2080–2100) were obtained from Ecoclimate, including four representative concentration pathways (RCPs; i.e., 2.6, 4.5, 6, and 8.5 W/m^2; here after numbers are shown without units) [32]. Each RCP scenario represents potential trajectories of greenhouse gas emissions projected to the future, ranging from the most optimistic (i.e., 2.6) to the worst-case scenario (i.e., 8.5) [32]. RCPs are the most updated climate scenarios from the Intergovernmental Panel on Climate Change (IPCC), Fifth Assessment Report (AR5), and replaced the SRES scenarios previously implemented by the IPCC AR4 [33]. The four RCP scenarios were estimated based on five different future general circulation models (GCM): CCSM, GISS, IPSL, MIROC, and MRI, allowing us to capture the variability in emissions (i.e., RCP scenarios) and climate simulations (e.g., CCSM vs. MRI).

Non-analogous climate evaluation

We explored areas with non-analogous (novel) climatic conditions between present-day climate in the calibration regions vs. future climate in the projection region of Minnesota. This resulted in a present vs. future comparison and calibration vs. projection regions. This analysis was done using the extrapolation detection (Exdet) tool developed by Mesgaran et al. [34]. Exdet identifies non-analogous environments between calibration and projection regions denoted as type I novelty [*sensu* 34]. Accounting for these non-analogous or novel environments enables a more confident interpretation of models [18,35,36].

Ecological niche models

Qiao et al. [37] proposed that multiple ecological niche modeling algorithms should be employed to identify the model that best fits with the available data, the study system, and the research question. We used Maxent to perform niche modeling because it enables the use of different variable transformations (features), i.e., linear (L), quadratic (Q), product (P), threshold (T), and hinge (H), and allows for different parameterizations (regularization values). In addition, Maxent allows automatic truncation in novel climates to avoid predictions in non-analogous environments.

Maxent is an occurrences-background algorithm, which estimates the most uniform probable distribution of the occurrences across a selected calibration region [13,38]. The background represents the summary of environmental conditions across the model calibration region. Because we explored two calibration regions (invaded range and two areas from the entire species' range) the available background varied. We developed models based on 5,000 and also 10,000 background samples.

Here, we tested 20 different regularization coefficient values ranging from 0.1 to 2. The regularization coefficients regulate the complexity of the model, higher values penalize for complexity and thus, produce simpler models (avoiding complex relationships between the data and the variables) that, in general, tend to have larger predictions [39]. Because assessing different configurations is recommended [39–41], we explored models based on six feature combinations reported in the literature: L, LQ, H, LQH, LQHP, and LQHPT [40].

We used raw values from Maxent to assess model fit according to Akaike's Information Criterion values corrected for small sample size (AICc), which ranks models based on their information content and complexity [42]; the model with the lowest AICc was selected (i.e, ΔAICc = 0) as best reconciling the goals of fitting occurrences with environmental input data and minimizing model complexity [41]. In addition, because low AICc does not represent the ability of the model to predict independent data, we also assessed predictive performance based on the full (AUC_{total}) and mean (AUC_{mean}) of the area under the curve of the receiver-operating characteristic (AUC) and the difference between training and testing AUC and its variability. These metrics assess if models can discriminate between occurrence and background points, with AUC values $\leq$0.5 consistent with randomly generated models unable to differentiate between backgrounds and occurrences. Because AUC has been questioned [43,44], we also used independent data to calculated mean omission rates (OR) from binary models based on using 100% ($OR_{100\%}$) and 90% ($OR_{90\%}$) of training occurrences as thresholds.

These metrics enable the proportion of independent occurrences predicted incorrectly to be quantified [40]. Evaluation of model predictions was performed using independent data obtained via dividing the occurrences in two sets, one for model calibration and one for evaluation. Calibration and evaluation data sets were developed based on four different data splitting configurations: (i) using one point at a time for model evaluation (i.e., Jackknife); (ii) apportioning the occurrences into four groups, each with an off-diagonal set for calibration and another for evaluation (i.e., block; as in [45]); (iii) selecting clusters of points and using half for calibration and the other half for evaluation (i.e., Checkerboard1 [40]), and (iv) partitioning the occurrences via cross-validation (*k*-fold; see [40]). Model evaluations were conducted using the R package ENMeval [40].

Model projection to Minnesota

Once the best regularization coefficient, feature configuration, and number of background points were determined for the calibration regions (Fig 2), the three selected models were projected to environmental conditions in Minnesota. Maxent allows strict model transference during model projection via 'extrapolation' and 'clamping' being deactivated [36,46]. This practice prevents unrealistic extrapolations of models into non-analogous (novel) environments that could be present in the projection region but absent from the calibration region [46].

In all, to identify the best model by calibration region ($\mathbf{M}_i$ vs. $\mathbf{M}_g$ vs. $\mathbf{M}_d$), we explored 120 parameter configurations (20 regularization coefficients × 6 feature combinations), and two background samples for each regions $\mathbf{M}_i$ and $\mathbf{M}_g$: 5,000 and 10,000; and 10,000 for $\mathbf{M}_d$ which was not explored due to the reduced extent of this calibration area (Table B in S1 File). The best models were projected to 20 future climate scenarios (4 RCP × 5 climate models). To inform interpretation of forecasts, we also estimated uncertainty of all final models. We parameterized final models based on our previous evaluations and generated surfaces of uncertainty using 80% of occurrences in Maxent and performed 25 bootstrap replications using random starting seeds. For final models, we selected the logistic output format in Maxent with clamping and extrapolation deactivated. We used the standard deviation of replicates as an indicator of uncertainty [38,47] (Fig 1) and developed a *t*-test ($\alpha = 0.05$) to compare the continuous suitability values of pixels among models in Minnesota.

Finally, we created an ensemble of models for different future climate scenarios in Minnesota. We averaged the final logistic models and calculated the standard deviations to identify areas where models were consistent (low SD) or diverged (high SD). There is debate about use of model ensembles, due to issues regarding interpretation of continuous units from different algorithms (e.g., general linear models vs. regression trees vs. Maxent) (see [13]). Here, we overcame such discrepancies by using the same suitability value (i.e., Maxent logistic), from the same parameterization so that differences only reflected differences in future climate models for Minnesota. We also estimated the number of lakes in Minnesota comprising the lowest and highest predictions of suitability using lake inventory data from the National Wetlands Inventory of the US Fish & Wildlife Service [48].

Results

Selected regularization coefficients differed by model calibration region: a regularization coefficient of 1.4 with LQHPT features provided the best fit (ΔAICc = 0) and good predictive performance ($AUC_{total} = 0.98$, $AUC_{mean} = 0.96$–0.97, $OR_{100\%} = 0.05$–0.09, $OR_{90\%} = 0.14$–0.16) for $\mathbf{M}_i$, 0.2 + LQ for $\mathbf{M}_g$ (ΔAICc = 0, $AUC_{total} = 0.97$, $AUC_{mean} = 0.95$–0.96, $OR_{100\%} = 0.01$–0.04, $OR_{90\%} = 0.12$–0.18), and 0.9 + LQ for $\mathbf{M}_d$ (ΔAICc = 0, $AUC_{total} = 0.89$, $AUC_{mean} = 0.85$–0.88,

$OR_{100\%}$ = 0.07–0.19, $OR_{90\%}$ = 0.01–0.02; Table B in S1 File). Our evaluations revealed that 10,000 background points provided good model fit and performance for the three model calibration regions explored. Logistic suitability values of starry stonewort models based on $\mathbf{M}_g$ (mean = 0.40, sd = 0.13) vs. $\mathbf{M}_i$ (mean = 0.13, sd = 0.07) were significantly different (t = 1098, df = 544500, $p < 0.001$), with higher suitability predicted when $\mathbf{M}_g$ was considered (Fig 3). Logistic suitability values of starry stonewort models based on $\mathbf{M}_d$ (mean = 0.30, sd = 0.13) vs. $\mathbf{M}_i$, and vs. $\mathbf{M}_g$ were also significantly different, with $\mathbf{M}_d$ showing higher suitability than $\mathbf{M}_i$ (t = 717.16, df = 551600, $p < 0.001$) but less than $\mathbf{M}_g$ (t = 315.76, df = 732220, $p < 0.001$; Fig 3). Model uncertainty was higher in the model calibrated in $\mathbf{M}_i$ ($\mathbf{M}_i$ vs. $\mathbf{M}_d$: t = 20.10, df = 592650, $p < 0.001$; sd $\mathbf{M}_i$ vs. $\mathbf{M}_g$: t = 79.35, df = 536950, $p < 0.001$; Fig 3). In present-day models, we found potential areas for starry stonewort distribution in southeast and central Minnesota and also in the Minneapolis-St. Paul metro region. The portion of Minnesota where starry stonewort has been confirmed to date was predicted to have high suitability for the model calibrated based on $\mathbf{M}_g$ and $\mathbf{M}_d$ (Fig 3).

The $\mathbf{M}_i$ model based on the invasive population in the US predicted only a small area of moderate suitability in central and southeastern Minnesota (Fig 3), while the model based on the entire species' range predicted a broad area of suitability across the state. Models from the global range $\mathbf{M}_g$ containing all the occurrences produced predictions with lower uncertainty. The $\mathbf{M}_d$ model calibrated based on the entire species range but with reduced dispersal potential predicted suitability resembling something between $\mathbf{M}_i$ and $\mathbf{M}_g$ (Fig 3). Prediction of starry stonewort suitability from $\mathbf{M}_d$ showed the highest uncertainty in western Minnesota.

Present-day climate across $\mathbf{M}_i$, $\mathbf{M}_g$, and $\mathbf{M}_d$ showed non-analogous environments across Minnesota under all RCP scenarios of the IPSL climatic model (Figs 4–6). All MRI emission scenarios showed Minnesota having analogous climates. Other climate models and emission scenarios showed different non-analogous climate configuration according to the **M** scenarios employed (Figs 4–6). For example, $\mathbf{M}_i$ under present-day climatic conditions overlapped with future climate conditions for all RCP scenarios in climate models GISS and MRI, RCP 2.6 and 4.5 in CCSM, and maintained environmental similarity in the northeastern part of Minnesota in the MIROC model (Fig 4). This pattern was similar for $\mathbf{M}_d$ (Fig 6) despite the lack of analogous environments in MIROC RCP 8.5. Models calibrated based on $\mathbf{M}_g$ included analogous environments except in the case of all RCP scenarios in the IPSL model and MIROC RCP 8.5, which showed non-analogous environments in a small region in southwestern Minnesota (Fig 5). According to Exdet, non-analogous conditions for the IPSL model were driven mainly by differences in mean diurnal range, while novel climates in the MIROC RCP 2.6, 4.5, and 8.5 and CCSM RCP 6 and 8.5 were driven by extreme values of maximum temperature of the warmest month (Figs 4–6). Novel climates in MIROC RCP 6 model were explained by the maximum temperature of the warmest month and by the mean temperature of wettest quarter.

Models calibrated based on $\mathbf{M}_i$ and $\mathbf{M}_d$ produced predictions with high uncertainties in Minnesota for all RCP scenarios (Figs 7 and 8). High suitability was predicted for $\mathbf{M}_i$ and $\mathbf{M}_d$ in scenarios CCSM RCP 2.6 and 4.5, MRI RCP 4.5, 6, and 8.5, and for $\mathbf{M}_d$ GISS RCP 6. Additionally, based on $\mathbf{M}_i$ and $\mathbf{M}_d$, models did not predict suitability under the IPSL climate model or predicted moderate suitability in small areas under the MIROC climate model (Figs 7 and 8), due to the absence of analogous environments (Figs 4 and 6).

The models from $\mathbf{M}_g$ transferred to future climate predicted an expansion of suitable areas under all GISS scenarios, with reduced suitability for future climate according to CCSM, IPSL, and MIROC (Fig 9). High variability was found for CCSM 2.6 and 8.5, GISS RCP 6, and all MRI scenarios. Some future climate scenarios indicated lack of suitability for starry stonewort throughout Minnesota (Fig 9). Suitability was not predicted for all IPSL scenarios due to non-

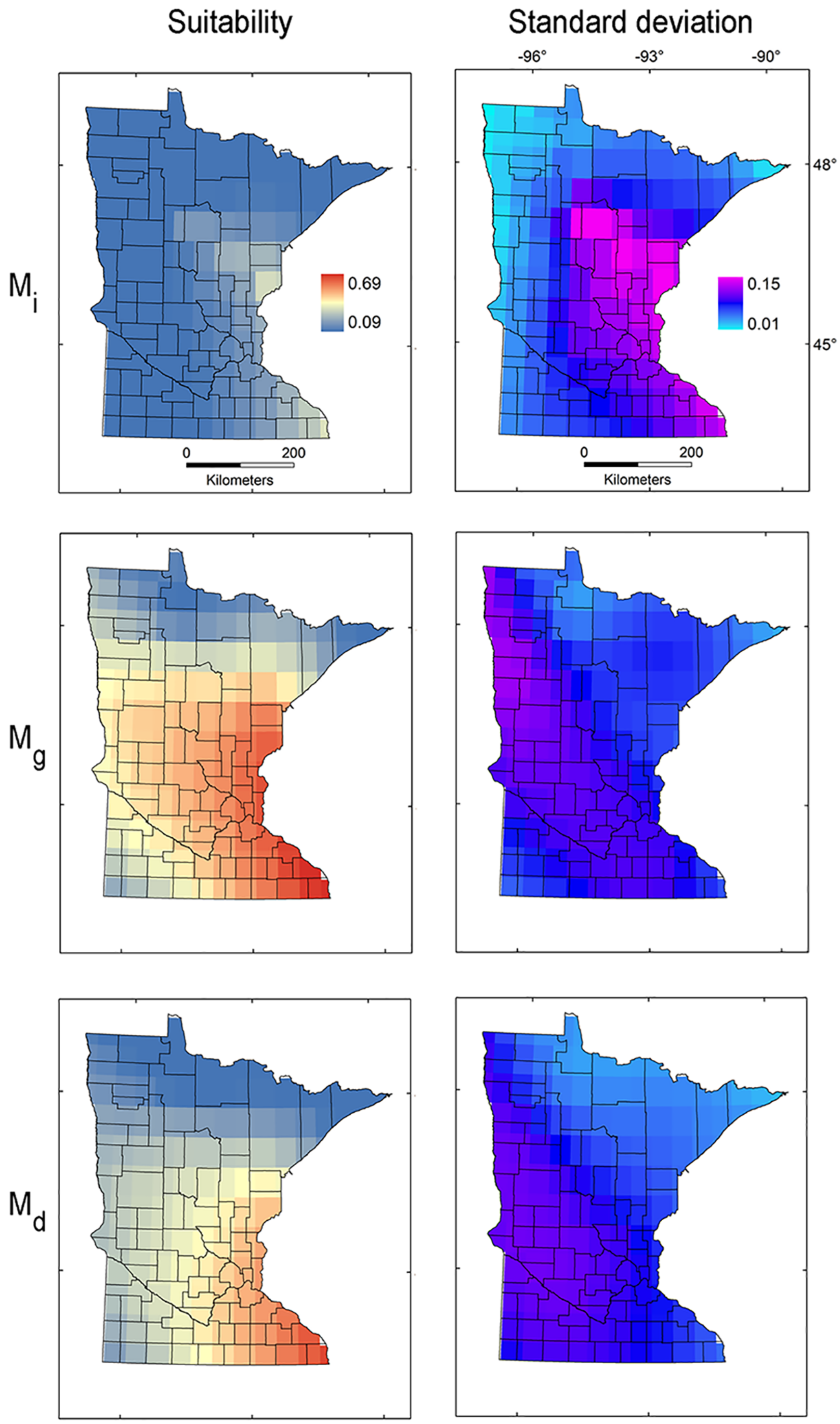

Fig 3. Ecological niche model transference to Minnesota under present-day climate. Ecological niche model predictions based on model calibration region in the invaded range with high dispersal (**M**$_i$; top), entire species' range with high dispersal (**M**$_g$; mid), and entire species' range with reduced dispersal (**M**$_d$; bottom) projected to Minnesota to identify areas with high (red) or low (blue) environmental suitability (left) and high (pink) or low (light blue) model uncertainty (right).

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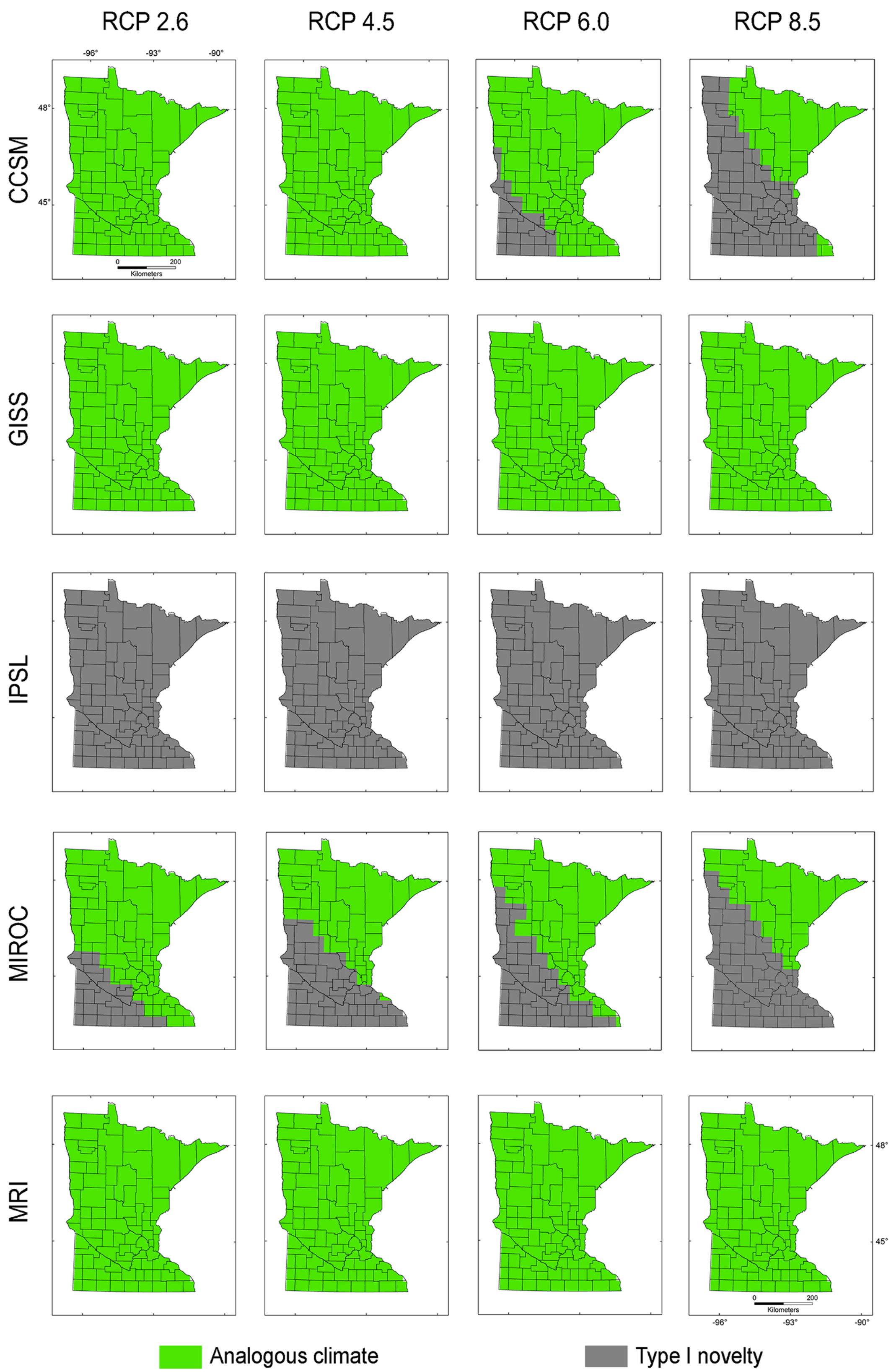

Fig 4. Environmental similarity comparison between the calibration M_i and the projection region of Minnesota. Exdet tool identified analogous climates between present-day climate in the calibration region from the

invaded range and future climate scenarios in the projection region of Minnesota. Areas with analogous (green) and non-analogous environments in Minnesota (grey) were identified for five future climate models (i.e., CCSM, GISS, IPSL, MIROC, MRI) and four RCP scenarios of CO_2 emissions (i.e., 2.6, 4.5, 6, and 8.5).

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analogous climates; while MIROC RCP 8.5 and CCSM RCP 8.5 showed unsuitability in analogous environmental conditions in all **M** scenarios. In general, climatic suitability is predicted to decrease under future climate conditions relative to present-day conditions (Fig 3 vs. Fig 10). The model ensemble showed a lack of agreement in predicted suitability among **M** calibration areas and RCP scenarios, with suitability values ranging from 0.01 to 0.12 for $\mathbf{M}_i$, 0.05 to 0.28 for $\mathbf{M}_g$, and from 0.06 to 0.30 for $\mathbf{M}_d$ (Fig 10). Areas with high values of suitability were also areas with high uncertainty in the model ensemble (Fig 10). In general, climatic suitability is predicted to decrease in the number of lakes of Minnesota under future climate conditions relative to present-day conditions except for the scenario RCP 2.6 from the climatic model CCSM and RCP 8.5 from MRI.

Discussion

Model predictions

We used a **BAM** ecological niche modeling framework to predict present-day and future climatic suitability throughout Minnesota for the aquatic invasive species starry stonewort. Under most future climate scenarios, the available range is predicted to shrink relative to present-day conditions. Based on the data available and the assumption of niche conservatism [49,50], all future climatic models under all RCP scenarios showed a decrease in suitable range relative to present-day conditions, with the exception of future climatic models: CCSM 2.6 and 4.5, and MRI RCP 4.5, 6, and 8.5 for $\mathbf{M}_i$, GISS RCP 6 for $\mathbf{M}_g$, and CCSM 2.6 and MRI 8.5 for $\mathbf{M}_d$, which showed increased areas of suitability with plausible range shifts. All these predictions, however, showed considerable uncertainty (Figs 7–9).

It is possible that our findings underestimate the potential invasiveness of starry stonewort by not capturing the full extent of its climatic tolerance [23]. Escobar et al. [8] recently described environmental tolerances of starry stonewort in its invaded and native ranges and found that invasion was associated with a shift in its realized niche, suggesting niche expansion, i.e., there were environmental conditions occupied by starry stonewort in the invaded range that lacked analogues in the native range [51]. This suggests that invasion potential may exceed what would be anticipated based on past performance alone, and starry stonewort may be able to expand into previously unoccupied environmental space [49,51]. Models could also be underestimating invasion due to overfitting from oversampled areas (i.e., sampling bias) and spatial autocorrelation in climatic variables; however, we minimized this risk by resampling occurrences to one per pixel and using coarse-resolution climatic variables, including data from remotely sensed imagery, to counter high spatial lag associated with data derived solely from climate stations [32,52,53].

The consensus areas of suitability across models (Fig 10) showed a pattern of reduced suitability across all **M** regions, suggesting a potential decline of the starry stonewort under warming climates in terms of the climates where the species is found to date. Model ensembles highlight areas of agreement across predictions, but their interpretation requires caution [17]. The lack of consensus of suitable areas for starry stonewort under future climate in Minnesota reflects the diversity of possible trajectories of future climate (Figs 7–9).

We note that our findings are based on estimated climatic tolerances and a proxy of establishment [23]. Numerous other factors, such as water chemistry, dispersal limitation, and

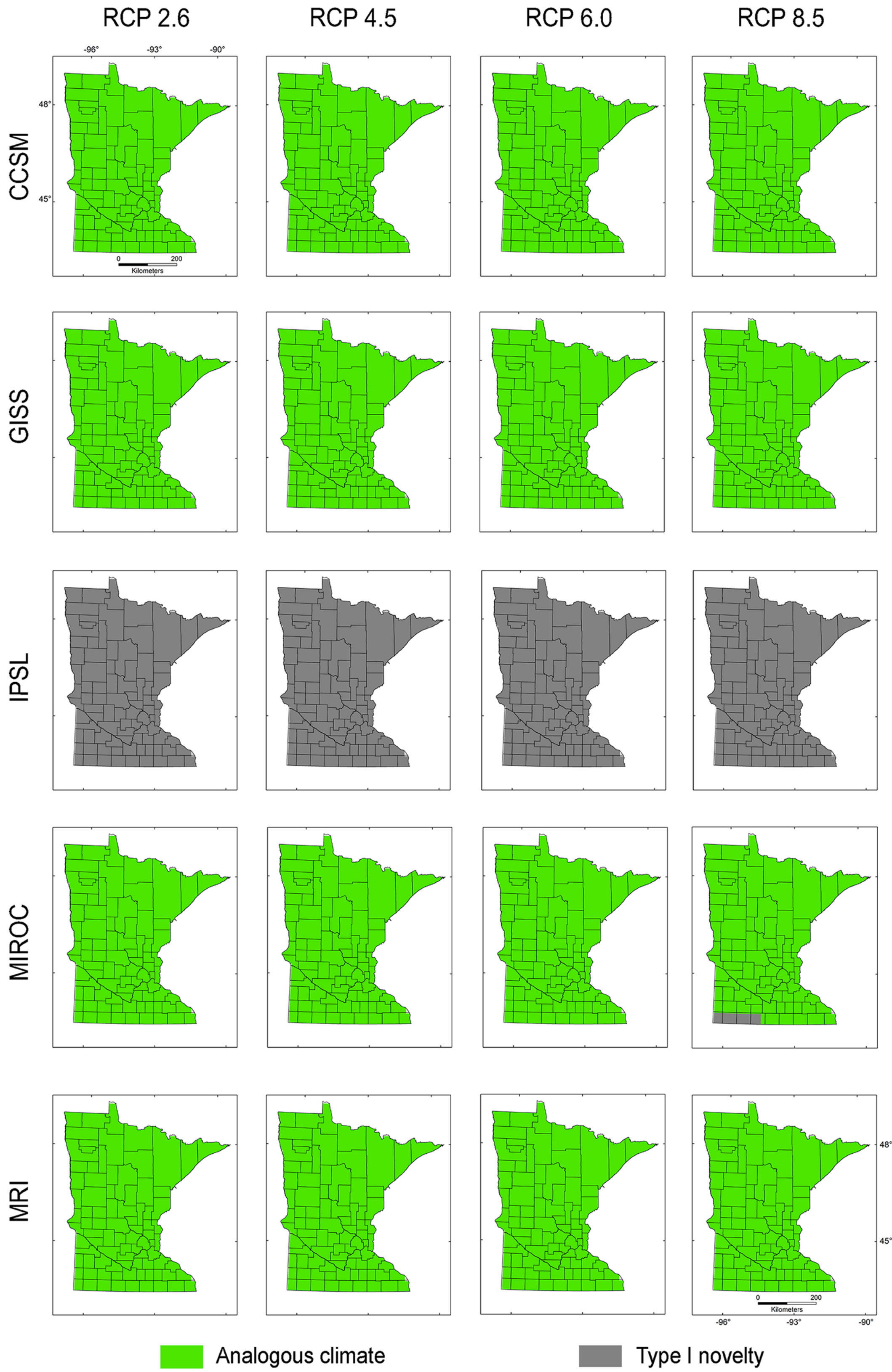

Fig 5. Environmental similarity comparison between the calibration M_g and the projection region of Minnesota. Legend as in Fig 3.

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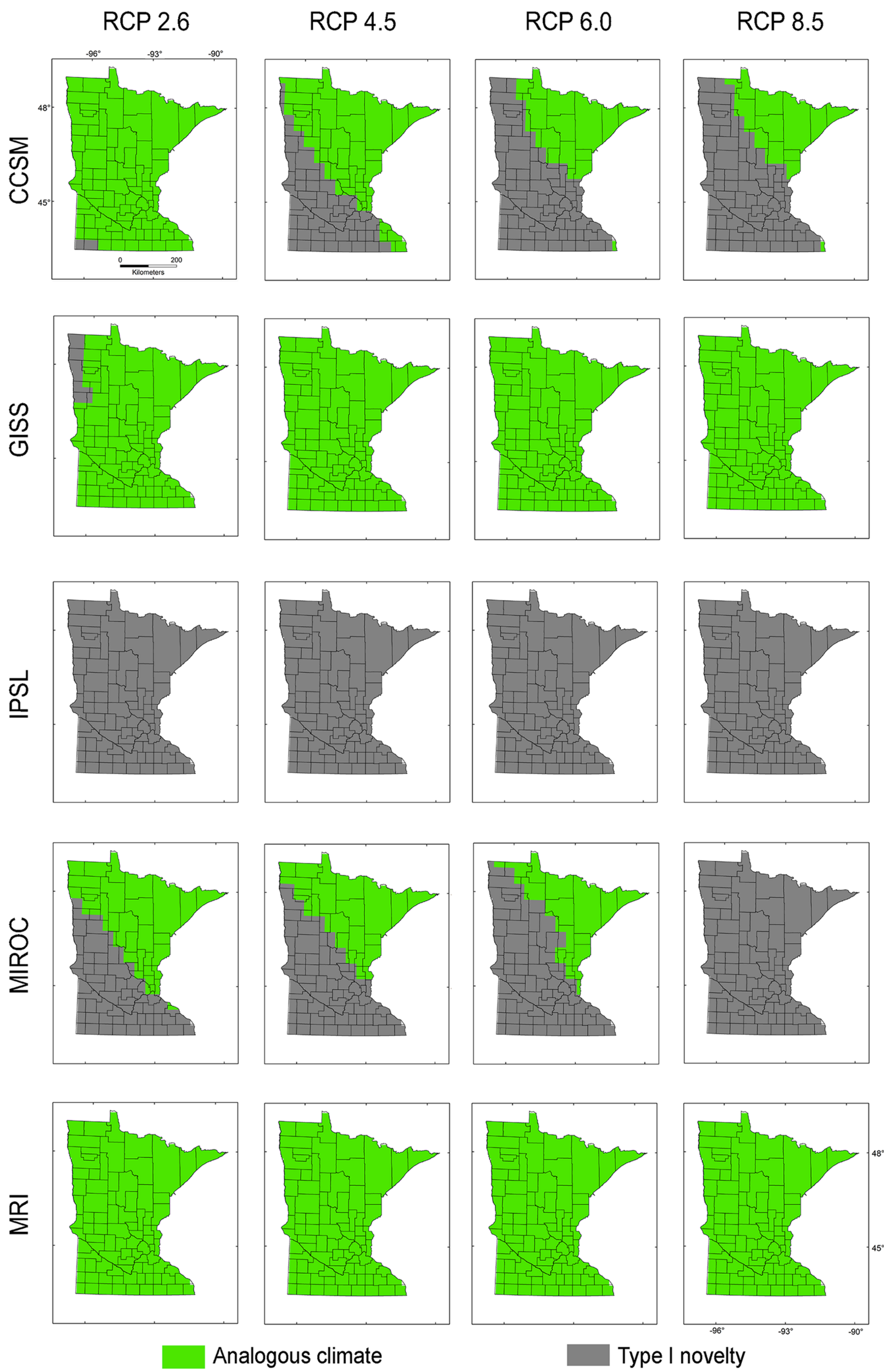

Fig 6. Environmental similarity comparison between the calibration M_d and the projection region of Minnesota. Legend as in Fig 3.

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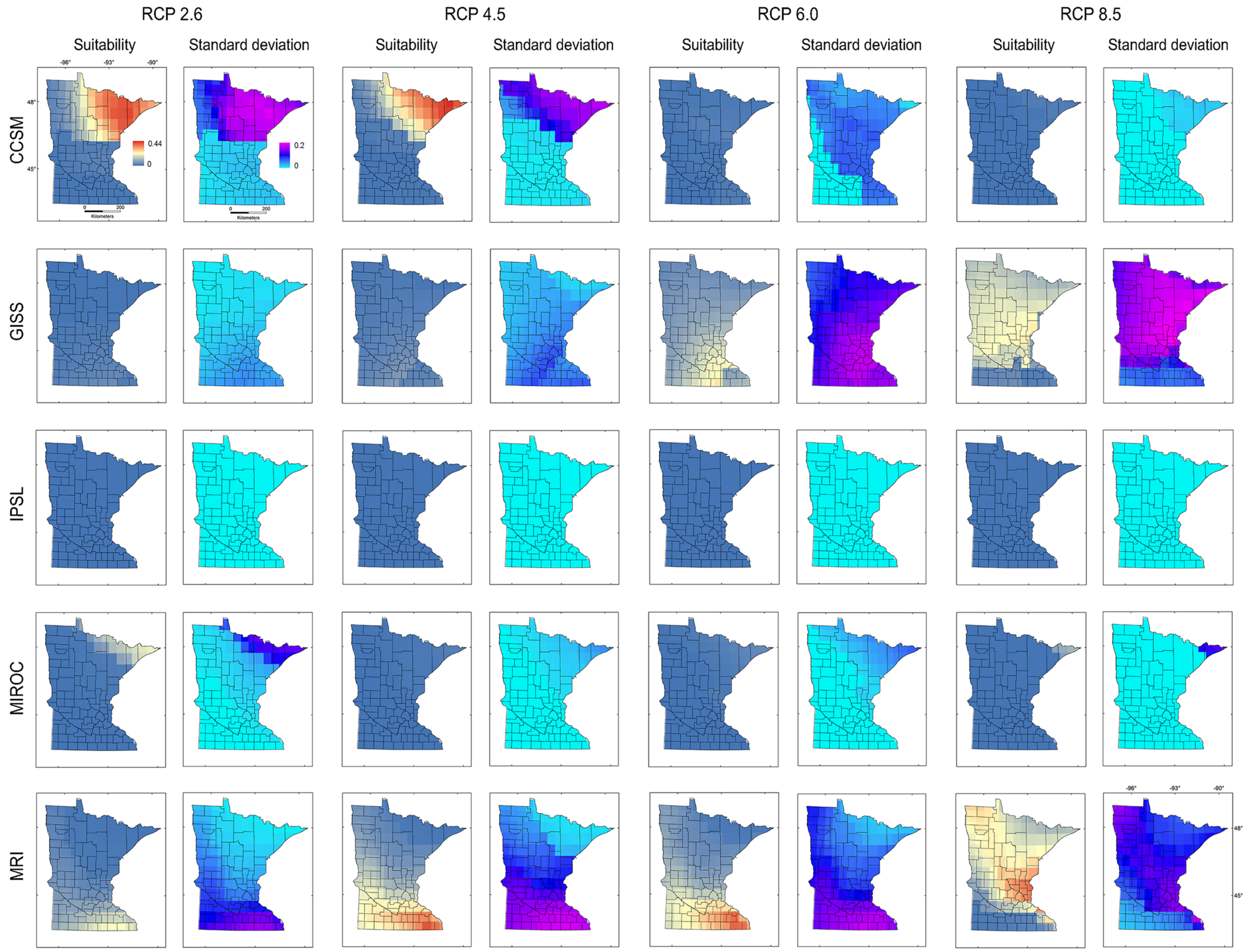

Fig 7. Ecological niche models of starry stonewort calibrated in M_i and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region M_i projected to Minnesota. Areas with high (red) or low (blue) environmental suitability (Suitability, left) and high (pink) or low (light blue) model uncertainty (Standard deviation, right) were identified for five future climate models (i.e., CCSM, GISS, IPSL, MIROC, MRI) and four RCP scenarios of CO_2 emissions (i.e., 2.6, 4.5, 6, and 8.5).

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agonistic interactions with resident biota, could limit starry stonewort expansion. However, a recent study of macrophyte communities in invaded lakes suggested plausible dominance of starry stonewort, with native species richness decreasing as starry stonewort increases in biomass [2]. These fine-scale, potentially complex and interacting factors cannot be accounted for in climate-based models, experiments would be needed to test the influence of these factors on starry stonewort population dynamics. Future research should assess how finer-scale abiotic variables (e.g., pH, conductivity, water clarity), biotic interactions, dispersal potential (via boater movement or natural water connectivity), and landscape factors (e.g., densities of roads and boat accesses) influence lake-level risk of starry stonewort invasion. Emergence of sexually reproductive populations could add new and longer-distance dispersal vectors due to small oospores that could potentially be spread by waterbirds or survive overland transport longer than bulbils [21].

Environmental variables

The environmental variables derived from the Ecoclimate repository are a promising alternative for modeling species distributed across inland and coastal/marine ecosystems [32], providing robust data on climatic variability needed for ecological niche models [54]. The 50-km spatial resolution of Ecoclimate variables mitigate the high spatial lag of finer-resolution climatic layers [52,53], which can produce flawed estimates due to high spatial autocorrelation from statistical downscaling [32,53]. We argue that during exploratory analyses, coarse-scale variables are useful for identifying plausible constraints for species establishment. Subsequent work can then incorporate finer-scale environmental variables (derived from remote sensing or habitat data) to complement climate-based models. Additionally, we developed analyses incorporating five future climate models: CCSM, GISS, IPSL, MIROC, and MRI, and four RCP emission scenarios: 2.6, 4.5, 6, 8.5. This allowed us to investigate a broader range of plausible climate scenarios. Ecological niche modeling of species invasions under future climates

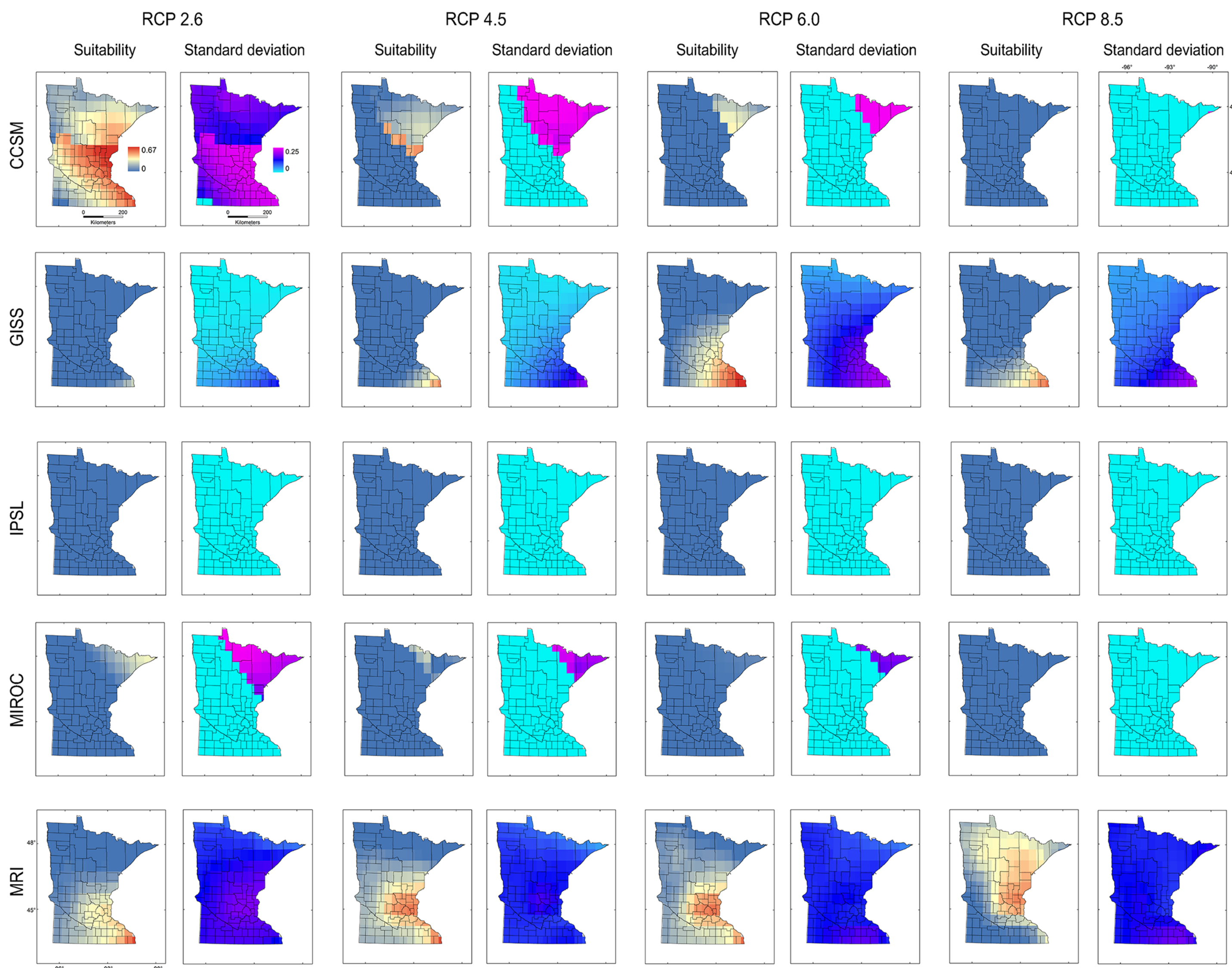

Fig 8. Ecological niche models of starry stonewort calibrated in M_d and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region $\mathbf{M_d}$ projected to Minnesota. Legend as in Fig 7.

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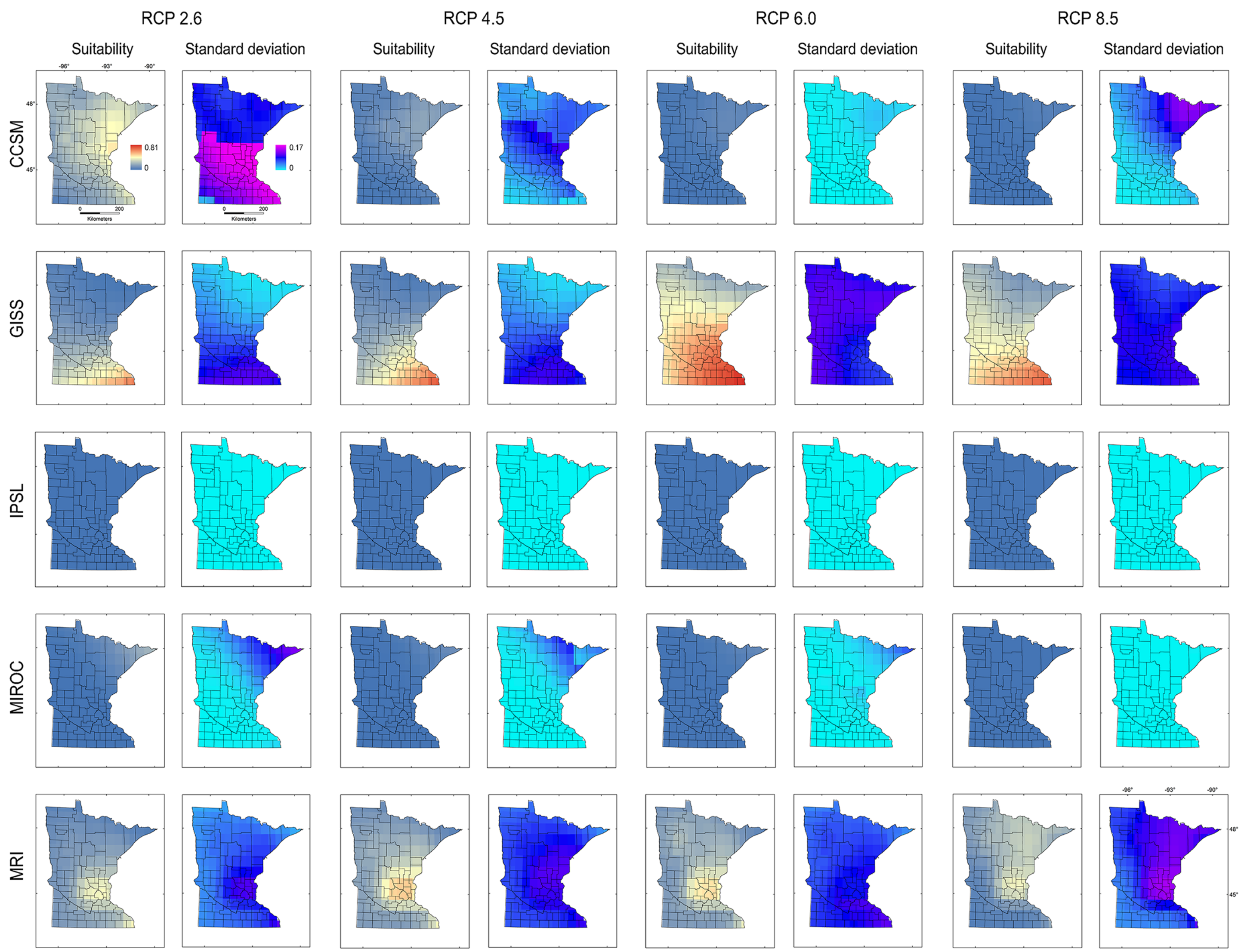

Fig 9. Ecological niche models of starry stonewort calibrated in $\mathbf{M_g}$ and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region $\mathbf{M_g}$ projected to Minnesota. Legend as in Fig 7.

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should incorporate alternative climate models and emission scenarios to reflect the uncertainty in future conditions.

The calibration region M

In agreement with previous studies using virtual species [29], our models based on empirical data suggest that a careless definition of the calibration region, **M**, may produce flawed results [23]. Restricting the model calibration region only to the invaded region, $\mathbf{M_i}$, in present-day climate (Fig 2), narrowed geographic predictions to southeastern Minnesota—all actual occurrences to date are outside of this region—as a result of the incomplete information provided to the algorithm (Fig 3). In contrast, considering the entire species' range for the two calibration regions $\mathbf{M_g}$ and $\mathbf{M_d}$ (Fig 2) included portions of central and central-north Minnesota where starry stonewort has known occurrences (Fig 3). We found that increasing the model calibration area generated an increase in AUC values, but from a practical perspective,

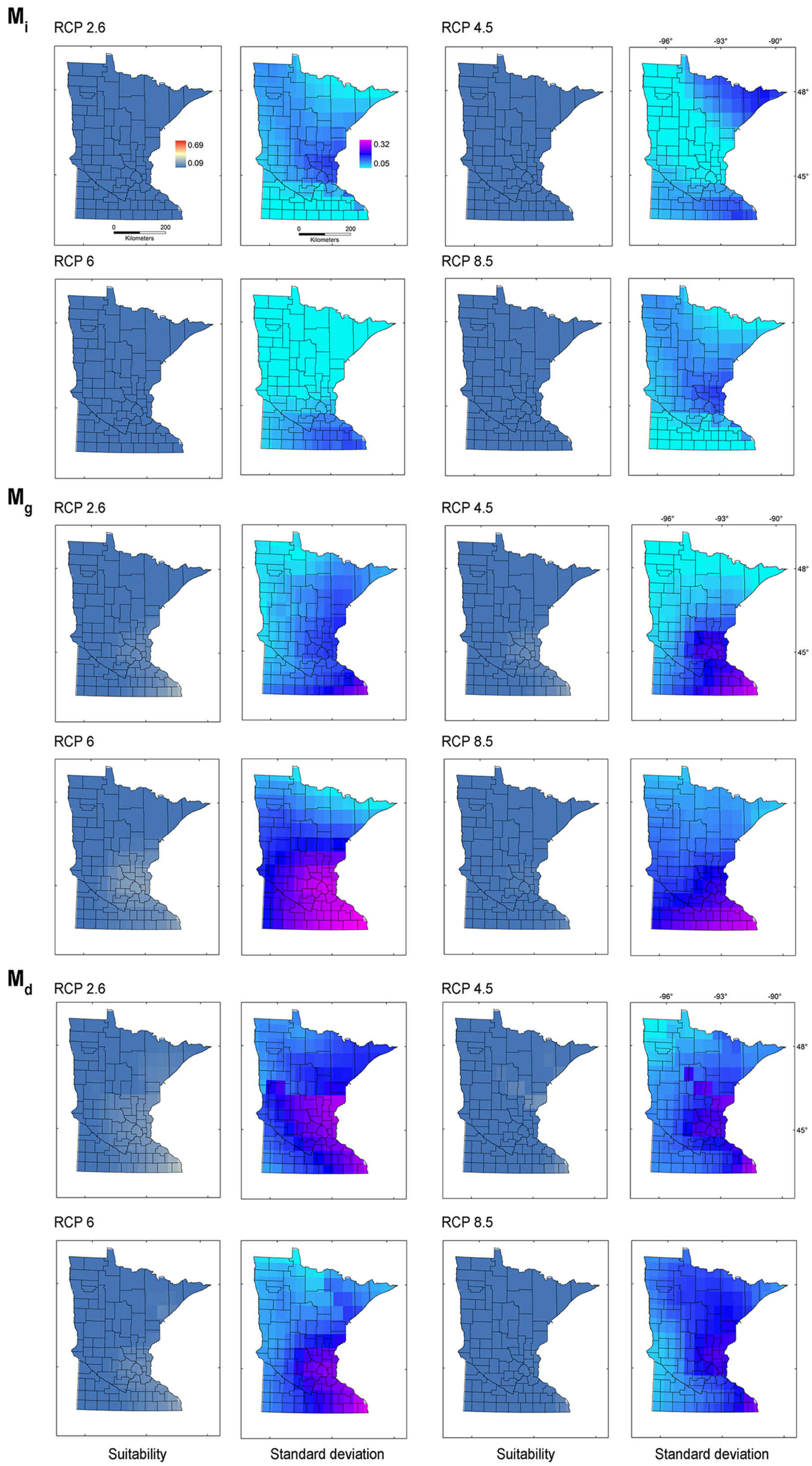

Fig 10. Starry stonewort future climate models ensemble. Model ensemble expressed as the average of continuous models in logistic format (left, 'Suitability'), showing areas with high (red) or low (blue) suitability

from all the RCP emission scenarios in comparison with the maximum range of suitability of climatic models projected to Minnesota in present environmental conditions (i.e., from the lowest [0.09] to the highest [0.69] suitability). Lack of agreement was estimated from the standard deviation of the final models (right, 'Standard deviation') and shows areas of high (pink) or low (light blue) disagreement among models. **Top**: Models calibrated in $\mathbf{M}_i$ and projected to future climate scenarios in Minnesota. **Mid**: Models calibrated in $\mathbf{M}_g$ and projected to future climate scenarios in Minnesota. **Bottom**: Models calibrated in $\mathbf{M}_d$ and projected to future climate scenarios in Minnesota.

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accounting for environmental conditions available in the entire range produced forecasts that were more reliable and more precautionary [30]; this suggests that AUC may not accurately reflect model performance due to high sensitivity of this metric to the extent of the model calibration region [29].

From a theoretical perspective, niche estimations should be guided by modern ecological niche theory [23]. According to Hutchinson [13,55], ecological niches occur in multidimensional environmental space, and species may not occupy all suitable abiotic environments (**A**) due solely to limiting biotic interactions (**B**; e.g., competition) (Fig 11 top). However, Soberón and Peterson [23] propose that Hutchinson's ideas were incomplete and that, in addition to **B**, a species can also be limited by its dispersal potential (**M**) (Fig 11 bottom). They propose that species rarely occupy their entire environmental potential and that the Hutchinsonian framework needs to be expanded. The **BAM** framework proposes that for a realistic **A** estimation for an invasive species, studies should include delimitations of **M** allowing a representative characterization of the dispersal potential of the species [23]. In other words, models aiming to estimate a good proxy of **A** should include all the areas where the species occurs, including the full native and invaded ranges. Thus, we stress that ecological niche modeling to forecast current and future biological invasions are dependent upon **M** (Fig 10 bottom). Ecological niche models calibrated in only a portion of the species' range or under a single **M** scenario may underestimate invasive potential (Fig 3). In this vein, our estimation of dispersal potential based on distance between populations in the invaded range may be confounded by search effort and may not reflect the actual directionality of spread. Genetic/genomic analyses could be used to reconstruct dispersal potential, invasion pathways, and directionality.

The extent of the calibration region was also crucial to establish the presence or absence of novel environments between calibration and projection regions, and between present-day and future climates [34,46]. Models $\mathbf{M}_i$ calibrated from the invaded range only, and models $\mathbf{M}_d$ calibrated based on a small dispersal potential (Fig 2), showed high levels of truncation of prediction in non-analogous novel climatic conditions across Minnesota, limiting our ability to project models to future scenarios (Figs 4 and 6). Conversely, $\mathbf{M}_g$ models from the entire species range with a hypothetical high dispersal identified suitable areas for starry stonewort in Minnesota under present-day and most future climate scenarios (Figs 5 and 9). This provides additional evidence that the calibration region extent plays a key role in ecological niche model projections for species invasions. Thus, model calibration regions should include the full distribution of the studied species under different **M** scenarios to capture the fullest possible set of environmental determinants of physiological tolerance of the organism, providing a firmer biological foundation for calibration region selection [13,31]. We urge researchers and reviewers to put special attention to the justification and biological support of the **M** area selected for model calibration in past and future ecological niche modeling studies.

Maxent and model evaluation

Current literature advocates Maxent for niche modeling due to its accessibility, user-friendly interface, and supporting literature [39]. However, the potential of Maxent to overestimate or

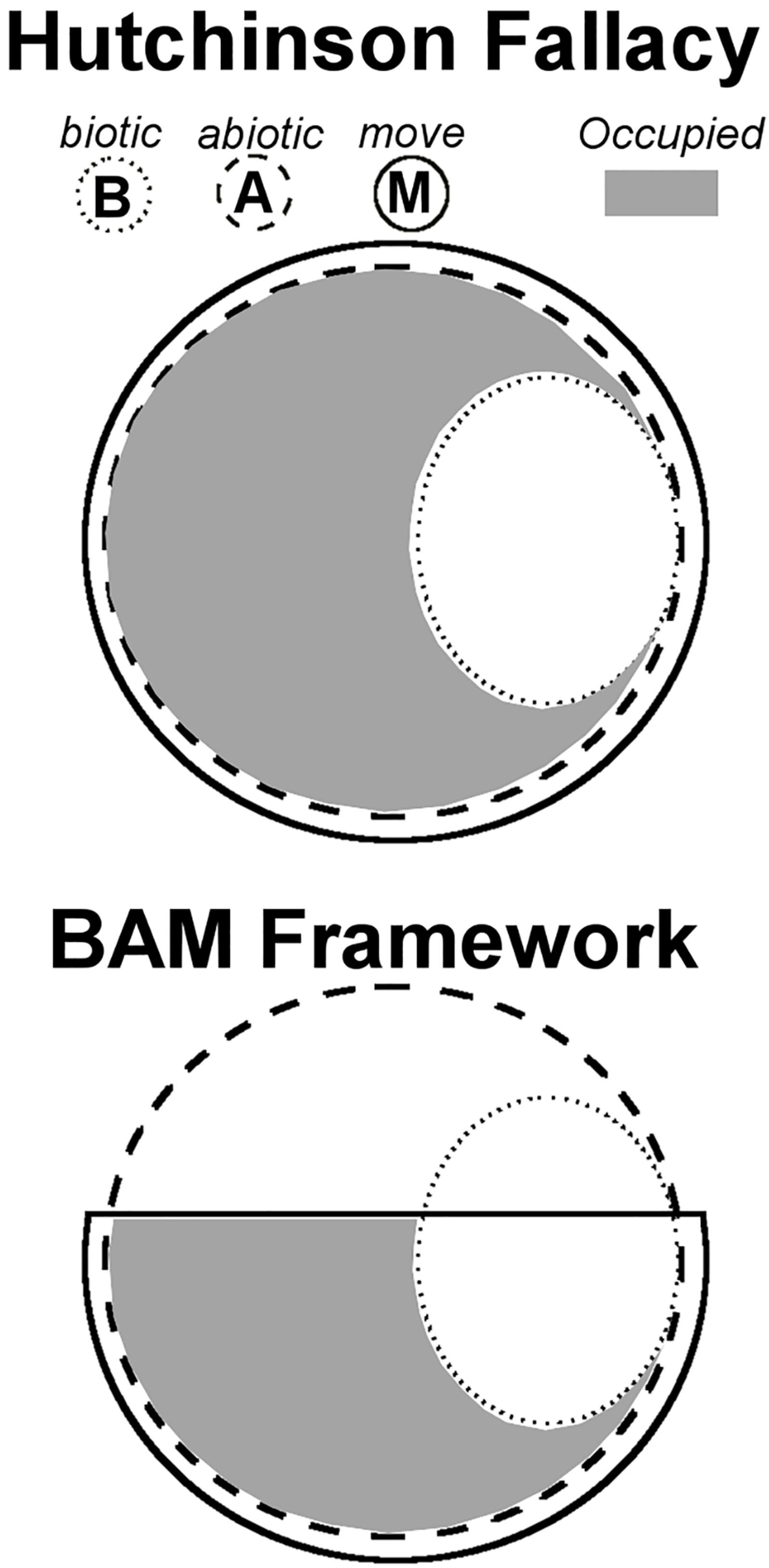

Fig 11. Conceptual framework used for interpretation of predictions. Top: The "Hutchinson Fallacy" expressed as the intersect of abiotic (**A**; dashed line) and biotic factors (**B**; dotted line) showing the

environments that a species can occupy (gray) or not (white area inside the dotted circle), based on biotic interactions solely (e.g., competitors). Note that under the Hutchinson's proposal, all the areas environmentally suitable can be reached by the species (i.e., entire circle), suggesting that the movement and dispersal potential of the species (**M**; solid line) is effective to occupy all the suitable conditions (i.e., **A** is contained in **M**). **Bottom**: The "BAM Framework" proposed by Soberón and Peterson [23] to explain that dispersal limitations (**M**) can also restrict the species to occupy (gray) only a portion of all the suitable environments (**A**). Note that in this example, the species can occupy a portion of the environmental conditions suitable due to the limited dispersal potential (i.e., half circle). **A** (abiotic) = environmental conditions suitable for the species; **B** (biotic) = interaction with other species; **M** (move) = movement or dispersal potential of the species.

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overfit predictions to the data available must be considered [18,27,38,39,41]. Maxent must be fitted for each study case considering the natural history of the species, the data available, and the assumptions involved. The results from our approach to control the extent of the calibration region, which included use of regularization coefficients, information-theory model selection, strict model evaluation, and strict model transference, support the contention that using the default parameterizations of Maxent, while convenient, is an inappropriate approach that can lead to inaccurate conclusions [29,41,46]. Thus, each modeling effort should include detailed individualized parameter selection, and model results should be critically assessed to determine if they are biologically sound, avoiding reliance on single model estimates [37].

Although predicted suitability from our present-day models ranged from minimal to broad across Minnesota (Fig 3), models with the two different calibration regions performed well in terms of omission rates and AUC values [40]. The heterogeneous suitability predicted under the two configurations reflects the sensitivity of ecological niche models to experimental design decisions (Fig 2) [13]; therefore, we propose that uncertainty estimation must be included as an essential component of ecological niche model estimations.

Conclusions

Starry stonewort is predicted to expand its current geographic range into novel areas across Minnesota under present-day climate conditions. Under future climate conditions, we estimate a reduction in suitability for the species. Our models are a step toward the development of management strategies to prevent and mitigate the spread of this species on the leading edge of its invasion. It is crucial to develop strategic interventions that target the role of human activities in starry stonewort spread. Further, our results suggest that sound forecasts require rigorous model design and evaluations to improve their reliability.

Supporting information

S1 File. Table A. Correlation matrix of environmental variables. **Table B.** Summary of model evaluations.
(DOCX)

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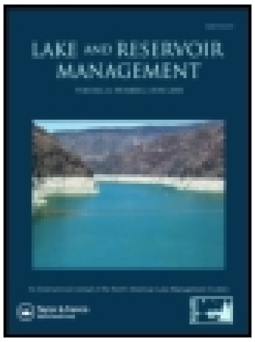

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Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota lake

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Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota lake

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ABSTRACT

Glisson WJ, Wagner CK, McComas SR, Farnum K, Verhoeven MR, Muthukrishnan R, Larkin DJ. 2018. Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota lake. Lake Reserv Manage. 00:00–00.

Starry stonewort (*Nitellopsis obtusa*), an invasive green macroalga in the family Characeae, has recently been found for the first time in several Midwestern states. This aquatic invasive species is of increasing concern to management agencies, lakeshore property owners, and other stakeholders. Starry stonewort has proven difficult to control, partly due to its ability to reproduce via bulbils (asexual reproductive structures). There has also been a lack of applied research addressing the efficacy of current management practices for controlling starry stonewort. We examined the effects of mechanical and algaecide treatments on starry stonewort biomass, bulbil density, and bulbil viability by monitoring treated areas and untreated reference locations concurrent with management implemented on Lake Koronis in Minnesota. Chelated copper algaecide applications alone and in combination with mechanical harvesting significantly reduced starry stonewort biomass, but algaecide treatment alone failed to reduce the capacity of starry stonewort to regenerate via bulbils. A second, granular algaecide application following an initial treatment with liquid algaecide did not further reduce biomass in any treated area and was associated with a substantial increase in bulbil density in an area treated with algaecide alone. Bulbil viability was greatest in the area treated only with algaecide (86%) and an untreated reference area (84%) and was lowest in an area treated with both mechanical harvest and algaecide (70%). The ability of starry stonewort to regenerate and persist following algaecide treatment is concerning. Multi-pronged management incorporating both chemical and mechanical approaches may improve outcomes of starry stonewort control efforts.

KEYWORDS
Algaecide; aquatic plant management; bulbil; Characeae; chelated copper; invasive species; macroalgae; mechanical removal

Control and management of aquatic invasive plants is challenging because many factors can influence treatment efficacy. As a result, a wide variety of approaches have been developed to achieve more effective control of aquatic invasive plants (Madsen 1993, Gettys et al. 2014, Hussner et al. 2017). Identifying control strategies for a species with little history of applied research or management can be difficult, as approaches that have been effective for other target species may have limited efficacy. Even closely related species can respond quite differently to the same treatments (Parks et al. 2016). Thus, it is particularly important to evaluate efficacy of management in the case of newly discovered or understudied invasive species, for which early treatment efforts are valuable opportunities to learn and update approaches to management.

In North America, starry stonewort (*Nitellopsis obtusa* [N.A. Desvaux] J. Groves) is an introduced macroalga in the family Characeae that is native to Europe and Asia. Starry stonewort was first found in the United States in the 1970s in the St. Lawrence River in New York (Geis et al. 1981) and then in the St. Clair–Detroit River system in Michigan 5 yr later (Schloesser et al. 1986). In just the past 5 yr, the species has been newly recorded in 5 US states (Pennsylvania, Indiana, Wisconsin, Vermont, and Minnesota) and Ontario, Canada (Kipp et al. 2017). New occurrence records and dense infestations have caused concern among lake

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users and resource managers (Pullman and Crawford 2010). Starry stonewort can produce dense beds and surface mats that interfere with boating and recreation, particularly at shallow depths. The ecological effects of starry stonewort invasion have received little investigation to date but there is evidence of negative effects on native aquatic plants; Brainard and Schulz (2016) found that native macrophyte species richness and abundance were negatively correlated with starry stonewort biomass in New York lakes. Moreover, starry stonewort may have a higher rate of carbon fixation (from HCO_3^-) in high pH conditions compared to other Characeae (Smith 1968), such as *Tolypella intricata*, which is native to the Great Lakes region. Higher rates of carbon fixation than native Characeae species could provide starry stonewort a competitive advantage in high pH lakes of the Midwest and Great Lakes region. Starry stonewort also appears to be exploiting novel niche space in the United States relative to its native range (Escobar et al. 2016), where it already exhibits fairly broad tolerance of environmental conditions (Rey-Boissezon and Auderset Joye 2015). Hence, there is cause for concern about the impacts of starry stonewort invasion, and research on the control of this introduced species is needed to guide management efforts.

Effective control of aquatic invasive plant species requires knowledge of individual species' biology (Hussner et al. 2017). For example, sexual reproduction has not been observed in populations of starry stonewort in North America due to an apparent absence of female individuals (Sleith et al. 2015). Instead, starry stonewort reproduction has been asexual, via the alga's nodes. Starry stonewort nodes are present aboveground along the stem and along rhizoids under the sediment, where they occur as specialized structures called bulbils (Bharathan 1983, 1987). Starry stonewort bulbils are white, multicellular, star-shaped structures (from which the species gets its common name) connected via rhizoids that help anchor starry stonewort in the substrate. Because new starry stonewort sprouts from bulbils (Bharathan 1987), management strategies need to target these structures to achieve effective control.

Another aspect of starry stonewort's biology that poses challenges for control is that, as an alga, it lacks a true vascular system (Raven et al. 2005). Hence, starry stonewort bulbils, which form beneath the sediment, are not connected by vascular tissue to aboveground structures. This limits the efficacy of herbicide treatment for starry stonewort control. For example, systemic herbicides that rely on transport through vasculature may not be able to translocate through starry stonewort to reach bulbils. Furthermore, even contact herbicides that do not rely on transport, but rather physical contact, may not be able to reach unexposed bulbils beneath the sediment. The capacity of herbicides to reach bulbils will limit treatment efficacy if bulbils can persist and remain viable following treatment.

Control of starry stonewort by current treatment approaches has proven difficult. Copper-based algaecides, including copper sulfate ($CuSO_4$) and chelated copper formulations, are contact herbicides widely used for algae control (Lembi 2014). Whereas these copper compounds have been used to manage starry stonewort in the United States, anecdotal observations indicate that these compounds may not achieve complete or sustained control of starry stonewort (Pullman and Crawford 2010). Mechanical harvesting has also been used for starry stonewort control, but anecdotal reports indicate that starry stonewort can regrow quickly following mechanical harvesting (Pullman and Crawford 2010). Compounding uncertainty about treatment effectiveness is a lack of research in this area; previous reports (i.e., Pullman and Crawford 2010) are qualitative and do not include a robust examination of treatment outcomes. We know of no published studies that have systematically evaluated outcomes of chemical or physical treatment options for starry stonewort management. Moreover, the few studies that have assessed the effect of treatment on other Characeae species either examined nontarget treatment effects (Hofstra and Clayton 2001, Wagner et al. 2007, Kelly et al. 2012), or were conducted in agricultural fields with limited application to natural lake systems (e.g., Pal and Chatterjee 1987, Guha 1991). This is a critical knowledge gap. The efficacy of current starry stonewort treatment practices must be addressed to better guide management decisions.

Observations from previous treatment efforts, combined with knowledge of starry stonewort biology, suggest that control of this species may be difficult, particularly because starry stonewort bulbils may persist and remain viable following treatments. We used a pilot treatment project for starry stonewort on Lake Koronis, the first lake in Minnesota found to have starry stonewort, to examine the response of starry stonewort to treatment by chelated copper algaecides and mechanical harvesting. We implemented a before-after-control-impact monitoring design in the field and laboratory tests of bulbil viability to evaluate management efficacy. Specifically, the objectives of our study were to evaluate the effects of mechanical and algaecide treatments on (1) starry stonewort biomass, (2) bulbil density, and (3) bulbil viability.

Study site

Lake Koronis is a 1201 ha lake on the border of Meeker and Stearns counties in central Minnesota that is part of the North Fork Crow River watershed (Fig. 1). The lake is classified as slightly eutrophic, with a Trophic State Index (Carlson 1977) of 54 (total phosphorus = 0.031 mg/L), and has a maximum depth of 40.2 m. Starry stonewort was discovered in Lake Koronis on 18 August 2015. The Minnesota Department of Natural Resources (MNDNR) conducted several surveys to delineate the extent of the infestation and found that, as of September 2015, it covered an area of ~100 ha.

Materials and methods

Treatments

In summer and fall of 2016, 3 infested areas of Lake Koronis were treated for starry stonewort control. These areas were designated for treatment by the Koronis Lake Association because they had large infestations of starry stonewort that interfered with navigation and recreational use. This ongoing treatment effort provided an opportunity to examine the subsequent response of starry stonewort. Hence, the 3 treated areas were the basis for our analysis and comprised the following: (1) a mechanically harvested channel (hereafter, mechanical area), (2) an area treated only with algaecide (algaecide area), and (3) an area that was first mechanically harvested and then treated with algaecide (mechanical + algaecide area; Fig. 1). To assess the efficacy of starry stonewort treatments, we also examined a 3.4 ha area invaded by starry stonewort that did not receive any treatment (untreated reference area) and compared this area to the treated areas. No algaecide or mechanical treatments were previously conducted in any of the treatment or reference areas that we evaluated.

Treatments were applied by independent contractors under the direction of the Koronis Lake Association. The mechanical area consisted of a 430 m linear channel (approximately 10 m wide) extending from a public water access that was mechanically harvested on 10 August 2016 using an Eco Harvester (Lake Weeder Digest LLC, New Hope, MN; Fig. 1). The Eco Harvester is a single-manned aquatic plant harvesting vessel that uses a large rotating drum designed to uproot plants and feed them onto a conveyor that pulls plants out of the water. The mechanical + algaecide area consisted of a separate 1.5 ha starry stonewort infested area that was mechanically harvested between 11 August and 9 September 2016 to completely cover the area (Fig. 1). This area and an adjacent unharvested 1.1 ha area (Fig. 1) were treated on 21 September 2016 with a liquid chelated copper formulation (Cutrine-Plus; copper ethanolamine complex, mixed; liquid) at 54.5 L/ha. Copper concentrations were measured at 1 h following this application with a colorimeter (Series 1200, LaMotte Company, Chestertown, MD). Average copper concentrations were 0.37 ppm at the surface and 0.45 ppm at the lake bottom. A second application was conducted in both the algaecide and mechanical + algaecide areas on 11 October 2016 with a granular formulation of the same compound (Cutrine-Plus; copper ethanolamine complex, mixed; granular) at 41.2 kg/ha. This second, granular treatment, was performed with the goal of destroying starry stonewort bulbils and remaining biomass by targeting the lake bottom. Average copper concentrations at 1 h following the granular application were 0.16 ppm at the surface and 0.15 ppm at the lake bottom. Treatments previously performed by MNDNR near the public water access in 2015 and 2016 were located >50 m from the mechanical treatment area, ≥1 km from the algaecide and mechanical + algaecide treatment areas, and >600 m from the untreated reference area and are thus presumed to have had no influence on these treatment areas. The untreated reference area was located >500 m from the algaecide and mechanical + algaecide treatment areas (Fig. 1). PLM Lake and Land Management Corporation (Brainerd, MN) applied algaecide treatments, and Dockside Aquatic Services (Mendota Heights, MN) performed mechanical harvesting.

Biomass and bulbil sampling

In the summer and fall of 2016, we sampled starry stonewort biomass and bulbil density and collected bulbils for laboratory evaluations of viability. We measured starry stonewort biomass prior to any treatments

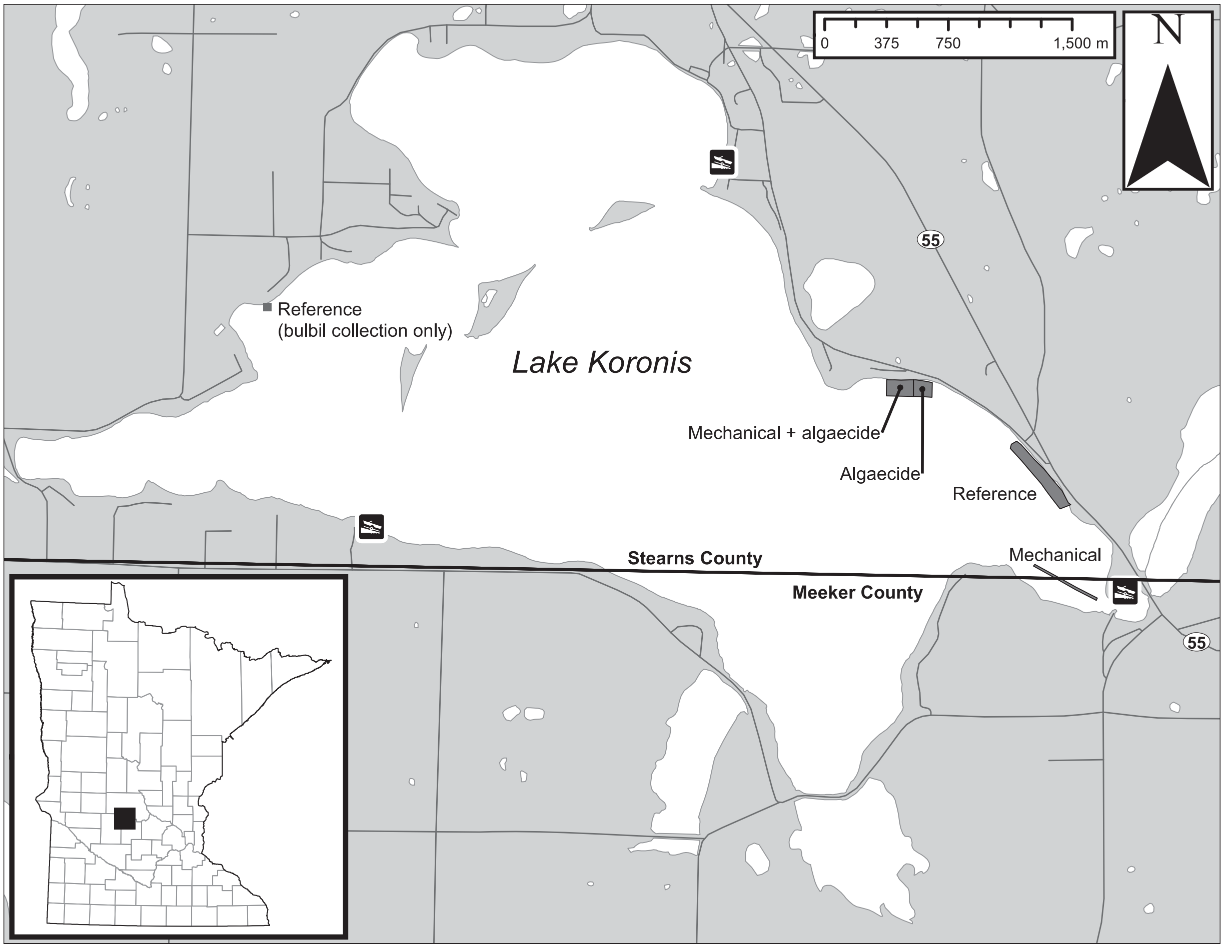

Figure 1. Map of starry stonewort (*Nitellopsis obtusa*) infested areas sampled July–October 2016 on Lake Koronis in Minnesota.

(19 Jul 2016 for treatment areas and 26 Jul 2016 for the untreated reference area) with grids of points distributed at 40 m spacing throughout each sampled area. Because our sampling comprised a uniform grid and treatment areas differed in size, we sampled different numbers of points in each area (mechanical, $n = 10$ points; algaecide, $n = 6$; mechanical + algaecide, $n = 8$; untreated reference, $n = 15$). At each point, we collected starry stonewort biomass by lowering a 7-tine rake (15 cm wide) attached to a telescoping pole to the lake bottom, making 3 rotations, and then pulling the rake and attached biomass to the surface (vertical rake method following Johnson and Newman 2011). We brought these samples to the lab, dried the samples to constant mass at room temperature in front of a fan, and weighed each sample. The vertical rake method can overestimate abundance for some aquatic plant species (Johnson and Newman 2011) and it is likely that we ensnared starry stonewort biomass from a greater area than that covered by the rake. Nonetheless, starry stonewort abundance values are comparable among samples in our study. We repeated this sampling procedure on 13 September, 7 October, and 28 October 2016 (all areas were sampled on all dates, except for the mechanical area, which was not sampled on 7 Oct). We estimated bulbil density using the 7 October and 28 October 2016 starry stonewort biomass samples, for which we counted all bulbils in each sample following drying. The vertical rake method was not designed to sample bulbils and may overestimate or underestimate bulbil density due to a number of potential factors (algal biomass, phenology, etc.); however, no accepted method exists and the vertical rake method provided an efficient and consistent option.

On 28 October 2016, we collected bulbils for viability testing. Bulbils were collected from the algaecide, mechanical + algaecide, and untreated reference areas, as well as a second untreated reference location. We haphazardly collected bulbils throughout each sampling area using 2 spins of a 14-tine rake (33 cm wide). We sampled until we were confident that we had collected ≥100 bulbils from each area (5—15 rake samples per area); however, bulbils were often small and obscured by plant material, so exact counts could not be determined in the field. Low bulbil density in the untreated reference area necessitated collection at a second untreated reference location ≥3.5 km from the algaecide and mechanical + algaecide areas (Fig. 1). We collected bulbils for viability testing at separate locations from sample points for bulbil density and biomass. We placed bulbils in plastic bags in a cooler for transport and returned the samples to the lab.

We counted bulbils in the lab and physically separated them from rhizoids. We examined bulbils for signs of sprouting, and did not observe sprouting in any of the bulbils used in our experiment. We placed bulbils from each sampling area into separate 11.4 L plastic tanks filled with 2 cm of topsoil overlain with fine-grained play sand to keep the sediment from entering the water column. We pressed each bulbil lightly onto the sediment surface and filled the tanks with dechlorinated water to a depth of 8 cm above the substrate. Water chemistry was within the range of northern tier lakes in which starry stonewort has been observed (Sleith et al. 2015, Midwood et al. 2016): pH = 8.65, conductivity = 253 µS/cm, alkalinity = 159 mg/L as $CaCO_3$, hardness = 145.4 mg/L as $CaCO_3$, total phosphorus = 0.042 mg/L, and total nitrogen = 0.34 mg/L. We maintained tanks under a 14 h/10 h light/dark schedule with multi-spectrum lights (RX30, Heliospectra AB; Göteborg, Sweden). We covered tanks with 50% black shade cloth to limit light intensity. Photosynthetically active radiation (PAR) at the water's surface, beneath the shade cloth, was 8 µmol/m^2/s. Mean temperature in the lab over the course of the experiment was 19.9 C, and mean water temperature in the tanks was 17.8 C. The total number of bulbils evaluated for each sampling area was: algaecide, n = 363 (2 tanks: n = 100, 263); mechanical + algaecide, n = 223 (2 tanks: n = 100, 123); and untreated reference, n = 100 (1 tank). One tank from each sampling area was planted on 28 October 2016 and one additional tank each for the algaecide and mechanical + algaecide areas were planted on 31 October 2016. The bulbil viability experiment began on 31 October 2016.

We checked bulbils for sprouting every 1–7 d for a total of 12 weeks (84 d). Bulbil viability was confirmed when we observed the emergence of a new shoot from a bulbil (i.e., sprouting). We used our own previous observations of bulbil sprouting and additionally followed Bharathan (1987) as a visual guide. Newly sprouting material was often conspicuously green (i.e., photosynthetic), which made determination of sprouting unequivocal. Occasionally, bulbils sank into the substrate before sprouting; these sprouting events were identified when green shoots emerged above the substrate. Once we observed a bulbil sprouting, we removed that bulbil from the tank to avoid duplicate counting. On the final day of the experiment, along with our regular examination, we used a fine-mesh strainer to sift through the substrate to collect and examine any remaining bulbils. We were not able to recover all bulbils that we had initially placed in tanks. Based on our observations, unrecovered bulbils were likely to have broken apart or decomposed over the course of the experiment; thus, we considered unrecovered bulbils as not viable.

Data analysis

Biomass

We examined differences in starry stonewort biomass among treatments using a before-after-control-impact (BACI) framework (Green 1979, Stewart-Oaten et al. 1986). Under this framework, we sought to determine whether the change in starry stonewort biomass in response to treatments significantly differed from changes in starry stonewort biomass that occurred naturally, as measured in the untreated reference area. Because treatments were implemented as pilot tests, each treatment was conducted in a single location and was not randomly assigned to a location, nor replicated. In order to take advantage of the data from Lake Koronis and make inferences about each treatment, sample points within each area were considered individual replicates, though we acknowledge that these points are not true replicates (Hulbert 1984, Stewart-Oaten et al. 1986). First, we used the BACI approach to examine overall treatment outcomes across the entire study. For this analysis, we included biomass data from sampling dates prior to any treatments being performed and from the final sampling date, after all treatments had been performed (Table 1 , Fig. 2). Then, to more closely inspect outcomes of individual treatments, we separately analyzed biomass data for (1) before and after the mechanical harvest, (2) before and after the first (liquid) algaecide treatment, and (3) before and after the second (granular) algaecide treatment (Table 1, Fig. 2). We examined treatments in this manner to isolate the effects of individual management actions

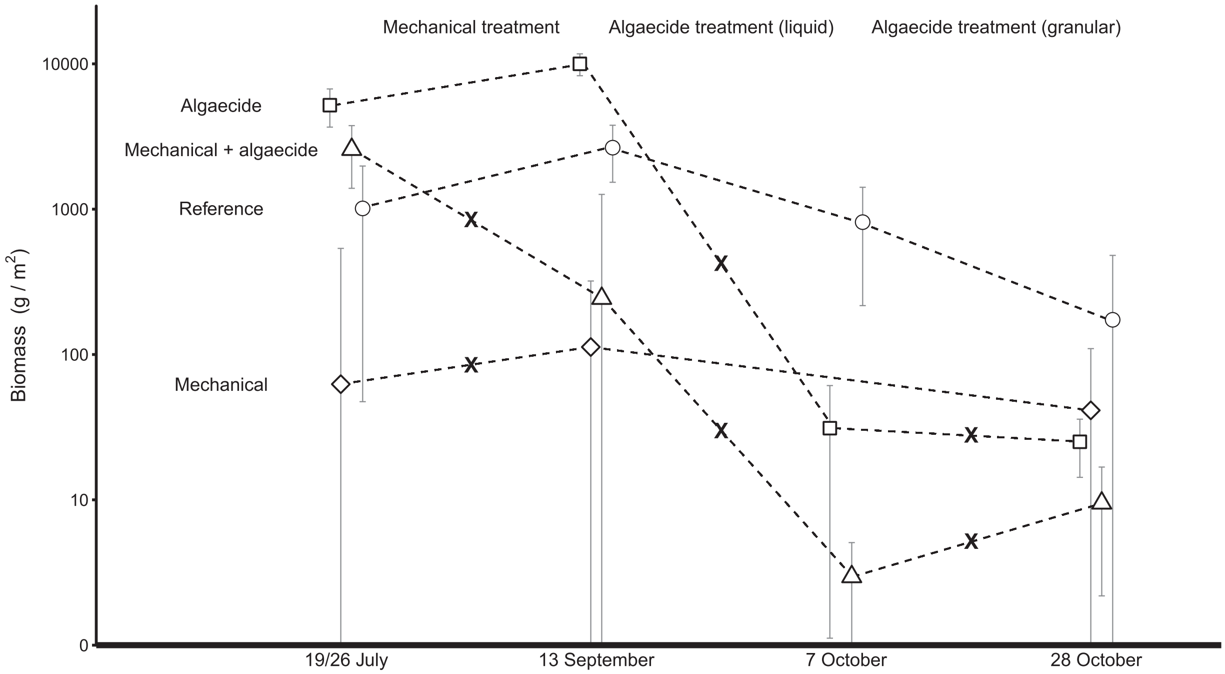

Figure 2. Starry stonewort (*Nitellopsis obtusa*) biomass July–October 2016. Biomass data are natural-log transformed. Each treatment area is represented with a different symbol. An X indicates that an area received the treatment designated at the top of the plot. Symbols and error bars are means ±1 SE.

in areas where multiple treatments were applied. For each of these individual analyses, we only included the treatment areas targeted with a given treatment and compared them to the untreated reference area.

We analyzed biomass data using linear mixed effects (LME) models with the nonlinear mixed-effects (nlme) package in R, version 3.3 (Pinheiro et al. 2017, R Core Team 2017), with point-level starry stonewort biomass (g/m^2) as the response variable. Predictor variables included sampling period (i.e., before or after treatment), treatment type (up to 4 levels: mechanical, algaecide, mechanical + algaecide, and untreated reference), and a sampling period × treatment interaction. In all models, we included sampling point as a random effect to account for repeated sampling of points over time (i.e., repeated measures). We natural-log transformed biomass data prior to analysis; this improved normality and resulted in greater homogeneity of variance among treatment types and sampling periods, as measured by the Fligner–Killeen test (Conover et al. 1981). Because there were some sampling points without starry stonewort, we added the minimum biomass value in the dataset (2.26 g/m^2) to all observations prior to natural-log transformation. For the analysis of biomass before and after the mechanical harvest, we combined data for the 2 mechanically harvested areas (mechanical and mechanical + algaecide). For the analysis of biomass before and after the first (liquid) algaecide treatment, we included data from the 2 sampling dates prior to algaecide treatment for the algaecide and untreated reference areas (Table 1, Fig. 2); hence, we included a random effect for sampling date in this model (within which the sampling point random effect was nested). Because we sampled an unbalanced number of points across sampling areas, we used Type III analysis of variance (ANOVA) to assess significance of our interaction term. A significant sampling period × treatment type interaction would indicate differences among treatments in terms of changes in biomass over time. To determine whether changes in biomass in the treatment areas differed from those in the reference area (and differed among treatment areas), we calculated the least-squares means for each sampling period × treatment type combination and used Tukey's honest significant differences (Tukey's HSD) tests of the least-squarest means.

Bulbil density

We tested for differences in the change in bulbil density among treatments using the same BACI framework as for biomass. Because we first measured bulbil density

Table 1. Before-after-control-impact (BACI) analysis of starry stonewort (*Nitellopsis obtusa*) biomass during management from July to October 2016 on Lake Koronis in Minnesota. Each row shows the mean biomass (±1 SE) of the treatment area before and after treatment (g/m^2), the change in biomass (g/m^2), the percent change in biomass, and the comparison of change in starry stonewort biomass in the treatment area versus the untreated reference area. *P* values with an asterisk (∗) indicate significant biomass change ($P < 0.05$) based on Tukey's honest significant differences test.

Treatment examined	Sampling period: Before	Sampling period: After	Treatment area	Biomass before (g/m^2)	Biomass after (g/m^2)	Biomass change (g/m^2)	Percent change	*P*
All	19 Jul	28 Oct	Mechanical	1144 (475)	157 (68)	−987	−86%	0.248
			Mechanical + algaecide	5250 (1183)	16 (7)	−5234	−100%	0.004∗
			Algaecide	6109 (1525)	30 (11)	−6079	−100%	0.013∗
			Reference	3623 (967)	807 (307)	−2816	−78%	—
Mechanical	19 Jul	13 Sep	Mechanical (combined)	2969 (753)	977 (438)	−1992	−67%	0.155
			Reference	3623 (967)	4527 (1124)	+904	+25%	—
First (liquid) algaecide	13 Sep	7 Oct	Mechanical + algaecide	1598 (1020)	2 (2)	−1596	−100%	< 0.001∗
	19 Jul, 13 Sep	7 Oct	Algaecide	8385 (1291)	59 (30)	−8626	−99%	< 0.001∗
	26 Jul, 13 Sep	7 Oct	Reference	4075 (733)	1992 (599)	−2083	−51%	—
Second (granular) algaecide	7 Oct	28 Oct	Mechanical + algaecide	2 (2)	16 (7)	+14	+700%	0.018∗
			Algaecide	59 (30)	30 (11)	−29	−49%	0.272
			Reference	1992 (599)	807 (307)	−1185	−59%	—

Initial biomass data for the untreated reference area were collected on 26 July. Mean biomass (±1 SE) in the before sampling period of the first (liquid) algaecide treatment is the mean biomass from both dates prior to the first (liquid) algaecide treatment.

after the mechanical treatment and the first (liquid) algaecide treatment, we could not compare bulbil density before and after all treatments were performed. However, we were able to test for evidence of a change in bulbil density from before to after the second (granular) algaecide application (7 Oct and 28 Oct 2016, respectively). We used a LME model with bulbil density (bulbils/m^2) as the response variable and sampling period, treatment type (3 levels: algaecide, mechanical + algaecide, and untreated reference; the mechanical area was not included because it was not sampled on 7 Oct), and sampling period × treatment interaction as predictor variables. We included sampling point as a random effect and used Type III ANOVA to assess significance of the interaction term, which would indicate differences among treatments in terms of change in bulbil density over time. We used Tukey's HSD of the least-squares means of each sampling period × treatment type combination to determine whether changes in bulbil density in the treatment areas differed from those in the reference area (and differed among treatment areas).

Bulbil viability

Lastly, we assessed bulbil viability based on data from the laboratory sprouting experiment. Each bulbil had a response of either *sprouted* (sprouted by the end of the experiment) or *unsprouted* (did not sprout by the end of the experiment). We used the summed counts of sprouted and unsprouted bulbils from each treatment type as the response variable in a generalized linear model (GLM) with binomial errors. We used treatment type as a categorical predictor variable (3 levels: algaecide, mechanical + algaecide, and untreated reference). With this model, we tested for differences in the proportion of viable bulbils among treatment areas. Additionally, as a metric for starry stonewort recovery potential via bulbils, we calculated the product of the proportion of bulbils sprouted from each area and bulbil density on the final sampling date (28 Oct 2016); this metric has units of viable bulbils/m^2.

Results

Biomass

Change in starry stonewort biomass over the course of the study (from before to after all treatments) significantly differed by treatment type (sampling period ×

treatment type interaction: $P < 0.001$, $X^2 = 21.993$, df $= 3$). Both the algaecide treatment alone (algaecide area) and the combined mechanical + algaecide treatment resulted in significantly greater biomass reduction than observed in the untreated reference area (Table 1, Fig. 2). Mechanical treatment alone did not result in significantly greater reduction in biomass than the untreated reference area, though we note that biomass in the mechanical area was initially much lower than in the reference area (Table 1, Fig. 2). Among treatments, reduction in starry stonewort biomass was significantly greater in the algaecide area and the mechanical + algaecide area compared to the mechanical area ($P = 0.002$ and $P < 0.001$, respectively; Table 1, Fig. 2).

To examine the effects of individual management actions, we analyzed change in starry stonewort biomass separately for each treatment: (1) mechanical harvest, (2) first (liquid) algaecide treatment, and (3) second (granular) algaecide treatment. Change in starry stonewort biomass from before to after mechanical harvest did not significantly differ from the untreated reference area when data from both mechanically harvested areas were combined (mechanical and mechanical + algaecide; Table 1, Fig. 2). However, we did observe an overall reduction in biomass among these areas (Table 1) and a large biomass reduction in the mechanical + algaecide area (Fig. 2).

Change in starry stonewort biomass from before to after the first (liquid) algaecide treatment significantly differed by treatment type (sampling period × treatment type interaction: $P < 0.001$, $X^2 = 23.134$, df $= 2$). Reduction in starry stonewort biomass was significantly greater in both the algaecide-only area and the mechanical + algaecide area, compared to the untreated reference area (Table 1, Fig. 2).

Lastly, change in starry stonewort biomass from before to after the second (granular) algaecide treatment significantly differed by treatment type (sampling period × treatment type interaction: $P = 0.039$, $X^2 = 6.472$, df $= 2$), with significantly greater biomass reduction in the untreated reference area compared to the mechanical + algaecide area (Table 1, Fig. 2). Given that the granular algaecide treatment was intended to reduce biomass, this result was unexpected, but should be interpreted with caution given that remaining biomass in the treated areas was very low at this time—and thus our ability to detect changes in biomass concomitantly low. Change in starry stonewort biomass

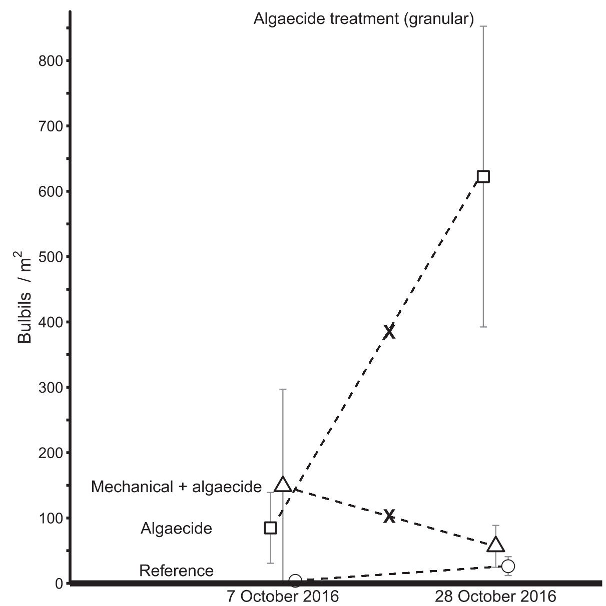

Figure 3. Starry stonewort (*Nitellopsis obtusa*) bulbil density before and after the second (granular) algaecide treatment. Each treatment area is represented with a different symbol. An X indicates that an area received the granular algaecide treatment. Symbols and error bars are means ±1 SE.

did not significantly differ between the algaecide and untreated reference areas (Table 1, Fig. 2).

Bulbil density

For the analysis of bulbil density, there was a significant interaction between sampling period and treatment type ($P = 0.002$, $X^2 = 12.941$, df $= 2$), indicating that change in bulbil density differed among treatments from before to after the granular algaecide treatment. The area treated with algaecide alone had a significantly greater *increase* in bulbil density than the untreated reference and mechanical + algaecide areas ($P = 0.005$ and $P = 0.002$, respectively; Fig. 3). There was no difference in change in bulbil density between the mechanical + algaecide and untreated reference areas ($P = 0.458$; Fig. 3).

Bulbil viability

Bulbils from all sampling areas began sprouting within 7 d (Fig. 4). At the conclusion of the experiment (12 weeks), 85.7% of bulbils had sprouted from the algaecide area, 84.0% from the untreated reference area, and 70.4% from the mechanical + algaecide area. Bulbil sprouting did not significantly differ between the algaecide and untreated reference areas ($P = 0.675$,

Figure 4. Sprouted starry stonewort (*Nitellopsis obtusa*) bulbils from the bulbil viability experiment. Pictured bulbils are ~5 mm in diameter.

deviance = 20.493, df = 2; Fig. 5a). Bulbil sprouting was significantly lower in the mechanical + algaecide area than both the algaecide and untreated reference areas ($P < 0.001$ and $P = 0.011$, respectively; Fig. 5a).

Our metric for starry stonewort recovery potential (viable bulbils/m^2) was 24 × greater in the algaecide area compared to the untreated reference area, 13.4 × greater in the algaecide area compared to the mechanical + algaecide area, and 1.8 × greater in the mechanical + algaecide area compared to the untreated reference area (Fig. 5b).

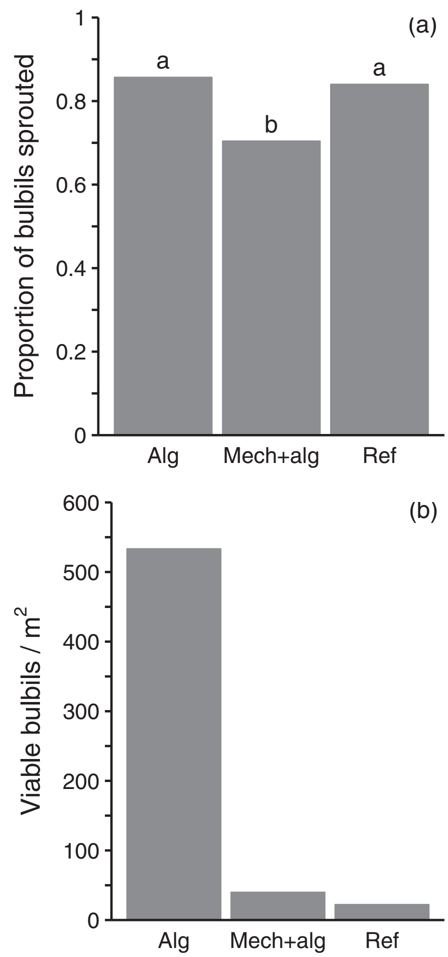

Figure 5. The proportion of starry stonewort (*Nitellopsis obtusa*) bulbils that sprouted from each treatment area (a), and starry stonewort recovery potential via bulbils following each treatment (b). Alg = algaecide treatment area, Mech + alg = mechanical + algaecide treatment area, Ref = untreated reference area. For proportion of bulbils sprouted (a), different letters indicate significant differences between treatment areas determined using a generalized linear model with binomial errors ($P < 0.05$).

Discussion

To our knowledge, this is the first study to report outcomes of *in situ* algaecide and mechanical treatments aimed at controlling starry stonewort and reducing its capacity to regenerate via bulbils. Chelated copper algaecide treatment and mechanical + algaecide treatment substantially reduced starry stonewort biomass. However, treatments did not eliminate the capacity of starry stonewort to regenerate via bulbils. Algaecide treatments alone did not reduce starry stonewort bulbil viability, and regardless of treatment, ≥70% of bulbils sprouted in our experiment. Furthermore, bulbil density substantially and significantly increased in the area treated with algaecide alone, a pattern not observed in an untreated reference area or areas that were also mechanically harvested. There was also no evidence that the second (granular) algaecide treatment further reduced starry stonewort biomass, nor the capacity of starry stonewort to regenerate via bulbils. These findings suggest high potential of starry stonewort to regenerate and persist via bulbils following algaecide treatment. The viability and density of bulbils following algaecide treatment is concerning and has implications for starry stonewort control that necessitate further investigation.

An important caveat of our study is that it was conducted in one lake over a single growing season. Furthermore, treatments were applied as large-scale pilot tests of alternative management options rather than being implemented as part of a designed experiment. As a result, treatments were not randomly assigned to experimental units, treatments were not replicated, and our replicate samples were not entirely independent; thus, treatments could have been confounded by unaccounted-for differences in environmental conditions in each area. These factors can limit the conclusions drawn from BACI analyses like the ones employed on our study (Stewart-Oaten et al. 1986, Underwood 1994). However, our findings reflect the outcomes of actual, hectare-scale, management efforts and provide valuable insights for future management, but should be interpreted in light of their limitations and viewed as a case study that illustrates patterns for further investigation.

Copper compounds have been used to successfully manage algae for decades (Netherland 2014) and provided substantial reductions of starry stonewort biomass in the present study, but failed to reduce the viability of starry stonewort bulbils. Failure of algaecide treatments to reduce bulbil viability could be because chelated copper simply does not destroy bulbils or inhibit sprouting. However, we consider this unlikely given the observed efficacy of algaecide treatments for destroying aboveground biomass and unpublished reports of effective bulbil control by copper compounds in laboratory trials. It is more likely that bulbils were not exposed to sufficient concentrations of chelated copper for sufficient lengths of time due to the physical barrier created by overlying sediment. Sufficient exposure is likely difficult to achieve when targeting bulbils under realistic *in situ* conditions. For example, Kelly et al. (2012) found that chelated copper did not prevent germination of oospores of the Characeae genera *Nitella* and *Chara* that were beneath the substrate. Similar results have been found with other aquatic plant species; for example, contact herbicides had little impact on growth and production of underground propagules (tubers) of hydrilla (*Hydrilla verticillata*; Steward 1969, Joyce et al. 1992). Following treatments with contact and systemic herbicides, underground propagules (turions) of curly-leaf pondweed (*Potamogeton crispus*) also remained viable at levels consistent with untreated lakes (Johnson et al. 2012). Thus, while our study is the first to document this pattern in starry stonewort, our findings are consistent with prior research on control of other submersed macrophytes that produce belowground asexual reproductive structures.

Chelated copper compounds that destroy bulbils or reduce bulbil viability *ex situ* may have limited effect on bulbils *in situ*. Laboratory studies evaluating effects of algaecides on starry stonewort bulbils should account for overlying sediment that protects bulbils in lakes (and realistic algaecide concentrations at or below the sediment) in order to better mimic field conditions. Depth profiles of starry stonewort bulbils beneath the sediment have not (to our knowledge) been reported, but *Chara* bulbils were at highest density 10−12 cm below the sediment surface and found at depths up to 29 cm (van den Berg 1999).

The potential for rapid, post-treatment recovery of starry stonewort by viable bulbils would be exacerbated by increased bulbil density. Hence, our finding that bulbil density significantly and substantially increased following granular algaecide application is concerning. We did not examine the causes of increased bulbil density in our study, but there are several explanations for our findings. For example, our results may be influenced by our ability to sample bulbils using the vertical rake method; this method was developed to sample aboveground biomass and may not accurately or precisely capture variation in bulbil density. Factors such as the amount of aboveground biomass, natural phenology (e.g., senescence and rhizoid formation), and overlaying sediment may affect the number of bulbils collected in a vertical rake sample. Nonetheless, redistribution of resources to rooting and reproductive structures following injury or damage is a well-documented phenomenon in plants (McNaughton 1983, Trumble et al. 1993, Lennartsson et al. 1997, Hawkes and Sullivan 2001, Schwachtje et al. 2006) and a similar process may drive the shifts in bulbil density we observed. For example, compensatory root production following substantial loss of aboveground biomass (as we observed in our treatments) has been shown in the invasive aquatic plant, alligatorweed (*Alternanthera philoxeroides*; Schooler et al. 2007). Moreover, stimulation of growth and reproduction following herbicide application—particularly at low doses—has been shown in numerous plant and alga species (Tiwari et al. 1981, Cedergreen et al. 2007, Cedergreen 2008, Calabrese and Blain 2009, Velini et al. 2010). Low algaecide exposure to starry stonewort rhizoids and bulbils beneath the sediment could have stimulated bulbil production through a direct growth-stimulation response. Alternatively, resources could have been reallocated through internal signaling to belowground biomass and reproduction following injury to aboveground structures. Chemical signaling following plant injury is well documented (Karban and Myers 1989, Walling 2000, Heil and Silva Bueno 2007) and, despite the lack of vasculature, intercellular transport of ions does occur in Characeae through plasmodesmata (Spanswick and Costerton 1967, Allen 1980, Franceschi et al. 1994). In addition, *Chara* spp. can take up and translocate nitrogen and phosphorus between aboveground and belowground structures (Littlefield and Forsberg 1965, Vermeer et al. 2003). Hence, nutrients, chemical compounds, and/or electrical signals stimulating bulbil growth may be able to travel through starry stonewort from exposed aboveground parts of the alga to belowground structures.

It is also possible that reductions in aboveground biomass could have created conditions that stimulated bulbil production from residual biomass. Removal of conspecific (same-species) neighboring plants can increase plant population growth rates by increasing propagule survival and growth (Gustafsson and Ehrlén 2003). Increased access to nutrients or light following aboveground biomass reduction may also have stimulated starry stonewort bulbil production. This effect has been shown in other Characeae; for example, increased light (UV-B radiation) from very low to ambient levels caused a substantial increase in the production of *Chara aspera* bulbils (de Bakker et al. 2001).

Mechanical harvesting was generally associated with better outcomes in terms of potential for reinvasion by bulbils. The mechanical harvest appeared to counter the increase in bulbil density observed in the algaecide-only treatment, as we observed no increase in bulbil density for the area that was mechanically harvested prior to algaecide treatments. These differences may be related to a large, rapid reduction in biomass in the algaecide-only area; prior to the initial algaecide treatment, biomass in the algaecide area was much greater (by >9 kg/m^2) than biomass in the mechanical + algaecide area. This substantially greater biomass was then rapidly reduced to levels similar to those in the mechanical + algaecide area (Table 1, Fig. 2). Such a large, rapid reduction in biomass may have stimulated bulbil production—by chemical signaling, reallocation of resources, and/or increased access to light or nutrients—to a greater degree in the algaecide area than in the mechanical + algaecide area, where comparable biomass had not accumulated. Furthermore, an increase in bulbil production in fall and winter, following senescence and biomass loss (Nichols et al. 1988), appears to be a natural component of starry stonewort phenology (McComas SR, Blue Water Science, Jun 2017, unpubl. data). Hence, sudden substantial losses of biomass associated with algaecide treatment may stimulate early onset of bulbil production. In other words, the large increase in bulbil density we observed in the algaecide area compared to the mechanical + algaecide area may have represented a hastening of an otherwise natural process rather than a net increase in bulbil production. Year-round sampling of starry stonewort biomass and bulbil density is needed to elucidate these patterns and clarify net effects of algaecide treatment on bulbil production.

An initial mechanical harvest to reduce biomass, followed by algaecide treatment of residual biomass, may be a means to reduce starry stonewort without triggering bulbil production. Our findings of lower bulbil density and reduced bulbil viability in the area that was initially mechanically harvested is encouraging for starry stonewort management (though high viability of starry stonewort bulbils remains a concern). Repeated mechanical and algaecide treatments may be a means to exhaust starry stonewort resources and bulbils over time. However, it should also be noted that harvesters can facilitate spread of aquatic invasive plants within water bodies (Anderson 2003, Hussner et al. 2017), and mechanical harvesting can be inefficient for small or low-density infestations. For small-scale starry stonewort infestations, manual hand-removal may be a better option. Continued hand-pulling of small starry stonewort infestations could reduce populations over time while engaging lake associations, volunteers, and other stakeholders in removal efforts.

Our study highlights the challenges associated with starry stonewort control efforts, particularly in large, dense infestations like the one in Lake Koronis. Therefore, measures should be taken to reduce starry stonewort spread in order to avoid dependence on difficult, costly, and resource-intensive management efforts. Where large infestations have established, starry stonewort is likely to persist for the foreseeable future and realistic, sustainable goals (e.g., reducing abundance and minimizing risk of spread) should be pursued.

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Realized niche shift associated with the Eurasian charophyte *Nitellopsis obtusa* becoming invasive in North America

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***Nitellopsis obtusa* (starry stonewort) is a dioecious green alga native to Europe and Asia that has emerged as an aquatic invasive species in North America. *Nitellopsis obtusa* is rare across large portions of its native range, but has spread rapidly in northern-tier lakes in the United States, where it can interfere with recreation and may displace native species. Little is known about the invasion ecology of *N. obtusa*, making it difficult to forecast future expansion. Using ecological niche modeling we investigated environmental variables associated with invasion risk. We used species records, climate data, and remotely sensed environmental variables to characterize the species' multidimensional distribution. We found that *N. obtusa* is exploiting novel ecological niche space in its introduced range, which may help explain its invasiveness. While the fundamental niche of *N. obtusa* may be stable, there appears to have been a shift in its realized niche associated with invasion in North America. Large portions of the United States are predicted to constitute highly suitable habitat for *N. obtusa*. Our results can inform early detection and rapid response efforts targeting *N. obtusa* and provide testable estimates of the physiological tolerances of this species as a baseline for future empirical research.**

Understanding how certain species experience great success outside of their native ranges, often becoming more ecologically dominant than their performance as native species would suggest[1] is a key challenge for invasion biology and has important implications for assessing risk associated with potential invaders. Examples of this phenomenon are numerous: Common reed (*Phragmites australis*) has suffered diebacks in Europe[2], even as Eurasian genotypes have expanded throughout North America[3]. Monterey pine (*Pinus radiata*) has been reduced to five native populations in California, United States (U.S.) and Baja California, Mexico[4], while being highly invasive in Chile, Australia, and New Zealand[5]. House sparrows (*Passer domesticus*) are extraordinarily successful as an introduced species despite declining in their native range[6]. Several mechanisms may drive these changes in fortune, including escape from natural enemies, altered population genetic structure, intra- and inter specific hybridization, novel allelopathic weapons, and unexploited resources[1,7–10]

Regardless of the underlying mechanisms, the success of some invasive species is attributable to their ability to occupy an ecological niche in their introduced range that is broader than or distinct from the niche realized in their native range[11]. It is true that many invasive species occupy niches very similar to those in their native ranges[12], but for others an expanded realized niche leads to greater dominance within communities[1], colonization of new types of habitats[13], or growth under novel climatic conditions[14]. The gap between the realized niche in a species' native range and its potential niche in a new range makes risk assessment more difficult, as even rare species can potentially become dominant under the right confluence of climatic, landscape, and biotic conditions[11,15,16].

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Nitellopsis Obtusa Invasion in North America

A recent example of a largely rare native species becoming an aggressive invasive species is the spread of *Nitellopsis obtusa* (N.A. Desvaux) J. Groves (starry stonewort) in North America. *Nitellopsis obtusa* is a dioecious green alga in the Characeae family that is uncommon across much of its native range in Europe and Asia[17,18] and is classified as a priority conservation species in the United Kingdom[19], near threatened in Switzerland[20], and endangered in Japan[18], though there is evidence of expanded distribution in parts of Europe over the past few decades[21]. It occurs in shallow, fresh to brackish water at depths up to 10 m and can reproduce asexually via fragments and star-shaped structures called bulbils[17]. *Nitellopsis obtusa* was first found in North America in the St. Lawrence River in 1978[22]; it is now widespread in Michigan, increasingly common in New York and, since 2012, has been recorded for the first time in Indiana, Wisconsin, and Minnesota[17,23].

Detection, impacts, and management. *Nitellopsis obtusa* is of increasing concern in the Great Lakes region of North America. It appears to spread readily via human-assisted movement of fragments and bulbils (only males have been found in North America to date, precluding sexual reproduction), with occurrences associated with boat accesses and high-use areas[17]. Where it invades, *N. obtusa* can spread rapidly, grow tall and dense, and form surface mats, interfering with boating and recreation and potentially displacing native plant species[17,24]. Where *N. obtusa* does invade, effective treatment can be difficult to achieve. Manual removal may leave behind fragments and bulbils that can lead to reinvasion[25]. Currently available chemical control methods have been subject to little rigorous testing, and anecdotal reports from herbidice applicators indicate that treatments can result in a "haircut" effect, with upper portions of plants killed but lower portions intact and able to resprout[24].

Challenges detecting *N. obtusa* and treating infestations compound the problem of its invasiveness. Charophytes are a taxonomically complex group and it can be difficult for non-experts to distinguish *N. obtusa* from other closely related, native muskgrasses and stoneworts (*Chara* and *Nitella* spp)[26]. Thus, it is possible that populations that are already established have not yet been detected. For example, when *N. obtusa* was first recorded in a Minnesota lake system in the summer of 2015, it was already present in an area >100 ha[27], suggesting that it may have established years prior to being identified. Sleith *et al.*[17] used a spatially stratified design to search for *N. obtusa* throughout New York State and found 18 previously unknown occurrences in a single field season.

Potential distribution. In light of the invasiveness of *N. obtusa*, uncertainty regarding its full distribution and physiological tolerance, and the limited toolkit available for its control, risk assessment to support prevention efforts is urgently needed. We performed ecological niche modeling to geographically evaluate invasion risk associated with *N. obtusa* and to investigate environmental conditions associated with its spread. Our approach is grounded in Hutchinson's framework that a species' niche comprises the confluence of suitable "scenopoetic" and "binomic" (biotic) factors[28]. In our niche model of *N. obtusa*, we focused on scenopoetic variables, defined as those abiotic environmental variables not consumed by the species and for which there is no competition among species[28,29]. Scenopoetic climatic variables, which operate at large spatial scales, are a robust source of information for characterizing multidimensional environmental space to estimate species' fundamental niches, and have the advantage of being stable even when species' abundances change[30]. Scenopoetic variables also help to define biomes, and are thus key components of species' biogeography[30]. We estimated the niche of *N. obtusa* based on scenopoetic variables associated with its global occurrences. Our goals were to: (1) determine whether *N. obtusa* was exploiting novel ecological niche space in its invaded range, (2) predict its potential for further expansion in North America, (3) identify priority regions for early detection and rapid response efforts targeting *N. obtusa*, and (4) estimate the physiological tolerances of the species as a baseline for future research. Our first three goals were addressed using occurrence records from the native and introduced ranges of *N. obtusa* coupled with climatic variables. We used these data to generate a binary (suitable/unsuitable) niche model of *N. obtusa* as a proxy for the species' fundamental niche. To estimate physiological tolerances (goal 4), we employed the binary ecological niche model and occurrence records as "masks" (i.e., spatial limits) to extract maximum and minimum values of climatic variables, and additional scenopoetic variables extracted from finer-scale, remotely sensed environmental data (Fig. 1).

Results

We identified 2,255 occurrences for *N. obtusa* distributed across France ($n = 1$), Switzerland (1), the United Kingdom (5), Germany (7), Japan (46), Sweden (116), and the Netherlands (1,776), as well as the US (303; Supplementary Material S1). After removing duplicates, 846 unique occurrences were used for modeling the species' native (Eurasia, $n = 575$) and invaded range (USA, $n = 271$; Fig. 2). Climate variables selected for model calibration included annual mean temperature, isothermality, minimum temperature of the coldest month, annual precipitation, precipitation seasonality, and precipitation of driest quarter. These variables were used because they represented the environmental information available throughout the entire study area and are likely to have biological significance for the species (Table 1). Using these climatic variables, we were able to generate a multivariate environmental space within which to estimate the ecological niche of the species for both native and invasive populations (Fig. 3).

We found generally high overlap in environmental conditions available in the native and invaded ranges (Fig. 3). However, there was evidence of some "novel" (non-analogue) environments in the invaded region (Fig. 4). *Nitellopsis obtusa* occurrences in North America were not distributed within the same environmental space occupied in the native range. For example, there was no overlap between native and invaded ranges in terms of the environmental space occupied based on three non-correlated, multivariate environmental axes (Fig. 3). The novel climates in the invaded areas were identified in land and estuarine areas; variables shaping conditions distinct from those found in the native range included isothermality, minimum temperature of the coldest month, precipitation

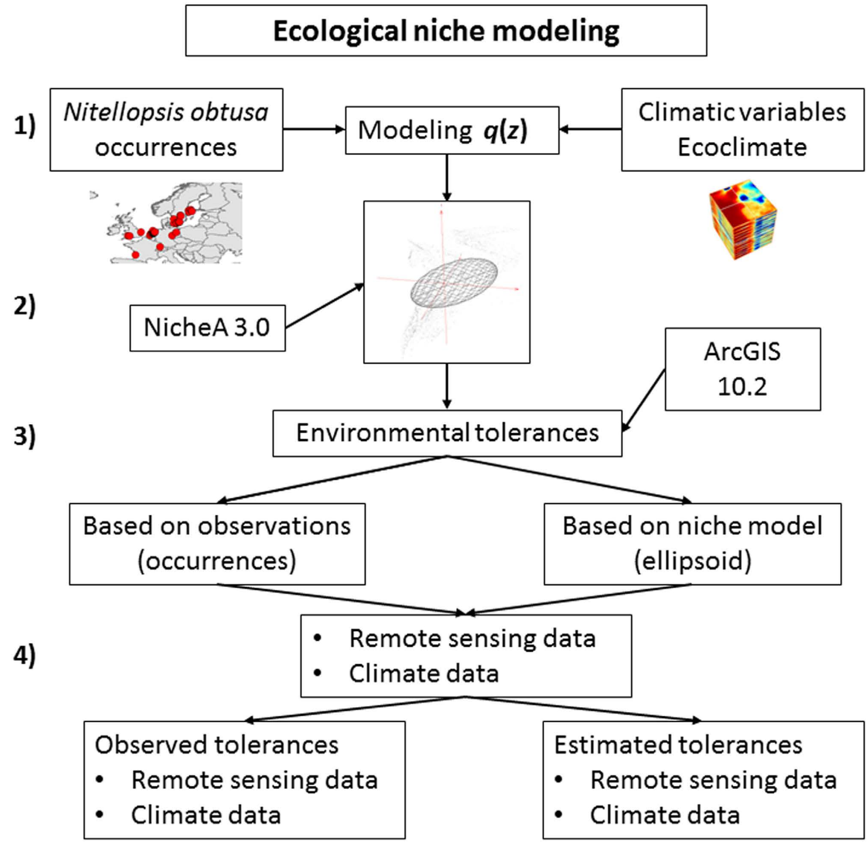

Figure 1. Framework to estimate the species' niche, the potential distribution, and the environmental tolerances. (**1**) *Nitellopsis obtusa* occurrences and scenopoetic variables at coarse scale were collected. (**2**) An ecological niche model based on occurrences and climate data was developed as a proxy of the species fundamental niche. (**3**) Raw occurrences and the niche estimated based on a minimum-volume ellipsoid were used to identify the range of environmental conditions wherein the species can occur based on observations and niche estimation respectively. (**4**) The environmental ranges were estimated using both climate data at coarse spatial resolution and remote sensing data at fine resolution. This figure was generated using ArcGIS 10.2 (ESRI, Redland, CA; www.esri.com) and NicheA 3.0 (Qiao, H. *et al.*[67]. NicheA: Creating Virtual Species and Ecological Niches in Multivariate Environmental Scenarios. Ecography: 10.1111/ecog.01961; http://nichea.sourceforge.net/).

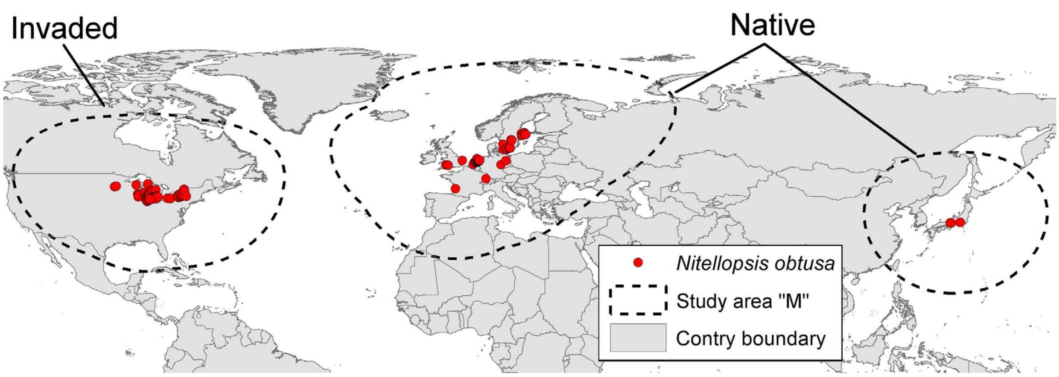

Figure 2. Study area and occurrences used in the ecological niche model of *Nitellopsis obtusa*. The model calibration areas, M, were estimated based on the maximum dispersal potential of the species in its largest geographic native range (Europe). We measured the maximum distance separating occurrences in Europe, resulting in a 2,150 km buffer; this distance (dashed line) was then applied across all available occurrences for the species (red points). This figure was generated using ArcGIS 10.2 (ESRI, Redland, CA; www.esri.com).

seasonality, and precipitation of the driest quarter (Fig. 4). To date, *N. obtusa* has not been recorded from these novel regions available in the invaded region. We were unable to reject the null hypothesis of similarity between the niche estimated in the invaded range and the environments available in the native range ($p > 0.05$; Fig. 5).

Variable/Range	Observed	Modeled
Annual mean temperature, °C (V1)	4.96–14.21	4.37–15.57
Isothermality, % (V3)	15.51–42.02	7.73–44.81
Minimum temperature of coldest month, °C (V6)	−18.68–5.53	−20.11–9.63
Annual precipitation, mm/m² (V12)	635.4–1819.69	396.66–1827.1
Precipitation seasonality, % (V15)	12.65–39.15	9.78–117.43
Precipitation of driest quarter, mm/m² (V17)	88.56–296.04	15.79–422.45

Table 1. Environmental variables used for the final niche model for *Nitellopsis obtusa*. Values based on known occurrences (observed) and those predicted by the ecological niche model (model).

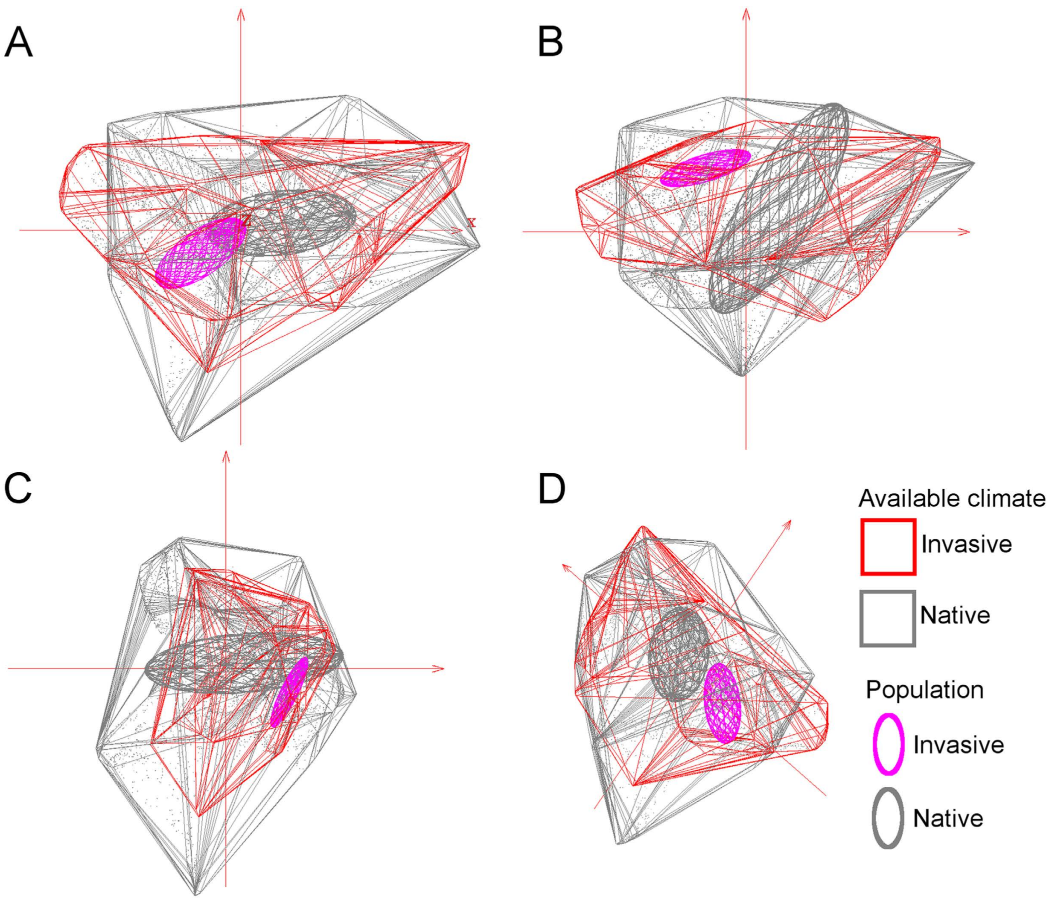

Figure 3. Native and invaded regions in (scenopoetic) environmental dimensions. Environmental conditions available in the native range (gray polyhedron) are compared with conditions available in the invaded range (red polyhedron). Environmental conditions under which *Nitellopsis obtusa* populations are found in the native range (gray ellipsoid) and the invaded range (pink ellipsoid) are also displayed. Visualizations of the: (**A**) first and second principal components (axes), (**B**) first and third principal components, (**C**) second and third principal components, and (**D**) three-dimensional visualization of the first three principal components. This figure was generated using NicheA 3.0 (Qiao, H. *et al.*[67]. NicheA: Creating Virtual Species and Ecological Niches in Multivariate Environmental Scenarios. Ecography: 10.1111/ecog.01961; http://nichea.sourceforge.net/).

Including occurrences from the invaded range expanded estimation of the fundamental niche of *N. obtusa*. The final model pooled native and invasive occurrences to estimate the species' fundamental niche (Fig. 6, gray minimum-volume ellipsoid), with areas of potentially high environmental suitability identified based on distance to the niche centroid (Fig. 7). The ecological niche model predicted suitability in some regions with novel environmental conditions, these were concentrated on the Atlantic coast of the U.S. Highly suitable conditions were identified along the Sea of Japan and Peter the Great Gulf in Asia, throughout much of Eastern Europe, and, within the US, portions of the Eastern Temperate Forest, Great Plains, and Intermountain West ecological regions (Fig. 7). The fundamental niche estimated using scenopoetic climate variables was then used to quantify environmental tolerance ranges based on additional abiotic variables extracted from remotely sensed environmental data.

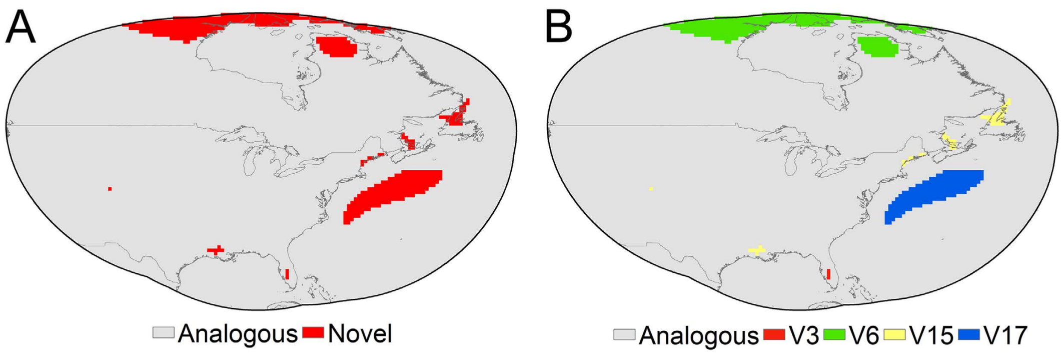

Figure 4. Exploration of novel environments in the invaded range. (**A**) Areas hosting novel environmental conditions not available in the native range (red) and analogous environments (gray) were identified. (**B**) Scenopoetic variables isothermality (V3; red), minimum temperature of coldest month (V6; green), precipitation seasonality (V15; yellow), and precipitation of driest quarter (V17; blue) were responsible of novel environments. This figure was generated in ArcGIS 10.2 (ESRI, Redland, CA; www.esri.com).

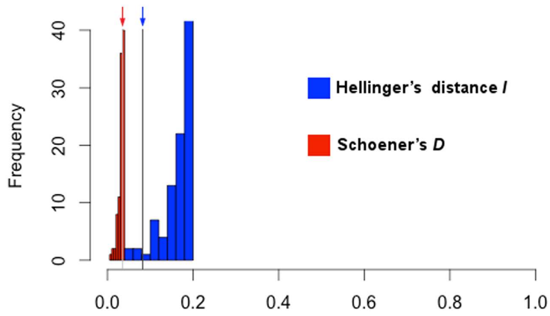

Figure 5. Background similarity test. Environmental conditions available in the native range and environments occupied by the species in the invaded range were compared using Hellinger's distance *I* (blue) and Schoener's D (red). Observed values (arrows) fall within expected values of similarity (null model distributions).

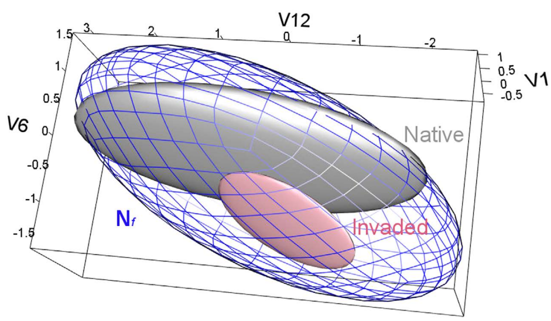

Figure 6. Ecological niche models for *Nitellopsis obtusa*. Models were estimated for the native (gray) and invaded (pink) populations, which resulted in non-overlapping niches. Thus, a final ecological niche model was generated by pooling all available occurrences (N_f; open blue ellipsoid). These models were generated using variables V1, V3, V6, V12, V15 and V17 (see Table 1); this figure depicts environmental space based on three dimensions (V1, V6, and V12). Figure done using R[76] (https://www.r-project.org).

Environmental tolerances of *N. obtusa* inferred from known occurrences were narrower than model predictions. For example, we found that *N. obtusa* occurred in areas with annual mean temperatures of 4.96–14.21 °C, but our niche model predicted that it could occur at a broader temperature range (4.37–15.57 °C; Table 1 and Supplementary Material S2–S6). From our estimation of the environmental ranges based on fine-scale variables, we found that *N. obtusa* reports from coastal areas are characterized by dissolved oxygen of 5.72–8.33 ml/l, however, niche modeling values proposed tolerances as low as 4.95 ml/l, suggesting tolerance to more eutrhophic coastal habitats. Observed values for pH ranged from 8.18–8.24, with a mean of 8.2, similar to the mean value predicted by the model (8.18). Observed salinity ranged between 5.5–31.8 PSS, while the model estimated 3.8–38.4. Other fine-scale variables showed considerable differences between observed and modeled values of

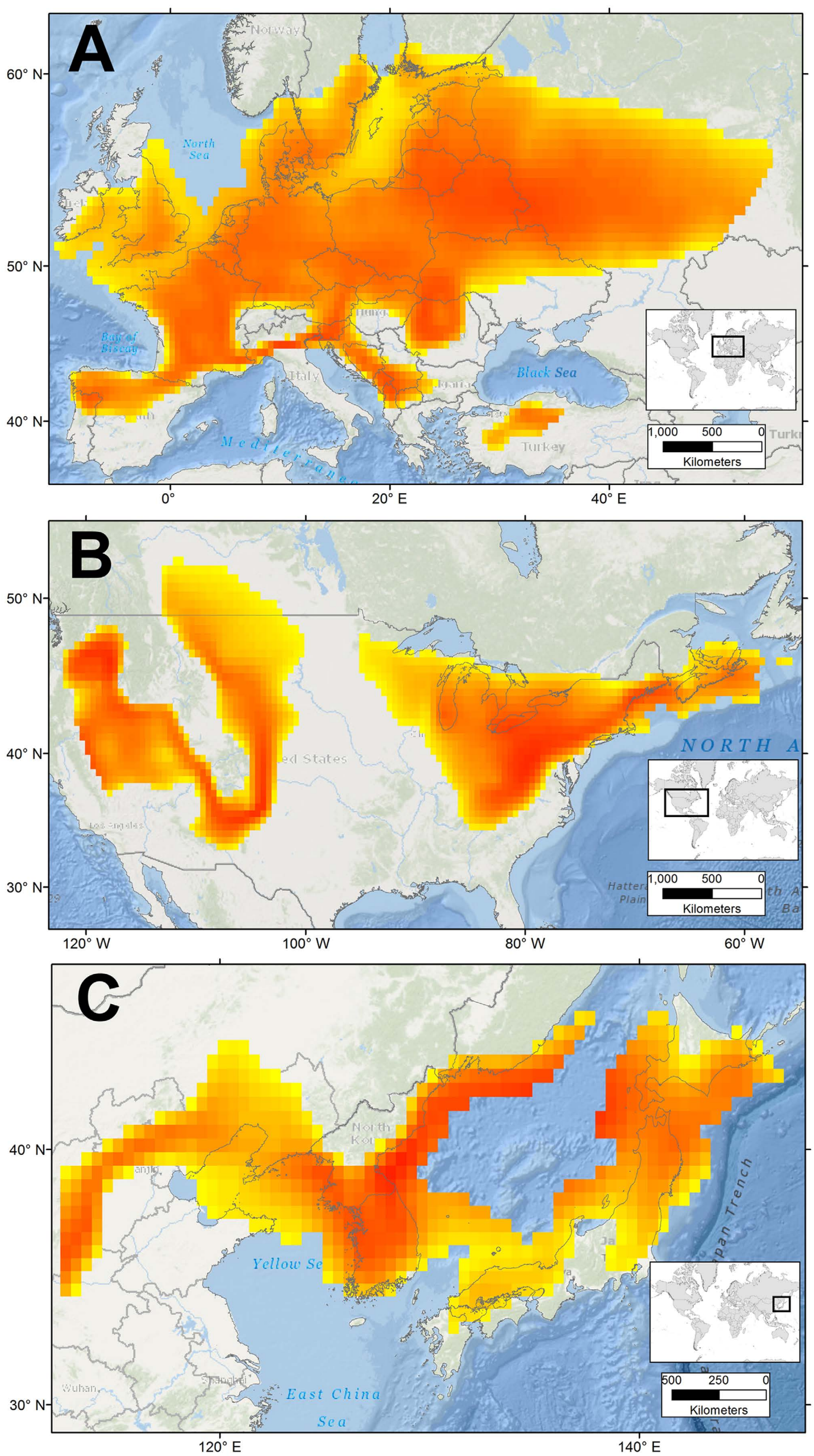

Figure 7. Geographically projected ecological niche model for *Nitellopsis obtusa*. Potential distribution of *N. obtusa* in coastal and inland waters in Europe (**A**), North America (**B**), and Japan (**C**). Shading is based on distance in multidimensional niche space to the niche centroid, and shows areas of relatively high (red) and low (yellow) environmental suitability restricted to coastal areas of 10-m water depth where the species is found. This figure was generated using ArcGIS 10.2 (ESRI, Redland, CA; www.esri.com).

N. obtusa tolerance. For example, mean nitrate was 19.57 and 3.42 µmol/l for the observed and predicted values, respectively (Table 2). Mean land surface temperatures (LST) observed in inland freshwater systems range from

Coastal	Observed Min	Observed Mean	Observed Max	Modeled Min	Modeled Mean	Modeled Max	Units
Calcite concentration	0	0.01	0.04	0	0	0.06	mol/l
Maximum chlorophyll *a*	8.12	40.47	64.57	0.33	5.38	64.57	mg/m^3
Mean chlorophyll *a*	8.12	29.46	47.81	0.22	3.04	53.65	mg/m^3
Minimum chlorophyll *a*	3.35	20.54	32.91	0.09	1.55	41.41	mg/m^3
Chlorophyll *a* range	0	26.2	35.47	0	3.83	53.63	mg/m^3
Cloud cover maximum	0.79	0.84	0.9	0.65	0.88	0.98	%
Cloud cover mean	0.72	0.76	0.78	0.49	0.77	0.92	%
Cloud cover minimum	0.62	0.67	0.7	0.27	0.65	0.84	%
Dissolved oxygen	5.72	6.03	8.33	4.95	6.44	8.4	ml/l
Nitrate	1.06	19.57	27.62	0.48	3.42	46.18	μmol/l
Maximum photosynthetically available radiation	41.4	45.85	48.53	39.46	46.75	59.72	Einstein/m^2/d
Mean photosynthetically available radiation	27.51	30.85	34.22	26.99	31.08	37.34	Einstein/m^2/d
pH	8.18	8.21	8.24	7.54	8.18	8.37	–
Phosphate	0.14	1.03	1.24	0.04	0.35	2.26	μmol/l
Salinity	5.49	28.16	31.81	3.83	30.05	38.42	PSS
Silicate	8.89	14.81	18.57	0.4	6.02	25.23	μmol/l
Maximum SST	17.07	19.91	23.5	13.01	20.12	31.85	°C
Mean SST	6.57	11.41	12.77	5.13	12.01	19.9	°C
Minimum SST	−1.15	2.63	7.28	−1.5	5.63	13.85	°C
SST range	11.37	17.28	24.64	4.87	14.49	28.48	°C
Inland	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Units**
Maximum value of the daytime LST	19	25.45	39	11	31.51	54	°C
Minimum value of the daytime LST	−21	−6.04	3	−30	−11.13	9	°C
Mean value of the daytime LST	8	12.44	23	−5	13.86	33	°C
Maximum value of the nighttime LST	13	18.18	26	4	18.23	27	°C
Mean value of the nighttime LST	1	6.69	13	−8	3.13	16	°C
Minimum value of the nighttime LST	−29	−10.97	0	−38	−16.98	4	°C

Table 2. Description of the environmental range of *Nitellopsis obtusa* based on fine-scale environmental variables. Values based on known occurrences (observed environmental range) and those predicted by the ecological niche model (modeled environmental range).

8–23 °C during daytime and 1–13 °C during nighttime. The niche model again predicted broader tolerances with mean LST of −5–33 and −8–16 °C during daytime and nighttime, respectively (Table 2).

Discussion

Main findings. We developed an ecological niche model for *N. obtusa* to assess its multidimensional climate tolerance and refined this information using biophysical variables derived from satellite imagery to characterize other environmental factors potentially associated with occurrence of this species. We then used the modeled niche of *N. obtusa* to predict which geographic areas likely contain environmental conditions suitable for this species. We found that, in its invaded range, *N. obtusa* is occupying environmental conditions not occupied in its native range (Fig. 3). However, a background similarity test showed that niche differentiation between the native and invaded ranges was not statistically significant.

Environmental tolerances. The environmental range predicted for *N. obtusa* based on scenopoetic variables (Table 1; Supplementary Material S2–S6) provides a baseline for finer-grained observational and experimental investigations of the species' biology. We found that minimum and maximum values of the scenopoetic climatic variables derived from the niche model were broader than the ranges observed based on locality information, suggesting *N. obtusa*'s potential expansion into new environments. For example, with respect to minimum temperature of the coldest month, occurrences correspond to a minimum temperature of −18.68 °C, but the model predicts that *N. obtusa* could occur in areas with temperatures as low as −20.11 °C, 1.4 °C below the minimum temperature observed to date (Table 1). However, this prediction was based on the assumption of a

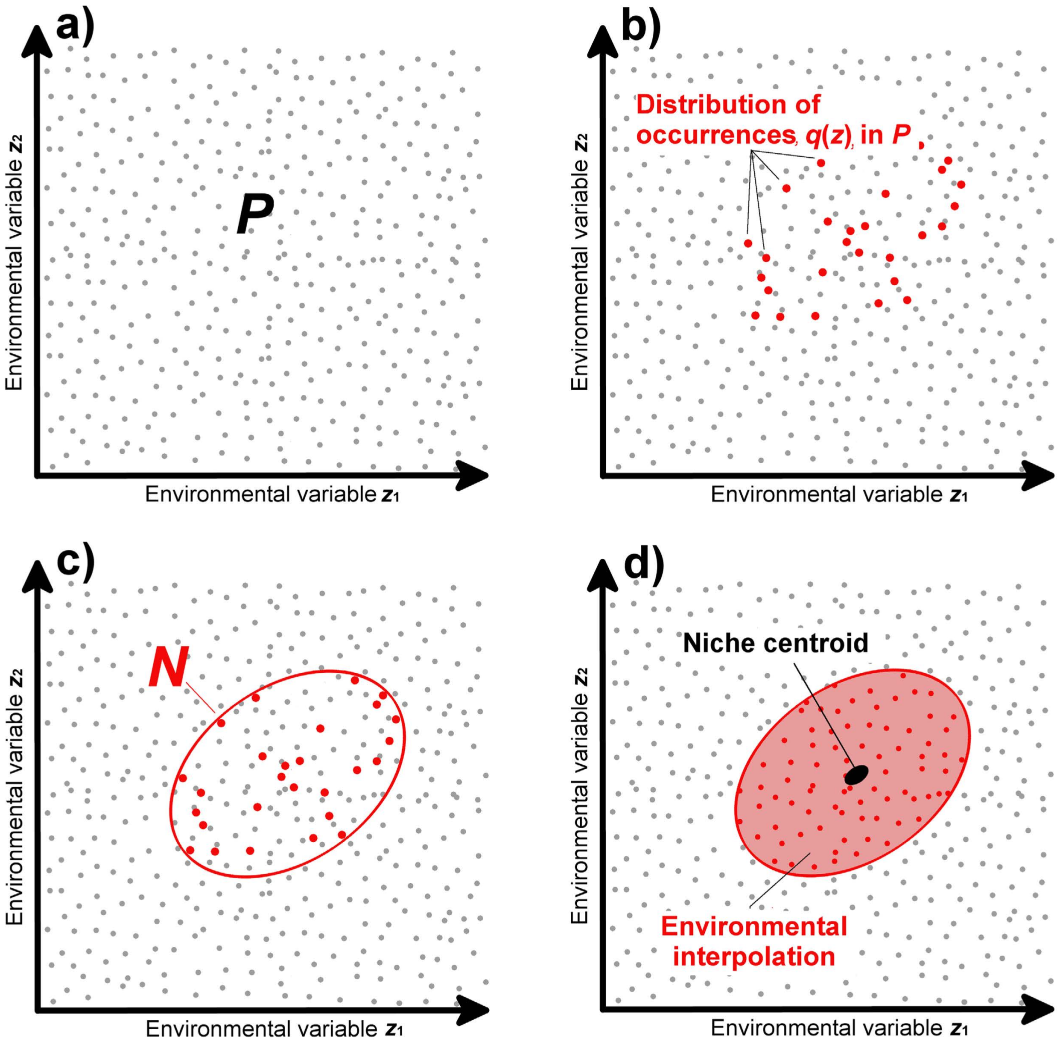

Figure 8. Ecological niche modeling framework. (**a**) Bivariate ($x = 2$) environmental space, *P*, constructed from environmental variables z_1 and z_2 (with values represented as gray points). (**b**). The distribution $q(z)$ of the species' occurrences *k* in the environmental space (red points). (**c**). Occurrences are used to build an existential niche model, *N* (red ellipsoid), as a proxy of the species fundamental niche, N_f (Drake[61]). (**d**). The niche model *N* uses interpolation of environmental values between occurrences (red areas within the ellipsoid). The niche centroid is estimated to identify the core of the niche, which is presumed to represent the most suitable environmental conditions.

Gaussian response to climatic variables, which has been supported by results from other species[31–35], but would need to be tested for *N. obtusa* specifically for robust validation.

Previous attempts to characterize the ecological niches of aquatic invasive species have generally focused on inland climate variables—even when focal species' ranges have extended to coastal or marine environments, which may limit full recognition of potentially invadable environments[36]. Our results suggest that incorporating environmental information from both inland and coastal sources provides a richer representation of the species' environmental niche. Integration of land and marine climate data in previous ecological niche models was limited by lack of availability of climate data layers covering both ecosystems. However, with the release of the Lima-Riberio *et al.*[37] dataset, this is no longer a constraint.

Realized niche shift. The presence of *N. obtusa* in broadly similar environments where it occurs as native or a non-native species suggests that its fundamental niche has been conserved during the invasion process in North America[38,39]. However, *N. obtusa* is using environments that, based on occurrence records we identified, are not occupied in its native range. This could arise due to human movement of *N. obtusa* to a new range, allowing it to overcome biogeographic barriers that constrained its potential distribution as a native species. Alternatively, *N. obtusa* may have expanded into new environments, occupying previously unfillied portions of its fundamental niche, as a result of release from natural enemies that may have limited its native range[30,40]. Occupancy of novel portions of a species' fundamental niche in separate geographic regions is termed a "realized niche shift"[16,41]. A realized niche shift does not suggest evolutionary adaptation of a species to novel environmental conditions, but

Variable	Bioclim	Description	Unit
V1	Bio1	Annual mean temperature	°C
V2	Bio2	Mean diurnal range	°C
V3	Bio3	Isothermality	%
V4	Bio4	Temperature seasonality	%
V5	Bio5	Maximum temperature of the warmest month	°C
V6	Bio6	Minimum temperature of the coldest month	°C
V7	Bio7	Temperature annual range	°C
V8	Bio8	Mean temperature of the wettest quarter	°C
V9	Bio9	Mean temperature of the driest quarter	°C
V10	Bio10	Mean temperature of warmest quarter	°C
V11	Bio11	Mean temperature of coldest quarter	°C
V12	Bio12	Annual precipitation	mm/m^2
V13	Bio13	Precipitation of the wettest month	mm/m^2
V14	Bio14	Precipitation of the driest month	mm/m^2
V15	Bio15	Precipitation seasonality	%
V16	Bio16	Precipitation of the wettest quarter	mm/m^2
V17	Bio17	Precipitation of the driest quarter	mm/m^2
V18	Bio18	Precipitation of warmest quarter	mm/m^2
V19	Bio19	Precipitation of coldest quarter	mm/m^2

Table 3. Bioclimatic variables used in this study.

rather an expansion into portions of the fundamental niche that potentially could have been (but were not) occupied in the native range[16,42]. This finding allowed us to identify uninvaded areas throughout the U.S. that could be at risk of *N. obtusa* invasion in the future (Fig. 7) —areas that could not have been identified based on occurrences from its native range alone.

We found that environments occupied by *N. obtusa* in its invaded range did not fundamentally differ from environments available—though not necessarily occupied—in its native range (Figs 3 and 4). However, lack of environmental overlap between extant native and non-native populations was observed in multivariate environmental space (Fig. 3). Such dissimilarity may be imperceptible in geographic space (Fig. 2), which can limit understanding of invasion dynamics and the potential for future spread. Previous models of biological invasions have invoked evolutionary changes in species' environmental tolerances to explain apparent fundamental niche shifts inferred based on models' failure to predict invaded ranges using native range data (e.g.[14,43,44]). However, failure to accurately forecast invaded ranges may arise from stochastic differences in species' environmental distributions that are not indicative of selection, and thus do not require niche evolution to be overcome[36]. In the present study, models of *N. obtusa* calibrated based on the native range alone would have failed to predict current occurrences of the species in North America due to non-analogous environmental conditions occupied by the species in the invaded range (Fig. 3).

Potential for future expansion. There has been relatively little investigation of the ecology of *N. obtusa*, particularly in its invaded range. Novel environmental conditions exploited by *N. obtusa* in North America provide insight into the process of invasion. The patterns we observed suggest that there are gaps in environmental occupancy for this species in North America, i.e., the potential niche is not filled[42]. Thus, it appears that this species has not reached equilibrium in its ecological distribution. Invasion of new geographic locations and currently uoccupied portions of the fundamental niche are likely to occur as dispersal barriers are overcome by unintentional human movement. The rapid spread and robust growth of *N. obtusa* in the Great Lakes region suggests that environmental conditions within this landscape constitute highly suitable habitat, and our ecological niche model predicts other, as yet uninvaded, hotspots elsewhere in the U.S.

Of the 29 states in the U.S. that contain at least a small area of moderate to high predicted suitability for *N. obtusa*, only 5 have known occurrences to date: Michigan, New York, Wisconsin, Indiana, and Minnesota. This suggests that there is substantial risk of *N. obtusa* expansion in the U.S., with the species perhaps at an early stage of progression toward becoming more widespread and dominant[45,46]. Detailed field sampling to characterize conditions associated with *N. obtusa* populations and controlled experiments assessing the influence of environmental parameters on fitness are needed to empirically explore this species' true environmental tolerance.

Ocean	Units
Calcite concentration	mol/l
Maximum chlorophyll a	mg/m^3
Mean chlorophyll a	mg/m^3
Minimum chlorophyll a	mg/m^3
Chlorophyll a range	mg/m^3
Cloud cover maximum	%
Cloud cover mean	%
Cloud cover minimum	%
Dissolved oxygen	ml/l
Nitrate	μmol/l
Maximum photosynthetically available radiation	Einstein/m^2/day
Mean photosynthetically available radiation	Einstein/m^2/day
pH	–
Phosphate	μmol/l
Salinity	PSS
Silicate	μmol/l
Maximum SST	°C
Mean SST	°C
Minimum SST	°C
SST range	°C
Land	**Units**
Maximum value of daytime LST	°C
Minimum value of daytime LST	°C
Mean value of daytime LST	°C
Maximum value of nighttime LST	°C
Mean value of nighttime LST	°C
Minimum value of nighttime LST	°C

Table 4. Remote sensing environmental variables used in this study.

Prevention of further spread could be supported by early detection and rapid response efforts. Increased awareness of and research on *N. obtusa* in North America will hopefully result in aquatic plant monitoring, early detection, and management professionals being more likely to identify relatively new infestations, when control is more feasible[24]. Our maps suggest areas without known occurrences where surveillance might be especially valuable, particularly in Western and Mid-Atlantic States (Fig. 7).

Finally, one implication of our findings is that climate change could have a large influence on the future distribution of *N. obtusa*[47]. Occurrences in both the native and invaded range are concentrated in northern latitudes (Fig. 2), which are expected to be subject to large changes in temperature and precipitation[48,49]. Our findings indicate that these climate variables are important components of the ecological niche for *N. obtusa*. To refine *N. obtusa* risk assessment, a critical next step is to predict the influence of climate change on future geographic distribution of the species. Such an investigation might, for example, indicate greater risk for expansion in Minnesota and Wisconsin and lower risk in Mid-Atlantic states than we have predicted here.

Methodological advances. Examination of both native and invasive populations in climate space expanded estimation of the niche of *N. obtusa*, enabling us to better approximate this species' fundamental niche. Our results reinforce that niche models for assessing invasiveness should not be calibrated based on populations defined by administrative areas of interest[50], instead models should be calibrated based on species' entire ranges to capture the most complete environmental information available.

In North America, *N. obtusa* has apparently been spreading only by asexual means[17], limiting genetic diversity of populations in the invaded range. Aggressive expansion of *N. obtusa* in the invaded range also contrasts with its rarity and conservation concern in much of its native range. The "niche centroid" hypothesis[51] proposes that species' populations that are nearest to the niche centroid (puatatively optimal environmental conditions) will have the highest population growth[52] and genetic diversity[53]. Evaluating the validity of this prediction for invasive species will inform understanding of the true dimensions of invasive species' niches, increasing fundamental biological understanding and supporting applied efforts to prevent further spread. *Nitellopsis obtusa* populations in the invaded range are occurring in a combination of climatic conditions not occupied in the native range, suggesting that dispersal limitation in the native range may be limiting filling of suitable portions of the niche. If the niche centroid hypothesis applies in the case of *N. obtusa*, populations closer to the niche centroid should have higher growth rates. This prediction requires empirical investigation.

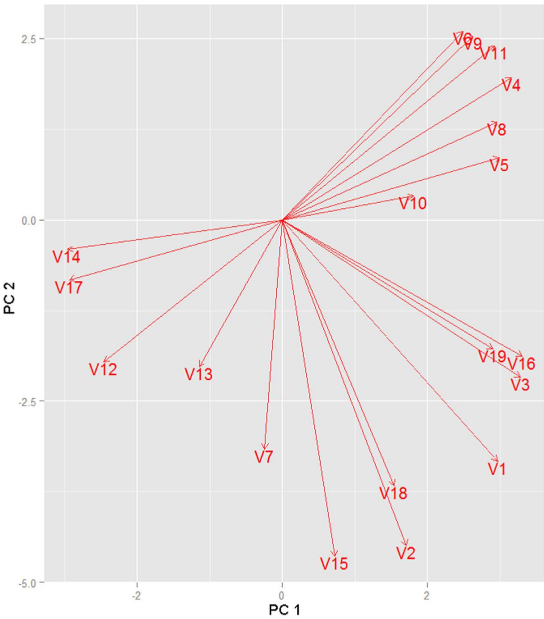

Figure 9. Principal components of the environmental variables used in the modeling process. Components are displayed in two dimensions, component one (PC1) and two (PC2), to show the association among variables. V1 = annual mean temperature; V2 = mean diurnal range; V3 = Isothermality; V4 = temperature seasonality; V5 = max temperature of warmest month; V6 = min temperature of coldest month; V7 = temperature annual range; V8 = mean temperature of wettest quarter; V9 = mean temperature of driest quarter; V10 = mean temperature of warmest quarter; V11 = mean temperature of coldest quarter; V12 = annual precipitation; V13 = precipitation of wettest month; V14 = precipitation of driest month; V15 = precipitation seasonality; V16 = precipitation of wettest quarter; V17 = precipitation of driest quarter; V18 = precipitation of warmest quarter; V19 = precipitation of coldest quarter.

Our model results should be viewed as baseline estimates of tolerance ranges for *N. obtusa*. Mean values of these ranges are approximations of conditions under which survival and growth should be high, i.e., environmental optima[52]. Alternatively, there may be biotic factors mediating *N. obtusa* invasion and population growth at finer scales that were not captured by our analysis. Competitive interactions with other macrophytes, depredation, and even pathogens or negative feedbacks with microbial communities may be more pronounced in the species' native ranges[40,54].

NicheA software added biological realism to our models by allowing us to: i) visualize the species distribution in environmental dimensions, ii) simulate the response of *N. obtusa* to environmental variables, and iii) predict invasion risk based on the niche centroid[52,55,56]. Areas predicted to be at high-risk based on environmental suitability were not clustered geographically, indicating the strong capacity of this approach to identify environmental suitability–relative to correlative methods that tend to interpret higher occurrence densities as necessarily indicating higher suitability, which can lead to spatial autocorrelation and model overfit[57,58]. This study prompted the development and release of new analytical tools: "*Generate Niches from Occurrences*" and "*Export Niche as Continuous Raster*"; these are now available within NicheA software 3.0 to facilitate the application of ecological niche modeling to predicting spread of other aquatic or terrestrial invasive species (http://nichea.sourceforge.net/).

Issues of scale in modeling aquatic invasive species. Scientific literature on modeling the ecological niche of aquatic invasive species is scarce, perhaps because resource managers are often more interested in finer-scale forecasts pertaining to the regions they manage, or becaue waterbody-specific environmental variables are of great importance but can be difficult to obtain[50]. Managers often require fine-scale models explaining potential expansion of aquatic invasive species, even being interested in suitability estimations for specific microhabitats within individual waterbodies, modeling at such scales can be difficult (but see[59]). Species' geographic distributions are the expression of complex interactions among abiotic tolerances, dispersal dynamics, and biotic interactions[60]. We limited our investigation to abiotic factors expected to shape *N. obtusa* current and potential distribution. Such coarser-scale, abiotic analyses for aquatic invasive species are critical for understanding biogeographic patterns of past invasions and for predicting areas at risk in the future[50]. Such analyses are a useful starting point for fine-grained modeling and empirical investigations.

Methods

Ecological niche modeling. We performed ecological niche modeling using an approach proposed by Drake[61] termed "range bagging." This is an ecological niche modeling approach that aims to characterize species' abiotic tolerances in multivariate environmental space from geographic locations of the species. A challenge for niche modeling is reliance on presence-only data, given lack of availability of robust species absence data[30].

No.	Climatic variable	Axis 1	Axis 2	Axis 3	Axis 4
V1	Annual mean temperature	0.268549	−0.30154	−0.04182	−0.06167
V2	Mean diurnal range	0.154837	−0.40671	0.153434	−0.0833
V3	Isothermality	0.296945	−0.19697	−0.16491	−0.0661
V4	Temperature seasonality	0.285549	0.178237	0.228065	0.026596
V5	Maximum temperature of the warmest month	0.27084	0.077764	0.233843	0.266797
V6	Minimum temperature of the coldest month	0.22525	0.235566	0.235308	−0.25582
V7	Temperature annual range	−0.02231	−0.28657	−0.05584	0.519083
V8	Mean temperature of the wettest quarter	0.268126	0.122484	0.232199	0.2296
V9	Mean temperature of the driest quarter	0.2369	0.228993	0.225927	−0.25839
V10	Mean temperature of the warmest quarter	0.16399	0.029729	0.409481	0.267514
V11	Mean temperature of the coldest quarter	0.264259	0.217343	0.02297	−0.1566
V12	Annual precipitation	−0.22286	−0.1774	0.317968	−0.22584
V13	Precipitation of the wettest month	−0.10389	−0.18329	0.143856	−0.44201
V14	Precipitation of the driest month	−0.26818	−0.03707	0.324793	0.041671
V15	Precipitation seasonality	0.065615	−0.42041	0.230196	−0.16373
V16	Precipitation of the wettest quarter	0.298626	−0.16991	−0.19483	−0.05618
V17	Precipitation of the driest quarter	−0.26579	−0.07492	0.334569	−0.03937
V18	Precipitation of the warmest quarter	0.139045	−0.33209	0.183675	0.184543
V19	Precipitation of the coldest quarter	0.262728	−0.161	−0.21275	−0.22105

Table 5. The eigenvector coefficients of a standardized principal component analysis of original climatic variables. Note: The eigenvalues of the first four axes are: axis 1 = 0.4453, axis 2 = 0.2112, axis 3 = 0.1651, and axis 4 = 0.0854 (sum = 90.73% of total variance explained).

Correlative presence-only models are strongly influenced by the study area extent used for model calibration[62]. Ecological niche modeling using range bagging requires presence data from the species of interest and a set of environmental factors defined by the researcher; the method is not considerably influenced by the study area extent in delineating the ecological niche and does not require absence data. Range bagging assumes that niches are convex and simply connected in a multidimensional environmental scenario, providing biological realism to estimations and reducing the effects of sampling bias.

The Drake[61] approach characterizes a species' multidimensional (n-dimensional) environmental space, $\boldsymbol{P}$, using *a priori* selected environmental variables, $\boldsymbol{z}$. The species' range for each environmental variable, $\boldsymbol{q}(\boldsymbol{z})$, is determined based on occurrence records, $\boldsymbol{k}$. Thus, $\boldsymbol{q}(\boldsymbol{z})$ is the environmental distribution of occurrences $\boldsymbol{k}$, within environmental space $\boldsymbol{P}$. We assume that $\boldsymbol{q}(\boldsymbol{z})$ is the set of environments in which the species' population can persist without further immigration being required, i.e., "fundamental niche," $\boldsymbol{N_f}$[30]. Because occurrences may include both imperfect and incomplete sampling, $\boldsymbol{q}(\boldsymbol{z})$ represents an approximation of $\boldsymbol{N}$—the "observable" or "existential" niche (*sensu* Peterson *et al.*[30]). Here we assumed that $\boldsymbol{k} \subseteq \boldsymbol{q}(\boldsymbol{z}) = \boldsymbol{N} \subset \boldsymbol{P}$[61]. We estimated $\boldsymbol{q}(\boldsymbol{z})$ separately for the native range (using native records, $\boldsymbol{k}_{\mathrm{n}}$) and introduced range (using $\boldsymbol{k}_{\mathrm{i}}$), to allow for the possibility that the realized niche would differ by range (Fig. 8).

Ecological theory proposes that niches have a Gaussian nature derived from species' physiological tolerances to multivariate environmental conditions[31,35,61,63,64]. A species' niche constitutes an n-dimensional "hypervolume" within a high-dimensional ecological space, i.e., $\boldsymbol{z} > 3$ [ref. 65]. Along each dimension, species are likely to show a bell-shaped fitness response (normal distribution with the left and right tails and peak representing suboptimal and optimal conditions, respectively[31,35,61,63,64]). Given these patterns, an ellipsoid shape provides a simple and reasonable proxy of a species' $\boldsymbol{Nf}$[61,66]. This approach adds biological realism to estimates of species' environmental tolerances and allows for interpolation along environments gradients, mitigating model overfit.

To perform this estimation using multiple environmental variables, we developed a novel tool "*Generate N(s) from occurrences*" which is now freely available in version 3.0 of the software NicheA[67]. NicheA generates a binary ecological niche model (suitable/unsuitable) via an environmental envelop algorithm that identifies space within a multi-dimensional environmental hypervolume occupied by occurrences of a given species. NicheA then generates a convex-polyhedron around all $\boldsymbol{k}$, allowing posterior estimation of minimum-volume ellipsoids circumscribing $\boldsymbol{q}(\boldsymbol{z})$, as a proxy of the species' niche. NicheA involves mapping occurrences into environmental space, such that occurrences that are geographically distinct may still share high environmental similarity.

Detail on the use of NicheA to generate ecological niches from species occurrences has been published elsewhere[66], detailed description of this process can be found at http://nichea.sourceforge.net/function_create_g4.html. The environmental scenario to estimate the species' niche was constructed based on scenopoetic (climatic) variables. We managed the ecological niche model as a climate envelope of ellipsoidal form. This provided a binary map of suitable (inside the ellipsoid) and unsuitable (outside the ellipsoid) climatic conditions.

This model was then projected to the geographic space as a binary species distribution model. This binary model was then used as a mask (i.e., geographic delimitation of the niche) to extract the environmental values from remote sensing data (Fig. 1).

We developed models for the native and invaded ranges and a final binary model pooling occurrences from both ranges. In the binary model, we quantified the distance to the niche centroid by dividing the minimum-volume ellipsoid by 100 units from the Euclidean distance of the ellipsoid centroid to its edge—where the ellipsoid centroid is zero and areas furthest from the ellipsoid centroid are 100—yielding an index characterizing the range of niche suitability[66]. We considered areas closest to the niche centroid to be most suitable for the species' population growth, abundance, and genetic diversity, based on prior empirical investigations of these relationships[52,53,55,68]. To perform this analysis, we developed the tool "*Export continuous ENM*," which is now available in NicheA 3.0.

Occurrences. Spatially referenced occurrence data were collected from herbarium databases accessed through the Global Biodiversity Information Facility[69] and the Global Invasive Species Information Network[70], using the keywords: "*Nitellopsis obtusa*", "*Nitellopsis obtusa* var. *ulvoides*," and "*Chara obtusa*". Additional occurrences for the United States were collected from published sources[17,23,24,71]. Geographic coordinates (latitude and longitude in decimal degrees) were compared with reported localities to identify and remove inaccurate records, final coordinates were then revisited and duplicate records removed.

Environmental variables. Given the breadth of *N. obtusa* occurrences, i.e., that it is found in inland to coastal and freshwater to brackish habitats, we used bioclimatic environmental variables capturing patterns for both land and coastal ecosystems. Bioclimatic variables are a robust representation of scenopoetic variables[28]. We began with 19 climate variables that reflect long-term values of temperature and precipitation at ~50 km^2 spatial resolution from the Ecoclimate repository[37] available at http://www.ecoclimate.org/ (Tables 3). We evaluated collinearity among these variables via principal component analysis using the software NicheA 3.0 [ref. 66]. Collinearity between pairs of variables was examined using bi-dimensional vector plots. Where collinearity was found to be high, the variables comprising greater information content, i.e., covering a longer gradient, and with clearer biological bases, were retained and the other variables excluded (Fig. 9). This resulted in six climate variables being used in the final model (Table 1).

We performed hierarchical post-processing to determine species' distribution in relation to other fine-scale environmental variables (Fig. 1). Briefly, the niche model developed using scenopoetic variables (i.e., climate) was employed to estimate *N. obtusa*'s niche. The resulting binary model was then used to extract values from all the climatic variables and also from remotely sensed environmental variables at ~9-km spatial resolution for coastal areas[72] and at ~1-km resolution for inland regions[73] (Table 4). Finally, we also used *N. obtusa* occurrences to extract the environmental values that it apparently tolerates under field conditions. Environmental values collected by occurrences were termed the "observed" environmental range and those derived from spatial masking of the binary ecological niche model were defined as the "modeled" environmental range (Tables 1 and 2). Predictions were constrained to areas $<$100 km off the coast to include brackish, coastal habitats up to 10 m water depth[17,74]. For niche model estimation, we developed the tool "*Occurrence statistics*," which is now available in NicheA. Data management and analyses were performed using ArcGIS 10.2 [ref. 75], R 3.2.1 [ref. 76], and NicheA 3.0 [ref. 66].

Study area. The extent of the geographic area considered influences ecological niche model outputs[62]; therefore, study area estimation should be based on the natural dispersal capacity of the species of interest[30]. We estimated dispersal distance using native populations in Europe, which are surrounded by biogeographic barriers (e.g., the North Atlantic Ocean and Tibetan Plateau) that separate them from other regions, including disjoint populations in Japan. We measured maximum distance separating occurrences in Europe as an indicator of intrinsic dispersal potential. This distance (2,150 km) was then used to generate a buffer around all occurrences. The resulting polygon constituting our study area was used to calibrated ecological niche models (**M** *sensu* Soberón & Peterson[60]; Fig. 2).

Invasion process. The multivariate environmental distribution of *N. obtusa* was explored using the first three orthogonal principal components (axes) of a principal components analysis of the bioclimatic variables (Table 5). Populations and available environments in the native and invaded ranges were displayed using the software NicheA 3.0 [ref. 66]. Additionally, to compare native and invaded environments for the original scenopoetic variables, we used the multivariate statistical tool ExDet[77]. Finally, we tested a one-way niche similarity using the Schoener's *D* and Hellinger's distance *I* metrics for background similarity testing. These analyses were performed using ENMTools 1.4.4 [ref. 78]. These similarity tests evaluate whether the invasive niche is more similar to the native niche than expected by chance[79].

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Author Contributions
L.E.E. conceived and designed the study, performed the analyses and wrote the paper; N.B.D.P. and D.J.L. provided guidance on selecting data, participated in technical discussions, and co-wrote the paper; H.Q. assisted in performing statistical analyses and co-wrote the paper; C.K.W. assisted in data collection and co-wrote the paper.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

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M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending December 31, 2018

SUBPROJECT TITLE: MAISRC Subproject 9.2: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol.
SUBPROJECT MANAGER: Dr. Michael McCartney
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WEBSITE: www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $380,318
AMOUNT SPENT: $380,318
AMOUNT REMAINING: $0

Overall Subproject Outcome and Results
Since arriving in Duluth Harbor in 1989, zebra mussels have infested more than 150 inland lakes and 17 rivers and streams in MN, with rising ecologic and economic costs. Efforts to block new invasions must be focused strategically on major sources of spread. To help achieve this, we used direct, forensic-like analyses to genetically identify waters from which mussels were carried to infest MN lakes. Using our new genome sequences and methods, we genetically classified mussels from more than 70 water bodies, with more than 6,000 DNA markers per mussel (compared to 9 markers/mussel in Subproject 9.1) – providing significantly increased clarity in the analysis. We found that lakes in the Detroit Lakes, Brainerd and Alexandria regions form large, unique genetic clusters found nowhere else. Additionally, mussels from the Mississippi and St. Croix Rivers, Lake Superior, and Lake Minnetonka (4 highly-likely source waters) are distinguishable from the clustered invasions with 6,000 genomic markers, but with our previous analysis of 9 markers, they were not. More research is needed across a larger, more regional landscape to determine the original sources of zebra mussels into Minnesota, but results reinforce the management message that prevention can work – there is no genetic information to support the hypothesis of a "super spreader" lake. Early and high profile infestations of zebra mussels appear to have been contained (e.g. Lake Millle Lacs). However, vectors that are moving mussels locally within lake-rich regions, need to be identified and blocked.

For the first time, we sequenced the entire zebra mussel genome, using state of the art technology that allowed mapping of genes to chromosomes with great confidence. We sequenced and measured expression of genes in tissues that control shell formation, byssal thread attachment, and survival in high temperatures—each are strong candidates for targeted gene modification. The results include a publicly accessible genome: a powerful tool for invasion biology and biocontrol researchers in Minnesota and worldwide.

Subproject Results Use and Dissemination
The results from this project were regularly communicated in presentations to public and professional audiences. McCartney delivered a total of 14 public presentations on research activities and outcomes at non-scientific meetings and events, and authored or co-authored a total of nine presentations on results of this work at professional conferences, meetings, and invited seminars, including talks at the University of MN Duluth, University of Montana Flathead Lake Biological Station, Montana Fish Wildlife and Parks, and the University of Iowa. As intended in the dissemination plan, outreach was accomplished at local, state and national levels with public talks in Douglas, Hubbard, Itasca, Meeker, Otter Tail, and Stearns Counties in MN, two in Wisconsin, two

in Montana and one in Iowa. Media attention on this project was high and resulted in three print news items, including two front-page feature articles in the Minneapolis Star Tribune. A highlight was two podcasts by Montana Public Radio in which both the population genomics of spread and the genome sequencing projects were covered in detail. Our research was regularly communicated in newsletter articles posted on the MAISRC website. Information about the zebra mussel genome project in the form of a white paper, written originally for a professional audience of scientists and managers in multiple disciplines (Activity 3), but accessible to members of the public with some background in AIS[1]. Two publications are in process (titles below)—one in revision[2] and the other to be submitted soon. Two other manuscripts are in preparation, one on invasion genomics (Activity 1), and the other reporting on sequencing and analysis of the zebra mussel genome (Activities 2 and 3). All Next Generation Sequence data from Activities 1 and 2 will be publicly available in the MAISRC Data Repository at the University of Minnesota or the National Center for Biotechnology Information database.

[1]McCartney, M.A., Mallez, S., Gohl, D. and K. Beckman (2018) The zebra mussel genome project: developing a new resource for invasion biology and biocontrol research. A white paper available from the author.

[2]McCartney, M.A., Mallez, S., Gohl, D. and K. Beckman (in revision) Genome projects in invasion and conservation genetics research programs. *Conservation Genetics*

Mallez, S. and McCartney, M.A. (in prep) Moving zebra mussels into the 'omics' era: SNPs from NGS-based genotyping outperform microsatellites in discerning invasion sources. *Ecology and Evolution*

Title: The zebra mussel genome project: developing a new resource for invasion biology and biocontrol research

Running header: Zebra mussel genome project

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ABSTRACT

Rapidly falling costs and advances in sequencing and informatics have made genome sequencing projects far more accessible to researchers in all of the life sciences, including invasion biology. A complete genome is now the most efficient way to identify and characterize genes controlling traits that contribute to invasiveness. At the genomic level, moreover, tremendous power is available to investigate fundamental questions in invasion science (e.g. the relative roles of pre-adaptations vs. post-colonization adaptive evolution in invasion success), and genomic analysis provides new options for development of control technologies. Yet relatively few invasive species genomes have been sequenced, and even fewer of these genomes have been put to use to study invasiveness. In this perspective, we describe an ongoing effort to sequence the genome of the zebra mussel and how this resource might aid in the development of future biocontrol strategies. We invite dreissenid biologists and others to join us in annotating and analyzing this genome, so that its full potential in understanding and controlling this highly destructive animal can be realized.

Introduction

A sequenced genome will soon become a routine part of any research program in biology. Costs drop almost monthly, and data collection and analysis technology development is moving so fast that projects often take advantage of new inventions while underway. One spectacular example (early 2018) is the Mexican axolotyl, an unprecedented long-read sequencing effort that required the creation of a new algorithm just to assemble its 32 gigabase genome, which is 10 times the length of *Homo sapiens* (Nowoshilow et al. 2018). Most of the life sciences can now benefit from the power of genomics and the new questions it can help to ask and answer. Invasion biology is no different. In this report, we briefly review contributions of genomics to the discipline to date, and describe our ongoing effort to sequence the zebra mussel genome.

Native to a small region of southern Russia and the Ukraine (Stepien et al. 2014), zebra mussels (*Dreissena polymorpha* Pallas 1771) have spread throughout European (Karatayev et al. 1997; Karatayev et al. 2003) and North American (Benson 2014) fresh waters to become one of the world's most prevalent and damaging aquatic invasive species (Karatayev et al. 2007). Fouling of water intake pipes costs the power generation industry over $3 billion USD from 1993-1999 in the Laurentian Great Lakes region alone (O'Neill 2008), where dreissenids are a large and complex economic burden to hydropower, recreation and tourism industries and lakefront property owners (Bossenbroek et al. 2009; Limburg et al. 2010). Ecological damage, the extent of which is just beginning to be understood, arises from the tendency for dense infestations to smother and outcompete native benthic species, and remove huge volumes of planktonic organisms from lakes and rivers. Among the noteworthy impacts are widespread population declines and local extinctions of native freshwater mussels and other invertebrates, damage to fish populations in some cases (Karatayev et al. 1997; Lucy et al. 2014; McNickle et al. 2006; Raikow 2004; Strayer et al. 2004; Ward and Ricciardi 2014), and dramatic restructuring of aquatic food webs (Bootsma and Liao 2014; Higgins and Vander Zanden 2010; Mayer et al. 2014). The congener *D. rostriformis "bugensis"* (or *D. bugensis*: the quagga mussel), while still nowhere near as widespread as the zebra mussel in North American lakes, has ecologically replaced zebra mussels in much of the Laurentian Great Lakes proper and in parts of Europe, and may lead to even greater ecological damage in those systems (Karatayev et al. 2011; Matthews et al. 2014; Nalepa and Schloesser 2014).

The ongoing European and North American invasions spurred an explosion in research effort on *Dreissena*—particularly focused on physiology, autecology, and ecosystem impacts (see (Schloesser and Schmuckal 2012) for a bibliography from 1989 - 2011). Aside from molecular systematic and population genetic studies (Brown and Stepien 2010; Gelembiuk et al. 2006; Mallez and McCartney 2018; May et al. 2006; Stepien et al. 2014), comparatively little genetic work has been accomplished, with transcriptomes from a few tissues (Soroka et al. 2018; Xu and Faisal 2010) the only genomic resources. With the sequence of the zebra mussel genome, we will provide a powerful new resource. We hope to bring together dreissenid mussel researchers and others who can analyze it in appropriate detail, and apply it to better understand and cope with this fascinating but highly destructive animal. Therefore, the first goal of this paper is to advertise the project and invite collaboration.

Our other goal is to consider more broadly the potential contributions of genomics to invasion biology. Three years ago, Rius et al. (2015) reviewed applications of Next-Generation Sequencing technologies to the study of biological invasions, and it is our intent to update their valuable review. In just 3 years, a sequenced reference genome has become an accessible goal or a resource that is an already available for the study of high-priority invasive species. In this paper, we describe some applications of genome projects to broader questions in invasion biology and towards the development of control technologies; some specific to dreissenids.

Genomics in invasion biology

To illustrate the availability of genomic resources to invasion biologists, we searched for assembled genomes from the 100 "world's worst" alien invasive species according to IUCN (Lowe et al. 2000) on Genbank's Genome resource (https://www.ncbi.nlm.nih.gov/genome/). Twenty-eight of these species have assembled genomes available of varying degrees of quality (Table 1)—a sizable resource for invasion biologists. Of course, there are many reasons for sequencing a genome, and several of these 28 projects were launched because of economic value or use as model species [e.g. *Oncorhynchus mykiss* (rainbow trout), *Sus scrofa* (pig), *Capra hircus* (goat), *Mus musculus* (mouse)]. Moreover, for only 8 of these species did we find that invasiveness was a topic of discussion in publications announcing the genome sequence. This is similar to what Rius et al. (2015) noted—I.e. that invasion biology *per se* has driven interest in genome projects projects in only a minority of cases.

Genomic studies of evolution of invasiveness

So then what does a complete genome provide for invasion biology research? For one, of great ongoing interest is whether and how invasions are facilitated by adaptive evolution (Cristescu

2015; Lee 2002; Sax et al. 2007). Genomic analysis provides unequalled power for identifying "invasiveness" genes and for characterizing their mode of evolution.

One good example is the Southeast Asian fruit fly *Drosophila suzukii*, which is rapidly expanding in Europe and North America since arriving about 2008 on these continents (Asplen et al. 2015). Unlike other (genetically more well-characterized) *Drosophila, D. suzukii* shows the unusual behaviors of egg laying and larval feeding on ripening rather than fermenting fruit, and as a consequence has become a damaging pest of soft fruits (e.g. blueberries, blackberries, strawberries). As part of research to develop integrated pest management, nuclear genomes, mitogenomes, and transriptomes were recently sequenced and analyzed (Ometto et al. 2013). To examine adaptive molecular changes associated with the ecological shift to ripening fruit, Ramasamy et al. (2016) analyzed the repertoire of 131 genes involved in olfaction throughout the genus—those encoding odorant receptors and other receptor proteins expressed in antennae, and the odorant binding proteins. They found several instances of gene loss, duplication and positive selection within these gene families along the *D. suzukii* lineage—candidate adaptations that facilitated the switch in larval feeding and egg laying behaviors and promoted the success of this host plant shift. This study could not have been accomplished without genomic resources.

The Asian longhorned beetle (*Anoplophora glabripenniss)* causes damage to > 100 tree species worldwide, and belongs to the beetle family containing the most species capable of feeding on woody plants. Its genome sequence (McKenna et al. 2016) included a large repertoire of enzymes that can digest wood, including several acquired through horizontal gene transfer from bacteria and fungi. The medfly *(Ceratitis capitata)* is able to locate and feed on a diversity of host plants, and its genome (Papanicolaou et al. 2016) shows “expansion” (by gene duplication) of chemosensory and visual genes, and others that encode detoxification of plant secondary compounds and synthetic pesticides. Similarly, expansions of gene families encoding immunity, diapause, and insecticide resistance are among the evolutionary changes within the Tiger mosquito (*Aedes ablopictus*) genome that may have promoted its range expansion throughout the world since the 1960’s (Chen et al. 2015).

In each of these cases, the extent of genomic changes involved (gene family expansions, changes in gene order and the like) suggests they arose prior to invasion. The issue of whether adaptations that favor invasiveness are pre-adaptive or whether they evolve rapidly, during and after establishment is of great academic and applied interest (Lee 2002; Ricciardi et al. 2017). Consider invasive plants, in which genomes of weeds have been shown to be smaller than genomes of non-weedy plants (Kuester et al. 2014). Shorter generation times, smaller seeds, and higher growth rates are associated with weediness and smaller genome size, but it is not clear whether small genomes promoted the evolution of weedy traits, or whether genome size reduction was selected for, post-invasion (Kuester et al. 2014). In each of the cases described above, comparative genomic analysis will allow future researchers to mine these genomes to learn much more about the rate and mode of the evolution of key invasiveness traits.

Genomics to study the invasiveness of dreissenids

It is clear that changes in transportation networks (e.g. canal building, opening of shipping channels, ballast water discharge) were the events that initiated primary invasions of European and North American waters (Karatayev et al. 2007; Pagnucco et al. 2015). Several biological characters, however, are responsible for the rate of spread of zebra and quagga mussels across both continents, while other traits have limited the range of suitable habitats. Genomics offers a path to understanding these traits at the genetic level and in the future, this understanding may provide the tools needed to develop control strategies.

The fibers that zebra and quagga mussels use to anchor themselves to hard surfaces are known as byssal threads. These are key innovations (unique in freshwaters) that allow dreissenids to attach to virtually any hard surface underwater (rocks, plants, woody debris, other mussels) and to boat hulls, plants entangled on boats and trailers, docks, boat lifts and other recreational equipment—allowing rapid rates of spread between water bodies (Collas et al. 2018; De Ventura et al. 2016; Johnson et al. 2001).

Byssal threads are complex extracellular fibers secreted by the bivalve foot, and their underwater adhesion properties and role in biofouling have motivated detailed study, with the marine blue mussels *Mytilus* being most well-characterized (Brazee, Carrington 2006; Lee et al. 2011; Peyer et al. 2009). Byssal threads in *Dreissena polymorpha* differ from those of *Mytilus* in fundamental ways, reflecting their deep convergent evolution in two different subclasses (Heterodonta and Pteriomorpha). First, the regions of the byssus—(a) the thread proximal and (b) distal to the foot, and (c) the plaques (structures that cement the thread to surfaces)—differ from each other in protein composition in *Mytilus* but not in zebra mussels, where each region shows a similar modified protein composition (Waite et al. 2005). Second, the rare amino acid 3,4-diphenylhydroxydoamine (DOPA) is an important modifier of proteins in the plaques and cuticle of *Mytilus* fibers, where it confers mechanical and adhesive properties; in zebra mussels

DOPA is present but in much lower quantities (Rzepecki, Waite 1993). Third, and unexpectedly, zebra mussel fibers (given their environment of less hydrodynamic stress), are stiffer and stronger than those of marine species (Brazee, Carrington 2006).

Quagga mussels are now numerically dominant in the Lower Great Lakes and have invaded a number of reservoirs in the Colorado River system in the southwest US (Benson 2014). While they dominate nearby lake bottom in eastern Lake Erie and western Lake Ontario, zebra mussels outnumber them on boats that have remained in the water for extended periods in harbors (Karatayev et al. 2013). This suggest that poorer attachment abilities may help explain why quaggas have invaded so many fewer inland water bodies in North America than have zebra mussels. Notably, quaggas build lower attachment-strength fibers than zebra mussels, and anchor them more slowly in flow (Peyer et al. 2009).

Expression of genes associated with byssogenesis has been studied in zebra mussels (Xu, Faisal 2010) but a majority of mRNAs that are either up or down-regulated during the synthesis of the byssus could not be identified. Comparative analysis of zebra and quagga mussels would provide testable hypothesis about genetic differences between the two species in the control of fiber synthesis and attachment. In both zebra and quagga mussels, we are using RNA sequencing (RNA-Seq) of transcripts from the foot (the byssus-secreting structure) following experimental induction of byssogenesis (Xu, Faisal 2010) to launch these comparative studies. A complete *D. polymorpha* genome and further annotation of genes expressed in the foot would benefit from ongoing *Mytilus* transcriptomic and proteomic analyses, which have discovered byssal thread foot proteins that were not found earlier in the fibers themselves (DeMartini et al. 2017; Qin et al. 2016). Recent work on proteins in the fibers of quagga mussels (Rees et al.

2016) and our transcriptomes will facilitate comparisons to zebra mussels.

Dreissena thermal biology has received some scrutiny by physiologists, and broad thermal tolerance and ability to adjust it to local conditions have clearly played a role in invasion success. Zebra mussels have higher lethal temperature limits, and they spawn at higher water temperatures in North America than in Europe (McMahon 1996, Nichols 1996). Populations in the Mississippi River provide a good illustration of their breadth of temperature tolerance. In the Lower Mississippi River zebra mussels are found south to Louisiana, where, without cooler water refuges within the river, they persist near their lethal limit of 29-30°C for 3 months during the summer, and for 3 months in the winter the river is at 5-10°C (Allen et al. 1999). In contrast, zebra mussels in the Upper Mississippi River encounter water temperatures > 25°C for just 1 month of the year, and < 2°C for about 3 months (data from USGS gauge from St. Paul, MN). Seasonal scheduling of growth and reproductive effort appears to be responsible for at least some of the adaptation/acclimation to conditions in the lower river, as populations in Louisiana shift their shell and tissue growth to the early spring and stop growing in summer (Allen et al. 1999) while more northerly populations grow tissue and spawn in summer months (e. g. Borcherding 1991; Claxton, Mackie 1998).

A properly annotated genome sequence could accelerate research on thermal adaptation in dreissenids. There is a vast literature on heat-inducible (e.g. “heat shock”) genes and proteins; in fact, marine bivalves and other intertidal invertebrates have been favored subjects (reviewed in Feder, Hofmann 1999). More recently, RNA-sequencing of transcriptomes in heat stressed animals has been accomplished in several invertebrate animals, including mollusks (Porcelli et al. 2015). The freshwater mussel *Villosa lienosa* was the subject of a small-scale study (5 heat-stressed animals, 5 unexposed), using RNA-Seq. The authors identified a diversity of expressed genes associated with heat stress, including each of the major components of a classic "heat shock" response pathway, and the endoplasmic reticulum protein unfolded protein response (UPR^{ER}), including molecular chaperones, antioxidants, immune factors, cytoskeletal elements and mediators of apoptosis (programmed cell death; Wang et al. 2012) Extensive transcriptome sequencing of stress genes in the Pacific oyster genome project (Zhang et al. 2012) revealed most of the same genes and a few others in temperature stress trials. It is possible that survival in high temperatures in natural environments could be related to genes not involved in thermal tolerance *per se*— immune-surveillance genes, for example. Studies of selective summer mortality in Pacific oyster compared gene expression profiles between genotypes that survived and died, and showed that a set of immune response genes was positively associated with summer survival (Fleury and Huvet 2012). To improve the genomic resources available for studying thermal tolerance in zebra mussels, we have generated transcriptomes from gill tissue in animals exposed to periods of low (24°C), medium (27°C) and high (30°C) chronic temperature stress.

Water chemistry plays a large role in limiting spread of zebra mussels and calcium concentration is the most important single water chemistry parameter (e. g. Mellina, Rasmussen 1994; Whittier et al. 2008). There is evidence that biomass of zebra mussels within water bodies is limited by ambient Ca^{2+} concentrations, and evidence for threshold concentrations below which populations cannot persist. In North America, few inland lake populations are found at concentrations below 20 mg/L Ca^{2+} (Cohen, Weinstein 2001). At several sites along the St. Lawrence River, Mellina and Rasmussen (1994) found no zebra mussel populations below 15 mg/L Ca^{2+}, while Jones and Ricciardi (2005) showed a decline in biomass of zebra and quagga mussels across a concentration range from 25 to 12 mg/L, with quagga mussel populations absent below 12 and zebra mussels absent below 7.5 mg/L. These thresholds are much higher than those for native sphaerid and unionid bivalves, which regularly occur at concentrations below 5 mg/L (McMahon 1996; McMahon 2002; Strayer 1993).

The mechanism(s) underlying poor tolerance of low Ca^{2+} in dreissenids have received relatively little study. Rearing success and percent of normal larvae were found to decline with Ca^{2+} concentration in laboratory studies of larval development (Sprung 1987). Vinogradov et al. (1993) showed that zebra mussel adults were unable to regulate Ca^{2+} concentrations in their circulatory fluid (hemolymph) at ambient concentrations < 12-14 mg/L (i.e. the animals lose Ca^{2+} to the surrounding water), and lower pH values further reduce their ability to regulate. Moreover, survival, reproductive output, somatic growth and shell growth have each been found to decline with calcium levels in experimental trials (Baldwin et al. 2012; Hincks, Mackie 1997).

In dreissenids and other bivalves, the shell is constructed of calcium carbonate of different crystal forms (typically calcite in adult and aragonite in larval shells) that are deposited in an organic matrix, either through an extracellular mechanism or one mediated by cells within the mantle tissue (Mount et al. 2004; Weiner, Traub 1984). Correlations between environmental Ca^{2+}, shell strength and calcification in some species, considered along with the evidence for selection on shell strength for predator defense in freshwater molluscs (Lewis, Magnuson 1999; Russell-Hunter et al. 1981 and references within), suggest that shell calcification may be the process responsible for low calcium sensitivity in dreissenids.

Genome sequences from bivalves have revealed a surprisingly large number of genes involved in shell formation. Searches of the complete Pacific oyster genome for similarity to known shell formation genes in other molluscs identified > 1,800 candidate genes, showed that some major genes are lacking, and revealed diversification of others, including a large variety of variants related to nacrein (Zhang et al. 2012), a component of the iridescent material inside the shell. Nacrein is also used to build pearls, and the pearl oyster (*Pinctada fucata martensii*) genome shows duplications in the nacrein gene family; one of the shell matrix-protein gene families whose diversity has been generated by tandem duplication to form gene clusters at 14 different loci (Takeuchi et al. 2016). Components of the shell-formation genome and proteome in *P. f. martensii* (Du et al. 2017) includes proteins related to collagen and others that are similar to the chondroitin sulfotransferase enzymes found in vertebrate bone.

For *D. polymorpha*, our specific interest would be in identifying genes related to calcification of the shell or "biomineralization" – the process whereby the protein-based shell matrix nucleates crystals of calcium carbonate, and orients their formation into the highly organized layers that compose the shell. Expressed Sequence Tags (ESTs) of messenger RNA's of the shell-building mantle tissue in the tropical pearl oyster *Pinctada margaritifera* (Joubert et al. 2010) were analyzed and a group of putative biomineralization-related genes were identified: 55 genes due to similarity to genes in other *Pinctada* species, 14 due to similarity to genes in more distantly related bivalves, and 13 due to similarity to genes in gastropods (a different class in Phylum Mollusca). For dreissenids, the most closely related bivalves for which sequence information is available at the genomic and/or transcriptomic levels are *Mytilus* (Murgarella et al. 2016), *Modiolus* and *Bathymodiolus* (Sun et al. 2017), all members of Family Mytylidae. We are currently using RNA-Seq of mantle libraries to identify biomineralization-related genes. Half of these libraries were prepared from mussels collected from calcium-rich (35 mg/L) and half from mussels collected from calcium-poor (13-14 mg/L) water bodies as a way to infer genes that may be up or down-regulated in response to calcium limitation. As sequenced genomes and other genomic resources from molluscs become increasingly available, comparative approaches in evolutionary developmental biology of shell formation and mineralization (Jackson, Degnan 2016) could be employed to investigate mechanisms of sensitivity of dreissenids to low calcium.

The zebra mussel genome sequencing project

Bivalves are a diverse Class of Mollusca with over 10,000 described species in marine and freshwater environments (Appeltans et al. 2012; Bogan 2008). As of this writing (April 2018), complete genomes have been sequenced and analyzed adequately in only 7 species—all of them marine and most of commercial harvest value (Table 2). Yet 21 invasive bivalve species cause damage to aquatic and marine ecosystems worldwide (Sousa et al. 2009), and only one—the golden mussel, *Limnoperna fortunei*— has so far been the subject of a genome sequencing project (Uliano-Silva et al. 2017).

Zebra mussel genome sequencing strategy

Genomes of eukaryotic organisms typically contain millions of DNA segments that do not code for genes and consist of repeated sequence motifs. In fact, over half the genome of humans and other mammals is comprised of repetitive DNA (de Koning et al. 2011) that arises from transposable elements and other unknown sources. Bivalve genomes are also highly repetitive, which makes assembly of raw data into contiguous sequences (contigs) challenging. The genomes of the two marine mussel species whose genomes have been sequenced – the deep sea *Bathymodiolus platifrons* and the intertidal *Modiolus philippinarum* – are highly repetitive, with 47.9% and 62%, respectively, being composed of repeats and transposable elements (Sun et al. 2017). Repeats are also common in oyster [36% of Pacific oyster *Crassostrea gigas* and 50% of pearl oyster *Pinctada fucata* (Li et al. 2017; Zhang et al. 2012)] and scallop genomes [39% of Yesso scallop and 32% of Chinese scallop (Li et al. 2017; Wang et al. 2017)].

To deal with its likely repetitive nature, we adopted the following approach to sequence the *D. polymorpha* genome*.* We generated preliminary short read data for a single zebra mussel by sequencing to a depth of approximately 100x on the Illumina HiSeq instrument. An assembly was performed in CLC Workbench (Qiagen Bioinformatics, Redwood City CA) which yielded ~500,000 contigs with an N50 (a measure of assembly contiguity roughly interpretable as a weighted median contig length) of 2.2 kilobases (kb). This is similar to the published assembly of the Mediterranean blue mussel (*Mytilus galloprovincialis*) genome, which was based only on short read data, with ~1.7 million contigs and an N50 of 2.6 kb (Murgarella et al. 2016). To generate a high-quality zebra mussel reference genome, we are obtaining 100x coverage with the Pacific Biosciences (PacBio) Sequel Single Molecule Real Time (SMRT) sequencing platform, which is capable of producing sequencing reads that are tens of kb in length. Such long reads resolve much of the ambiguity in repetitive regions as the reads are long enough to span many repeats and anchor them to unique sequences. A sequencing depth of 100x PacBio combined with Illumina short read data has been shown to be effective for high-quality assembly of eukaryotic genomes, including the completion of a single 25 Mb contig that spans all of *Drosophila melanogaster* chromosome arm 3L (Berlin et al. 2015).

The technologies for obtaining long-range genomic scaffolding information are rapidly evolving. Additional technologies such as nanopore sequencing (Jain et al. 2018), Hi-C (Burton et al. 2013), optical mapping, and synthetic long read approaches employed by 10x Genomics (Zheng et al. 2016) have been successfully used to improve genome assemblies and for long-range mapping of polymorphisms to parental chromosomes [i.e. haplotype phasing (Moll et al. 2017; Seo et al. 2016)]. We are also planning to incorporate Hi-C to further improve long-range scaffolding of the zebra mussel genome.

With the ability to generate increasingly long sequencing reads, a major challenge is isolating high-quality DNA of sufficient length, in quantities large enough to take full advantage of these technologies. We isolated >100 ug of genomic DNA from an individual zebra mussel from Duluth/Superior Harbor in Lake Superior using a Qiagen Genomic Tip 100/G kit. Pulsed-Field Gel Electrophoresis indicated a broad size distribution from 20-120 kb (not shown). To create a PacBio library, the genomic DNA was needle sheared to an average size of approximately 40 kb, SMRTbell adapters were ligated, and the final library was size selected for molecules >20 kb on the PippinHT (Sage Science). An Agilent TapeStation Genomic DNA assay indicated that the average size of the final sequencing library was >20 kb.

To date, we have generated 168.97 gigabases (Gb) of sequencing data on the PacBio Sequel. This represents an estimated coverage of 77-105x, based on estimates of genome size ranging from 1.6-2.2 G. The N50 for subreads (PacBio terminology for sequence read partitions that can be used, in our case, for assembly) is 16,524 bp, validating the high quality of our input DNA and PacBio sequencing library. In order to build gene models and to functionally annotate the zebra mussel genome, we have also acquired expression data from 3 different adult tissues (mantle, foot, and gill) using RNA-Seq, and are continuing to collect RNA-Seq data from embryos and larvae spanning a range of developmental stages. In addition to its utility in gene modeling efforts, studying a large proportion of the expressed transcriptome will also provide information about tissue and stage-specific gene expression patterns that may help inform bio-control efforts.

Applications of genomics: Development of biocontrols

In a few cases, invasive species genome projects have been motivated by the goal to discover new biocontrol strategies. For example, vector-directed biocontrol drove the sequencing of the genomes of the invasive mosquito species that carry malaria (*Anopheles gambiae*: Holt et al. 2002) and those that carry yellow fever, dengue and Zika viruses (*Aedes aegypti:* Nene et al. 2007). Sequencing of the genome of the crown-of-thorns sea star (*Acanthaster planci* spp. group) identified the genes for an array of molecules released when animals aggregate to spawn—including a large number of unique ependymin-family proteins active in the central nervous system of many animals and their putative receptors (Hall et al. 2017). This communication system may be a target for biocontrol using synthetic peptides that mimic aggregation cues. With the exception of attempts to identify parasites and other natural enemies (Molloy 1998), no biological control efforts have been attempted against zebra or quagga mussels. Below we describe technologies under development for genetic modification that could, given our new genomic resources, potentially be applied to dreissenids for control.

Genetic modification biotechnologies

Molecular biologists have invented several techniques with which they can deliver foreign DNA, or make precise edits in the native DNA of organisms. The CRISPR/Cas9 gene editing system has received the greatest recent attention for applications in biological conservation, including control of invasive species—due to low cost, rapid experimental turn-around time, and potential for spreading genetically edited alleles throughout wild populations—even when they lower fitness—through a mechanism known as a "gene drive" (Burt 2003; Gantz, Bier 2015; Gantz et al. 2015). The CRISPR/Cas9 system works by using a Cas9 endonuclease that can be directed, by a gene-specific guide RNA included in the engineered construct, to cleave a 20-basepair-long DNA sequence in virtually any genome (Fig. 1). Flanking the guide RNA is the payload sequence that contains the desired gene edit. Cas9 cleavage of the non-engineered homologous chromosome initiates a DNA repair process that, in the properly engineered construct, will convert the non-engineered into the engineered copy, making the edited gene homozygyous. This allows for super-Mendelian inheritance that has been demonstrated in laboratory studies (Gantz, Bier 2015; Gantz et al. 2015; Hammond et al. 2015), and that can, in theory, rapidly drive the edited gene to high frequencies in natural populations (Champer et al. 2016; Esvelt et al. 2014). Laboratory demonstrations of how this might be used in control, to date, all come from mosquito vectors of disease—including edits that confer host resistance to carrying malarial parasites (Gantz et al. 2015), and others that code for female sterility mutations to lower host fitness (Hammond et al. 2015)—but the possible applications are virtually limitless.

Nonetheless, there is considerable recent discussion and controversy about the release of CRISPR/Cas9 into the environment, with two issues of concern. The first is biosafety and the regulatory oversight of the technology. Several members of the scientific community, including some who developed the technology, have made pleas to strictly control technology development until the ecological and ethical risks of gene drives can be adequately addressed (Akbari et al. 2015; Bohannon 2015; Caplan et al. 2015; Oye et al. 2014). As a consequence, protocols for ecological risk evaluation by the international system that regulates testing and release of genetically modified live organisms are now being developed more formally (Hayes et al. 2018). With the risks come enormous potential benefits, so the creation of a framework for ecological risk assessment of CRISPR/Cas9 and similar technologies is essential.

The second issue, ironically, is whether CRISPR/Cas9 gene drives will ever impact natural populations enough to create risk (or benefit). Using both mathematical population genetic theory (Deredec et al. 2008; Drury et al. 2017; Noble et al. 2017a; Noble et al. 2017b; Unckless et al. 2017) and direct characterization of mutations (Champer et al. 2017; Drury et al. 2017), several recent studies have examined the evolution of resistance to gene drives. The extent to which resistance will affect prospects for CRISPR/Cas9-based control is not entirely clear. One study predicts that CRISPR/Cas9 gene drives are too efficient for resistance mutations to slow their propagation throughout the range of invasive species—and that unintended transmission (e.g. to native-range populations) remains likely (Noble et al. 2017a). Several other studies, however, suggest that resistance will hamper the spread of a gene drive unless, beforehand**,** constructs are carefully designed (Noble et al. 2017b; Unckless et al. 2017), and focal populations are screened for Cas9 target sequence polymorphisms (Drury et al. 2017). It may be that resistance evolves so readily that environmental risk has been overestimated, but research on the fate of CRISPR/Cas9 gene drive in natural populations is just beginning.

A still more-recent but promising approach to biocontrol uses components derived from the CRISPR/Cas9 system described above—in this case, to create synthetic barriers to reproduction of invasive species in the wild. It uses a modified protein (dCas9) that, rather than being used to edit genes and initiate a gene drive, allows for control of gene expression (Qi et al. 2013). Maselko et al. (2017) developed a system in which dCas9, paired with a guide RNA molecule, precisely locates a target gene in the genome (as in the CRISPR/Cas9 system above), binds to its promoter sequence and drives the target gene to overexpress its gene product. Target genes, for which overexpression is known to be lethal, can then be chosen to control invasive populations (Fig. 1).

When an engineered strain mates with a wild type, the heterozygous offspring die from overexpression of the gene, off the wild type promoter. The result is synthetic incompatibility, or immediate "post-zygotic" reproductive isolation between engineered and wild type, with the proof of concept demonstrated in yeast (Maselko et al. 2017). Mating between individuals of the engineered strain produce offspring that can survive because the promoter has been mutated to prevent the dCas9/guide RNA construct from binding to it. The use of the system in invasive species could involve releases of engineered individuals, that by mating with wild type, would suppress population mean fitness as in sterile insect biocontrol designs (Maselko et al. 2017). Since dCas9 does not cleave the homologous chromosome, this system does not cause gene conversion leading to a gene drive, thus avoiding any increased environmental risk of that outcome. But since there is a fitness deficit for the engineered strain (incompatibility) and no gene drive to counter it, the down side would be a need to periodically release engineered individuals. Determining how often and how large these releases would need to be requires population genetic modeling, which remains to be done. This technology is not immune to some forms of resistance (e. g., survival of individuals due to mutation(s) in the promoter sequence that prevents the dCas9/guide RNA construct from binding to it) and this also needs consideration.

Target genes for genetic modification

The first step forward in research on genetic modification requires selection of target genes and biological processes that, when modified, will produce the desired fitness effect (lethality, reduced viability, infertility). Availability of genome sequences is essential for selecting target genes and designing constructs. For example, Drury et al. (2017) generated genomic sequences from 4 global populations of the flour beetle *Tribolium castaneum* to examine population variation in Cas9 sites in target genes. Edits in these genes are expected to produce a range of fitness costs from their effects on eye pigmentation, female and male fertility, and insecticide sensitivity. Maselko et al. (2017) used the yeast genome to search for target genes that when modified would produce lethal overexpression, then searched population genomic data from rice and fruit flies to look for variants in dCas9 target sites within promoter regions.

Among possible targets in *Dreissena* are genes controlling byssal thread synthesis, thermal tolerance, and shell formation and mineralization—all processes with data available to advise homology searching and with clear biological significance. Genes controlling embryonic development are also prime targets. The Pacific oyster genome project (Zhang et al. 2012) produced data on gene expression across 38 embryonic and larval stages—for example it showed that about 800 genes start their transcription between the last embryonic and 1st larval stage. The project provided functional studies of specific genes expressed across stages, information on genetic regulation of organogenesis, and on male and female-specific genes expressed in gonad. A large number of developmental genes were also identified from the Pearl oyster and Yesso scallop genomes (Wang et al. 2017; Zhang et al. 2012).

Developmental genes or domains can be conserved at the sequence level, sometimes across broad phylogenetic distances (e.g. *Hox* gene homeodomains), which will aid in their identification. A recurring theme is "co-option" for new functions of genes in animal evolution, and this is seen in mollusks. For example, a *nanos* gene copy controls germline differentiation in *Drosophila*, and the *Tis11* gene is not involved in embryogenesis in vertebrate animals from which it was isolated, yet both genes have been recruited to control spiral cleavage divisions in mollusk embryos (Chan, Lambert 2011; Rabinowitz et al. 2008). Studies of spatial pattern of expression also implicated *vasa* and *nanos* gene family members in germ cell development in oysters, snails and other animals (Dill, Seaver 2008; Rabinowitz et al. 2008); knockdown of *vasa* expression by RNA interference was later confirmed to lower oyster fertility by inhibiting gonad development (Fabioux et al. 2009). The arthropod segmentation gene *engrailed* controls embryonic shell (protoconch) formation throughout molluscs, as does *dpp-BMP2/4*, a gene that specifies the dorso-ventral axis in arthropods and vertebrates (Jackson, Degman 2016; Nederbragt et al. 2002; Wanninger, Haszprunar 2001).

The zebra mussel genome: a community resource

It is impossible for us to envision; let alone to take advantage of the range of applications of this genome to research and management. We recognize, moreover, that to properly analyze it we will need assistance from experts from a number of unrelated disciplines—biomineralization, comparative and evolutionary genomics, developmental biology, materials science, physiology and physiological ecology—to name some that come to mind. With this review, we encourage interested individuals to collaborate on a cross-disciplinary effort to annotate and analyze the genome, and to formulate research on applications. Worldwide the dreissenid mussel research community is large and diverse, and we need its help in this important effort.

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Figure legend

Figure 1. Strategies of zebra mussel genetic modification. The strategies on the left involve genomic editing to interupt the biological function of target genes; with or without gene drives to spread the modification throughout populations. The strategy on the right involve insertion of a gene activator to drive over-expression of genes that create post-zygotic barriers to reproduction. This would lower fitness via “gamete wastage” in engineered populations.

Table 1. Sequenced genomes available from 100 of the world's worst alien invasive species. The five columns with bold italic headings provide descriptions of the length and quality of the sequenced genomes**.** Assembly level: contig is a term for an assembled contiguous stretch of DNA sequence; scaffold refers to when a set of contigs is ordered and placed in the correct orientation; chromosome level is when biological chromosomes are assembled is relative completion (some gaps may remain). The number of contigs provides a metric for the assembly quality; in general the smaller the number the larger the contig length. Contig N_{50} is roughly a measure of the shortest contig length in the data encompassing 50% of the genome in basepairs (bp). Genome length is the total length of the assembled genome in gigabase pairs (Gb).

Common name	**Taxon or group**	**Strain/isolate**	**Impacts**	***Assembly level***	***Number of scaffolds***	***Number of contigs***	***Contig N50 (bp)***	***Genome length (Gb)***	**Year submitted**
Rabbit	Mammal		Degrades biodiversity, particularly in introduced areas that lack predators	Chromosome	3,318	84,024	64,648	2.737	2005
Frog chytrid fungus	Fungus	JAM81	Cause of many amphibian declines and extinctions	Scaffold	127	510	318,114	0.024	2011
Comb jelly	Aquatic invertebrate		Invasive carnivore that consumes zooplankton	Scaffold	5,100	24,927	11,914	0.156	2011
Argentine ant	Terrestrial invertebrate		Often displaces native ants	Scaffold	3,030	18,227	35,858	0.220	2011
Red imported fire ant	Terrestrial invertebrate		Highly damaging nuisance species and pest of crop plants, livestock	Scaffold	69,511	90,219	14,677	0.396	2011
Mouse	Mammal	C57BL/6J	Economic pests, carriers of human disease, several negative impacts on invaded ecosystems	Chromosome	262	750	32,273,079	2.794	2012

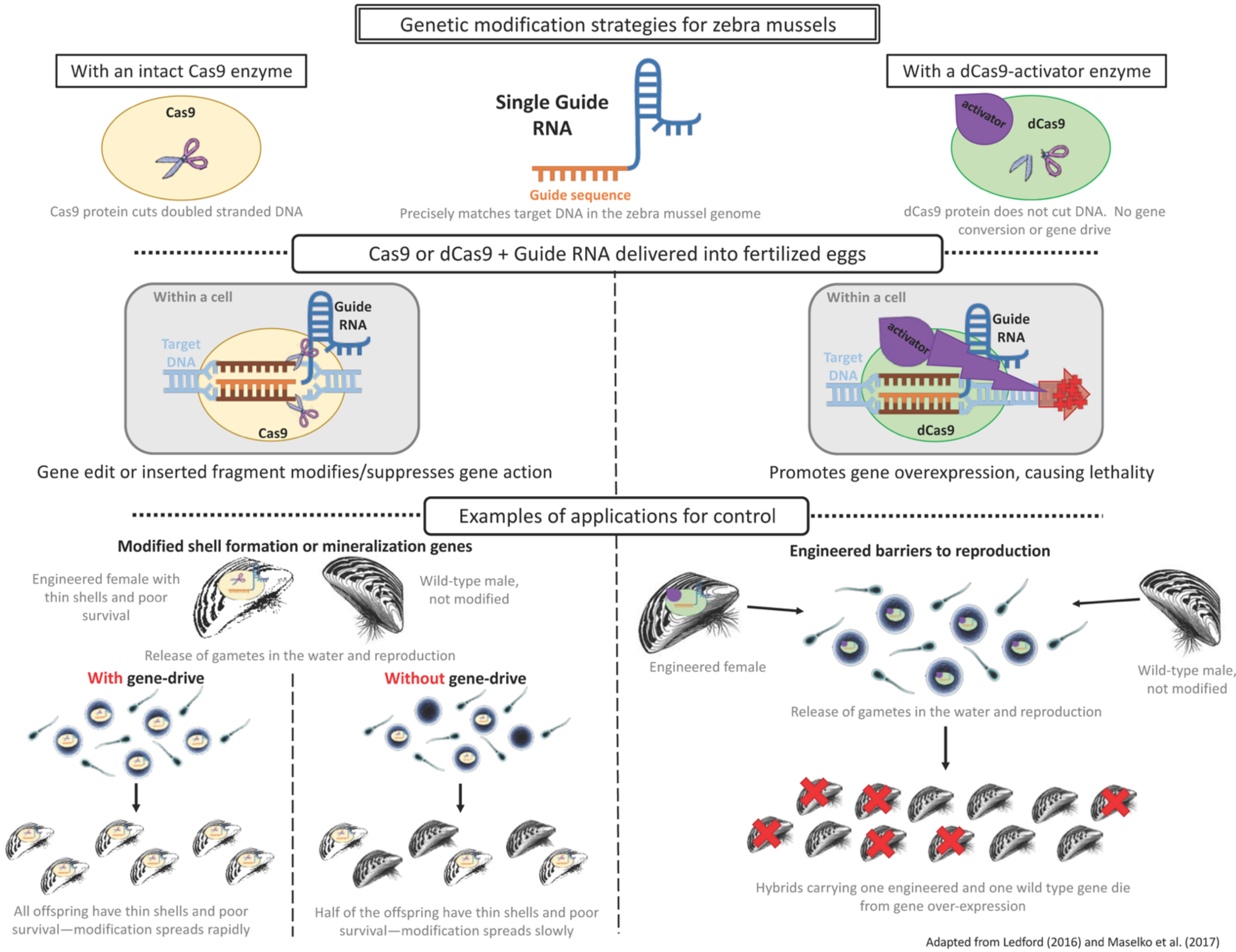
Macaque	Mammal		Lower native bird diversity by eating eggs and chicks, and competing for food	Chromosome	7,625	87,764	86,040	2.947	2013
Crayfish plague	Protist	APO3	Water mold lethal to European crayfish	Scaffold	835	4,659	36,439	0.076	2014
Common carp	Fish		Uproots aquatic vegetation, causing declines in, plants, other fishes and water quality	Chromosome	9,378	53,088	75,080	1.714	2014
Phytophthora root rot	Fungus	MP94-48	Highly damaging with broad host range	Scaffold	5,777	5,831	24,715	0.054	2015
Little fire ant	Terrestrial invertebrate	WASHAW1	Stinging ants that displace native species and harm crop plants	Scaffold	77,788	103,610	37,912	0.324	2015
Starling	Bird	715	Outcompetes native birds for nesting sites and damages fruits and other crops	Scaffold	2,361	22,666	151,865	1.037	2015
Asian tiger mosquito	Terrestrial invertebrate	Foshan	Widespread vector of yellow, dengue and Chikungunya fever viruses	Scaffold	154,782	355,061	18,430	1.923	2015
Avian malaria	Protist	SGS1	Parasites of birds, causing wide-ranging levels of mortality	Chromosome	514	724	583,861	0.023	2016
Sweet potato whitefly	Terrestrial invertebrate	MEAM1	Pest of vegetable crops and ornamentals with vast host range	Scaffold	19,751	31,571	84,501	0.615	2016
Goat	Mammal		Voracious grazers with great impacts on vegetation and	Chromosome	29,907	30,399	26,244,591	2.923	2016

			cascading effects, particularly on islands						
Asian longhorned beetle	Terrestrial invertebrate	ALB-LARVAE	Wood feeding pest of trees in forests and urban settings	Scaffold	9,867	26,749	80,490	0.707	2017
Mediterrenean blue mussel	Aquatic invertebrate		Marine mussel that displaces native species	Scaffold	1,002,334	1,136,100	2,627	1.500	2017
Rainbow trout	Fish	Swanson	Preys upon and outcompetes native fishes, and hybridizes with native trout	Chromosome	139,800	559,855	13,827	2.179	2017
Domestic cat	Mammal	Cinnamon	Voracious predators on native birds, reptiles and mammals responsible for several extinction events	Chromosome	4,525	4,909	41,915,695	2.522	2017
Pig	Mammal	201423004	Feral pigs are pests of crops and property, dig up native vegetation, prey on several native species	Chromosome	14,157	14,818	6,372,407	2.755	2017
Red deer	Mammal	*hippelaphus*	Strong impacts on native forest flora and fauna in invaded range	Chromosome	11,479	406,637	7,944	3.439	2017
Bullfrog	Amphibian	Bruno	Preys upon and outcompetes native amphibians	Scaffold	1,544,635	2,124,505	5,415	6.250	2017
Golden apple snail	Aquatic invertebrate	SZHN2017	Voracious feeder on crops and native vegetation	Chromosome	24	746	1,072,857	0.440	2018
Western mosquito fish	Fish	NE01/NJP1002.9	Causes decline and extinction of other small	Scaffold	2,943	73,682	17,511	0.599	2018

		native fishes through competition						
Leafy spurge	Land plant	Aggressive weed	Scaffold	1,633,094	2,242,201	605	1.125	2018
Cane toad	Amphibian	Toxic skin glands poison predators upon ingestion, endangering native species	Contig	N/A	31,391	167,498	2.552	2018

Table 2. Sequenced genomes from bivalve molluscs

Species	Family	Common name	Commercial interest	Assembly level	Number of scaffolds	Number of contigs	Contig N50 (bp)	Genome length (Mb)	Reference
Bathymodiolus platifrons	Mytilidae	Hydrothermal vent mussel	None	Scaffold	65,662	272,497	12,602	1,658.2	Sun et al. 2017
Chlamys farreri	Pectinidae	Zhikong (Chinese) scallop	Wild harvest and culture	Scaffold	96,024	148,999	21,500	779.9	Li et al. 2017
Crassostrea gigas	Ostreidae	Pacific oyster	Hatchery culture—leads aquatic animals in global harvest	Scaffold	7,659	30,460	31,239	557.7	Zhang et al. 2012
Crassostrea virginica	Ostreidae	Eastern oyster	Wild harvest and hatchery culture	Chromosome	11	669	1,971,208	684.7	Gómez-Chiarri et al. 2015
Mizuhopecten (Patinopectin) yessoensis	Pectinidae	Yesso scalllop	Culture from wild seed	Scaffold	82,659	120,022	65,014	987.6	Wang et al. 2017
Modiolus philippinarum	Mytilidae	Phillipine horse mussel	None	Scaffold	74,573	301,873	18,389	2,629.6	Sun et al. 2017
Pinctada martensii	Pteriidae	Akoya pearl oyster	Cultured pearls	Chromosome	5,039	85,944	21,518	991.0	Unpublished


Genetic modification strategies for zebra mussels
With an intact Cas9 enzyme
Cas9
Cas9 protein cuts doubled stranded DNA
Single Guide RNA
Guide sequence
Precisely matches target DNA in the zebra mussel genome
With a dCas9-activator enzyme
activator
dCas9
dCas9 protein does not cut DNA. No gene conversion or gene drive
Cas9 or dCas9 + Guide RNA delivered into fertilized eggs
Within a cell
Guide RNA
Target DNA
Cas9
Gene edit or inserted fragment modifies/suppresses gene action
Within a cell
activator
Guide RNA
Target DNA
dCas9
Promotes gene overexpression, causing lethality
Examples of applications for control
Modified shell formation or mineralization genes
Engineered female with thin shells and poor survival
Wild-type male, not modified
Release of gametes in the water and reproduction
With gene-drive
Without gene-drive
All offspring have thin shells and poor survival—modification spreads rapidly
Half of the offspring have thin shells and poor survival—modification spreads slowly
Engineered barriers to reproduction
Engineered female
Wild-type male, not modified
Release of gametes in the water and reproduction
Hybrids carrying one engineered and one wild type gene die from gene over-expression
Adapted from Ledford (2016) and Maselko et al. (2017)

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 10: Citizen Science and Professional Training Programs to Support AIS Response
SUBPROJECT MANAGER: Daniel Larkin
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $525,389
AMOUNT SPENT: $520,850
AMOUNT REMAINING: $4,539

Sound bite of Subproject Outcomes and Results

We developed the AIS Detectors program to train volunteers to be "eyes on the water" for AIS detection and response. 299 people are certified and have contributed 10,000+ hours of work. The AIS Trackers program has been piloted and will launch next year. This project also launched Starry Trek.

Overall Subproject Outcome and Results

Early detection of invasive species is critical. However, there are few professionals addressing aquatic invasive species (AIS) in Minnesota relative to our state's vast water resources. Furthermore, while many efforts each year seek to control AIS, there are gaps in synthesizing treatment outcomes. These gaps limit our ability to improve management and contribute to uncertainty for lake associations and others tasked with management decision-making. We developed AIS citizen science and training programs to address these challenges. Specifically, AIS Detectors trains volunteers as "eyes on the water" for AIS detection and response, and AIS Trackers educates non-professionals on AIS management and leverages monitoring data to refine management guidance. Over 820 Minnesotans have participated; more have been reached through presentations, media, and publications. To date, 299 people have become certified AIS Detectors and gone on to contribute >10,000 hours to outreach, stewardship, citizen science, and other volunteer activities, a service value >$273,000. Outgrowths of Detectors have led to additional service, including "Starry Trek", which annually draws ~200 volunteers statewide for targeted searches for the invasive alga starry stonewort. This event, in partnership with the Minnesota DNR and colleagues from Wisconsin, has led to identification of two new starry stonewort populations and associated opportunities for rapid response; over 500 people have participated. Through AIS Trackers, we developed a new online course to educate people about AIS management and new mechanisms for analyzing AIS treatment outcomes. Over 70 people have piloted this program, which will open in 2020 to a wide audience in Minnesota and beyond. Minnesotans benefit from our work through enhanced capacity for AIS surveillance and robust training that helps professionals and non-professionals alike make better-informed management decisions. Results show that natural resources benefit when we empower Minnesotans to contribute to AIS prevention efforts through rigorous, science-based training and service programs. These programs are now well-established and will continue to be implemented under support from MAISRC, UMN Extension, and program revenue.

Subproject Results Use and Dissemination

Information from our project has been disseminated through 2 publications (attached), 16 invited talks, 11 contributed presentations, 5 webinars, 69 media stories, and online resources. This project has also contributed significantly to MAISRC Subproject 8 (“Risk assessment, control, and restoration research on aquatic invasive plant species”).

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Flipping the Classroom to Train Citizen Scientists in Invasive Species Detection and Response

Abstract

Extension educators are increasingly using flipped classrooms, wherein online content delivery precedes in-person learning. We have applied this approach to two Extension programs in which citizen scientists are trained in early detection of invasive species. Our goal in using the tool of flipped classrooms is to accommodate large amounts of content while focusing classroom time on skills development. In 2017, we assessed efficacy of the flipped classroom through knowledge tests and surveys completed by 174 participants and 106 participants, respectively. Results demonstrated large knowledge gains and high participant satisfaction. We encourage Extension professionals to consider whether use of the flipped classroom format could advance achievement of their programs' learning objectives.

Keywords: adult learners, citizen science, flipped classroom, invasive species

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Introduction

Research on adult learners has identified key differences between adult and traditional student–aged audiences

(Knowles, 1980; Merriam, 2001), providing blueprints for effective andragogy in Extension programming (Strong, Harder, & Carter, 2010). In particular, adult learners have greater capacity to direct their own learning, have problem-oriented learning goals, wish to immediately apply new knowledge, and are more self-motivated than externally motivated (Knowles, 1980).

These characteristics of adult learners align well with flipped classroom teaching methods. In this format, the traditional in-class lecture components of teaching occur prior to face-to-face meetings via self-paced, independent learning focused on knowledge and comprehension (Anderson & Krathwohl, 2001; Milman, 2012), freeing in-class time for higher level, more active modes of learning that leverage the presence of instructors and peers to facilitate application, analysis, and synthesis (Anderson & Krathwohl, 2001; Mazur, 2009).

Researchers have advocated the flipped classroom as a means for improving Extension programming. Strong, Rowntree, Thurlow, and Raven (2015) argued for more community-centric rather than content-centric approaches to Extension and cited the flipped classroom as a tool for advancing that shift. Others have documented the efficacy of flipped classroom approaches in Extension for internal staff development (Burns & Schroeder, 2014; Franz, Brekke, Coates, Kress, & Hlas, 2014) and youth programs (Garst, Baughman, & Franz, 2014; Weitzenkamp, Dam, & Chichester, 2015).

We employed flipped classrooms in two University of Minnesota Extension programs focused on increasing capacity for invasive species early detection and rapid response through citizen science. We used flipped classrooms to accommodate the large amount of content delivery these programs required and to reserve face-to-face time for participants to practice, implement, and demonstrate competency with newly gained knowledge and tools.

Descriptions of Programs

AIS Detectors (AISD) (www.aisdetectors.org) targets detection and control of plant and animal aquatic invasive species (AIS) and was launched in the flipped classroom format in spring 2017. Forest Pest First Detectors (FPFD) (www.myminnesotawoods.umn.edu/forest-pest-first-detector/), which focuses on terrestrial invasive insects and plants, had launched in 2008 and was switched to the flipped classroom format in spring 2017. Both programs engage adults as citizen science volunteers and place high expectations on participants' capacity to (a) identify numerous invasive species and native look-alikes, (b) use a smartphone app to report suspected new infestations, and (c) communicate responsibly and effectively with professionals from resource management agencies and the public.

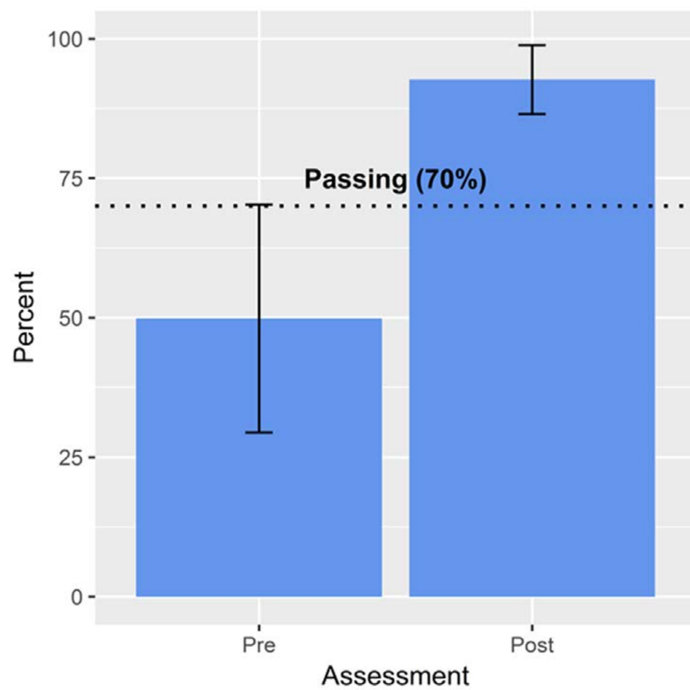
Evaluation of Flipped Classroom Effectiveness

We used knowledge tests and participant surveys to evaluate the effectiveness of the flipped classroom approach for participants in seven AISD workshops and two FPFD workshops held across Minnesota in spring 2017. For AISD, we tested participants' understanding of key issues and concepts at three time points through testing administered before and after exposure to the online curriculum and a postworkshop knowledge exam. For FPFD, we assessed content knowledge through testing administered after the online curriculum. In addition, we used postworkshop online surveys, created via Qualtrics online survey software, to solicit anonymous evaluations from participants. For AISD, we asked students to rate the effectiveness of the flipped classroom format using a multiple-choice question and sought comments through an open-ended question. For FPFD, we used a multiple-choice question to gauge participant agreement with the statement "The flipped classroom approach worked well for me." Additionally, in response to a general request for comments, several participants commented on the flipped classroom.

Results from knowledge testing of 174 participants (AISD *n* = 123, FPFD *n* = 51) and survey responses from 106 participants (AISD *n* = 66, FPFD *n* = 40) showed the flipped classroom to be highly effective. For AISD, testing indicated that prior to completing the online curriculum, only 18% of participants had satisfactory knowledge of AIS (based on a passing grade of 70%). After completion of the online curriculum, all participants passed (*M* = 93%) (Figure 1). In the AISD postworkshop exam, all but one participant passed (*M* = 88%), the lone exception being the only person who had not completed the online curriculum. For FPFD, all participants scored 95% or higher on knowledge assessments following completion of the online curriculum.

Figure 1.

Before-and-After Assessment of Knowledge Gain Related to the AIS Detectors Program's Online Curriculum

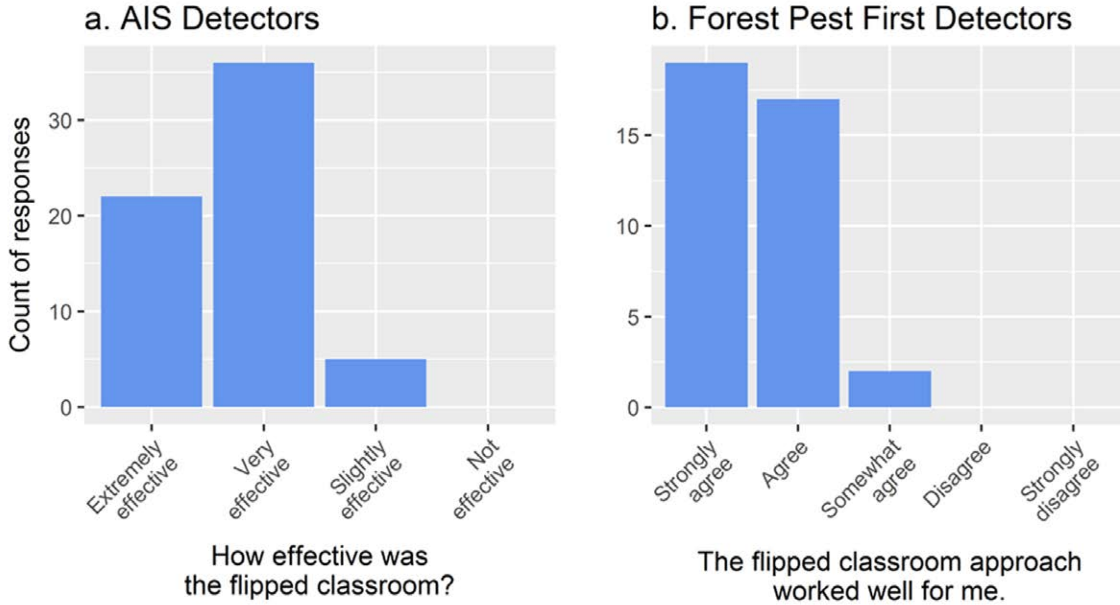


Note: Bars show means; error bars are ±1 standard deviation.

Participants also reported high satisfaction with the format: 92% of AISD respondents considered the format to be very or extremely effective; 95% of FPFD survey respondents agreed or strongly agreed that the flipped classroom approach worked well for them (Figure 2).

Figure 2.

Summary of Participant Evaluations of the Effectiveness of the Flipped Classroom Approach



Participants' qualitative survey responses highlighted factors that contributed to their satisfaction with the flipped classroom format. In particular, participants reported that the flipped classroom helped them understand the material, enjoy the learning experience, and make the most of their in-person time (Table 1).

Table 1.

Selected Participant Comments on Efficacy of the Flipped Classroom Approach

Program	Participant comments
AISD	Knowing the material before class actually makes the class more productive. Not concerned as much about learning the material because you know the basics. Because of this you can ask better questions that will expand your knowledge.
	It was much more interactive and thus easier to learn the material. It is hard to listen through and retain knowledge from multiple PowerPoint presentations.
	It was very well done! No boredom whatsoever!
FPFD	I really benefited from the flipped classroom approach and enjoyed the small group sessions.
	Loved it. Much more conducive to really learning and using the material.
	I really liked this format better and enjoyed the small group discussions upstairs.

Note. AISD = AIS [Aquatic Invasive Species] Detectors program. FPFD = Forest Pest First Detectors program.

Conclusion

Extension professionals are increasingly using the flipped classroom in their programming. We found it to be an effective and enjoyable means of teaching challenging content to adult learners and training them to implement new skills. In particular, it allowed us to make the most of our limited in-person time with participants. We encourage others in Extension to ask whether a flipped classroom could benefit their programs and to consider this approach when designing new courses or updating existing ones.

Acknowledgments

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2013 Project Abstract

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project #11: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods – Phase 1: Problem Formulation
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APPROPRIATION AMOUNT: $93,343

Overall Project Outcomes and Results

Individual [invasive] carp continue to be found in Minnesota waters, and there remains pressure for sound statewide management to address this potential threat. To help advance the management of [invasive] carp in Minnesota and inform the initial problem formulation step in a risk assessment, this project conducted focus groups and in-depth interviews to: 1) identify potential adverse effects from [invasive] carp to inform a subsequent risk assessment, and 2) characterize the tensions and conflicts that are hampering [invasive] carp management. First, we conducted 5 focus groups with 20 individuals, including MN-DNR managers and stakeholders involved with invasive carp. During these focus groups, participants created a list of potential adverse effects that could occur if invasive carp were to establish in Minnesota, and discussed the importance and potential causes of these adverse effects. The resulting potential adverse effects were associated with 26 valued and potentially affected entities. Focus group participants also discussed what could and should be done to manage invasive carp, including where improvements in existing management efforts are needed. The results from this work were summarized in the report *Potential adverse effects and management of Silver & Bighead carp in Minnesota: Findings from focus groups*, informed the in-depth interviews on management, and will inform the risk assessment to be conducted in Phase 2 of the project. Second, to study and help address the tensions and conflicts impeding management we conducted 16 in-depth interviews with individuals who have been involved with [invasive] carp management in Minnesota, including state and federal agency officials, University researchers, and representatives from non-governmental organizations. As presented in the report *Exploring tensions and conflicts in invasive species management: The case of* [*invasive*] *carp,* we found three areas of tension and conflict impeding [invasive] carp management: 1) scientific uncertainty (concerning the impacts of [invasive] carp in Minnesota and the impacts of barriers on [invasive] carp and native fish species), 2) social uncertainty (concerning the divergent views of what, if anything, should be done to manage [invasive] carp), and 3) the needed approach to [invasive] carp research and management. Findings point to the need for the right relationship to uncertainty and for reflexive deliberation on the judgments informing research and management decisions.

Project Results Use and Dissemination

The potential adverse effects described in the report *Potential adverse effects and management of Silver & Bighead carp in Minnesota: Findings from focus groups* will be used in the Phase 2 project to inform the analysis phase of the risk assessment for bigheaded carp in Minnesota. Project findings were shared via presentations. First, findings were shared at the 2015 Association for Environmental Studies and Sciences conference in a presentation titled, "How to prevent harm: Exploring conflicts within invasive [invasive] carp management." Findings were also presented at the MAISRC 2015 Research Showcase in a presentation titled, "Advancing [invasive] carp management using risk analysis: Findings from year one." Findings from phase 1 will also be shared at the 2016 Minnesota Invasive Carp Forum. Project findings were summarized and distributed in two written reports: 1) *Potential adverse effects and management of Silver & Bighead carp in Minnesota: Findings from focus groups*, and 2) *Exploring tensions and conflicts in invasive species management: The case of* [*invasive*] *carp*. These reports were made available online and provided to stakeholders and managers involved with [invasive]carp.

MINNESOTA AQUATIC INVASIVE SPECIES RESEARCH CENTER
UNIVERSITY OF MINNESOTA

Minnesota Bigheaded Carps Risk Assessment

A report for the Minnesota Department of Natural Resources

-Final-

May 12, 2017

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Executive Summary

Introduction

Aquatic natural resources are ecologically, culturally, economically, and politically important to the state of Minnesota. Two aquatic invasive species that pose a threat to these resources are bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), which are collectively referred to as bigheaded carps. Bigheaded carps are native to East Asia and were introduced into the southern United States during the early 1970's, where they were promoted by state and federal agencies as a nonchemical way to improve water quality in retention ponds, sewage lagoons, and aquaculture operations. Subsequent unintentional release and large flood events allowed these species to escape into the Mississippi River, where they began reproducing and spreading. They are considered invasive species in the United States because of their potential to disrupt ecosystems by consuming large amounts of plankton and, in the case of silver carp, the ability to jump up to 10 feet in the air and create a recreation hazard. In Minnesota, 33 individual bigheaded carp have been captured through 2016, varying from 0 to 6 individuals per year. However, all of the captures have been adults and there is not thought to be a reproducing population of bigheaded carps in the state. The nearest reproducing population of bigheaded carps is thought to be in southern Iowa.

Project Need and Purpose

Bigheaded carps pose a threat to the state of Minnesota, but there has yet to be a systematic study of how their arrival would impact different waterbodies across the state. This project helps fill this gap by assessing the risks from bigheaded carps to the waterbodies of Minnesota. Specifically, this risk assessment estimates both the likelihood that bigheaded carps would establish in 4 select watersheds and the resulting severity of 4 salient potential adverse effects. The findings from this risk assessment can help the management context in Minnesota in many ways. First, these findings can help prioritize areas of the state for management actions by determining which watersheds are at higher risk. Second, these findings can help justify reasoned management actions by estimating the likely impacts of bigheaded carps if no additional management actions are taken. Third, this risk assessment can help refine societal expectations for what the arrival of bigheaded carps would look like.

Methodology

The risk assessment was completed using a multi-step process. First, focus groups and a survey were conducted to determine which potential adverse effects – i.e., potential undesirable changes caused by bigheaded carps – were most important to examine in the risk assessment. Second, a two-day expert, deliberative workshop was held to complete the major analytical portion of the risk assessment. After the workshop, project researchers and a self-selected group of workshop participants authored this report based on the results from the workshop.

Finally, in March 2017 a draft version of this report was presented and discussed during a meeting exploring the findings and implications of the risk assessment. This final report was revised based on the feedback from that meeting.

Step #1: Identifying potential adverse effects & Narrowing scope

During the first step of the risk assessment process, five focus groups were conducted to create a comprehensive list of potential adverse effects. Three focus groups were held with personnel from the Minnesota Department of Natural Resources (MNDNR) and two with individuals active in the non-governmental organization stakeholder community in Minnesota. Due to the large list of potential adverse effects that was generated during these focus groups, a survey was conducted to prioritize those considered most important for Minnesota. The survey was completed by those who took part in the focus groups and the participants of the subsequent deliberative risk assessment workshop.

The four potential adverse effects that emerged from the survey and were studied in the risk assessment are: 1) decrease in non-game fish populations; 2) decrease in game fish populations; 3) reduction in species diversity and ecosystem resilience; and 4) decrease in recreation quality from the jumping silver cap hazard. For the scope of the risk assessment, the following watersheds were selected in consultation with the MNDNR: Sand Hill River Watershed, Nemadji River Watershed, Lower St. Croix River Watershed, and the Minnesota River – Mankato Watershed. These watersheds were chosen to represent a diversity of basins and river types, to be relevant to the state's current decision making context, and, when possible, to be worst-case scenarios – watersheds in each basin that are likely to be most favorable to bigheaded carps.

Step #2: Risk assessment workshop

The second step of the risk assessment process was the two-day expert, deliberative risk assessment workshop held in March 2016. Twenty-three individuals with expertise on bigheaded carps and/or Minnesota's waterways participated in the risk assessment workshop, including individuals from 5 federal agencies, 5 academic institutions, MNDNR, natural resource agencies from 2 other states, and a stakeholder group. A combination of facilitated small and large group discussions was used to characterize the risk of the four potential adverse effects in each of the four watersheds. This was done by sequentially characterizing: 1) the likelihood that bigheaded carps would establish in each watershed if they arrived there, 2) the resulting abundance of bigheaded carps in each watershed, and 3) the severity of the potential adverse effects caused by the resulting abundance of bigheaded carps. The time scale considered for each step was within 10 years of arrival. The overall risk was a product of the likelihood of establishment and the severity of the potential adverse effect.

Important methodological considerations

This assessment estimated the risks from bigheaded carps assuming they arrive in each watershed considered. It was outside the scope of this assessment to examine how likely it is that bigheaded carp will arrive in each watershed. There continues to be important management and research taking place to slow the spread of bigheaded carps, so that arrival is prevented. This risk assessment estimates what would happen if bigheaded carps do arrive in these different watersheds, helping to make clear where to prioritize, and what is at stake in, management actions.

For the game fish and non-game fish potential adverse effects, risk assessment workshop participants selected one important fish species to focus on for each watershed. Although the study of additional fish species is warranted, it fell outside the scope of this assessment. The fish species that were selected, however, provide useful insights on the risks posed to game and non-game fish in Minnesota.

Throughout this project, there was an explicit effort to involve a breadth of resource managers and stakeholders from Minnesota. These participants provided needed local expertise on the state's waterways and ensured that the value judgments within the risk assessment were informed by stakeholders and managers.

Risk Assessment Findings

The findings from this assessment reveal that the risks posed by bigheaded carps vary across watersheds and potential adverse effects. Figure E1 summarizes the estimated establishment probabilities (size of square) and consequence levels (color of square) generated by the participants. The Minnesota River-Mankato watershed was estimated to have the highest probability of establishment (70%), followed by the Lower St. Croix River (45%) and Nemadji River watersheds (38%), with the lowest probability for the Sand Hill River watershed (22%). The consequence levels varied across watersheds and potential adverse effects, with lower consequence levels generally for the Nemadji River and Sand Hill River watersheds and for the non-game fish and game fish potential adverse effects.

Given that overall risk is a product of the probability of establishment and consequence level, the larger the square and the more red the color, the higher is the risk. The highest estimated risk, therefore, was for Species diversity/Ecosystem resilience and Recreation jumping hazard for the Minnesota River – Mankato watershed, and the Recreation jumping hazard for the Lower St. Croix River watershed. The certainty for the risk characterizations were generally low, due largely to the lack of data concerning invasions of bigheaded carps in waterbodies similar to those found in Minnesota.

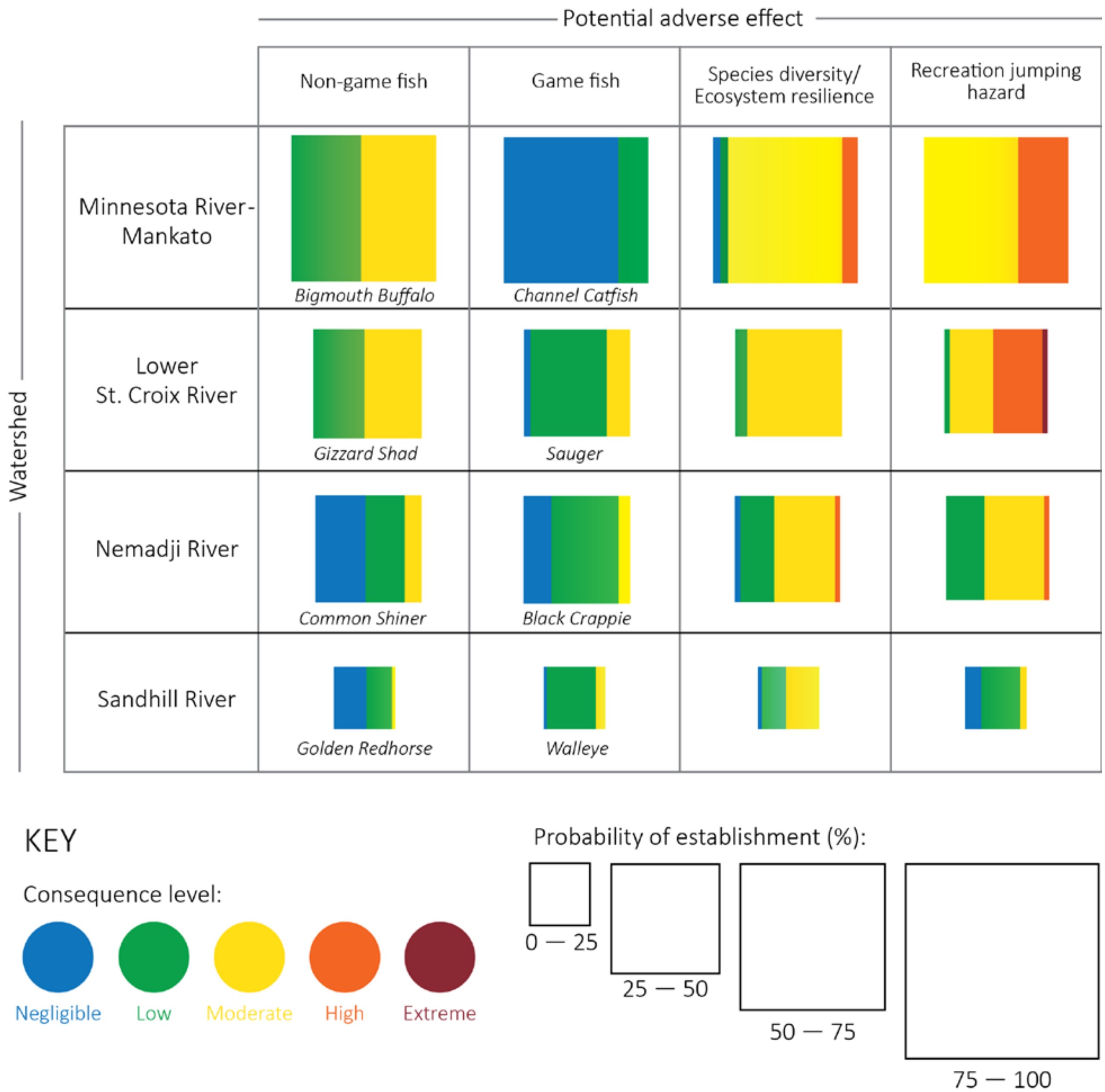

Figure E1: Summary of Minnesota Bigheaded Carps Risk Assessment findings. The size of the squares corresponds to the estimated probability of establishment for bigheaded carps in that watershed. The color of the squares corresponds to the consequence levels that participants deemed to be most likely for each potential adverse effect, with the width of the color proportional to the number of participants who chose that consequence level as most likely. Also provided for each watershed are the common names for the fish species considered.

A variety of factors influenced the characterizations of risk. Overall, the major determinants of establishment likelihood involved factors affecting the probability of successful spawning by bigheaded carps and the survival of their young-of-the-year. These included several biotic and abiotic factors, such as spawning habitat, water temperature, flow regime, nursery habitat, food resources, and potential predators. With regards to the non-game and game fish potential adverse effects, the non-game fish species considered for the Minnesota River - Mankato and the Lower St. Croix were planktivores (Bigmouth Buffalo and Gizzard Shad), and the expected dietary and habitat overlap with bigheaded carps led about half of participant to select a moderate consequence level. Non-planktivore fish species were generally considered to have a low or negligible consequence level. The severity of potential adverse effects are also likely to vary within a watershed with, for example, areas of greater severity in the shallows and backwaters of rivers where bigheaded carps are more likely to reach higher densities and take part in jumping behavior.

Discussion & Implications

These risk assessment findings support the need for a reasoned and timely response to the threats posed by bigheaded carps. First, the findings show that the Minnesota River – Mankato and similar watersheds are at a higher risk, followed by the Lower St. Croix River and similar watersheds. Unfortunately, these two watersheds are found in the southern and eastern parts of the state, which are closest to the current invasion front. These findings support the need to prioritize management that can slow or prevent the spread into these areas, or that can lessen the consequence levels of any resulting adverse effects.

Second, the risks posed by bigheaded carps are not uniformly high or uniformly low across potential adverse effects and watersheds. Because there is not uniformly low risk, it is important to take reasoned action in response to the threat. Because there is not uniformly high risk, it is important to consider the collateral damage of possible management actions, to ensure actions do less harm to native species than bigheaded carps would. For example, non-selective barriers on rivers have been shown to cause extirpations of native fish species. Species-selective deterrents, however, such as those using sound, provide the potential to slow the spread of bigheaded carps while not hurting native fish populations. While research is still advancing on such deterrents, the potential is promising. Other possible management actions that don't harm natives include improving ecosystem resilience, restoring top native predators such as flathead catfish, and eliminating cross-watershed connections.

To pursue a balanced and reasoned approach to management, it is important that decisions weigh: 1) the potential effects if no management actions are taken (i.e., risks from bigheaded carps); 2) the efficacy of management actions on bigheaded carps; 3) the effects of management actions on native species (i.e., collateral damage). The goal is to pursue research and management that can prevent the spread of bigheaded carps and reduce the severity of any adverse effects, while avoiding disproportionate harm to native species.

This risk assessment provides one part of the equation to determine the desired response to bigheaded carps in Minnesota, a response that should not be based on either reactionary apathy or fear. While this assessment is a necessary first step, additional work is required. First, looking explicitly at the economic aspects of bigheaded carp risks and of management actions would also help inform decision making, and the risks characterized here provide a good starting point for that effort. Second, the approach to, and findings from, this risk assessment can be built upon to examine the risks to other watersheds in Minnesota or the region. Finally, there is a need to regularly update these findings to keep up with the relevant scientific literatures.

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1 Introduction

1.1 Minnesota context

Aquatic natural resources are ecologically, culturally, economically, and politically important to the state of Minnesota. Minnesota has an abundance of surface water, more than 11,000 lakes and 69,000 miles of rivers and streams. Those waters are vitally important to both recreation and commerce within the state (MNDNR 2013). About 800,000 watercraft are registered in Minnesota, which is the most per-capita of any state in the nation (Kelly 2014). There are 1.3 million licensed resident anglers and the state attracts another 259,000 non-resident anglers each year. Fishing related expenditures total an estimated $2.4 billion annually (USFWS 2011), and when recreational boating is added to those expenditures, the economic impact is approximately $5.5 billion annually (2015 National Marine Manufacturers Association).

Lake Superior and the Mississippi River also serve as important waterways for shipping in Minnesota. Minnesota's portion of the Mississippi River system is used to move more than half of Minnesota's agricultural exports, which in 2013 was 9.2 million tons of freight valued at nearly $2 billion. In 2015, 11.6 million tons of freight traveled on the Mississippi River system (MNDOT 2016). Minnesota's portion of Lake Superior was used to move 58 million tons of freight in 2013, which was valued at $7.2 billion (MNDOT 2016b). Commercial fishing is another economic use of Minnesota's waterways, with an estimated 3.5 million pounds of fish harvested annually (MNDNR 2016).

Protecting the waterways of Minnesota from the threats posed by aquatic invasive species falls under the authority of the Minnesota Department of Natural Resources (MNDNR) and a host of federal agencies, such as the United States Fish and Wildlife Service (USFWS), the United States Geological Survey (USGS), the National Park Service (NPS), and the United States Army Corps of Engineers (USACE).

1.1.1. Bigheaded carps

Bighead carp (Hypophthalmichthys nobilis) and silver carp (Hypophthalmichthys molitrix), (collectively referred to as bigheaded carps[1]) are native to East Asia and considered invasive species in the United States, where they are listed as injurious species under the United States Lacey Act. These species were introduced into the southern United States during the early

[1] Concerning terminology, in this document "bigheaded carps" will be used to refer to bighead and silver carp. "Asian carp" is used to refer to bighead, silver, grass (*Ctenopharyngodon idella*), and black (*Mylopharyngodon piceus*) carp. "Invasive carp" is also used to refer to the four Asian carp species, as that is the terminology used by the Minnesota Department of Natural Resources.

1970's when they were promoted by state and federal agencies as a nonchemical and environmentally friendly way to improve water quality in retention ponds and sewage lagoons, and to aid in fish aquaculture operations (Kelly et al. 2011). Subsequently, unintentional release and large flood events allowed these species to escape into the Mississippi River drainage, where they began reproducing and expanding their distribution (Kelly et al. 2011). Bigheaded carps have migrated up into portions of the Mississippi and Missouri rivers, and adjoining tributaries, dispersing into new habitats and ecosystems (Asian Carp Regional Coordinating Committee 2014). Bigheaded carps are considered one of the most concerning aquatic invasive species in North American because of their potential to disrupt ecosystems from the bottom up and, in the case of silver carp, to cause a recreational hazard by jumping up to 10 feet in the air when startled (USFWS 2014).

Silver carp can exceed 3.5 feet in length and weigh up to 60 pounds, while bighead carp can exceed 5 feet in length and weigh over 100 pounds (USFWS 2014, Kolar et al. 2007). In US waters, silver carp generally have a lifespan of 5 to 7 years and reach sexual maturity between 2 and 4 years of age, whereas bighead carp generally have a lifespan of 8 to 10 years and reach sexual maturity between 2 and 4 years of age (Kolar et al. 2007); however, some individuals have been known to live more than 25 years (Duane Chapman, personal communication). Bigheaded carps consume phytoplankton and zooplankton; silver carp consume mainly phytoplankton, while bighead carp consume zooplankton and other microorganisms. Both species can also consume detritus (Kolar et al. 2007). Individuals grow rapidly and can quickly become too large for most piscivorous North American fish to consume. Bigheaded carps spawn in turbulent flowing water once water temperatures exceed 18 ºC and spawning is typically triggered by rising water levels (Abdusamadov 1987, Kolar et al. 2007). Eggs are semi-buoyant but, if not kept in suspension by currents, they will settle to the bottom, which is detrimental to their survival (George et al. 2016). This means a minimum length of river is required for embryos to develop successfully (Garcia et al. 2013, Kolar et al. 2007, Krykhtin and Gorbach 1981). After hatching, larval bigheaded carps move into backwater areas. Many native large river fish are dependent on backwater resources (especially as nursery habitat) and so bigheaded carps' use of backwaters may be particularly impactful.

Both bighead and silver carp have high fecundity (Kolar et al. 2007) and the potential to populate new areas and reach high abundances, given favorable environmental conditions (Asian Carp Regional Coordinating Committee 2014). The ability to reach high abundances contributes to the impacts bigheaded carps can have on North American river ecosystems as well as on recreational river use. Silver carp jump from the water and can strike and injure recreational users (Spacapan et al. 2016). Additionally, bigheaded carps can disperse over great distances, contributing to their spread throughout North America (Degrandchamp et al. 2008;

Coulter et al. 2016a). The overlap in food resources and feeding efficiency of bigheaded carps lead them to be successful competitors with native planktivores such as gizzard shad (Dorosoma cepedianum) and bigmouth buffalo (Ictiobus cyprinellus) (Irons et al. 2007, Sampson et al. 2009) and the young of native species that also consume planktonic resources (USFWS 2014, Kolar et al. 2007). Bigheaded carps can also alter plankton communities and increase production of undesirable cyanobacteria, further altering invaded ecosystems (Radke and Kahl 2002). Increases in bigheaded carp abundance have been correlated with changes in the relative abundance of native fishes (Solomon et al. 2016). The rapid growth of bigheaded carps means that they are only consumed by native predators at small sizes (i.e., young-of-year). The high fecundity, rapid growth, feeding habits, mass spawning events, and dispersal capacity all contribute to the invasion success of bigheaded carps (DeGrandchamp et al. 2008, Carlson and Vondracek 2014).

As of November 2016, 33 individual bigheaded carp have been captured in Minnesota, varying from 0 to 6 individuals per year (Figure 1-1). Captured silver carp have weighed between 15.8 and 19.1 pounds, averaging 17.9 pounds. Captured bighead carp have weighed between 21.3 and 47.5 pounds, averaging 31.7 pounds. Most of these bigheaded carp have been captured on the Mississippi River, with some captured on the St. Croix and Minnesota Rivers (Figure 1-2). All captures have been adults, and therefore the population of bigheaded carps is considered a non-reproducing population at this time in Minnesota. The nearest reproducing population in the Mississippi River system is thought to be in southern Iowa (Figure 1-2). For the Missouri River watershed, which includes far southwestern Minnesota, the nearest reproducing population is below Gavins Point Dam on the mainstem, and in the James River, which is a tributary.

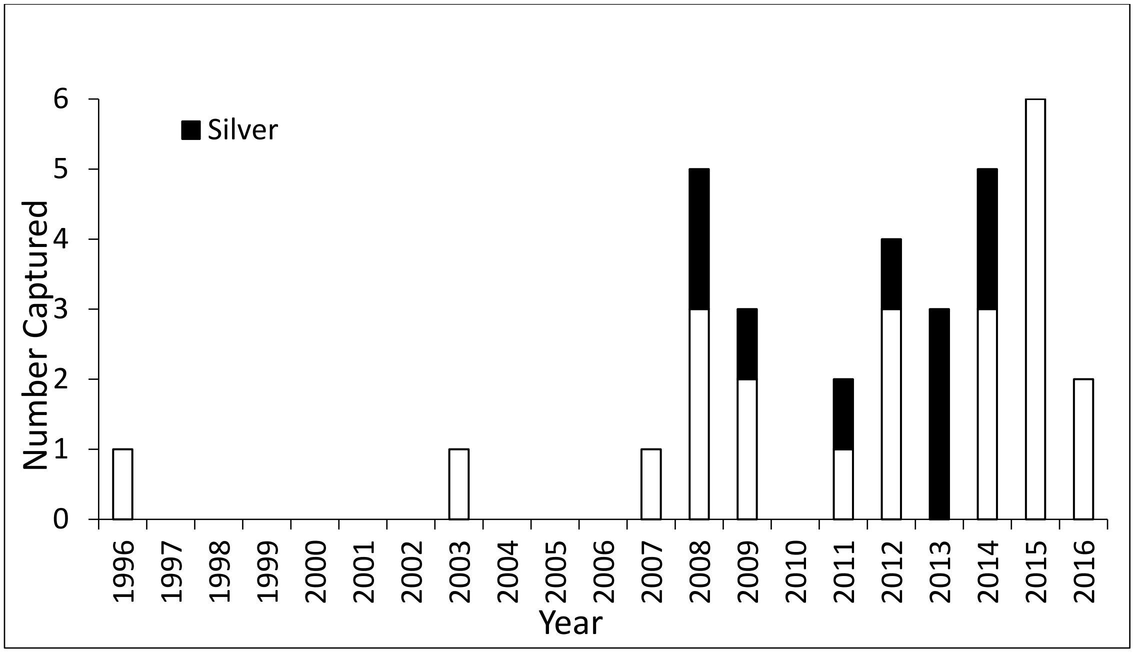


Figure 1-1. Number of individual silver (shown in black) and bighead (shown in white) carp captured per year in Minnesota as of November 2016.

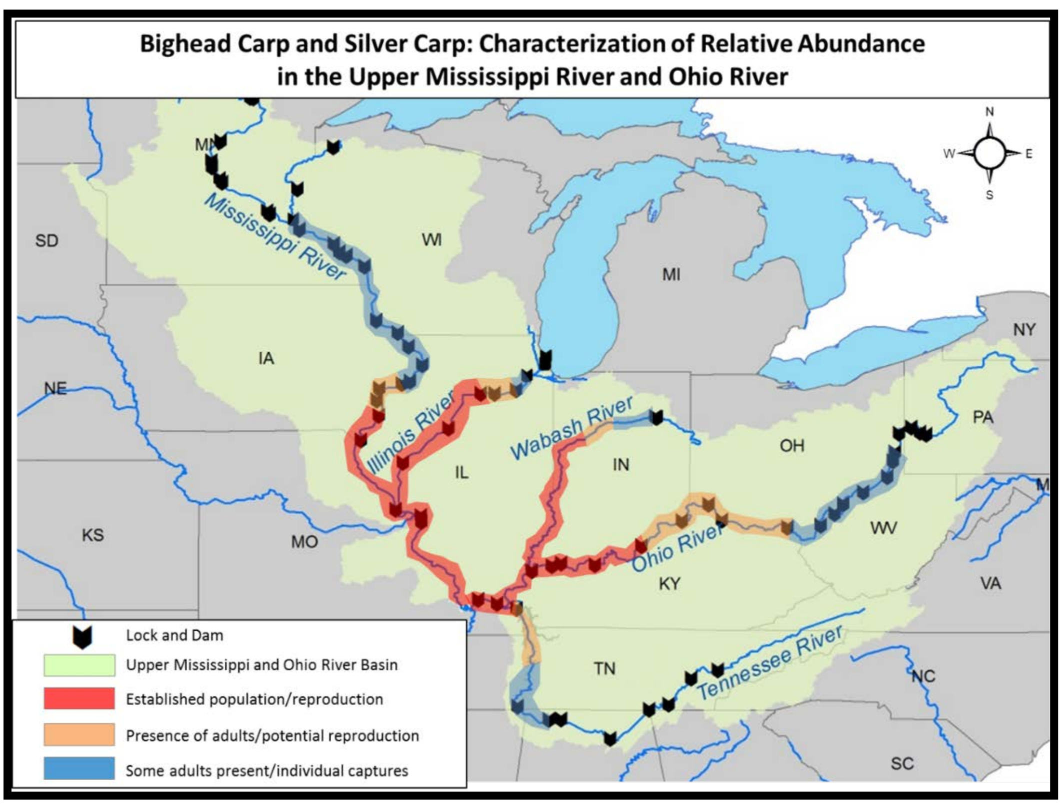


Figure 1-2. Characterization of Relative Abundance of bigheaded carps in the Upper Mississippi River and Ohio River. (Figure from USFWS 2015).

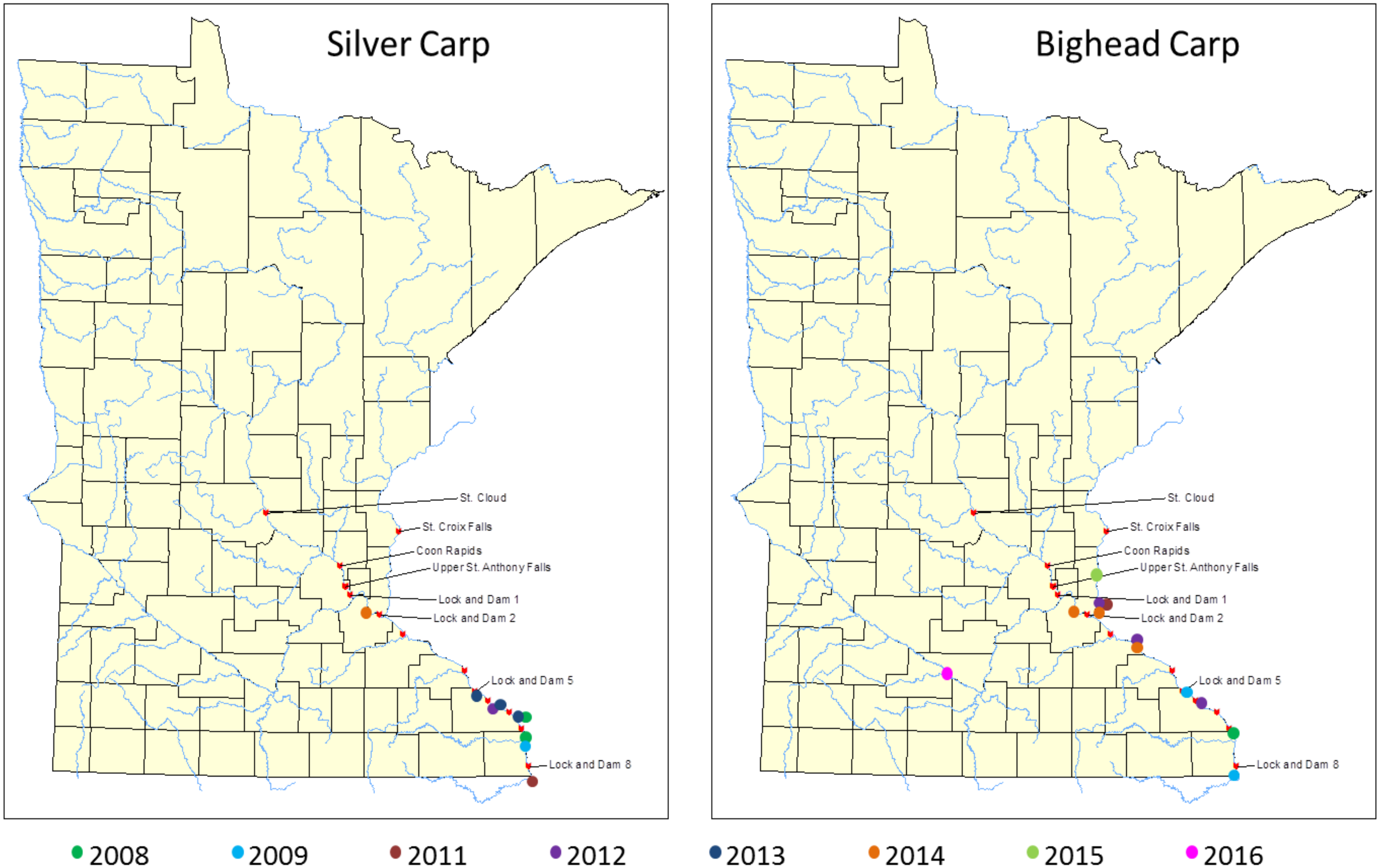


Figure 1-3. Locations that individual bigheaded carps have been found in Minnesota since 2008.

1.1.2. Existing management of bigheaded carps in Minnesota

Given that individual bigheaded carp are being captured in Minnesota but there is not yet an established (i.e., self-sustaining reproducing) population, there is a need to pursue and explore management to address this potential threat. The MNDNR is highly engaged with the management of bigheaded carps in Minnesota. The agency uses the Minnesota Invasive Carp Action Plan (MNDNR 2014) to guide activities. Plan elements include: 1) early detection and monitoring of susceptible waters; 2) prevention and deterrence; 3) response preparation; 4) management and control; and 5) outreach and communication. More specifically, the MNDNR is actively engaged in monitoring Minnesota waters for changes in bigheaded carp population size, range expansion, and reproduction; preventing or limiting range expansion at strategic locations; and accelerating research on control strategies. The MNDNR publishes an annual invasive species report that highlights invasive carp management activities (2011, 2012, 2013, 2014, 2015 Invasive Species Annual Report).

1.1.2.1. Assessment, detection, and monitoring of Invasive Carp

MNDNR Fisheries released a GIS spatial map depicting where invasive carp may spread by their own swimming capabilities in November 2013 (MNDNR 2013b). This included assigning relative risk of invasive carp passage at stream barriers and identification of potential watershed breaches. Since publication, work has been done to verify watershed breaches. The MNDNR invasive carp monitoring program was established in 2012. The MNDNR relies on six methods to detect and monitor the expansion and population changes of invasive carp in Minnesota: traditional fisheries monitoring programs; targeted sampling; contracted commercial fishing; monitoring the commercial catch; reported sightings; and environmental DNA (eDNA) sampling by the USFWS. The monitoring program targets all life stages of carp: egg, larval, juvenile, and adult. MNDNR fisheries began a fish telemetry study in spring of 2013 to understand fish movement around lock and dams and in the Mississippi River system. The USFWS also connected the receiver system with one located in Missouri to help monitor carp movements throughout potions of those two rivers.

1.1.2.2. Preventing upstream movement into northern Minnesota

The MNDNR believed that the best way to keep bigheaded carps out of the Upper Mississippi River watershed was to close the Upper St. Anthony Falls Lock. It required an act of Congress to close the lock, which is administered by the United States Army Corps of Engineers (USACE). Lock closure provisions were included in the Water Resources Reform and Development Act (WRRDA) bill which was signed into law by President Obama on June 10, 2014. The lock was closed on June 10, 2015. Additionally, the Minnesota Legislature approved $16 million in 2011 to fund improvements to the Coon Rapids Dam, including features to make it a more effective barrier against passage by bigheaded carps. Based on a 79-year flow record, fish passage through the dam would be possible an average of 4-5 days every ten years. Although the Coon Rapids Dam may be passable by invasive carp in rare high-water conditions, it provides important redundancy to the barrier at Upper St. Anthony Falls.

1.1.2.3. SW MN barriers

In 2011, the Iowa DNR captured two bighead carp with a bag seine in East Okoboji Lake, Iowa. The following year, a commercial fishing seine haul captured both bighead and silver carp from Iowa's Big Spirit and East Okoboji lakes. If bigheaded carps are able to swim upstream from Big Spirit Lake, they have the potential to reach lakes in southwest Minnesota. In fiscal year 2013, the MNDNR received funding from the Outdoor Heritage Fund (OHF) to place barriers in this region to limit invasive carp expansion. To help prevent the migration of invasive carp into southwest Minnesota, the MNDNR partnered with Iowa DNR to install an electric deterrent at the outlet of the Iowa Great Lakes, located on Lower Gar Lake. This deterrent became operational in May 2013. The area fisheries office in Windom, MN also identified seven sites where barriers could be installed to prevent the spread of invasive carp into high value lakes or between watersheds. Work was completed at these sites in November 2015.

1.1.2.4. Minnesota and Mississippi Rivers

The MNDNR is partnering with Minnesota State University - Mankato to evaluate invasive carp deterrents in the Minnesota River. University partners will collect and analyze data on hydrologic and geomorphic characteristics to determine potential locations and feasibility for deterrent measures. The project also will examine biological data to identify habitats that are highly suitable for invasive carp. Lastly, in spring 2015 researchers began investigating the Minnesota River - Red River watershed boundary to determine if the two watersheds can become connected during high water events. The MNDNR is beginning to look at potential actions at Lock and Dam 5 on the Mississippi River to slow the upstream expansion of carp. The installation of an acoustic/bubble deterrent has been proposed as a possible action.

1.1.2.5. Partnerships

In 2012, the Minnesota legislature appropriated funds to create an Aquatic Invasive Species Research Center at the University of Minnesota, in collaboration with the Commissioner of Natural Resources. The research center is pursuing a number of research initiatives, including:

1. Understanding and developing strategies for implementing eDNA as a molecular technique to assess potential presence of invasive carp in large Minnesota rivers;
2. Evaluating the potential to detect and locate invasive carp through the use of "Judas fish," a new behavioral tool to locate aggregations of invasive fish so they might be tracked and/or removed;

3. Developing food, pheromone, and hormone attractants for invasive carp to induce high-density aggregation for the purposes of fish detection, measurement, control and removal;
4. Conducting an assessment of effectiveness of enhanced bubble curtains as deterrents of invasive carp movement into small tributaries;
5. Installation of sound deterrents to deter invasive carp in the Mississippi River;
6. Assessing the potential use of native pathogens as invasive carp control agents;
7. Conducting risk analyses to identify invasive carp control priorities and methods.

In addition, the Sorensen laboratory at the University of Minnesota is continuing with LCCMR and MNDNR funding to study fish and carp passage around and through locks and dams in the Mississippi River, and ways the locks and dam operations might be safely altered to prevent the invasion and establishment of silver and bigheaded carp. The possibility of altering gate operations at specific structures to hold back carp at these locations without effecting scour is the focus of various types of numeric modeling. Results are promising and suggest carp passage is already very low at some key structures and might be reduced to a few percent of present values at no cost and in ways that do not appear to enhance scour or affect lock usage and thus might be acceptable for management (Peter Sorensen, personal communication). In addition, laboratory research with specific sounds that also appear unlikely to strongly affect many native fishes suggests that they could be placed into locks to prevent most carp passage. This scheme has been described but field tests have not yet been funded.

1.1.3. Tensions and conflicts facing management and the need for risk assessment

Even with many management actions already taking place in Minnesota, there is a need for work to help prioritize future management actions. Informational interviews with state and federal agency personnel during the scoping of this project indicated support for a bigheaded carps risk assessment that could identify areas of the state most at risk from bigheaded carps, characterize factors influencing the level of risk, and help prioritize management. Research on the tensions and conflicts facing the management of invasive carp in Minnesota also supports the need for a bigheaded carps risk assessment in Minnesota (Kokotovich and Andow 2017). Kokotovich and Andow (2017) conducted 16 in-depth interviews with state and federal agency officials, researchers, and stakeholders involved with invasive carp management in Minnesota to learn about the tensions and conflicts impacting management. Findings from these interviews reveal a complex set of issues revolving around three areas of tension and conflict: 1) scientific uncertainty concerning the effects of Asian carp in Minnesota and the efficacy and non-target effects of possible management actions; 2) social uncertainty concerning both the lack of societal agreement on how to respond to Asian carp and the need to avoid acting from apathy and/or fear; and 3) the desired approach to research and management. Scientific uncertainty and social uncertainty were seen to reinforce each other and complicate efforts to determine the desired approach to invasive carp research and management.

The scientific uncertainty surrounding the likely effects of invasive carps in Minnesota emerged as an important area of tension and conflict hampering management, both because it was seen as complicating decisions on individual management actions and because it was seen as potentially reinforcing apathy- and fear- based societal responses. A risk assessment was seen as a way to help address this area of tension and conflict. Knowing more about the likely effects of invasive carp in Minnesota could help identify reasoned management actions and prevent societal reactions based on apathy or fear. For example, interviewees stated that the decision making about management actions such as species-selective deterrents or non-selective barriers should be based on both the likely consequences from invasive carps and the likely effects of the deterrent or barrier, including its efficacy on invasive carps and its non-target impacts on native ecosystems. Without both sides of the equation, it is difficult to pursue well-informed decision making. Interviewees also described how individuals and institutions will be less likely to act from apathy (e.g., believing invasive carp will cause no impacts and therefore management is unimportant) or fear (e.g., believing invasive carp will cause catastrophic impacts and management actions should be taken regardless of their collateral damage) if the likely effects of bigheaded carps in MN are better understood (Kokotovich and Andow 2017). As a result, the risk assessment presented here – characterizing the risks from bigheaded carps for Minnesota – will be useful to the current decision making and societal context.

It is important to explicitly note that the risk assessment findings reported here provide information that is at once necessary and insufficient to inform the management of bigheaded carps in MN. Any decision about a particular management action, such as a deterrent or barrier, must be based on the likely effects of bigheaded carps as well as on careful scrutiny of the proposed action itself. Decision making regarding management actions should take into account the ecological, social, and economic impacts of bigheaded carps and of the proposed action, including consideration of the probabilities and conditions of those impacts. This work, due to necessary limitations of scope, only partially addresses the host of factors needed to inform a potential management decision, and should be used in a way that acknowledges this.

1.2. National context

1.2.1. Existing effects and management efforts

Many other areas of the United States have experienced invasions from bigheaded carps. Insights emerging from studies of these areas are important to efforts to predict and avoid consequences from bigheaded carps in Minnesota.

1.2.1.1. Illinois River

The Illinois River is a highly modified waterway that is the direct connection between the Mississippi River basin and the Great Lakes Basin, via the Chicago Area Waterway System. Since the early 1990's bigheaded carps in the Illinois River have gradually expanded their range and continued to increase in numbers such that they currently dominate the fish biomass (nearly 70%) in some navigation pools. Prior evidence has demonstrated significant declines in body condition of gizzard shad (−7%) and bigmouth buffalo (−5%) following the bigheaded carps invasion (Irons et al. 2007).

Beginning in 2009 the Illinois Department of Natural Resources and several agencies took an aggressive approach to inhibit the expansion of bigheaded carps into the Great Lakes. The overall goal of the Asian Carp Regional Coordinating Committee (ACRCC) is to prevent Asian carp from establishing self-sustaining populations in the Chicago Area Waterway System (CAWS) and Lake Michigan. Efforts to prevent the spread of bigheaded carps to the Great Lakes have been underway for over 6 years (see Asian Carp Monitoring and Response Plan, Interim Summary Reports 2010, 2011,2012,2013,2014, and 2015 (asiancarp.us)). In response to threats posed to the Great Lakes by bigheaded carps, the ACRCC and the Asian Carp Monitoring and Response Workgroup have identified the following projects to gain further understanding of Asian carp, improve methods for capturing Asian carp, and directly combat the expansion of Asian carp range. During this time, goals, objectives, and strategic approaches have been refined to focus on five key objectives in the Monitoring and Response Plan (see 2016 Monitoring and Response Plan for Asian Carp in the Illinois River and Chicago Area Waterway System (asiancarp.us)):

1. Determination of the distribution and abundance of any Asian carp in the CAWS, and use of this information to inform response removal actions;
2. Removal of any Asian carp found in the CAWS to the maximum extent practicable;
3. Identification, assessment, and reaction to any vulnerability in the current system of barriers to prevent Asian carp from moving into the CAWS;
4. Determination of the leading edge of major Asian carp populations in the Illinois River and the reproductive successes of those populations; and

5. Improvement of the understanding of factors behind the likelihood that Asian carp could become established in the Great Lakes.

1.2.1.2. Wabash River

The Wabash River, a large tributary to the Ohio River, originates in western Ohio before flowing west and south through Indiana to form the border between Indiana and Illinois. The watershed is 85,326 km^2 (Gammon 1998) and is > 60% agriculture. The river has one mainstem dam in the upper reaches, creating > 600 km of free-flowing river. Bighead carp were first detected in the Wabash River watershed in 1995 and silver carp in 2003 (USGS NIS 2016). Bigheaded carps are considered established although they occur at lower abundances than in other North American invaded rivers (i.e., Illinois River; Stuck et al. 2015). The Wabash River watershed contains a potential pathway for bigheaded carps to the Great Lakes basin via the Little River and Eagle Marsh (USACE 2010). However, this hydrological connection has since been blocked with the construction of an earthen berm (NRCS 2016]). In addition to hydrologic separation, management of bigheaded carps in the Wabash River watershed has focused on monitoring and angler education to prevent spread into areas not already invaded (D. Keller, Personal communication). Monitoring activities include acoustic telemetry (including in the Little River to monitor the Eagle Marsh pathway; Coulter et al. 2016b), pathogen surveys (Thurner et al. 2014), spawning surveys (e.g, Coulter et al. 2013; Coulter et al. 2016a), and eDNA surveys (e.g., Erickson et al. 2016). Some commercial fishermen harvest bigheaded carps but there is not currently an effort to deplete the population (D. Keller, personal communication). Since the invasion of bigheaded carps, the Wabash River fish assemblage showed increased efficiency in energy transfer, and a change in the dominant functional feeding group (planktivore-omnivores to benthic invertivore; Broadway et al. 2015). Abundance of low trophic level fishes has increased, a change likely driven by increasing numbers of bigheaded carps (Broadway et al. 2015).

1.2.1.3. Mississippi River – South of Minnesota

The Mississippi River Basin is the largest drainage basin in North America and covers approximately 3,225M square kilometers and includes all or parts of 31 states and two Canadian provinces. Throughout much of the Mississippi River and many of its associated tributaries, bigheaded carp populations are considered established. However, relative abundance or biomass is lower in the northern reaches of the Mississippi River (i.e., Minnesota, Wisconsin, and Iowa). Bigheaded carps were first observed in lower portions of the Mississippi River in the 1970s and 1980s but recently have been documented at locations in the upper reaches of the Mississippi River. Despite the well-established naturally recruiting populations particularly in the southern reaches (below Keokuk, Iowa) of the Mississippi River, extremely limited empirical evidence on the effects of Asian carp exists in the Mississippi River basin.

Mississippi River Basin (further south than Minnesota) fish community data collected from 2003-2015 by the Long Term Resource Monitoring program and the Missouri Department of Conservation suggest that the relative abundance of bigheaded carps has increased exponentially, while relative abundance and condition of some native fishes has declined (Phelps et al. In Review). Standardized sampling evaluations of floodplain lakes of the Mississippi River yielded similar results; floodplain lake fish communities were drastically altered by abundant bigheaded carps after their invasion (Phelps et al. In Review). Furthermore, laboratory experiments corroborated field evidence, showing that bigheaded carps reduced native fishes abundance through competition for prey. To this end, multiple lines of evidence suggest bigheaded carps are reducing the abundance of native fishes in the Mississippi River south of Minnesota (Phelps et al. In Review). Reductions in bigheaded carps in the Mississippi River (south of Minnesota) could reduce the decline in native fish abundances and prevent further expansion throughout North America (Seibert et al. 2015). Currently, minimal harvest occurs but efforts are in place to inform constituents about Asian carp through outreach and education.

1.2.2. Previous risk assessments and the need for a MN risk assessment

There have been two primary bigheaded carps risk assessments conducted in North America (Kolar et al. 2007; Cudmore et al. 2012). Kolar et al. (2007) provided a summary of the biology, distribution, and organismal risk of the bighead, silver, and largescale silver carp for the United States. The judgment of risk was for the overall risk potential of these species, based on the probability of establishment and the consequences of establishment. The authors assessed seven elements of risk, using a risk scale of low, medium, or high, with a 5-point certainty scale (Very certain, Reasonably certain, Moderately Certain, Reasonably Uncertain, Very uncertain). The seven elements assessed were: 1) Estimated probability of the exotic organism being on, with, or in the pathway; 2) Estimated probability of the organism surviving in transit; 3) Estimated probability of the organism successfully colonizing and maintaining a population where introduced; 4) Estimated probability of the organism spreading beyond the colonized area; 5) Estimated economic impact if established; 6) Estimated environmental impact if established; and 7) Estimated impact from social and/or political influences. These seven elements of risk were assessed at the scale of the entire United States.

The risk for silver and bighead carp for the first 4 elements having to do with establishment were all characterized as high – very certain, the highest risk and certainty ratings possible. The 5th and 6th element, for economic and environmental effect, were both characterized as medium to high risk – reasonably certain, for both bighead and silver carp. The 7th element, for social and/or political influences, was characterized as medium risk – reasonably certain. The overall risk potential for both bighead and silver carp was considered high. This level of risk was deemed unacceptable for the United States and one that "justifies mitigation to control negative effects" and means that silver and bighead Carp are "organisms of major concern for the United States" (Kolar et al. 2007, p. 155).

Cudmore et al. (2012) conducted a binational risk assessment of bigheaded carps for the Great Lakes basin to provide advice for management actions. The scope of the risk assessment was determined during a workshop of Great Lakes researchers, managers, and decision makers. The focus was on assessing, for each one of the Great Lakes, the likelihood of arrival, survival, establishment, and spread, and the magnitude of ecological consequences, given the current management context. Five-point scales were used for characterizations of likelihood, consequence, and certainty. The overall characterization of risk was a function of the probability of introduction and the magnitude of ecological consequence. Probability of introduction was characterized as:
Probability of Introduction = Min [Max (Arrival, Spread), Survival, Establishment]

Based on the agreed upon scope, a draft risk assessment was created by the authors and presented to a larger expert peer review group that came to consensus on the all of the risk assessment rankings (Cudmore et al. 2012).

For the Minnesota context, it is especially useful to review the findings of Cudmore et al. (2012) for Lake Superior, because that Great Lake borders the state. Lake Superior received overall risk scores that were lower than the other Great Lakes because of a lower likelihood of introduction and a lower likely ecological effect (Table 1-1) (Cudmore et al. 2012).

Table 1-1. Risk characterization for Lake Superior from binational risk assessment. (From Cudmore et al. 2012).

Element	Rank	Certainty
Arrival	Very Unlikely	Moderate
Spread	Very Likely	High
Max (Arrival, Spread)	Very Likely	High
Survival	Very likely	High
Establishment	Moderate	Moderate
P(Introduction)	Moderate	Moderate
Ecological Impact ~20 years	Low	Moderate
Ecological Impact ~50 years	Moderate	Moderate
Overall risk ~20 years	**Low-Moderate**	**Moderate**
Overall risk ~50 years	**Moderate**	**Moderate**

Kolar et al. (2007) and Cudmore et al. (2012) characterized the potential risks from bigheaded carps for the US and the Great Lakes, yet these risk assessments are not sufficient to inform decision making in Minnesota. There is a need for a risk assessment that has an appropriate geographic scale, that is informed by the MN decision making context, and that involves people knowledgeable of the ecology and decision making context of Minnesota. First, a risk assessment with the correct geographic scale would provide the specificity necessary to help identify which parts of Minnesota are most at risk and what adverse effects are most likely in different parts of the state. Second, people involved with the MN decision making context, such as state and federal agency personnel and local stakeholders, should be involved in the risk assessment scoping process to determine, for example, which watersheds and potential adverse effects are most important to study. Third, there is a need to involve people in the risk assessment with the right expertise to assess the risks for particular watersheds within Minnesota. This local expertise is key to being able to apply the findings from other areas impacted by bigheaded carps to the Minnesota context. A risk assessment focused on Minnesota can provide the level of detail and nuance to be most useful for the local decision making context.

2 Methodology

The methodology for this risk assessment followed a deliberative approach (NRC 1996) and contained three major steps. First, the specific scope of the risk assessment was determined by state agency personnel and local stakeholders. Second, a two-day expert workshop was held to characterize the risk to Minnesota from bigheaded carps. Finally, project researchers and a select group of workshop participants created this report that summarizes the outcomes from the workshop.

2.1 Defining scope

Initial informational interviews and project research (Kokotovich and Andow 2015; Kokotovich and Andow 2016) revealed one overarching goal and two objectives to guide the risk assessment. The overarching goal was to characterize the risks from bigheaded carps to Minnesota to inform management and research. The two objectives for the risk assessment were: 1) determine what areas of the state are most at risk; and 2) determine which potential adverse effects are most likely to result from an invasion and their level of consequence. Given the constraints of this project, it was not possible to assess all watersheds of the state and all potential adverse effects. Because of this, state agency personnel and stakeholders were engaged to help determine two foundational parts of the scope: the watersheds and potential adverse effects to be studied. MNDNR personnel and stakeholders were asked to help define the scope given their knowledge of the state's water resources and the current bigheaded carps decision making context.

An important assumption of this risk assessment involves its focus on the establishment and effects of bigheaded carp, and not on their spread. Classically, the assessment of invasive species risk involves two steps, exposure analysis and effects analysis. Exposure analysis includes estimating the likelihood of introduction, establishment and spread, while effects analysis includes estimating the likelihood and severity of the ecological, economic, or social consequences from that exposure (Anderson et al. 2004). This risk assessment focuses on characterizing the likelihood of establishment and the consequence of resulting effects, assuming bigheaded carps arrive in each watershed. Work has been conducted to understand the spread potential (MNDNR 2013b), and research and management continue to help slow the spread (Zielinski & Sorensen 2016; Kennedy 2016). Ideally, management actions will be successful in slowing or stopping the spread of bigheaded carps into the state. However, an understanding of whether and how bigheaded carps will negatively impact watersheds if they do arrive can help prioritize management, determine what collateral damage from management actions are justified, and help inform societal expectations on bigheaded carps.

The process to select the potential adverse effects – i.e., potential consequences from bigheaded carps in need of evaluation – for the risk assessment had two parts. First, 5 focus groups were held to create a list of all potential adverse effects, 3 with personnel from the MNDNR and 2 with stakeholders involved with bigheaded carps in Minnesota. Focus group participants created a list of all potential adverse effects that could result from the establishment of Asian carp in Minnesota (Kokotovich and Andow 2015). Second, in advance of the risk assessment workshop, an online survey was conducted to decide which potential adverse effects were most important to study. The survey was conducted with 30 people who were either taking part in the risk assessment workshop or had participated in one of the focus groups. From these survey findings, four potential adverse effects were identified: decrease in non-game fish populations, decrease in game fish populations, reduction in species diversity and ecosystem resilience, and decrease in recreation quality due to the silver carp jumping hazard. In addition to being highly ranked individually, these potential adverse effects are consequential to other highly valued aspects of Minnesota's waterways: 1) overall ecological health, 2) public attitudes towards waterways, and 3) opportunities for, safety of, and quality of recreational boating and fishing.

The watersheds were chosen to represent a diversity of basins and river types, to be relevant to the state's current decision making context, and, when possible, to be worst-case scenarios – watersheds in each basin that are likely to be most favorable to bigheaded carps. Minnesota has eight major watersheds that drain the state's waters and the Minnesota River, St. Croix River, Red River, and Great Lakes basins were prioritized for this project. To help select the specific watershed within these basins, a ranking process based on measurable variables was used to select the watersheds that were most likely to be favorable to bigheaded carps. Factors generally seen as correlating to establishment and effect that were used in this estimation included: perennial cover; fish species richness; phosphorus risk; and aquatic disruptions/dams. The four watersheds selected to be the focus for this risk assessment were: Sand Hill River Watershed (HUC 09020301), Nemadji River Watershed (HUC 04010301), Lower St. Croix River Watershed (HUC 07030005), and Minnesota River - Mankato Watershed (HUC 07020007) (Figure 2-1). For the purposes of this report we will sometimes shorten the names of these watersheds to, for example, St. Croix River and Minnesota River.

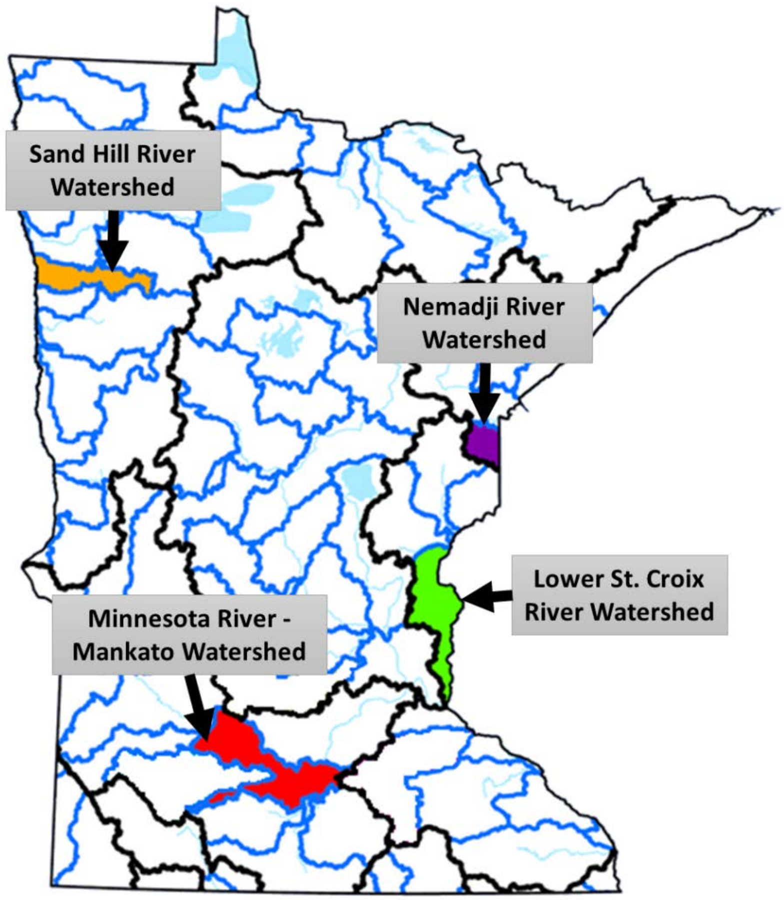


Figure 2-1. Map of watersheds selected for risk assessment.

2.2 Risk Assessment Workshop

On March 8th and 9th 2016 a workshop was held at the University of Minnesota to conduct the main parts of the risk assessment. Twenty-three experts on bigheaded carps and Minnesota's waterways participated in the risk assessment workshop, including individuals from 5 federal agencies, 5 academic institutions, the MNDNR, natural resource agencies from 2 other states, and a stakeholder group. The attendees were selected to ensure the needed expertise on both bigheaded carps and Minnesota's waterways was present to deliberate on and characterize the risk. A mixture of small and large group discussions was used to characterize the overall risk, which was characterized in three steps: the likelihood that bigheaded carps would establish in each watershed, the resulting abundance of bigheaded carps in each watershed, and the severity of adverse effects caused by the resulting abundance.

2.2.1 Workshop day 1: Likelihood of establishment and resulting abundance

Day one started with a large group discussion to create a list of biotic and abiotic factors that influence whether bigheaded carps establish in a particular watershed and their resulting abundance (see Section 3). This large group discussion helped identify important principles to inform the establishment and abundance characterizations that would be taking place during the remainder of the first day. Each participant was then assigned to one of four small groups, and each group was associated with one of the selected watersheds. Each small group had a graduate student facilitator who was familiar with the workshop process and had expertise in fisheries or risk assessment. Selected participants from the MNDNR began the small group session by describing the watershed and its relevant characteristics. The facilitators then guided each group through their two objectives for the first day.

First, each group characterized the likelihood that bigheaded carps would establish in their particular watershed, given arrival. Specifically, they estimated the likelihood that bigheaded carps would establish in their watershed within 10 years of their arrival, assuming they arrive with enough individuals to where establishment would be possible under ideal conditions. Also, it was assumed that the current management context would not change. Groups were not taking into account how likely it is that bigheaded carps arrive in the watershed, but were only focusing on what the risk would be if they arrive. The goal was to identify the watersheds that are most at risk if bigheaded carps arrive. Each participant used 5-point scales to characterize the likelihood of establishment (Table 2-1) and the certainty of their characterization (Table 2-2). These scales were adapted from previous Asian carp risk assessments (Cudmore et al. 2012).

Table 2-1. Establishment likelihood scale and percentages range.

Establishment likelihood scale	**Establishment likelihood range (%)**
Very unlikely	0 – 5%
Low	5 – 40%
Moderate	40 – 60%
High	60 – 95%
Very likely	95 – 100%

Table 2-2. Certainty scale and definition.

Certainty Scale	**Definition of scale**
Very low	±90%; E.g., little to no information to guide assessment
Low	±70%; E.g., based on ecological principles, life histories of similar species, or experiments
Moderate	±50%; E.g., inference from knowledge of species
High	±30%; E.g., primarily peer reviewed information
Very high	±10%; E.g., extensive, peer-reviewed information

After characterizing the likelihood of bigheaded carp establishment, each small group characterized the resulting abundance of bigheaded carps in their watershed, assuming they were to establish. Five-point scales were used to characterize the resulting abundance (Table

2-3) and the certainty of their characterization (Table 2-2). This abundance level was used in Day 2 to characterize how severe the adverse effects would be. For example, a very high resulting abundance of bigheaded carps would be expected to lead to more severe adverse effects than a very low resulting abundance.

Table 2-3. Resulting abundance scale and definition.

Resulting abundance scale	**Definition of scale**
Very low	Few individuals, <1% of total fish biomass
Low	1 – 5% of total fish biomass
Moderate	5 – 25% of total fish biomass
High	25 – 60% of total fish biomass
Very high	>60% of total fish biomass

With each of these characterizations, participants also characterized their justifications, areas of disagreement, and research needs. The small group did not need to come to consensus on the characterizations; in fact, they made each characterization individually. Participants were encouraged to explore and record any differences in reasoning that led to divergent characterizations. The small group format allowed groups to become familiar with their watershed and to discuss issues in much more detail than would be possible if the large group addressed each watershed.

After the small groups made their characterizations, all participants reassembled for the final large group discussion of Day 1. This discussion consisted of three parts that were repeated for each small group: 1) the small group presented their characterizations of establishment likelihood and resulting abundance for their watershed and summarized their justifications; 2) other workshop participants asked questions and raised any concerns about the characterizations to the small group; 3) all workshop participants then characterized the establishment likelihood and abundance for the watershed in question based on the small group's report and subsequent discussion. These characterizations provided by all workshop participants based on the recommendations of the small group were the ones that informed the subsequent overall characterization of risk. Both the small group and large group characterizations were recorded and are presented in each of the watershed sections within this report.

2.2.2 Workshop day 2: Adverse effects

Day 2 started with a large group discussion where participants created a list of potential risk pathways that could lead from bigheaded carps to the adverse effects being analyzed (see Section 3). Participants also discussed the key biotic and abiotic factors that influence whether an adverse effect is likely to take place as a result of a particular risk pathway. The small groups from Day 1 met again, this time to discuss and characterize each potential adverse effect for each watershed. Small groups began by characterizing the potential impact on plankton within the watershed, as that was deemed an important intermediary step for some of the other potential adverse effects. For the potential adverse effects, participants used a 5-point scale to describe the consequence level (Negligible; Low; Moderate; High; Extreme) and certainty (Table 2-2) of their characterization. Precise definitions were provided for the consequence scale specific to each adverse effect (see Appendix B). Small groups characterized the severity of an adverse effect based on the likely resulting abundance of bigheaded carps in that watershed. These resulting abundances were the ones determined by the large group characterization on Day 1. Small groups characterized the adverse effects twice, once for the most likely abundance and a second time for the second most likely abundance. Due to time limitations, however, the large group characterizations were only conducted for the most likely resulting abundance. The difference between a small group's adverse effects characterization for the most likely and second most likely resulting abundances was used to understand how the overall characterization of risk would change if the second most likely resulting abundance was achieved (Section 8.3). The process for the large group characterizations of adverse effects was the same as Day 1: small group report back, discussion, and characterization of each adverse effect for the particular watershed. The characterizations of the adverse effects are presented in each subsequent watershed section within this report.

2.3 Overall Risk Characterization

At the end of the workshop, participants had characterized the likelihood that bigheaded carps would establish in each of the four watersheds and the likely severity of the resulting adverse effects. In order to determine the overall risk for each watershed, the characterizations of establishment and adverse effects needed to be combined. These overall risk characterizations for each watershed are presented in Section 8. They were arrived at by turning the establishment characterizations from the workshop into a single percentage for each watershed and combining it with the adverse effect characterizations. The likelihood of establishment for each watershed was turned into a single percentage using the following calculation: First, the individual likelihood characterizations were weighted based on the certainty scores provided by the participants. The weighting factors were assigned as $\frac{\mathbf{1}}{\boldsymbol{Certainty}\,\%}$ as shown in Table 2-4.

Table 2-4. Weighting factor provided to establishment likelihood

Certainty Score	Weighting factor provided to establishment likelihood
Very High (±10%)	1/.1 = 10
High (±30%)	1/.3 = 3.33
Moderate (±50%)	1/.5 = 2
Low (±70%)	1/.7 = 1.43
Very Low (±90)	1/.9 = 1.11

Second, the overall likelihood of establishment was then calculated using the following equations, where ERHi = high value of the establishment likelihood range for category i, and ERLi = the low value of the establishment likelihood range for category i:

$$Overall\ Likelihood\ of\ Establishment$$

$$= \sum_{i=Very\ unlikely}^{Very\ likely} \frac{Sum\ of\ weighted\ scores\ in\ category\ i}{Sum\ of\ weighted\ scores\ across\ all\ categories} * \frac{ERHi + ERLi}{2}$$

An example calculation for the Sand Hill River is provided in Table 2-5.

The weighting factor allowed us to incorporate the certainty expressed by the participants into the establishment scores, thereby incorporating the certainty into the overall characterization of risk. Participants were not told that their certainty scores would be used as a weighting factor, so there was no motivation to change their certainty scores to influence the weighting of their characterization. Given that most certainty scores ranged between Very Low and Moderate, this weighting factor did not have a significant effect on the overall likelihood of establishment for each watershed. The overall likelihood of establishment calculated with and without the weighting factor differed by less than 2% for each watershed.

The overall risk characterization score was calculated as the Probability of Consequence Level Given Arrival and combined the overall establishment likelihood with the adverse effect characterizations. An example of this calculation for the Minnesota River is shown in Table 2-6.

This means that if bigheaded carps were to arrive in the Minnesota River (with enough individuals to make establishment possible), participants thought there was a 70% chance that they would establish. If they were to establish, 47.6% of participants thought bigheaded carps would have a low impact on Bigmouth Buffalo and 52.4% of participants thought bigheaded carps would have a moderate impact on Bigmouth Buffalo. So the probability of a low consequence given arrival is (.476)(.70) = .33 or 33% and the probability of a moderate consequence given arrival is (.524)(.70) = .37 or 37%. The remaining probability equals the estimated likelihood that bigheaded carps would not establish in the Minnesota River watershed (30%).

Table 2-5. Calculation for overall establishment percentage for the Sand Hill watershed. Initial = Number of participants who characterized the likelihood and certainty. W.S. = Weighted scores, based on the weighting factor in Table 2-4.

		Likelihood of establishment							
		Very unlikely (.00-.05)		Low (.05-.40)		Moderate (.40-.60)		High (.60-.95)	Very likely (.95-1.00)
		Initial	W.S.	Initial	W.S.	Initial	W.S.		
Certainty of assessment	5 – Very high certainty								
	4 – High certainty			4	13.33				
	3 – Moderate certainty	2	4	9	18				
	2 – Low certainty	1	1.43	3	4.29	1	1.43		
	1 – Very low certainty					1	1.11		
Overall Likelihood of Establishment Calculation:									
A	Calculate proportion of weighted scores in each likelihood category	.12 = (4+1.43)/43.59		.82 = (13.33+18+4.29)/ 43.59		.06 = (1.43+1.11)/ 43.59			
B	Calculate midpoint of each likelihood range	.025 = (.05+.00)/2		.225 = (.40+.05)/2		.5 = (.60+.40)/2		.775	.975
C	$\sum A * B$	(.12*.025)+(.82*.225)+(.06*.5) = .22 = Overall Likelihood of Establishment							

Table 2-6. Calculation used for overall risk characterization score.

MN River Game fish: Bigmouth Buffalo – Adverse effect characterizations	Negligible	Low	Moderate	High	Extreme
		.476	.524		
MN River – Establishment Likelihood for MN River	.70				
Overall risk characterization = Probability of consequence level given arrival	Negligible	Low	Moderate	High	Extreme
		.33 = (.476)(.70)	.37 = (.524)(.70)		

2.4 Risk Assessment Report

The writing of this risk assessment report had multiple steps and involved project researchers and workshop participants. At the workshop itself individual workshop participants volunteered to help with the writing of this report (Appendix A). This group of authors included representatives from each watershed/small group. Notes from the small group workshop sessions were provided to the authors from each group. The authors from each watershed used those notes to draft the section describing the characterizations of their watershed. This included the following sub-sections: an introduction to the watershed; the final characterizations (i.e., establishment likelihood, resulting abundance, adverse effects); justifications for the characterizations; and research needs. In addition to these sections on the watersheds, certain workshop participants contributed to other sections of the report, mainly the introduction. After the report was compiled, it was provided to all workshop participants for review. Comments from the workshop participant reviews were incorporated into the March 15th, 2017 draft version of the report. This March 15th draft of the report was then presented to state and federal agency officials, representatives from local units of government, stakeholders, and members of the public at the March 2017 “Risk-based management for bigheaded carps workshop” held at the University of Minnesota (for outcomes from the meeting, see Appendix C). This 2017 workshop provided an opportunity to discuss the findings and management implications of the risk assessment. Feedback from this workshop helped inform this final version of the risk assessment report.

Project researchers (Adam Kokotovich & David Andow) assembled and revised the different sections of the report and wrote the Executive Summary, Methodology, Overall Risk Characterization, Discussion, and Appendices. The overall conclusions in this report are based on the findings that emerged from the risk assessment, but represent the views of the project researchers.

3 Possible biotic and abiotic factors and pathways to adverse effects

During the workshop, participants spent parts of each morning in a large group discussion addressing pertinent issues for each day's objectives. On Day 1 participants produced a list of possible biotic and abiotic factors impacting establishment and abundance (Table 3-1). On Day 2 they produced a list of possible risk pathways to potential adverse effects and the factors affecting them (Table 3-2).

Table 3-1. Biotic and abiotic factors that may possibly influence the likelihood of establishment and resulting abundance of bigheaded carps (BC).

Factors	*Description*

Suitable flow and thermal conditions	• Hydrology: Flow and depth of system – habitat suitability
	  o Fragmentation & Impoundment – Needed length of suitable flow for successful reproduction
	  o River discharge during and immediately after peak spawning (during suitable thermal window) – temporal flow suitability
	  o Existence of sustained flood pulse
	• Thermal regimes (climate suitability)—habitat suitability
	  o Timing of necessary thermal conditions
	  o Thermal window contracts moving northward
	  o Climate change may influence this
	• Frequency of suitable conditions

Morphological alterations	• Channelization and channel sinuosity o Channel sinuosity and lack of channelization could improve availability of backwater habitat

Water quality	• Water clarity
	  o Turbidity (organic & inorganic) & Color (e.g. tannins) – Improves larval survival
	  o Clarity for feeding/adult habitat
	• Dissolved oxygen
	• Extent to which waterbody is impaired
	  o Ability of BC to exploit impaired waterbodies

Conditions for larval development	• Conditions that prevent settling of eggs • Turbid conditions to prevent predation of larvae
Habitat diversity for use by various BC life stages	• Backwater habitat for adults and young of year • Timing of connectivity between backwater habitat and main channel • Alternate flow sources/mixing
Adequate food source	• Plankton • Prevalence of cyanobacteria • Nutrient concentration
BC adult population	• Density (positive effects on establishment, could have density dependent effects on abundance) • Age composition • Condition

Possible changes to BC	• Hybridization • Adaptation
Existing fish community and impacts on various life stages of BC	• Impacted community vs. intact community • Predation/predator community and spatial distribution • Alternate prey community structure • Competition • Effects from fragmentation on native community
Other possible predation	• Bird community
Current management of fisheries	• Commercial fishing harvest rates (downstream) for BC and other fish that could serve as competitors • Flow management

Table 3-2. Potential risk pathways from bigheaded carps to adverse effects and the factors affecting them. ↑ = Increase in; → = Leads to.

<table>
<tr><td>↑BC→Plankton (reduction in abundance or quality)→Shift in native fish feeding pathways to less preferred foods→Game & non-game fish (reduction in abundance or quality)
Emerald shiner changed to benthic feeding</td></tr>
<tr><td>↑BC→Plankton (reduction in abundance or quality)→Planktivores (reduction in abundance or quality)→Piscivores (reduction in abundance or quality)→ Game & non-game fish (reduction in abundance or quality of both planktivores and piscivores)

Factors

Planktivores could be adults or juveniles
Competition with and predation on larval fish
Bigger effect in lakes/pools/backwaters where plankton are more likely to be affected
Decrease in omega-3 levels in pelagic fish

Comments on specific species

Walleye

EcoSim modelling on Lake Erie
Cladocerans important for larval walleye
Emerald shiner loss

Paddlefish (nongame)

Eating BC larvae?
Loss of plankton forage

Crappies in Mississippi River could eat juvenile BC

</td></tr>
<tr><td>↑BC (taking up physical space)→Displacement of native fish →Game & non-game fish (reduction in abundance or quality)
Limited spawning and nursery habitat</td></tr>
<tr><td>↑BC (silver carp)→Jumping hazard→ Impacts on recreation

At 40% CPUE (~60% biomass) boat electrofishing in James River saw jumping

Might differ for larger river (less effect on silver carp, less likely to jump)
Patchiness—more concentrated areas (high biomass category) have jumping; backwaters specifically

Peoria (75% biomass) saw extreme impacts
</td></tr>
</table>

<table>
<tr><td>

At low abundances of silver carp there are occasional jumpers
Boat traffic levels influence detection and effects
In the Iowa Lakes area, there are silver carp and lots of boat traffic, but no reported jumping
Harder to get them to jump in deep water, more likely to jump in shallow water
In 1-1.5 m, silver carp jump even with non-motorized boats (Wabash, low abundance)
In IL River, silver carp can jump even without boat noise (could be from other threat)
Impacts on fishing opportunities (Positive? Negative?)
Loss of fishing tournaments
Bass in IL River doing well in absence of fishing
Risk/ hassle for anglers

</td></tr>
<tr><td>
↑BC→Plankton (reduction in abundance or quality)→Planktivores (reduction in abundance or quality)→Piscivores (reduction in abundance or quality)→ Species that depend on plankton and fish (reduction in abundance or quality)→Species diversity/resilience reduction

Forcing native species into smaller feeding niches
Less able to cope with additional stressors, e.g.: fragmentation; other AIS; habitat loss
Bald eagles, river otters, pelicans, other terrestrial piscivores
Cormorant biomass increased in EcoSim model with BC
Increased IL River use by pelicans
Loss of bald eagle prey
Impacts on mollusk

</td></tr>
<tr><td>
↑BC→Plankton (reduction in abundance or quality of crustacean zooplankton)→Increased light penetration→Chlorophyll a increase → Game & non-game fish (reduction in abundance or quality)

Fish impacts unknown
Changes in rotifers/phytoplankton

</td></tr>
<tr><td>
↑BC→Bioturbation from bottom feeding→Algae bloom → Decreased oxygen → Game & non-game fish (reduction in abundance or quality)

Only when very low abundance of food in water column

</td></tr>
</table>

4 Minnesota River

4.1 Introduction to watershed

The Minnesota River has a total length of 668 kilometers from the headwaters of the 115 km-long Little Minnesota River along the Coteau des Prairies, to the 42 km-long Big Stone Lake, before 511 km of the Minnesota River proper to its confluence with the Mississippi River in the Twin Cities. The Minnesota River Valley was carved by the much larger Glacial River Warren at the end of the last ice age when it was the primary outlet of Glacial Lake Agassiz.

The river's 44,800 km^2 watershed was primarily tallgrass prairie prior to European settlement but is now dominated by row-crop agriculture. Extensive wetland drainage and stream channelization has resulted in increased runoff and channel erosion (Schottler et al. 2013). The Minnesota River now carries the largest sediment load to the Mississippi River of any tributary north of Illinois (Lenhart et al. 2013) and is a major contributor of phosphorous and nitrates to downstream waters including Lake Pepin and the anoxic Mississippi Gulf Dead Zone.

Despite water quality impairments and habitat degradation, free-flowing reaches of the Minnesota River and its tributaries have diverse fish assemblages. The lower 386 kilometers of the Minnesota, from the Mississippi confluence to Granite Falls Dam, represents the longest dam-free river reach in Minnesota. At Granite Falls a 6 meter high hydropower dam creates a barrier to fish passage. Forty of the 97 native species documented in the Minnesota River watershed are absent upstream of the Granite Falls Dam. The lake sturgeon (Acipenser fulvescens), Minnesota's largest fish species, was historically found to the river's headwaters in Big Stone Lake but now ends its range at the Granite Falls dam. Following the 2013 removal of the Minnesota Falls dam (5.6 km downstream of Granite Falls), 15 native fish species have returned that had not been found upstream of that dam. These included rare (SGCN - Species in greatest conservation need) species like paddlefish (Polyodon spathula), lake sturgeon, blue sucker (Cycleptus elongates), and black buffalo (Ictiobus niger), as well as important game species like flathead catfish (Pylodictis olivaris) and sauger (Sander canadensis). Similar recolonization of native fishes has followed removal of dams on Minnesota River tributaries like the Pomme de Terre, Cottonwood, and Lac qui Parle rivers.

The species richness of native mussels has declined significantly in the Minnesota River watershed. Of the 43 native mussels historically found in the Minnesota River watershed, 20 species have been extirpated from the basin (Sietman 2007). Water quality impairments, sedimentation, zebra mussels, fragmentation and other factors can adversely affect native mussel populations. Nationally, 22 of 26 extinctions of native mussels have been attributed to dam construction (Haag 2009). Skipjack herring, (Alosa chyrsochloris) the sole host of ebonyshell (Fusconaia ebeba) and elephant ear mussel (Elliptio crassidens), were also found to Big Stone Lake but were extirpated from the upstream Mississippi watershed shortly after construction of Lock and Dam 19 near Keokuk, Iowa (Tucker and Theiling 1999; Fuller 1980; Fuller 1974). This subsequently led to functional extirpation of the two mussel species. Ebonyshell mussels were historically the most abundant mussel in the Upper Mississippi and Lower Minnesota Rivers. Conversely, dam removals have resulted in returns of native mussels following the return of host fish species. Removal of the Appleton Milldam on the Pomme de Terre river resulted in the recolonization of three native mussels that had been extirpated upstream of the dam.

Several characteristics of the Minnesota River are specifically relevant to bigheaded carp life history, habitat requirements, and interrelationships with other fish species. Relevant attributes of bigheaded carps include:

1) Juvenile bigheaded carp likely require backwater habitat, particularly those that have periodic anoxic conditions and low predator abundance.
2) Bigheaded carps spawn in flowing water at warmer water temperatures, usually when temperatures reach 20^{o} C and when current velocities exceed 15-25 cm/s.
3) Bigheaded carps have plantivorous feeding habits including the ability to consume and digest cyanobacteria.
4) Young bigheaded carps are highly susceptible to predation.

The 175 km reach of the Minnesota River between Redwood Falls and St. Peter drops 26 meters in elevation for an average slope of 0.0015 percent. The reach has a sinuosity of 1.5 with numerous oxbow backwaters. The Minnesota River has increased in width by 52% and shortened by 7% since 1938 and by 12% since 1854 due to hydrologic changes (Lenhart et al. 2013). The decline in sinuosity of the Minnesota has resulted in the addition of new backwaters due to meander cutoffs, but bed incision resulting from increased slope or increases in fine sediment supply can isolate or fill these backwaters. A few bedrock outcrops and riffles with coarse substrates exist near Redwood Falls but most of the reach has a sand or silt bed.

River flows and their seasonal variations are critical in defining available habitat as well as species interactions (Aadland 1993). Water levels of the Minnesota River at Mankato have nearly 4 meters of average annual fluctuation and low flows dewater a significant proportion of the river channel (Table 4-1; Figure 4-1; Figure 4-2). As flows fall, backwaters drain and many are disconnected from the main channel. This contrasts with impounded rivers like the Illinois and Upper Mississippi which are held at a normal pool elevation during low flows maintaining static water levels and lateral connectivity to many of the backwaters.

Table 4-1. Flow statistics for the Minnesota River at Mankato for the period 1902 to 2016 (USGS gage 05325000). Flood recurrence intervals are Log Pearson Type II regressions for annual peak flow data 1903 through 2015).

Annual mean flow	110 m^3/s
Record peak flow	2625 m^3/s in 1965, est. 3115 m^3/s in 1881
Lowest daily mean flow	0.9 m^3/s in 1934
Record peak stage	9.2 m
Minimum stage (gage control)	Near zero gage depth tied to riverbed
Annual minimum median daily flow	10.6 m^3/s
Annual maximum median daily flow	196 m^3/s
1.5 year flood (instantaneous peak)	325 m^3/s
2-year flood (instantaneous peak)	504 m^3/s
10-year flood (instantaneous peak)	1368 m^3/s
100-year flood (instantaneous peak)	2717 m^3/s

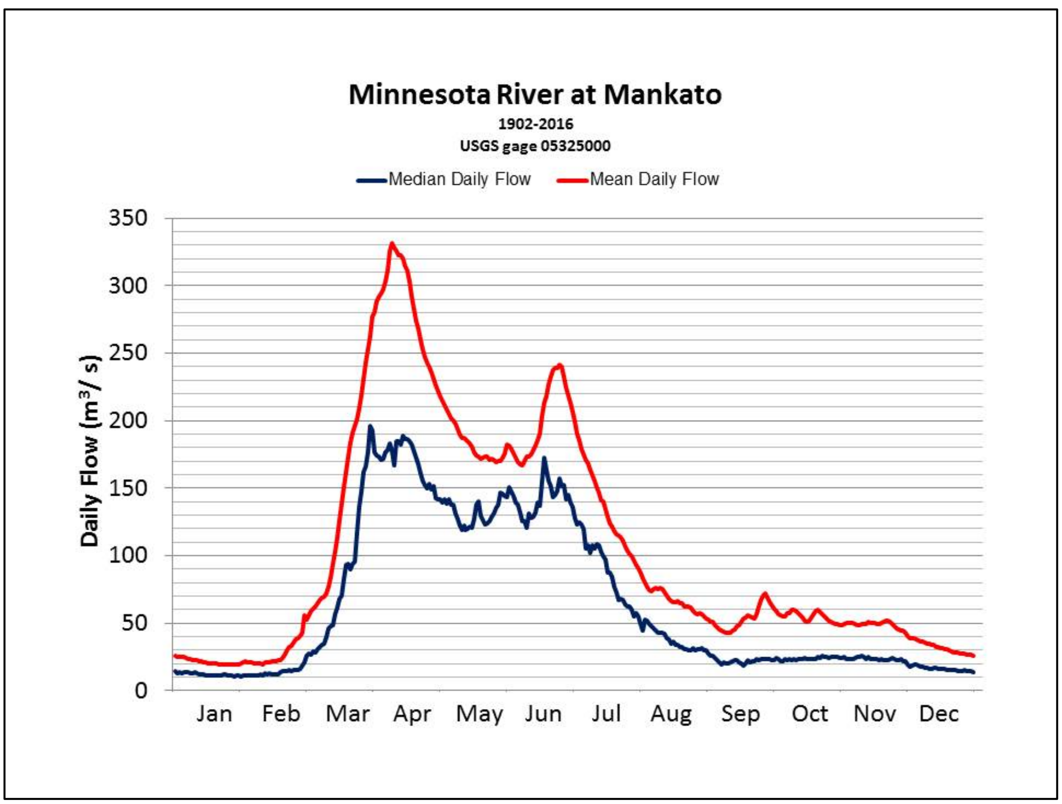


Figure 4-1. Median and mean daily flows over the period of record (1902-2016) for the Minnesota River at Mankato (USGS gage 05325000).

Figure 4-2. The Minnesota River downstream of Mankato near the median peak flow and the median annual minimum daily flow. The median peak flow shown in top photo (487 m^3/s - June 23, 2010) and the median annual minimum daily flow shown in bottom photo (11 m^3/s – November 5, 2003). Note differences in wetted area, backwater area and connectivity at the two flows.

4.2 Likelihood of establishment

4.2.1 Justifications

The entire small group characterized the likelihood of establishment in the Minnesota as high (Table 4-2), and the large group characterizations largely aligned (Table 4-3). The justification for this characterization included that the Minnesota has characteristics that would support establishment including extensive oxbow backwaters, suitable temperature regimes, eutrophic water quality, and adequate size. The small group concluded that the climate of the Minnesota River would support establishment since silver carp colonized and reproduced in the James River upstream to North Dakota at latitudes north of the Minnesota River. In addition, since bigheaded carps are long-lived fish, they do not need to successfully reproduce every year to maintain a population.

Key areas of uncertainty stemmed from the fact that to date, only one grass carp, one bighead carp and no silver carp have been documented in the Minnesota despite direct connections to the Mississippi River. Access is limited during low flows by the upper locks and dams but the Tainter gates of these dams are open during floods which allows fish passage. The lack of recruitment of grass carp (Ctenopharyngodon idella) that have been present in low numbers in northern parts of the Mississippi River for a longer period of time may suggest unfavorable conditions for bigheaded carps due to similar spawning habits. Although it is unclear whether the scarcity of bigheaded carps suggests that the watershed has limiting factors or if establishment will simply take more time, the group felt that is was more likely the latter.

4.2.2 Final characterizations

Table 4-2. MN River Likelihood of Establishment – Small Group Final Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)				J, D, F	
	Low certainty (+/- 70%)				A, C, E	
	Very low certainty (+/- 90%)					

Table 4-3. MN River Likelihood of Establishment – Large Group Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)			2		
	Moderate certainty (+/- 50%)			1	11	
	Low certainty (+/- 70%)			1	5	
	Very low certainty (+/- 90%)					

4.2.3 Research needs

Research needs discussed included: 1) Total biomass of bigheaded carps and native species in impounded and free-flowing rivers; 2) Information on the limnology, water quality (including dissolved oxygen), seasonal connectivity, coverage and relationships to flow, fish assemblages and resident predators of backwaters; 3) Changes in growth rates where high biomass exists and long-term effects on populations; 4) Native predators and fish communities, limnology, and influence of hypoxia in backwaters; and 5) Hypoxia tolerance of bigheaded carps at each life stage and during winter ice cover.

4.3 Resulting abundance

4.3.1 Justifications

The small group discussion reflected that it is difficult to predict the resulting abundance of bigheaded carps if they become established in the Minnesota River. This is because the resulting abundance would be dependent on a number of abiotic and biotic factors including seasonal variations in flow, temperature regimes and associated growth rates, water chemistry and dissolved oxygen, winter mortality, suitability of habitat for the suite of life history stages, predation mortality from other fish species and piscivorous birds, competition by native planktivores, and disease-related mortality. After discussing these factors, the small group's characterization of resulting abundance was moderate (5/6) with low or very low certainty, while one member chose high resulting abundance (Table 4-4). The large group was split between moderate (12/20) and high (8/20) resulting abundance (Table 4-5).

Factors influencing this characterization included that during low flow conditions, fish can become concentrated at high densities in remaining pools. While this may lead to higher local abundance, it may also affect predation mortality, interspecific and intraspecific competition, disease transmission, and stress.

Since juvenile bigheaded carps depend heavily on backwater habitat, the dynamics of these backwaters are important. Juvenile silver and bighead carp are able to survive low dissolved oxygen due to a vascularized lower jaw extension that enables respiration at the water surface (Adamek and Groch 1993). This adaptation facilitates predator avoidance in anoxic backwaters where less tolerant predators may not exist. Hypoxia is common in backwaters of agricultural rivers (Shields et al. 2011). During drought conditions, hypoxia in pools in the Minnesota River has also been observed.

Although water quality data in backwater habitats of the Minnesota River is limited, early observations have indicated the use of backwaters by a variety of predatory fish species. Most shallow eutrophic water bodies in Minnesota are also vulnerable to winter hypoxia. Under these conditions, respiratory adaptations of juvenile bigheaded carps to hypoxia may not apply due to ice cover. During low flows, fish would be forced out of dewatered backwaters and concentrated in the remaining wet parts of the main channel. This may influence predation mortality of all life stages of bigheaded carps.

For predators to control fish populations, they must be abundant enough to cause significant mortality. Predation of adult silver carp estimated at up to 2 kg by increasing numbers of white pelicans (Pelecanus erythrorhynchos) has been observed on the Illinois River by one of the small group members. Marsh Lake in the upper Minnesota River has the largest white pelican rookery in North America and could help to control bigheaded carps in the Minnesota River (Wires et al. 2005).

The Minnesota River is noted for its flathead catfish, a species that can reach weights of over 23 kg and is capable of consuming individual fish up to 30% of their own body weight (Davis 1985). Flathead catfish may be a significant predator on bigheaded carps, as they have been shown to be an effective predator on common carp (Cyprinus carpio) (Davis 1985). While Flathead catfish are found in the Illinois River where bigheaded carps are very abundant, they are heavily exploited and the Illinois River has no harvest limit on flathead catfish for either commercial or recreational fisheries. The Minnesota River has no commercial harvest on flatheads and a limit of two fish for recreational harvest with only one fish over 24 inches.

Small-bodied fish species may also be important predators on bigheaded carps by feeding on eggs, larvae, and juveniles (Johnson and Dropkin 1992). In the Susquehanna River, spotfin shiners are an important predator on American shad (Alosa sapidissma) eggs and larvae. Like the bigheaded carps, American shad are pelagic spawners. Spotfin shiners are one of the most abundant cyprinids in the Minnesota River and its tributaries.

There were disagreements about the role of impoundments, suspended sediment, available plankton resources and predators in determining the abundance of bigheaded carps.

4.3.2 *Final characterizations*

Table 4-4. Resulting abundance – Small Group Final Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)				J	
	Low certainty (+/- 70%)			D, F, E		
	Very low certainty (+/- 90%)			C, A		

Table 4-5. Resulting abundance – Large Group Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)			4	4	
	Low certainty (+/- 70%)			6	4	
	Very low certainty (+/- 90%)			2		

4.3.3 *Research Needs*

Research needs discussed included: 1) the role of refugia from predators on existing bigheaded carp populations and their abundance; 2) relationships of river stage to backwater connectivity and coverage area; 3) effects of latitude, climate and interactions of climate and habitat on the abundance of bigheaded carps; and 4) the timing and duration of backwater connectivity as well as coverage area relationships to river stage and the hydrology of the Minnesota River.

4.4 Adverse Effects

During the characterization of potential adverse effects, the small group characterized the consequence of each adverse effect for the likely abundance of bigheaded carps that was determined in the previous step. The small group also characterized the consequence resulting from the second most likely abundance of bigheaded carps. For the Minnesota River small group, the first abundance was “Moderate” and the second abundance was “High”. In the tables below, the characterization for the “Moderate” abundance is noted with “A”, “B”, “C”, etc. whereas the characterization for the “High” abundance is noted with “A_H”, “B_H”, “C_H”. The letters represent different individuals within the small group.

4.4.1 Change in plankton

4.4.1.1 Justifications

The small group acknowledged that observed shifts in plankton species composition and size structure are typical where bigheaded carps have become established and abundant. Effects on phytoplankton have been variable but often associated with smaller algal fragments. Xie and Lui (2001) found increases in water clarity and cessation of blooms due to grazing by bigheaded carps on cyanobacteria while Carruthers (1986) found no significant effect on cyanobacteria blooms or water clarity and Lieberman (1996) found increased turbidity in a pond stocked with silver and bighead carp. A number of studies have shown a decline in cladocerans and a shift to a smaller size structure of zooplankton (Radke 2002; Cooke et al. 2009; Garvey et al. 2012) with one study showing an opposite shift to a larger size structure in cyanobacteria dominated subtropical Asian lakes (Zhang et al. 2013). To capture the nuance within the changes to plankton community, the small group characterized both the change in total biomass of plankton and the consequence from the change in plankton community composition.

4.4.1.2 Final characterizations

Table 4-6. MN River Change in total biomass of plankton – Small group characterizations.

		Change in total biomass of plankton						
		Large increase	Moderate increase	Small increase	No change	Small decrease	Moderate decrease	Large decrease
Certainty of assessment	Very high certainty (+/- 10%)							
	High certainty (+/- 30%)							
	Moderate certainty (+/- 50%)			C C_H	A, J A_H, J_H			
	Low certainty (+/- 70%)				F F_H			
	Very low certainty (+/- 90%)				E E_H			

Table 4-7. MN River Change in plankton community composition – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				C	
	Moderate certainty (+/- 50%)				E, F, J E_H, J_H	C_H
	Low certainty (+/- 70%)				A	A_H, F_H
	Very low certainty (+/- 90%)					

4.4.2 Consequence for non-game fish

4.4.2.1 Justifications

The small group chose spotfin shiner and bigmouth buffalo as example nongame species to assess potential effects of bigheaded carps due to their relative abundance and potential for competition and resource limitations. Bigmouth buffalo are planktivores, while spotfin shiners are invertivores.

Spotfin shiners are generalized invertivores primarily consuming insects (Dobie et al. 1956) but Becker (1983) also notes consumption of small fishes, carp eggs, plankton, and other items. Johnson and Dropkin (1992) and Johnson and Ringler (1998) found spotfin shiners to be a major predator on American shad fry in the Susquehanna River. Like the bigheaded carps, American shad are pelagic spawners. As a result, spotfin shiners may actually benefit by preying on the eggs and fry of bigheaded carps. Spotfin shiners spawn in crevices, are often associated with riffles, and prefer slow riffle habitat as both juveniles and adults (Aadland 1993; Aadland and Kuitunen 2006). The small group considered the likely adverse effect consequence level for spotfin shiners to be negligible (4/5) or low (1/5) since dietary and habitat overlap with bigheaded carps is limited (Table 4-8), and the large group also characterized the consequence level as between negligible and low (Table 4-9).

The small group considered the consequence of invasion by bigheaded carps to bigmouth buffalo to be more significant since they are planktivorous and have dietary and habitat overlap with that of bigheaded carps (Table 4-10). The large group also considered the consequence to bigmouth buffalo to be more significant than for spotfin shiner, characterizing the adverse effect consequence level between low and moderate (Table 4-11). Irons et al. (2007) found a 5% decline in condition factor for bigmouth buffalo in the Illinois River associated with increased abundance of bigheaded carps. Bigmouth buffalo consume zooplankton as well as benthic invertebrates. Bigmouth buffalo also have habitat overlap with bigheaded carps since they spawn in flooded backwaters and floodplains. As discussed above, the evaluated reach of the Minnesota River is not impounded so feeding ecology of bigmouth buffalo may be different due to differences in the density and composition of zooplankton, and feeding strategies of native fishes. Commercial harvest of bigmouth buffalo in the Minnesota River is limited to one commercial fisherman with an annual catch of 450 to 1360 kg. Bigmouth buffalo is also targeted by an unknown number of bow-fisherman.

The small group determined that the greatest potential for interaction between bigheaded carps and native fishes is for species with the greatest dietary and habitat overlap. Sampson et al. (2009) evaluated dietary overlap of bigheaded carps with 3 plantivorous fishes and determined it to be greatest for gizzard shad, less for bigmouth buffalo, and least for paddlefish. These species are the most prominent planktivores in the Minnesota River. In addition to species that are planktivorous as adults, early life stages (particularly larvae) of most fish species feed on meiofauna (invertebrates generally between 45 μm and 1 mm in size) that can include species consumed by bigheaded carps.

While dietary overlap by bigheaded carps could adversely affect growth and survival of native planktivorous species and early life stages of other fishes, available bigheaded carp eggs and fry could provide a new food source. Predation on bigheaded carp fry or juveniles by sauger and black crappie (Pomoxis nigromaculatus) was indicated by group members familiar with examples from the Illinois River. Unlike most native fish species, bigheaded carps are capable of feeding on and digesting cyanobacteria, thus tapping into a relatively unexploited resource. Juvenile channel catfish (Ictalurus punctatus) and blue catfish (Ictalurus furcatus) consumed and increased body mass when fed silver carp fecal pellets (Yallaly et al. 2015).

Several studies have shown downward trends in commercial harvest, relative abundance, or catch per unit effort for certain native fish species concurrent with increases in the abundance of bigheaded carps. However, determining mechanisms, cause, and effect is complicated by the dynamic nature of fish populations (particularly lotic species) that cycle with annual variations in hydrology, climate, harvest, and other factors. In the Illinois River, Garvey et al. (2012) found declines in standardized catches of bigmouth buffalo, white bass, freshwater drum, sauger, black crappie, and common carp concurrent with increases in bigheaded carps but these trends could not be directly attributed to bigheaded carps since the downward trends began prior to bigheaded carps establishment. For example, a sauger stocking program began in in the Illinois River in 1990 following declining abundance from the 1970s to 1990s which was prior to establishment of bigheaded carps (Heidinger and Brooks 1998). Both sauger and black crappie fisheries were reportedly doing well by group members familiar with the Illinois River.

Relative abundance trends must be evaluated with the recognition that the addition of bigheaded carps can result in large increases in total biomass that are not necessarily associated with declines in native species biomass. A controlled study by Arthur (2010) using 46 sites in Southeast Asia with paired wetlands, controls and replicates found no changes to native species richness or biomass despite a 180% increase in total biomass resulting from stocked bigheaded carps. This may be due to the unique ability of bigheaded carps to digest cyanobacteria including toxic Microcystis (Chiang 1971) which enables them to take advantage of a food resource that most native fishes cannot.

Attributing declines in native species richness associated with invasive species is complicated by concurrent declines associated with water pollution, land-use changes, overfishing and other factors (Gurevitch and Padilla 2004). This is especially true for effects of non-predatory species like bigheaded carps on native species in river systems. A number of papers associating native fish species declines with bigheaded carps have been based on heavily stocked fish culture basins where alterations by fertilization, habitat alteration, nutrients, fragmentation and predator removal were implemented; and, in some cases, reported impacts were to other artificially maintained fish stocks. For instance, a paper by Barthelmes (1984), widely cited as evidence of effects on percids, reported a decline in zooplankton abundance (except in the littoral zone) and an unsuccessful year class of stocked zander (Sander lucioperca) in a 20 hectare German Lake following extreme stocking rates of 10,000 silver carp per hectare. While this research has some applications for pond culture of food fish as intended, it has limited implications for wild native fish populations in a connected watershed. Donghu Lake, China has also been cited as an example of native species extirpation related to bigheaded carps (Kumar 2000). However, native fishes were actively removed after the lake was designated as a fish farm lake, separated into a series of ponds and heavily stocked with bigheaded carps, severely polluted by raw sewage and industrial waste, and separated from the Yangtze River by dike construction. Natural lakes connected to the Yangtze typically have 100 fish species but only 30-40 species in lakes where connections have been blocked (Ping and Chen 1997). Fu et al. (2003) identified separation of Donghu from the river as a primary factor in the loss of native fish species, and identified reconnection of the Yangtze River to its lakes as the most immediate restoration need to mitigate loss of fish biodiversity.

Reproduction of many Minnesota fish species has been associated with seasonal spawning migrations up higher gradient tributaries (Aadland et al. 2005) where the habitat of bigheaded carps is marginal. Large migrations and associated reproduction have been documented in the Yellow Medicine River and other Minnesota River tributaries. The reproductive contributions of these tributaries to the Minnesota River fish community may limit the competition effects of bigheaded carps on associated native species.

4.4.2.2 Final characterizations

Table 4-8. MN River Consequence for non-game fish (Spotfin shiner) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	A, F A_H, F_H	J_H			
	Low certainty (+/- 70%)	E, C E_H, C_H	J			
	Very low certainty (+/- 90%)					

Table 4-9. MN River Consequence for non-game fish (Spotfin shiner) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	9	2			
	Low certainty (+/- 70%)	2	8			
	Very low certainty (+/- 90%)					

Table 4-10. MN River Consequence for non-game fish (Bigmouth buffalo) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)		F	C_H, E_H, F_H	J_H	
	Very low certainty (+/- 90%)		A	C, J, E A_H		

Table 4-11. MN River Consequence for non-game fish (Bigmouth buffalo) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		2	4		
	Low certainty (+/- 70%)		7	3		
	Very low certainty (+/- 90%)		1	4		

4.4.3 Consequence for game fish

4.4.3.1 Justifications

The small group evaluated important game species in terms of abundance and potential interactions with bigheaded carps. Important game species of the Minnesota River included flathead catfish, channel catfish, walleye, smallmouth bass, and sauger. Most game species in the Minnesota River have low dietary overlap with bigheaded carps as juveniles and adults but may have some overlap as larvae. However, many of the game species have reproductive strategies that limit this potential. Walleye (Sander vitreus) and sauger spawn primarily in riffles which are most available in steeper tributaries to the Minnesota River where habitat for bigheaded carps is marginal. Flathead catfish spawn in nest cavities and guard their eggs and fry. Centrarchids like smallmouth bass (Micropterus dolomieu) spawn in backwaters in cleared out nests and also guard their eggs and early fry stages, but would have some potential for interactions in these backwaters. Northern pike also spawn in backwaters and floodplains but spawn very early and young may benefit from predation on bigheaded carp fry.

The group chose channel catfish (Ictalurus punctatus) as an example game species to assess potential effects of bigheaded carps due to their relative abundance and importance as a game fish.

Channel catfish are generalized invertivores as juveniles with increasing fish, crayfish, frogs and other items in their diets as adults (Becker 1983). Channel catfish spawn in cavities like muskrat tunnels and guard their fry for about a week after they hatch. Age-0 channel catfish prefer riffle mesohabitat with shallow to moderate depths and moderate velocities but are widely distributed across habitat types. Both juvenile and adult catfish prefer pool habitat (Aadland 1993; Aadland and Kuitunen 2006). Since there is relatively little dietary overlap with bigheaded carps, there is low potential for competition. Adult channel catfish may prey on juvenile bigheaded carps. Juvenile channel catfish ate and increased body mass when fed silver carp fecal pellets (Yallaly et al. 2015). The small group determined that bigheaded carps would have negligible adverse consequences for channel catfish due to the low dietary and habitat overlap (Table 4-12), while the large group characterized the consequence level between negligible and low (Table 4-13).

4.4.3.2 Final characterizations

Table 4-12. MN River Consequence for game fish (Channel catfish) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)	A A_H				
	High certainty (+/- 30%)	C, E, F, J C_H, E_H, F_H, J_H				
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)					
	Very low certainty (+/- 90%)					

Table 4-13. MN River Consequence for game fish (Channel catfish) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)	1				
	High certainty (+/- 30%)	7				
	Moderate certainty (+/- 50%)	7	1			
	Low certainty (+/- 70%)		2			
	Very low certainty (+/- 90%)		1			

4.4.4 Consequence for species diversity/ecosystem resilience

4.4.4.1 Justifications

Predicting effects of bigheaded carps on species richness and ecosystem resilience was particularly challenging for the small group since species diversity and ecosystem resilience, while related, constitute complex and somewhat different questions. Effects on species richness could be habitat-specific and localized or at the watershed scale. Ecosystem resilience, or the ability of the system to recover from disturbance, was assessed as it pertains to colonization by bigheaded carps. In terms of species invasions, the entire species assemblage of the Minnesota River is comprised of species that invaded since the last ice age. As each of these species colonized the watershed they likely had variable effects on the biotic community by altering competition, predation, and food web structure. While river systems are dynamic, connections in the stream network allow migrations across a broad range of available habitats for reproduction, changing habitat needs with season, optimal foraging, recolonization following drought, hypoxia, and catastrophic events, and habitat partitioning in response to competition and predation pressures. The question is whether the addition of bigheaded carps would significantly alter this resilience.

Group predictions on the effects of bigheaded carps on species richness and ecosystem resilience ranged more widely among group members than other variables. The range of these predictions were likely related to differences in the way members viewed this topic and spatial scales of effect. Some individuals indicated the potential for localized, habitat specific changes in species richness especially in backwaters, while others responded in terms of projected watershed scale effects. Combining species richness effects with ecosystem resilience may also have affected variability in predictions. The majority of participants of both the small and large groups rated consequences for species richness/ecosystem resilience as moderate (Table 4-14; Table 4-15).

One of the problems in evaluating effects of bigheaded carps on native species is that most of the literature is from impounded and regulated systems like the Illinois River, so group discussions evaluated important differences in free-flowing rivers like the Minnesota River. Pelagic plankton production in free-flowing rivers is limited since plankton are continually swept downstream by flowing water and due to suspended sediment that limits light penetration. Reservoirs increase phytoplankton production by increasing residence time and by increasing light penetration as suspended sediment fall out of suspension (Søballe and Kimmel 1987). Algal concentrations at several sites on the Upper Mississippi River increased

40-fold following dam construction (Baker and Baker 1981). Like phytoplankton, zooplankton abundance in the pelagic zone also increases with increasing residence time (Reckendorfer et al. 1999), decreasing velocity (Walks 2007) and increasing water clarity (Hart 1986). Zooplankton biomass increased approximately 19-fold following impoundment of Cat Arm Lake in Newfoundland (Campbell et al. 2011). Havel et al. (2009) concluded that reservoirs were the primary source of cladocerans and copepods in the Missouri River due to exponential declines in abundance with distance from mainstem dams. Conversely, Santucci et al. (2004) found that low-head dams adversely affected macroinvertebrates and stream fishes by degrading habitat, water quality, and fragmentation.

Interactions of bigheaded carps with early life stages of native fishes were a particular concern raised in small group discussions due to potential dietary overlap. Since bigheaded carps have been shown to affect abundance and composition of pelagic meiofauna, it is important to evaluate this in the context of its potential impact on native fish species. While it is often assumed that meiofauna, the food of most larval fish species, exists primarily in the water column, this is not typically true of unimpounded rivers. King (2004) found meiofauna densities to be 100 times greater in the epibenthic zone (upper 1 cm of sediment and lower 11 cm of water column) than in the pelagic zone of all habitat types in a floodplain river. Shiozawa (1991) also found high microcrustacean densities in the benthos of slow-water habitats in Minnesota streams. Therefore, while native larval fish depend on meiofauna, much of it exists at the river bed rather than in the water column. In contrast to the bigheaded carps that are adapted to feeding in the water column but poorly adapted to feeding on benthos due to their upward directed supra-terminal lower jaws, most native fishes of the Minnesota River have downward directed sub-terminal lower jaws adapted to benthic feeding. The effects of bigheaded carps on epibenthic meiofauna are a research need.

Due to the inability to swim in strong current, most species of larval and age-0 fish tend to congregate in low velocity areas (Aadland and Kuitunen 2006) including backwater habitats. Shifting to shallow habitats can also be a means of predator avoidance for small-bodied fishes (Schlosser 1987). Quantitative prepositioned electrofishing sampling provides some perspective on the distribution of age-0 fish. In the Yellow Medicine River (1988-2008) age-0 fish densities were highest in sampled shoreline habitat in 11 years, riffles in 5 years, backwaters in 2 years and run habitat in 1 year (Figure 4-3). Year to year density was extremely variable due to differences in flow, geomorphic change to the site, flood magnitude, and other factors. Connected backwaters were not present in the study reach in all years. Drought in 1988 concentrated fish in remaining habitat and provided suitable conditions for age-0 fish across habitat types, particularly backwaters.

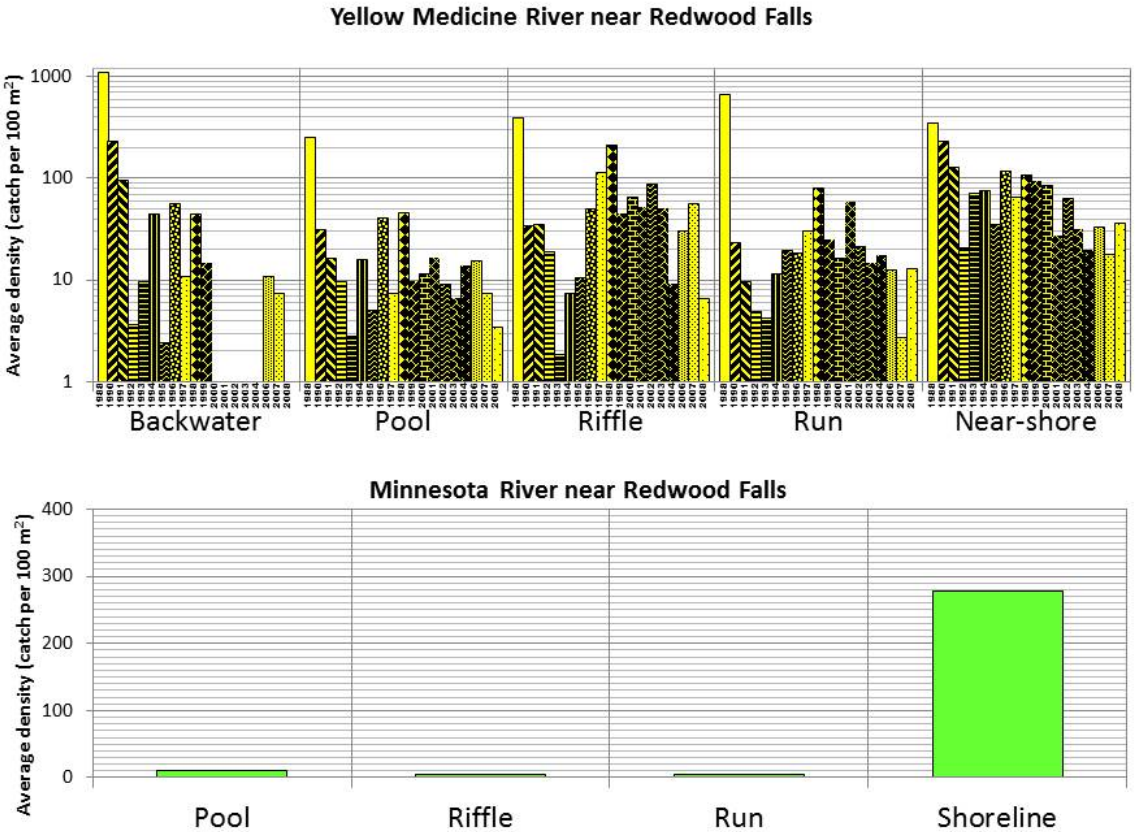


Figure 4-3. Density of age-0 fishes in sites on the Minnesota (1990) and Yellow Medicine (1988-2008) Rivers. Based on quantitative electrofishing gear across habitat types. Connected backwaters were not present during sampling in the Minnesota River reach or in some years on the Yellow Medicine River reach. Near-shore was within 2 meters of the edge of water.

Densities of larval fishes (cyprinids, catastomids and centrarchids, <25 mm) in 17 rivers across Minnesota were highest in close proximity to the stream bed in very shallow water less than 10 cm deep (Figure 4-4). Age-0 fish (all species) in the Minnesota and Yellow Medicine rivers were highest in water less than 20 cm deep. The use of very shallow water by age-0 fishes and close proximity to the stream bed support the importance of epibenthic meiofauna as a food resource. Since native species of free-flowing rivers are adapted to feeding on epibenthic meiofauna, the free-flowing Minnesota River is likely to respond differently than impounded and fragmented systems like the Illinois and Upper Mississippi rivers to colonization by pelagic feeding bigheaded carps.

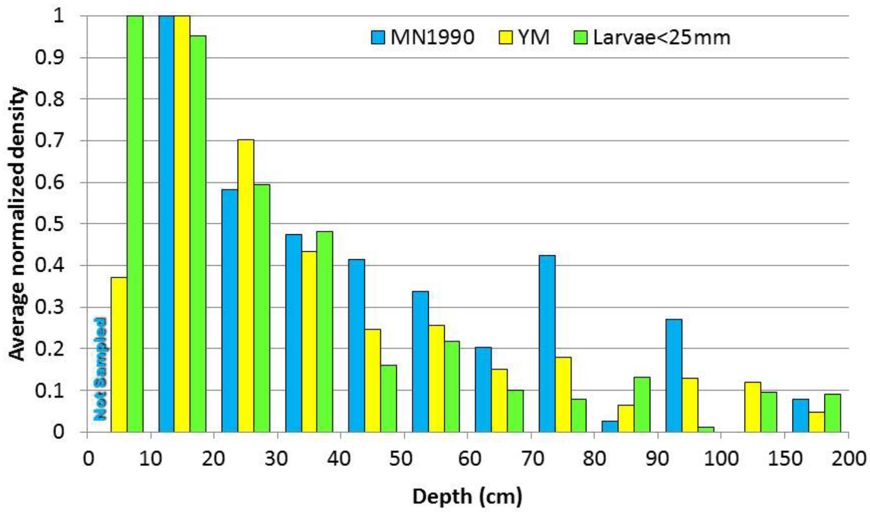


Figure 4-4. Distribution of age-0 fish of all species in the Minnesota River (1990) and Yellow Medicine River (1988-2008) and for larval fish across 17 rivers in Minnesota. Based on quantitative prepositioned electrofishing samplers.

The potential abundance of the bigheaded carps and resulting effects on native species in the assessed reach of the Minnesota River may also be limited by that fact that it is free-flowing. Stuck et al. (2015) found silver carp abundance of the impounded Illinois River to be over three times higher than that in the free-flowing Wabash River. The potential of bigheaded carps to alter plankton composition and affect native species in the Minnesota River was considered to be most likely in backwater habitats, which bigheaded carps prefer. Competition with native species in hypoxic backwaters is likely to be limited to tolerant species.

4.4.4.2 Final characterizations

Table 4-14. MN River Consequence for species diversity/ecosystem resilience – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)			D D_H, E_H	C_H, J_H	
	Very low certainty (+/- 90%)	A A_H		E, F, J	C F_H	

Table 4-15. MN River Consequence for species diversity/ecosystem resilience – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)			4		
	Low certainty (+/- 70%)			6	1	
	Very low certainty (+/- 90%)	1	1	5	1	

4.4.5 Consequence for recreational boating and fishing from jumping silver carp hazard

4.4.5.1 Justifications

This question assumes colonization of the Minnesota River by silver carp (bighead carp do not tend to jump) at moderate and high densities, those characterized as the most likely resulting abundances for the Minnesota River-Mankato watershed in Day 1 of the workshop. The small group considered use of the river and silver carp densities to be primary variables in determining hazards to boaters. Much of the use of the Minnesota River is from river banks due to navigational hazards and limited access points. Bank anglers would be less vulnerable to hazards from jumping silver carp than boat anglers. Silver carp tend to jump where they exist at high densities or when they are confined in a narrow channel or shallow water and are startled by approaching boats. While motor boats tend to startle and elicit jumping by greater numbers of fish, canoes can also elicit jumping.

The small group characterized the consequence to recreational boating and fishing from jumping silver carp at a moderate (5/6) to high (1/6) consequence level (Table 4-16), and the large group characterization was also split between moderate (13/20) and high (7/20) consequence (Table 4-17). When the small group considered a high, instead of moderate, resulting abundance of bigheaded carps in the Minnesota River-Mankato watershed, the consequence level was split between high (4/6) and extreme (2/6).

Hazards associated with jumping carp have not necessarily resulted in a reduction in recreational fishing in rivers with high silver carp densities like the Illinois River since determined anglers are not deterred. However, a change in demographics or strategies of users may exist. Some boaters have made modifications such as protective netting or changes in operation to reduce risks while others are likely to go elsewhere. The group considered that some people may come to the river specifically to see silver carp.

4.4.5.2 Final characterizations

Table 4-16. MN River Consequence for recreational boating and fishing from jumping silver carp hazard – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				J F_H	J_H
	Moderate certainty (+/- 50%)			C, D	D_H	C_H
	Low certainty (+/- 70%)			A, E, F	A_H, E_H	
	Very low certainty (+/- 90%)					

Table 4-17. MN River Consequence for recreational boating and fishing from jumping silver carp hazard – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)			2	3	
	Moderate certainty (+/- 50%)			5	4	
	Low certainty (+/- 70%)			5		
	Very low certainty (+/- 90%)			1		

4.4.6 Adverse Effects: Research needs

Research needs include baseline data for diversity and biomass of native species in the Minnesota River, including for phytoplankton and zooplankton abundance and composition in the main channel and backwater habitats of the Minnesota River. In addition, there is a need for a better understanding of meiofauna densities in the pelagic and epibenthic zones in the Minnesota River across habitat types including backwaters and main channel riffles, runs, pools, and near-shore areas.

To further understand potential interactions between bigheaded carps and native fishes, research needs include: 1) comparative lateral and vertical distributions of native fishes, particularly the larval life stage, across backwaters and other habitats; 2) the relative contributions of tributaries to the recruitment of native fishes in the Minnesota River; 3) the comparative abundance of bigheaded carps in tributaries of rivers (with established populations) of different sizes and habitat characteristics (slope, backwater habitat, etc.); and 4) the effects of bigheaded carps on meiofauna in free-flowing rivers.

Research needs concerning the jumping hazard include incidence rates of silver carp related injuries for boaters, paddlers, and shore anglers on a similar river system with moderate or high silver carp abundance.

4.5 Overarching uncertainties, research needs & areas of disagreements

Predicted effects associated with bigheaded carps in the Minnesota River are heavily dependent on how abundant they become. There was general agreement within both the small and large group that bigheaded carps have a substantial probability of becoming established at some level in the Minnesota River. There was progressively less agreement and certainty on predicted abundance and effects on native species. Since establishment, abundance, effects on plankton community and, ultimately, interactions with native species have compounding uncertainty, this is to be expected.

5 St. Croix River

5.1 Introduction to watershed

The lower St. Croix River is a 6th order river that borders Minnesota and Wisconsin and flows into Pool 3 of the Mississippi River. The 2370 km^2 watershed is a mix of agricultural, forested, and urban land use. The upper portion of the watershed is primarily forested, with agriculture and urban use becoming more prevalent in the lower portion of the watershed. The watershed contains numerous lakes and wetlands that reduce flooding and sediment transfer in the St. Croix River. As such, water clarity is generally high. The lower St. Croix River starts at the confluence of the Snake River and is characterized by a meandering and braided channel before widening into Lake St. Croix. Lake St. Croix is a 3115 ha widening of the river that is 42km in length and a maximum depth of 24m. Given that it has long retention times, it has many lake characteristics such as wave action, internal production, and thermal stratification. Water clarity is relatively high for a large river system (2.5m). There is an impassable dam near Taylors Falls, 84km from the convergence with the Mississippi River. The St. Croix River has a diverse fish community with nearly 100 fish species recorded. Imperiled large river fishes such as lake sturgeon, paddlefish, and blue sucker (Cycleptus elongates) are routinely collected during MNDNR fish sampling. Primary game fish include white bass (Morone chrysops), walleye, smallmouth bass, and sauger (MNDNR 2014b). Forage base for these sportfish include gizzard shad, emerald shiners (Notropis atherinoides), and spottail shiners (Notropis hudsonius). Three aquatic invasive species, Eurasian watermilfoil (Myriophyllum spicatum), rusty crayfish (Orconectes rusticus), and zebra mussel (Dreissena polymorpha), are already established in the St. Croix River.

5.2 Likelihood of establishment

5.2.1 Justifications

The likelihood of bigheaded carps establishment in the Lower St. Croix Watershed was characterized by the small group as mostly moderate (3/5), with one person characterizing it as high and one characterizing it as low (Table 5-1). The large group characterization of establishment likelihood was mainly moderate (15/21), but ranged from low (5/21) to high (1/21). For the establishment likelihood characterization a closed system was assumed (i.e., no open connection with the Mississippi River). The resulting abundance was characterized for both a closed and open system, and the effects characterizations were all for an open system – i.e., one that took into account the connection with the Mississippi River. Participants thought the study area provided suitable food resources, water temperature, and flows (for reproduction) for bigheaded carps, but thought it lacked in nursery areas, spawning habitat, and turbidity. Because of the widening of the river and decreased flows, zooplankton is presumed to be abundant as a food source in Lake St. Croix. In addition, increasing phosphorous loads to the St. Croix River are likely to increase overall productivity.

Historical peak flows and water temperatures in the St. Croix River are conducive as spawning cues for bigheaded carps. Specifically, occasional increased flows in July were noted in the historical hydrograph that match current spawning conditions observed in Midwest US rivers. However, there was uncertainty as to whether eggs would be able to hatch before settling out into the slow flowing portion of the river because the distance from St. Croix Falls dam to Lake St. Croix is only 39km. This distance is considerably shorter than the 100km reported in the literature that is thought to be needed for successful spawning (Kocovsky et al. 2012). Participants were uncertain as to whether carp actually needed 100km of free flowing river as stated in the literature, or whether this distance could be considerably less based on anecdotal evidence. The group also questioned whether the area below Taylors Falls would provide a suitable spawning area given the water depth and area (i.e., is it large enough to support mass spawning of bigheaded carps). Another factor limiting the recruitment of bigheaded carps is the lack of suitable nursery areas. There are few turbid backwater habitats available in the St. Croix River. The primary nursery habitat would be Lake St. Croix, but eggs may not develop fully before they settle out into the lake portion. Water clarity is high throughout the river and in Lake St. Croix, which participants also thought would reduce recruitment through increased predation of carp eggs and larvae.

The St. Croix River is unlike systems where bigheaded carps are currently found in terms of water clarity and species diversity. In the Midwest US, bigheaded carps are typically found in abundance in turbid river systems. There was uncertainty as to what affect clear water would have on egg and larval survival in terms of predation. Also, the number of potential fish predators on bigheaded carps was considered higher than in systems where they are currently found. Whether the high abundance of predators could control bigheaded carp populations was unknown.

5.2.2 *Final characterizations*

Table 5-1. St. Croix River Likelihood of Establishment - Small Group Final Characterization (Closed System Assumptions).

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				R	
	Moderate certainty (+/- 50%)			P, O, M		
	Low certainty (+/- 70%)		Q			
	Very low certainty (+/- 90%)					

Table 5-2. St. Croix River Likelihood of Establishment – Large Group Characterization (Closed System Assumptions).

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		2	7	1	
	Low certainty (+/- 70%)		3	7		
	Very low certainty (+/- 90%)			1		

5.2.3 *Research needs*

Participants disagreed on the length of free flowing river needed for egg development of bigheaded carps; however, models exist to help determine the length of river needed based on water temperature and velocity (FluEgg model; Garcia et al. 2013). Better information on temperature and flows are needed in this area to input into the FluEgg model to determine whether the area is suitable for spawning.

Research is needed on whether adult bigheaded carp avoid clear water habitats and what affect clear water has on the recruitment of bigheaded carps. Recruitment of bigheaded carps could be reduced in clear water due to increased predation on their eggs and larvae.

5.3 Resulting abundance

5.3.1 Justifications

The small group determined that carp would likely sustain themselves at a low abundance in the St. Croix River when considered a closed system (Table 5-3). The group was between low and moderate certainty in this prediction. Participants justified this low abundance in that there would be low recruitment, but growth of individuals would be high because of high zooplankton densities. A diverse fish community should keep numbers low due to predation and no available niches for carp to fill. The group thought that the systems in which bigheaded carps have become abundant were heavily disturbed before invasion and had numerous open niches for bigheaded carps to fill. Under an open system scenario, immigration from the Mississippi River could be large and there are no deterrents to adult carp survival in terms of prey and water temperature in the St. Croix River. As a result the large group, considering the open system scenario, largely characterized the resulting abundance of bigheaded carps as moderate (13/21), the second most characterized abundance being low (5/21) followed by high (3/21) (Table 5-4). The open system scenario is assumed for the remainder of the characterizations to take into account the connection between the St. Croix and Mississippi rivers.

5.3.2 *Final characterizations*

Table 5-3. St. Croix River Resulting Abundance – Small Group Final Characterization (Closed System Assumptions).

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		P, O			
	Low certainty (+/- 70%)	Q	M, R			
	Very low certainty (+/- 90%)					

Table 5-4. St. Croix River Resulting Abundance – Large Group Characterization (Open System Assumptions)

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		3	9	1	
	Low certainty (+/- 70%)		2	3	2	
	Very low certainty (+/- 90%)			1		

5.3.3 *Research needs*

Group members identified several research needs. There was a large need in determining adult preference for clear or turbid waters. The question of whether bigheaded carps would actively avoid the St. Croix River due to clear water and select the Minnesota River because of its turbid conditions was unknown. There was also uncertainty in how well we understood the fish community in the St. Croix River in terms of food webs and available niches. A better monitoring program of the fish community in the St. Croix River was considered necessary to identify any impacts from an established population of bigheaded carps. The group thought more research was needed on predation of bigheaded carps by native fish in terms of what sizes could be preyed upon and by which species.

5.4 Adverse Effects

During the characterization of potential adverse effects, the small group characterized the consequence of each adverse effect for the likely abundance of bigheaded carps that was determined in the previous step. The small group also characterized the consequence resulting from the second most likely abundance of bigheaded carps. For the St. Croix River small group, the first abundance was “Moderate” and the second abundance was “Low”. In the tables below, the characterization for the “Moderate” abundance is noted with “P”, “Q”, “R”, etc. whereas the characterization for the “Low” abundance is noted with “P_L”, “Q_L”, “R_L”. The letters represent different individuals within the small group.

5.4.1 Change in plankton

5.4.1.1 Justifications

At a moderate abundance scenario, the majority of panelists thought there would be a small decrease in plankton abundance after the establishment of bigheaded carps (Table 5-5). In the low abundance scenario, the panel unanimously thought there would be no change in plankton abundance. The decrease was predicted to be small given that there is ample prey in the system that could potentially accommodate another planktivore species such as bigheaded carps. Participants thought that a more likely scenario was a community shift from larger to smaller bodied zooplankters. As a result, overall zooplankton biomass may only decrease slightly, but quality zooplankton (e.g., larger cladocerans) may experience a more significant decrease. Also, rotifer abundance may increase from a decrease in predation from larger zooplankters.

5.4.1.2 Final characterizations

Table 5-5. St. Croix River Change in total biomass of plankton – Small group characterizations.

		Change in total biomass of plankton						
		Large increase	Moderate increase	Small increase	No change	Small decrease	Moderate decrease	Large decrease
Certainty of assessment	Very high certainty (+/- 10%)							
	High certainty (+/- 30%)				P_L			
	Moderate certainty (+/- 50%)				O_L, R_L	P		
	Low certainty (+/- 70%)				R Q_L	O, Q		
	Very low certainty (+/- 90%)							

5.4.2 Consequence for non-game fish

5.4.2.1 Justifications

Gizzard shad, a planktivorous fish species, was chosen as the non-game fish for this watershed because they are a common forage fish in the St. Croix River and play an important role in structuring predator populations. There is also evidence from the literature that diet overlap is high between bigheaded carps and gizzard shad (Irons et al. 2007). Three of four small group members believed that the consequence of a moderately abundant population of bigheaded carps would be low for gizzard shad, and one thought it would be moderate (Table 5-6). The large group characterizations were divided between low (9/19) and moderate (10/19) consequence (Table 5-7). This is primarily due to the fact that the panel concluded that there would only be small effects on the overall zooplankton biomass after the establishment of bigheaded carps. Also, the group thought that gizzard shad could switch food resources (e.g. detritus) and continue to maintain their current abundance. The group did concede that habitat overlap would be high and there was some discussion on the potential for reduced fitness of gizzard shad and potential for this to lower overall abundance. Body condition of gizzard shad has decreased in the Illinois River after establishment of bigheaded carps, which led some participants to predict a moderate negative consequence on gizzard shad in the St. Croix River. Effects on gizzard shad in a low abundance scenario were predicted to be negligible.

5.4.2.2 Final characterizations

Table 5-6. St. Croix River Consequence for non-game fish (Gizzard Shad) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)	O_L, P_L, R_L				
	Moderate certainty (+/- 50%)	Q_L	O	Q		
	Low certainty (+/- 70%)		P, R			
	Very low certainty (+/- 90%)					

Table 5-7. St. Croix River Consequence for non-game fish (Gizzard Shad) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		3	6		
	Low certainty (+/- 70%)		6	4		
	Very low certainty (+/- 90%)					

5.4.3 Consequence for game fish

5.4.3.1 Justifications

The small group chose sauger as its game species as this species is commonly targeted by anglers in the St. Croix River and is sampled in relatively high abundance in MNDNR sampling. The small group predicted a low level of consequence from bigheaded carps on sauger populations with moderate certainty (Table 5-8). The large group also characterized the level of consequence for sauger as low (13/18), followed by moderate (4/18) and negligible (1/18) (Table 5-9). The effect on sauger populations would largely result from a decrease in abundance and condition of prey (primarily gizzard shad). However, small group members thought that sauger could switch to alternate prey such as young-of-year freshwater drum. Sauger may also prey on young-of-year bigheaded carp as an alternative to gizzard shad. The group thought that negative effects of bigheaded carps could be partially offset by a potential decrease in angler pressure on sauger if bigheaded carps were to establish – a result of fewer anglers wanting to be on the river if a moderate population of bigheaded carps were present. However, it was unknown if angler pressure would decrease with a moderate population of bigheaded carps. Effects on sauger were negligible for the low abundance of bigheaded carps scenario.

5.4.3.2 Final characterizations

Table 5-8. St. Croix River Consequence for game fish (Sauger) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)	Q_L, R_L				
	Moderate certainty (+/- 50%)	O_L, P_L	O, P, R			
	Low certainty (+/- 70%)		Q			
	Very low certainty (+/- 90%)					

Table 5-9. St. Croix River Consequence for game fish (Sauger) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		9	3		
	Low certainty (+/- 70%)		4			
	Very low certainty (+/- 90%)	1		1		

5.4.4 Consequence for species diversity/ecosystem resilience

5.4.4.1 Justifications

The small group thought that a moderate change in species diversity would take place under a scenario with moderate carp abundance, ranging from high to low certainty (Table 5-10). The group agreed that species diversity would be most affected at lower trophic levels, with changes in zooplankton communities. Group members thought there would be a potential shift from large-bodied cladocerans to higher abundances of rotifers. There was high certainty regarding this shift in lower trophic levels, but changes in higher trophic levels were uncertain. Although the group was less certain about effects on fish diversity, the high number of intolerant fish species in the St. Croix River may make it easier to detect a change in species diversity. The large group also characterized the consequence largely as moderate (17/19) (Table 5-11).

5.4.4.2 Final characterizations

Table 5-10. St. Croix River Consequence for species diversity/ecosystem resilience – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)			Q		
	Moderate certainty (+/- 50%)	R_L	O_L, P_L	P, R		
	Low certainty (+/- 70%)			O Q_L		
	Very low certainty (+/- 90%)					

Table 5-11. St. Croix River Consequence for species diversity/ecosystem resilience – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)			1		
	Moderate certainty (+/- 50%)		1	8		
	Low certainty (+/- 70%)		1	7		
	Very low certainty (+/- 90%)			1		

5.4.5 Consequence for recreational boating and fishing from jumping silver carp hazard

5.4.5.1 Justifications

The small group characterized the jumping hazard impact of a moderate population of bigheaded carps on recreational boating and fishing at both a high consequence level (3/4) and low consequence level (1/4), with varying degrees of certainty (Table 5-12). Although the overall chance of getting struck by a silver carp was considered low, the reactions by the public to such events was predicted to be high. Given that there are abundant alternative water resources around the area, small group members thought people would rather go elsewhere to recreate than risk being struck by a silver carp. However, because most of the boating traffic occurs in the lake portion of the river, encounters between bigheaded carp and boats maybe rare given the depth and area of the lake portion and that silver carp are more likely to jump in shallow or confined waters. Group members thought it was more likely to encounter jumping silver carp in a confined area as opposed to the open expanse of Lake St. Croix. The large group characterized the consequence level of the jumping hazard to recreational boating and fishing as predominantly high (9/19) and moderate (8/19), and also extreme (1/19) and low (1/19) (Table 5-13).

5.4.5.2 Final characterizations

Table 5-12. St. Croix River Consequence for recreational boating and fishing from jumping silver carp hazard – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)		P_L, R_L	O_L	O	
	Moderate certainty (+/- 50%)				R	
	Low certainty (+/- 70%)		P	Q_L	Q	
	Very low certainty (+/- 90%)					

Table 5-13. St. Croix River Consequence for recreational boating and fishing from jumping silver carp hazard – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				4	1
	Moderate certainty (+/- 50%)			2	4	
	Low certainty (+/- 70%)		1	5	1	
	Very low certainty (+/- 90%)			1		

5.4.6 Adverse Effects: Research needs

Group members thought that a food web study would be beneficial to understanding the potential role bigheaded carps would play in the system. A potential energy pathway study using stable isotope analysis would be beneficial to understanding food webs in the St. Croix River before and after establishment by bigheaded carps. There was disagreement as to whether comprehensive studies currently exist examining zooplankton community response to invasions by bigheaded carps in other rivers. Data on zooplankton communities in rivers is sparse compared to lakes and reservoirs. The group also wanted more information on current zooplankton communities to know whether prey resources were sufficient to maintain gizzard shad abundance when resources are also in demand by bigheaded carps.

The group wanted better estimates of species richness and diversity in the St. Croix River. A more intense monitoring program is needed to detect any changes in diversity as a result of establishment by bigheaded carps. In addition panelists thought it would be difficult to detect changes in gizzard shad and sauger abundance given current fish sampling protocols.

Panelists wanted more information on what influences sauger recruitment in the St. Croix River and thought that recruitment might be driven more by hydrology than prey availability. If hydrology drove recruitment success, than a decrease in prey resulting from bigheaded carps may not have a negative effect on sauger. However, hydrology and other environmental conditions could also be driving available prey resources for sauger, and panelists thought additional research was needed in this area. The group was unsure how anglers would respond to different levels of bigheaded carps abundance. Would angler pressure on sauger decrease because there would be fewer anglers on the river, or would it increase if there were fewer recreational boaters for the anglers to compete with?

Panelists were uncertain as to whether bigheaded carps would be at the water's surface near boats given the clear water of the St. Croix River. It is possible that bigheaded carps would stay in deep water to avoid sunlight and not have many encounters with boats. The group also was uncertain as to the density of bigheaded carps needed for jumping behavior. There were also questions surrounding how the public would react to jumping bigheaded carps and what factors would influence differences across reactions. Whether anglers would become acclimated to this new phenomenon and eventually return to boating on the St. Croix River was unknown.

5.5 Overarching uncertainties, research needs & areas of disagreements

Because the St. Croix River system is different than systems where bigheaded carps are currently found, participants had difficulty determining whether or not they would succeed in such an environment. The effects of water clarity and aquatic species diversity on the establishment of bigheaded carps and their effects on the system was a common uncertainly throughout the scenarios. Bigheaded carps are currently found in high abundance in impaired river systems, such as the Illinois River. Whether the St. Croix River would be more resilient to invasion given that it is less impaired is unknown. Research into how bigheaded carps react to clear water is needed to accurately determine the potential risk of invasion into these low turbidity systems.

Another common theme across scenarios was the need for baseline information (fish diets, zooplankton, etc.) to detect future changes. Fish sampling is currently conducted every 3 to 6 years on the St. Croix River by the MNDNR. Sampling gear has varied across years from electrofishing, trap nets and gill nets. A more rigorous and standardized sampling protocol for both fish and zooplankton is needed to address potential changes in these aquatic communities.

6 Nemadji River

6.1 Introduction to watershed

The Nemadji River flows 111 km from its headwaters at Maheu Lake in Pine County to Allouez Bay in the St. Louis Estuary, which covers 4,856 ha at the west end of Lake Superior. The Nemadji River watershed covers 112,260 ha on the southwest corner of Lake Superior. The Nemadji watershed includes numerous streams, 17,141 ha of wetlands (National Wetlands Inventory Data), and 35 lakes greater than 4 ha located mostly in the watershed's headwaters area. Land use in the watershed's Minnesota portion is mostly related to rural forestry, pasture production for hay cutting, and some beef cattle. Lakeshores are developed, although not as intensively as is typical in northern counties. The watershed is in the Northern Lakes and Forest Ecoregion, which is dominated by glacial till in ground moraines and drumlins and highly erodible clay soils. Glacial till occurs throughout the upper watershed, whereas the lower one-third of the watershed is covered in red clay from Quaternary geology, sometimes up to 61 m thick; this layer was deposited during a geologic period when glacial lakes covered the region (MPCA 2014).

The Nemadji River is famous for its turbid, clay-filled water which is visible as a large plume in the western end of Lake Superior after any significant rain event. Though red clay erosion is natural, human activities on the land in the last century have accelerated the natural process, and as a result the river has cut deep valleys into the surrounding bluffs. During the pre-settlement era the landscape was covered with mature coniferous trees that stabilized the riparian areas near the rivers and streams. During the mid 1800s loggers removed the forest in the watershed and coarse woody structure in streams. Logging converted forest to permanent agriculture, streams were cleared to efficiently transport logs to sawmills, and many roads and railroads were cut through the basin. This all led to efficient hydrologic pathways for water to get to the river quickly (Natural Resources Conservation Service and U.S. Forest Service 1998). While 69% percent of the watershed is now reforested, the deciduous trees adjacent to streams may not be an effective sediment filter, or may not form a sturdy or deep enough root system to hold soils in place in currently downcut channels. Many red clay slumps in the watershed move downhill despite tree cover, likely due to shallow groundwater movement beneath the root zone. The riparian areas along the stream vary greatly in width and quality (Natural Resources Conservation Service and U.S. Forest Service 1998). Nearly 90% of the fine sediment in the river is due to bluff erosion and slumping, and 74% of this sediment ultimately ends up in Lake Superior (CSWCD 2017).

Despite substantial impairment from turbidity and siltation, the Minnesota portion of the Nemadji watershed contains 40% of Lake Superior's migratory trout and salmon spawning habitat in Minnesota (Minnesota Department of Natural Resources, unpublished information). Streamflow is somewhat stable compared to the much more dynamic streams of the North Shore of Lake Superior in Minnesota. Mean discharge during the warmer summer months varies from 0.14-0.42 cubic meters per second (cms) in upstream reaches to an annual average of 6.48-21.12 cms during June-September in 2011-2015 at the lower Nemadji River gauge (U.S. Geological Survey 2016). Average precipitation in the area is about 76.2 cm per year. The upper reaches remain cool enough during the summer months to support growth for brown trout (Salmo trutta), which requires temperatures of 5-23 C. The long-term mean air temperature in summer is 16.7 C. The watershed contains numerous beaver dams and man-made impoundments, which block movements of anadromous steelhead rainbow trout; 4-8 beaver dams are removed annually in a major tributary, the Blackhoof River, to maintain anadromous passage. The upstream reaches contain limited numbers of brook, brown, and rainbow trout and also small populations of suckers, chubs, and minnows. In the upstream reaches the stream gradient averages 2.5 m/km and the stream is 4.9 m wide on average. At the downstream end of the Nemadji River, stream gradient drops to less than 1.3 m/km and widens to 18.2 m on average. Near the river mouth gravel bars can prevent some canoe and kayak traffic during summer months, and the fish species composition is similar to that in the St. Louis Estuary. The mouth of the Nemadji River is an area of side-channel wetlands that extend for about 1.6 km upstream. Wetlands at the mouth of the Nemadji cover about 26.4 ha and support the spawning beds of over 60 warm water fish species, including muskellunge, perch, bass, walleye, and northern pike. Lamprey also occur in the river, and are actively controlled by the US Fish and Wildlife Service. This area is identified by the Lake Superior Binational Program as important habitat to the Lake Superior ecosystem for coastal wetlands as well as fish and wildlife spawning and nursery grounds. The St. Louis Estuary supports diverse recreational activity including boating, fishing, canoeing and kayaking, and also a considerable amount of barge and large vessel traffic, as the Duluth/Superior Port is one of the busiest ports in the world.

The fish community of the St. Louis Estuary system is composed of a diverse mix of warm and cool-water species that are common to many Minnesota lakes. Several of these fishes support an active fishery, including walleye, northern pike, muskellunge, lake sturgeon, channel catfish, black crappie, and smallmouth bass. The fishery has developed over the past 20 years as the waters have become less contaminated; however, fish consumption advisories are still in place for larger predatory fishes. Summer angling effort has ranged from 93,315 hours in 2015 to 295,621 hours in 2003 (Minnesota DNR unpublished documents; Lindgren 2004a). For comparison, the highest recent angling effort on the Minnesota waters of Lake Superior proper was 204,881 hours in 2015. In the Estuary, anglers prefer walleyes, accounting for 86% of the targeted summer effort in 2003 (Lindgren 2004a). In recent years, the adult walleye population has varied between 60,070 ($\pm$ 24,484) in 1981 to 97,887 ($\pm$ 24,484) in 1993. Lake sturgeon abundance has increased to the point that a catch-and-release season was implemented in 2015 to protect the populations (Minnesota DNR unpublished data). Minnesota and Wisconsin stocked muskellunge annually from 1983 through 2005 and both states actively managed muskellunge by regular fish surveys. Regarding other fishes, yellow perch and black crappie are sought almost exclusively during the winter Lindgren 2004b). Winter anglers sought yellow perch 18.7% of the time and black crappie 42.1% of the time in the winter of 2002/2003, whereas anglers did not target yellow perch and only targeted black crappie 1.6% of the time in the summer of 2003. Anglers also targeted northern pike 13.1% of the time during winter and 7.2% of the time during summer. The other fishes are targeted by less than 5% of all other anglers yet add to the unique diversity of the fishery in the St. Louis Estuary.

The primary prey fishes in the Estuary are trout-perch (Percopsis omiscomaycus), yellow perch, white sucker, and redhorse (Moxostoma sp), and also juveniles of many predators and numerous cyprinids including common carp. Yellow perch growth rates are relatively fast and survival to larger sizes is low, which indicate that predation on yellow perch is intense. Boygo (2015) surveyed open water areas of the Estuary in 2015 with a bottom trawl and caught a wide variety of small fishes, including black crappie (27%), trout-perch (23%), and yellow perch (17%). Spottail shiners were also common, occurring at lower densities in 77.5% of the trawl samples. The abundance of a new invasive fish, white perch (Morone americana), may be increasing (Boygo 2015).

The Estuary contains several aquatic invasive fishes, including sea lamprey, eurasian ruffe (Gymnocephalus cernuus), common carp, white perch, rainbow smelt (Osmerus mordax), round goby (Neogobius melanostomus), and tubenose goby (Proterorhinus semilunaris). Eurasian ruffe were first observed in Wisconsin DNR seines in 1986, and expanded quickly in Minnesota DNR gill nets, increasing from 0 fish/net in 1987 to 16.3 fish/net in 1992. Catches subsequently declined to less than 4 fish/net in 1994-2005. Boygo (2015) observed a 10-fold decrease in bottom trawl catches compared to 1989-2004. Catches may have declined due to small mean length, a possible consequence of intensive predation following intensive predator stocking by both Wisconsin and Minnesota in 1989 to 1993 and from other fishes whose populations expanded as Estuary conditions improved. Other invasive fishes appear to be at low levels in the Estuary, possibly due to the Estuary's high fish diversity. No native species appear to be recently extirpated or in danger of being imperiled due to the high diversity; rather, continued improvements to the Estuary have improved the habitats for many fishes.

The lower Nemadji system has suffered many abuses and yet retains many natural features and is now being protected and rehabilitated because the system contains ecologically rich mesic hardwood forests, floodplain forests, and marshes. The marshes are diverse, contain mostly native species, function well ecologically, and provide summer residency for some uncommon resident birds. Invasive plants are still quite localized in disturbed areas such as levees and formerly dredged areas. The Nemadji River Bottoms at the lower end of the river are also identified as a Lake Superior Basin Priority Site due to the high quality floodplain wetlands and the erodibility of the soils in this area. Continued improvements to the Nemadji River and the St. Louis Estuary will benefit native fishes, however the reduction in sedimentation may also provide additional nursery habitat for newly invading species. Species that are produced in the Nemadji River and are not transported by high currents into Lake Superior can spread out into the St. Louis Estuary. That estuary contains an abundance of shallow, productive, backwater habitat for juvenile fishes and a variety of habitats and substrates for adult fishes to grow and reproduce.

6.2 Likelihood of establishment

6.2.1 Justifications

Members of the Nemadji River small group thought that bigheaded carps would have a relatively high (60-95 %) likelihood of establishment, and most (3 of 5) members were highly certain of this assessment (Table 6-1). Differences of opinion were wider with the larger group, where most (11 of 20) characterized bigheaded carps as having a low likelihood of establishment, while 6 of 20 thought there was a moderate likelihood of establishment (Table 6-2). Most members of the larger group were moderately certain of this assessment. These and all subsequent characterizations considered the Nemadji estuary along with the larger St. Louis Bay estuary, because of their physical connection.

Discussion around likelihood of bigheaded carps establishment in the Nemadji River included the variability in habitat suitability for bigheaded carps spawning, feeding and growth. Although much of the upper Nemadji River is trout habitat that is cold, clear and unlikely to support growth of bigheaded carps, it also provides over 48 km of free flowing potential spawning habitat for bigheaded carps, and the productive St. Louis Bay Estuary at the downstream end of the Nemadji River provides suitable habitat for juveniles and adults. Earlier studies of bigheaded carps spawning in China (Yi et al. 1988, reviewed by Kolar et al. 2007) suggested that bigheaded carps required specific hydrologic and thermal requirements to spawn successfully, and a minimum of 161 km for eggs to drift downstream, hatch and settle into favorable backwater nursery habitats. However, recent research by Kocovsky et al. (2012), Garcia et al. (2013), Deters et al. (2013), and Coulter et al. (2013) suggests that reproductive ecology of introduced bigheaded carps is more plastic. Bigheaded carps can spawn successfully at lower temperatures, and in less turbid water and shorter river habitats (<26km) than previously thought. Some group members thought bigheaded carps may not be able to spawn in spring when river flows are cold and fast, but could spawn during August as temperatures increase and flows decline. Nursery habitat for young bigheaded carps was thought to be poor in the upper river where plankton biomass is low and predation from trout and gobies would be high, but would be suitable in the lower river and estuary which are productive, turbid environments. As an example, the group noted that cisco (Coregonus artedi), a native planktivore inhabits the St. Louis estuary in summer. Other members noted that bigheaded carps inhabit multiple habitat types in China's Yangtze River, including colder streams. Members considered uncertainty associated with climate warming that could improve thermal habitat quality for bigheaded carps, and presence of other invasive species such as round goby that have thrived in the Nemadji River.

6.2.2 Final characterizations

Table 6-1. Nemadji River Likelihood of Establishment - Small Group Final Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				S, U, X	
	Moderate certainty (+/- 50%)				W	
	Low certainty (+/- 70%)				T	
	Very low certainty (+/- 90%)					

Table 6-2 Nemadji River Likelihood of Establishment – Large Group Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		9	4	2	
	Low certainty (+/- 70%)		1	1		
	Very low certainty (+/- 90%)		1	1	1	

6.2.3 Research needs

Key research needs were to understand why bigheaded carps are not abundant in coldwater streams, or why they are present but not established. Some small group members thought the water would be too cold for reproduction or growth, or that river flows may be too high, and predation by coldwater fish communities may be too intense. The small group felt it would be useful to investigate other watersheds where there are enough adults to establish but no evidence that bigheaded carp have successfully established. Members felt that some uncertainties regarding the establishment of bigheaded carps could be answered by development and application of temperature and flow models for the Nemadjii River, application of bioenergetics and stock-recruit models to predict growth potential and reproductive success, respectively, and further studies of juvenile bigheaded carp movement patterns.

6.3 Resulting abundance

6.3.1 Justifications

Most (4 of 5) small group members were moderately certain that bigheaded carps would comprise a moderate (5-25% of total fish biomass) level of abundance, with one member being highly certain (Table 6-3). Members felt that bigheaded carps abundance would fall on the low side (5-10% of total fish biomass, including anadromous fishes) of this abundance category. Half (10/20) of the larger group felt that bigheaded carps would reach a moderate level of abundance, with 8 of 20 assessing bigheaded carps abundance as low and 2 of 20 individuals assessing potential abundance as very low (Table 6-4). Most (14 of 20) large group members were moderately certain of their assessment, while certainty of other members ranged from very low or low (5 of 20 individuals) to high (1 of 20 individuals).

Factors affecting the assessment of potential abundance of bigheaded carps were similar to those mentioned for their establishment. The small group felt that bigheaded carps would not have enough plankton to support growth in the upper watershed, so would be confined to the St. Louis Bay Estuary which is more productive. The group thought that the ability of bigheaded carps to persist would rely on their ability to feed on alternative food sources in the lower river and estuary, including detritus and fish larvae. Therefore the whole estuary, including the Nemadji River and St. Louis Bay, would need to be managed as one system. Western Lake Superior zooplankton abundance has varied, between 1996 and 1997, from 20 to 55/L (Johnson et al. 2004), whereas zooplankton abundance in the lower Missouri River varied, between habitats, from 5 (chute habitat) to 45/L (backwater habitat) (Dzialowski et al. 2013). Zooplankton densities were significantly higher in the backwaters habitat than the chute habitat of the lower Missouri River. Rotifers dominated (30/L) the zooplankton community in the lower Missouri River, while adult copepods density was measured at about 0.9/L, and no cladocerans were documented there. In contrast, cladoceran density in Western Lake Superior ranged from 0.3 to 1.2/L, while adult and juvenile copepod density ranged from 10 to 14/L, and rotifer density ranged from 9 to 39/L. Thus, density of large zooplankton has been somewhat higher in western Lake Superior than in the lower Missouri River. Zooplankton density in western Lake Superior historically supported a population of cisco from which commercial landings exceeded 1 million pounds annually (Anderson and Smith 1971). Diets of the cisco and bigheaded carps are similar—both are often zooplanktivorous. Thus, if the cisco can sustain a fishable population in the Lake Superior's Duluth-Superior area, which includes the St. Louis River estuary and connected, nearshore lake habitat, then bigheaded carps may find adequate food resources also establish self-sustaining populations there. Also, thermal habitat in the nearshore waters of western Lake Superior is likely more suitable to growth and feeding than the colder waters of the upper Nemadji River. Thus, food and thermal habitat combined may be suitable, in portions of western Lake Superior, to enable populations of bigheaded carps to establish there, if introduced.

Several studies of the diet of bigheaded carps indicate they can readily consume a variety of prey types that may be available in St. Louis Bay estuary. Chen (1982) found diet of bigheaded carps in China included bacteria, detritus, phytoplankton and zooplankton. The ability of bigheaded carps to consume small plankton is related to their gill raker size. Bighead carp have average gill raker widths ranging from 20-60 µm, and can consume particles down to 17 µm, while pore size of silver carp gill rakers ranges from 20-25 µm and can allows them to consume particles down to 8 µm (Opuszynski 1981; cited in Sampson et al. 2009). Sampson et al. (2009)

found that the diet of bigheaded carps in backwater lakes of the Illinois and Missouri River was dominated by rotifers, and cautioned that the competition for prey may be greatest in less productive habitats of the Great Lakes. Cooke and Hill (2010) used bioenergetics modeling to investigate the potential for bigheaded carps to grow at ambient temperatures and prey densities in Great Lakes habitats. They found bigheaded carps would not show positive growth in open water habitats of the Great Lakes, but would grow well in productive embayments, estuaries and wetland habitats. They noted that bigheaded carps could achieve positive growth in habitats with lower prey densities and temperatures, owing to lower metabolic costs. Bigheaded carps diet flexibility, potential availability of suitable prey, and cooler water temperatures in the St. Louis Estuary may combine to support positive growth and low to moderate abundance of bigheaded carps.

6.3.2 Final characterizations

Table 6-3. Nemadji River Resulting Abundance – Small Group Final Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)			T		
	Moderate certainty (+/- 50%)			X, U, S, W		
	Low certainty (+/- 70%)					
	Very low certainty (+/- 90%)					

Table 6-4. Nemadji River Resulting Abundance – Large Group Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)		1			
	Moderate certainty (+/- 50%)	2	7	5		
	Low certainty (+/- 70%)			3		
	Very low certainty (+/- 90%)			2		

6.3.3 Research needs

The small group identified research needs to better evaluate potential abundance of bigheaded carps in the Nemadji River estuary, and connected, nearshore areas of western Lake Superior. Needs included a desire for case histories of establishment by bigheaded carps in ecosystems similar to the Nemadji River watershed; estimates of straying rates of bigheaded carps from connected systems such as the St. Louis estuary; studies of flexibility in bigheaded carps feeding behavior; homing tendencies of bigheaded carps; and minimum habitat requirements for bigheaded carps in free-flowing waters.

Areas of disagreement and uncertainty about bigheaded carps potential abundance included whether water flows and temperature were too cold to support successful reproduction and recruitment of carp, whether to consider only habitat in the St. Louis Bay Estuary or within the whole watershed, and what types of food were available to support bigheaded carps growth.

6.4 Adverse Effects

During the characterization of potential adverse effects, the small group characterized the consequence of each adverse effect for the likely abundance of bigheaded carps, arrived at earlier in the process. The small group also characterized the consequence resulting from the second most likely abundance of bigheaded carps. For the Nemadji River small group, the first abundance was "Moderate" and the second abundance was "Low." In the tables below, the characterization for the "Moderate" abundance is noted with "S", "T", "U", etc. whereas the characterization for the “Low” abundance is noted with “S_L”, “T_L”, “U_L”. The letters represent different individuals within the small group.

6.4.1 Change in plankton

6.4.1.1 Justifications

In its first characterization of the effects of bigheaded carps on plankton, the small group largely believed (4 of 5 individuals) that consumption by a moderately abundant bigheaded carps population would cause a moderate decrease in plankton biomass (Table 6-5). One individual felt that bigheaded carps would cause a large decrease in plankton biomass. For the second characterization for a low resulting abundance of bigheaded carps, most (3 of 5 individuals) thought plankton biomass would show a small decrease, with a range from no change in biomass to a moderate decrease in biomass.

The groups identified several potential adverse effects resulting from a reduction in quality or abundance of plankton due to bigheaded carps consumption. Reduced quality or abundance of plankton may cause a shift in native fish diets to less preferred foods, resulting in reduced fish abundance, growth or condition. Reduced abundance of plankton could cause a reduction in abundance of native planktivores, which potentially would reduce abundance of piscivores and/or game fish. The groups recognized that planktivores could be either larval or juvenile stages of piscivorous fish (e.g., walleye) or adult stages of prey fish such as common shiners, gizzard shad or cisco. Native planktivores also may experience a reduction in habitat in competition with bigheaded carps, making them less able to cope with additional stressors (other aquatic invasive species, habitat fragmentation) or more available to predators. Bigheaded carps’ consumption of plankton in the water column could increase light penetration, which may reduce densities of game and non-game fish. Bioturbation by bighead carps feeding on the bottom could stimulate algal blooms, reduce water column oxygen concentrations, and potentially reduce abundance or quality of game and non-game fishes.

Empirical studies of bigheaded carp effects on fishes in the Illinois and Missssissippi River indicate that bigheaded carp consumption has reduced biomass of large zooplankton, which coincided with reduced condition of native planktivores including gizzard shad and bigmouth buffalo (Irons et al. 2007). A modeling study to project impacts of bigheaded carp invasion in Lake Erie found a reduction in biomass of large zooplankton, with a decline in biomass of native planktivores (Zhang et al. 2016).

6.4.1.2 Final characterizations

Table 6-5. Nemadji River Change in total biomass of plankton – Small group characterizations.

		Change in total biomass of plankton						
		Large increase	Moderate increase	Small increase	No change	Small decrease	Moderate decrease	Large decrease
Certainty of assessment	Very high certainty (+/- 10%)							
	High certainty (+/- 30%)							
	Moderate certainty (+/- 50%)					S_L, U_L	S, U, W	X
	Low certainty (+/- 70%)				W_L	T_L	T X_L	
	Very low certainty (+/- 90%)							

6.4.2 Consequence for non-game fish

6.4.2.1 Justifications

Common shiner was chosen as the non-game species because of its high relative abundance in the watershed compared to other species.

The small group varied from low to moderate certainty in their judgment that if bigheaded carps reached a moderate level of abundance, they would have a negligible to moderate effect on common shiner abundance through a reduction in plankton biomass (Table 6-6). At a low abundance, the group felt that bigheaded carps would have a negligible to low adverse effect on common shiner. The larger group also largely felt that bigheaded carps would have a negligible (9 of 19 individuals) to low (7 of 19 individuals) effect on common shiner, with 3 of 19 individuals predicting a moderate effect (Table 6-7). As justification for their decision, the small group members stated that common shiner is an omnivore, and could switch to other prey sources if bigheaded carps depleted the available biomass of plankton. The small group also mentioned that in the Illinois River where bigheaded carps are abundant, few examples have been reported of detectable effects of bigheaded carps on native fishes. On the other hand, two individuals mentioned that even a modest decrease in plankton biomass could have moderate effects on common shiners in a low productivity system like the Nemadji River.

6.4.2.2 Final characterizations

Table 6-6. Nemadji River Consequence for non-game fish (Common Shiner) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	W W_L	X_L	X		
	Low certainty (+/- 70%)	S S_L, T_L, U_L	T, U			
	Very low certainty (+/- 90%)					

Table 6-7 Nemadji River Consequence for non-game fish (Common Shiner) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	3	1	3		
	Low certainty (+/- 70%)	6	6			
	Very low certainty (+/- 90%)					

6.4.3 Consequence for game fish

6.4.3.1 Justifications

Black crappie is one of the most targeted sportfish in the Nemadji River during both open water and ice covered periods. Thus, the Nemadji River small group chose to evaluate the potential effects of bigheaded carps on black crappie to forecast potential effects on this important fishery. The small group predicted that a moderate abundance of bigheaded carps in the Nemadji River watershed would have a negligible (undetectable changes; 2 of 5 participants) to low (small decrease in the population leading to minor reduction in angling quality; 3 of 5 participants) effect on black crappie but the group only had low (4 of 5 participants) to moderate (1 of 5 participants) certainty (Table 6-8).

Justifications for the small group's predictions focused largely on the group's previous predictions that bigheaded carps would reach a fairly low total biomass (5-25% of total fish biomass) and would only reduce plankton resources by 5-15% in this system, which would have a minimal effect on black crappie. The group also discussed how the Nemadji River's heterogeneous habitats may allow for habitat separation between the two species. Other justifications for the small group participants' predictions included the higher trophic position of black crappie compared to bigheaded carps, low diet overlap between species as adults, and lack of evidence that high densities of bigheaded carps have negatively affected sportfishes in other areas of invasion (e.g., Illinois River). However, there was concern that black crappie early life stages may compete with bigheaded carps for plankton, potentially resulting in reduced survival of larvae and recruitment. Under the scenario of low bigheaded carps abundance in the Nemadji River, the small group predicted a negligible (5 of 5 participants) effect on black crappie and the members had low (3 of 5 participants) to moderate (2 of 5 participants) certainty. Uncertainties recognized by the group when making this decision included how successful and abundant bigheaded carps would be in a coldwater environment, and the ability of black crappie to move around to microhabitats within the Nemadji River to reduce spatial overlap with bigheaded carps and adapt to changing environmental conditions. The group also identified that their prediction could be improved by reviewing pre- and post-bigheaded carp invasion data on black crappie populations in other locations (e.g., lower and middle Mississippi River, Illinois River).

The large group characterization for bigheaded carps adverse effect on black crappie in the Nemadji River varied from negligible (5 of 19 participants), to low (12 of 19 participants), and moderate (2 of 19 participants). The large group's certainty level concerning black crappie ranged from very low (3 of 19 participants), to low (15 of 19 participants), and moderate (1 of 19 participants) (Table 6-9).

6.4.3.2 Final Characterizations

Table 6-8. Nemadji River Consequence for game fish (Black Crappie) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	W U_L, W_L				
	Low certainty (+/- 70%)	X S_L, T_L, X_L	S, T, U			
	Very low certainty (+/- 90%)					

Table 6-9. Nemadji River Consequence for game fish (Black Crappie) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	1				
	Low certainty (+/- 70%)	3	11	1		
	Very low certainty (+/- 90%)	1	1	1		

6.4.4 Consequence for species diversity/ecosystem resilience

6.4.4.1 Justifications

Beyond their potential impacts on individual fish species in the Nemadji River, bigheaded carps also may affect species diversity and ecosystem resilience. The small group predicted that a moderate abundance of bigheaded carps in the Nemadji River watershed would have a low (minimal change in ecosystem structure or function; 2 of 5 participants) to moderate (detectable change in ecosystem structure, function, and ability to withstand stressors; 3 of 5 participants) effect on species diversity and ecosystem resilience and the small group had low confidence in their prediction (5/5 participants)(Table 6-10).

Although the small group recognized several mechanisms by which bigheaded carps could affect the ecosystem (e.g., competition with native planktivores), participants generally predicted a low to moderate effect of bigheaded carps on the Nemadji River ecosystem due to 1) predicted changes in native species distributions instead of biomass following bigheaded carps invasion and 2) bigheaded carps would likely only occupy the lower portion of the watershed, leaving the upper reaches intact. The small group also discussed the large number of invasive species already present within the Nemadji River watershed (e.g., round goby (Neogobius melanostomus), spiny water flea (Bythotrephes longimanus), alewife (Alosa pseudoharengus), sea lamprey) and was uncertain how another invasive species would interact with or change the current ecosystem structure and function. The small group then predicted a low abundance of bigheaded carps population would have a negligible (undetectable changes in ecosystem structure and function; 2 of 5 participants) or low (3 or 5 participants) effect on the Nemadji River ecosystem, but the small group still had low certainty in their decision (5 of 5 participants). The small group desired additional information on effects of bigheaded carps on ecosystem structure and function in other invaded ecosystems and how they may interact with other invaders at higher (e.g., sea lamprey, salmonids) and lower (e.g., zebra mussels, spiny water flea) trophic levels to alter ecosystems.

The large group predicted more substantial effects of bigheaded carps on the Nemadji River structure and function compared with the small group, with individuals anticipating negligible (1 of 19 participants), low (6 of 19 participants), moderate (11 of 19 participants), and high (significant changes to ecosystem structure, function, and ability to withstand stressors; 1 of 19 participants) effects. The large group had very low (4 of 19 participants), low (7 of 19 participants), and moderate (8 of 19 participants) certainty (Table 6-11).

6.4.4.2 Final characterizations

Table 6-10. Nemadji River Consequence for species diversity/ecosystem resilience – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)	T_L, W_L	T, W S_L, U_L, X_L	S, U, X		
	Very low certainty (+/- 90%)					

Table 6-11. Nemadji River Consequence for species diversity/ecosystem resilience – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	1	3	4		
	Low certainty (+/- 70%)		3	4		
	Very low certainty (+/- 90%)			3	1	

6.4.5 Consequence for recreational boating and fishing from jumping silver carp hazard

6.4.5.1 Justifications

Bigheaded carps also pose a risk to humans due to the leaping behavior of silver carp that could disrupt boating activities and result in collisions and physical injury. The small group predicted that a moderate abundance of bigheaded carps would have a moderate (occasional sightings of jumping carp and minor changes in boating and fishing; 3 of 5 participants) to high (regular sightings of jumping carp, occasional collisions, and changes in boating and fishing; 2 of 5 participants) effect on recreational opportunities in the Nemadji River watershed but had very low (±90%; 2 of 5 participants) or low (±70%; 3 of 5 participants) certainty (Table 6-12).

The small group discussed the morphology of the Nemadji River and recreational boating in the area. Those familiar with the system indicated that most recreational boating occurs at the confluence of the Nemadji River with Lake Superior which is generally very shallow with the exception of a shipping channel that is maintained at a deeper depth. Recreational boating is perceived to be low in general, resulting in low probability of boater interactions with a moderate abundance of bigheaded carps. However, people who do recreate in this area often use the shallow confluence flats which might increase interactions and collisions with silver carp. This could alter recreational boater and angler behavior, resulting in increased use of the deeper shipping channel that may increase interactions between recreational and commercial boaters. The small group then predicted that a low abundance of bigheaded carps would have a low (rare sightings of jumping carp but does not cause change in boater behavior; 3 of 5 participants) to moderate (2 of 5 participants) effect on recreational boating and fishing but participants had very low (±90%; 2 of 5 participants) to low (±70%; 3 of 5 participants) certainty. The large group generally agreed with the small group (Table 6-13). The large group predicted that bigheaded carps would have a low (7 of 19 participants), moderate (11 of 19 participants) or high (1 of 19 participants) effect on recreational boating and fishing in the Nemadji River.

6.4.5.2 Final characterizations

Table 6-12. Nemadji River Consequence for recreational boating and fishing from jumping silver carp hazard – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)		T_L, W_L	T, W X_L	X	
	Very low certainty (+/- 90%)		S_L	S U_L	U	

Table 6-13. Nemadji River Consequence for recreational boating and fishing from jumping silver carp hazard – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)			3		
	Low certainty (+/- 70%)		6	6	1	
	Very low certainty (+/- 90%)		1	2		

6.4.6 Adverse Effects: Research needs

The Nemadji River small group identified several research needs to better predict potential adverse effects of a bigheaded carps invasion. The small group recognized that pre- and post-invasion data would valuable for monitoring and understanding the effects of a bigheaded carps invasion. The group identified a suit of unique native species (e.g., cisco, lean lake trout, kiyi (Coregonus kiyi)) in the Nemadji River watershed that could be affected by a bigheaded carps invasion and recommend long-term monitoring of these populations to potentially assess pre- and post-invasion population changes. The small group noted that most monitoring to date in other regions of bigheaded carps invasion has focused on plankton and planktivorous fishes: the small group saw a need to better understand how bigheaded carps may affect native piscivores (either positively or negatively). The small group also saw value in better understanding metabolic processes, growth, and consumption demands of bigheaded carps in coldwater, oligotrophic systems where growing degree days and food resources are limited in order to better understand their potential ecosystem effects. Finally, little is known regarding environmental conditions and stressors that trigger silver carp jumping behavior. The small group thought an experiment identifying factors resulting in jumping behaviors would improve communications between recreational boaters, fishers, and biologists regarding risks associated with recreating in areas invaded by bigheaded carps.

6.5 Overarching uncertainties, research needs & areas of disagreements

The Nemadji River small group generally agreed on the effects, or lack thereof, of bigheaded carps on native fishes, ecosystems, and recreational boaters and fishers, and had no major areas of conflict or disagreement. However, the certainty level was low and the small group identified several areas where additional research would improve their understanding of the ecosystems effects of bigheaded carps, with a focus on the Nemadji River. To date, most work on bigheaded carps is being conducted on large, warmwater rivers (e.g., Mississippi, Illinois, Ohio, Missouri). In contrast, little is known if bigheaded carps could successfully invade a small, cool/coldwater river, and if so, what effects they would have on these systems. Further, the small group discussed the suite of invasive species that currently occupy the Nemadji River watershed, including round goby, spiny water flea, zebra and dreissenid mussels, salmonids, and sea lamprey. The group desired information on how existing invaders may compete with or facilitate the invasion of bigheaded carps, how populations of existing invaders may change through the establishment of a new invader, and resulting impacts to ecosystem structure, function, and resilience. The small group also discussed the opportunity and ability of organisms to move within the Nemadji River watershed in response to a bigheaded carps invasion and desired information on movement rates of fishes between the Nemadji River, St. Louis Estuary, and Lake Superior.

7 Sand Hill River

7.1 Introduction to watershed

The Sand Hill River Watershed drains approximately 1259km^2 of northwestern Minnesota (Erickson et al. 2015), and spans parts of two Level III Ecoregions: the North Central Hardwoods and the Lake Agassiz Plain (Omernik et al. 1988). The upper and eastern 10% of the Sand Hill River Watershed lies within The North Central Hardwood Forests Ecoregion, in which Omernik et al. (1988) characterized land cover and land use as a mosaic of forests, wetlands, lakes, crops, pastures, and dairies. In contrast, the Lake Agassiz Plain that underlies the lower and western 90% of the Sand Hill River Watershed is a flat agricultural area, formerly covered by tallgrass prairie and dominated presently by rowcrops such as soybeans, sugar beets, and corn (Omernik et al. 1988).

The majority (71%) of the Sand Hill River waterway is altered (Anderson et al. 2014). Sand Hill Lake is the headwaters of the Sand Hill River. The Sand Hill River has one noteworthy tributary, Kittelson Creek, which begins as the outlet of Kittelson Lake, and flows nearly 20km to its confluence with the Sand Hill River. In the upper and eastern reaches that flow through glacial moraine and the beach ridge regions, the Sand Hill River generally follows its natural course, but in the lower and western reaches that flow across the Lake Agassiz Plain, the river was ditched by the US Army Corps of Engineers in the late 1950s, removing 18 miles of channel (USACE 2013). These alterations were in addition to four drop structures and two dams that were added to the mainstem to reduce flooding and improve drainage (Anderson et al. 2014). Most of the tributaries in the lower half of the watershed are ditches.

The Minnesota Pollution Control Agency sampled 19 biological monitoring sites for fish and macroinvertebrates in the Sand Hill River Watershed. Forty-five species of fish were detected throughout the watershed (Anderson et al. 2014) with most of these being smaller and/or benthic species. No imperiled species were present in the watershed but a variety of small-bodied species are abundant, and some minnow species characterized as sensitive in this ecoregion (e.g., longnose dace (Rhinichthys cataractae)) were present in the upper reaches of the watershed. Several game fish are present in this watershed including yellow perch (Perca flavescens), walleye, northern pike, and several Ictaluridae catfish. Common carp is the only aquatic invasive species known to occur in this watershed. Fish biotic integrity generally improved from headwaters to confluence, which was largely a result of connectivity of the lower half of the watershed maintaining connectivity with the Red River of the North and barriers (i.e., grade improvement structures and dams) preventing movement into the upper half of the watershed. This is supported by the macroinvertebrate data which indicated greater proportions of tolerant taxa in the lower and channelized reaches, relative to the upper, more natural reaches of the watershed (Anderson et al. 2014).

7.2 Likelihood of establishment

7.2.1 Justifications

The small group believed that there is a low likelihood of establishment of bigheaded carps in the Sand Hill River Watershed and a majority of the large group (16/21) felt similarly (Table 7-1; Table 7-2). Justification for this characterization included, first, native fishes in the lower part of this watershed are unable to recolonize above the grade improvement structures and dams, so it is reasonable to assume that it would be similarly difficult for bigheaded carps to expand upstream as well. Second, establishment implies self-sustaining populations, which are unlikely given the overall scarcity of rearing habitat for juvenile bigheaded carps.

7.2.2 Final characterizations

Table 7-1. Sand Hill River Likelihood of Establishment - Small Group Final Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)		K			
	Moderate certainty (+/- 50%)		L, H			
	Low certainty (+/- 70%)		G, I			
	Very low certainty (+/- 90%)					

Table 7-2. Sand Hill River Likelihood of Establishment - Large Group Characterization.

		Likelihood of establishment				
		Very unlikely (.00-.05)	Low (.05-.40)	Moderate (.40-.60)	High (.60-.95)	Very likely (.95-1.00)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)		4			
	Moderate certainty (+/- 50%)	2	9			
	Low certainty (+/- 70%)	1	3	1		
	Very low certainty (+/- 90%)			1		

7.2.3 Research needs

Research needs identified include: identification of settling areas and the development of a Fluvial Egg Drift Simulator model. Second, there is little documentation of bigheaded carps using shallow, flashy, channelized or ditched habitats, so experimentation in artificial streams would benefit our ability to predict their establishment in watersheds like the Sand Hill River, where those habitat conditions are abundant.

7.3 Resulting abundance

7.3.1 Justifications

Given that bigheaded carps establish in the Sand Hill River, the small group estimated they would reach moderate to high abundances (Table 7-3). The large group estimated the likely abundance of bigheaded carps in the Sand Hill River would be very low to high, with varying levels of certainty but the majority of experts estimated that bigheaded carps abundance would be moderate (Table 7-4). The fish assemblage in the Sand Hill River is dominated by small- to medium-bodied fishes (e.g., central mudminnow (Umbra limi), creek chub (Semotilus atromaculatus)) with low abundances of medium and large fishes (e.g., white sucker (Catostomus commersonii), yellow perch) and no planktivores (MPCA 2014b) that may directly compete with bigheaded carps (e.g., bigmouth buffalo) (Irons et al. 2007; Sampson et al. 2009). Therefore, it is expected that bigheaded carps will be able to establish an ecological niche.

Sand Hill River waters are nutrient-rich (MPCA 2014b) which could provide abundant resources for bigheaded carps. Additionally, bigheaded carps are large-bodied relative to many Sand Hill

River species meaning that, at low densities, bigheaded carps could compose a high percentage of total fish biomass. The Sand Hill River is separated hydrologically by four dams that restrict lateral connectivity (MPCA 2014b) and may restrict movement and spawning of bigheaded carps to the lower Sand Hill River. However, plans to remove these dams in the near future (Sand Hill River Fish Passage Project 2016) could increase connectivity to backwater and low flow habitats; areas preferred by bigheaded carps (Kolar et al. 2007; Calkins et al. 2012) that could lead to higher abundances in the Sand Hill River. Overall, it is expected that the lower Sand Hill River would have the highest abundances of bigheaded carps due to emigration from the Red River and low velocity habitats at the Red – Sand Hill River confluence.

7.3.2 Final characterizations

Table 7-3. Sand Hill River Resulting Abundance – Small Group Final Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)				G	
	Moderate certainty (+/- 50%)			H, K	I	
	Low certainty (+/- 70%)				L	
	Very low certainty (+/- 90%)					

Table 7-4. Sand Hill River Resulting Abundance – Large Group Characterization.

		Resulting abundance (% of total fish biomass)				
		Very low (Few individuals, <1%)	Low (1-5% of total fish biomass)	Moderate (5-25% of total fish biomass)	High (25-60% of total fish biomass	Very high (>60% of total fish biomass)
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)		1			
	Moderate certainty (+/- 50%)		3	7	2	
	Low certainty (+/- 70%)	1	1	4	1	
	Very low certainty (+/- 90%)			1		

7.3.3 Research needs

The Sand Hill River is dissimilar from most rivers in which bigheaded carp populations have been observed (e.g., Illinois River, Middle Mississippi River) contributing to uncertainty around the abundance they may achieve but hydrology, resource availability, and thermal regime have all been examined as factors that can influence the establishment and abundance of bigheaded carps (Kolar et al. 2007; Calkins et al. 2012; Kocovsky et al. 2012). Modeling efforts, coupled with hydrological surveys, could help resolve uncertainty (e.g., Kocovsky et al. 2012; Garcia et al. 2013; Garcia et al. 2015) surrounding availability of adequate habitats for all life history stages. Additionally, surveys could reveal the presence of backwater and nursery habitats. It is also unknown whether backwater habitats are a necessity for bigheaded carps or simply a preferred habitat, and whether bigheaded carp populations can reach high abundances in rivers lacking slackwater areas. Thus, information on habitat use of bigheaded carps and ecosystem characteristics that contribute to different abundances of bigheaded carps would be vital in adding certainty to predictions of post-invasion abundance in the Sand Hill River.

7.4 Adverse effects

During the characterization of potential adverse effects, the small group characterized the consequence of each adverse effect for the likely abundance of bigheaded carps that was determined in the previous step. The small group also characterized the consequence resulting from the second most likely abundance of bigheaded carps. For the Sand Hill River small group, the first abundance was “Moderate” and the second abundance was “Low”. In the tables below, the characterization for the “Moderate” abundance is noted with “G”, “H”, “I”, etc. whereas the characterization for the “Low” abundance is noted with “G_L”, “H_L”, “I_L”. The letters represent different individuals within the small group.

7.4.1 Change in plankton

7.4.1.1 Justifications

One of the most well documented consequences of invasion by bigheaded carps is a decline in abundance of larger crustacean zooplankton and an increase in the plankton proportions that are composed by rotifers (e.g., Sass et al. 2014). However, the Sand Hill River currently does not likely support a large plankton community due to light limitations from turbidity and a rapid flushing rate despite high nutrient run-off. Small-bodied plankton that are not consumed by bigheaded carps may benefit from nutrients imported by migrating bigheaded carps (e.g., Polis et al. 1997) or from predatory release as bigheaded carps consume larger, predatory zooplankton. Additionally, bigheaded carps migrate over long distances (DeGrandchamp et al. 2008; Coulter et al. 2016b), and so individuals may move into or out of the Sand Hill River from the Red River seasonally, moving nutrients and seasonally altering food web dynamics. Feces from bigheaded carps may result in more bioavalaible nutrients in the water column which may stimulate phytoplankton growth. Excretion from bigheaded carps may compensate for their feeding activities. Therefore, the small group estimated that there would be a small decrease in plankton biomass at a moderate abundance of bigheaded carps with low to high certainty (Table 7-5). At low densities of bigheaded carps, the small group estimated that there would be either no change in plankton biomass or a slight increase (Table 7-5). However, there was uncertainty regarding the current abundances and assemblage of plankton in the Sand Hill River. If there are few crustacean zooplankton currently present, bigheaded carps may have less of an impact on plankton biomass.

7.4.1.2 Final characterizations

Table 7-5. Sand Hill River Change in total biomass of plankton – Small group characterizations.

		Change in total biomass of plankton						
		Large increase	Moderate increase	Small increase	No change	Small decrease	Moderate decrease	Large decrease
Certainty of assessment	Very high certainty (+/- 10%)							
	High certainty (+/- 30%)				I_L, K_L	H, I	G	
	Moderate certainty (+/- 50%)				H_L	K		
	Low certainty (+/- 70%)			G_L	L_L	L		
	Very low certainty (+/- 90%)							

7.4.2 Consequences for non-game fish

7.4.2.1 Justifications

In addition to altering plankton composition, bigheaded carps may also affect native fish species in the Sand Hill River. Many of the species that compose the fish assemblage in the Sand Hill River Watershed rely on benthic resources; therefore golden redhorse (Moxostoma erythrurum) was selected as a representative species to evaluate the potential impacts of bigheaded carps. The small and large groups estimated negligible to low impacts of a moderate abundance of bigheaded carps on golden redhorse with large differences in certainty (Table 7-6; Table 7-7). The impacts of bigheaded carps on the planktonic community and native planktivores are well established (Radke and Kahl 2002; Sass et al. 2014), but there have only been limited studies on their potential effects on the benthic fish community (e.g., Yallaly et al. 2015). Impacts on the benthic community would be indirect and, therefore, difficult to distinguish from other sources of change. Overall, group members agreed that there would be little direct competition for food resources but that bigheaded carps could physically displace golden redhorse from some habitats. Bigheaded carps present in a low abundance would likely have a negligible to low impact on golden redhorse because it would be less likely golden redhorse would be displaced and other impacts from bigheaded carps would also be reduced. Bigheaded carps may consume eggs or larvae of benthic species during routine feeding activities, which could negatively impact golden redhorse populations. However, this has yet to be documented. Bigheaded carps may potentially stimulate the benthic food web because food items being digested by bigheaded carps have a short retention time in the digestive tract (Kolar et al. 2005). Therefore, excreted items may be only partially digested and could be a food resource for benthic fishes (Yallaly et al. 2015).

7.4.2.2 Final characterizations

Table 7-6. Sand Hill River Consequence for non-game fish (Golden Redhorse) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)	K_L, L_L, G_L	I_L			
	Moderate certainty (+/- 50%)	G H_L	K			
	Low certainty (+/- 70%)	L	H	I		
	Very low certainty (+/- 90%)					

Table 7-7. Sand Hill River Consequence for non-game fish (Golden Redhorse) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)	1				
	Moderate certainty (+/- 50%)	7	3			
	Low certainty (+/- 70%)	2	4	1		
	Very low certainty (+/- 90%)		1			

7.4.3 Consequences for game fish

7.4.3.1 Justifications

Bigheaded carps may affect game fish populations in the Sand Hill River through several mechanisms. Piscivorous game fish may consume young-of-year or juvenile bigheaded carps but bigheaded carps may also compete with larval and juvenile game fish for planktonic resources which could decrease condition and impact recruitment. Schools of young bigheaded carps may displace young game fish from refuge or nursery habitats resulting in increased predation on native species as they are forced into open habitats. Bigheaded carps may also indirectly produce changes in the food web that would decline forage fish abundance, negatively impacting piscivorous game fish. The Sand Hill River contains several game fish species (MPCA 2014b) and impacts of bigheaded carps on two species were evaluated: northern pike (Esox lucius) and walleye (Sander vitreus). Northern pike spawn earlier than bigheaded carps (northern pike: 8 - 12ºC, Casselman and Lewis 1996; bigheaded carp: 17 - 28ºC, Coulter et al. 2016a) and shift from planktivory to piscivory rapidly (beginning around 4 cm in total length, Frost 1954). As a result, young northern pike may be piscivorous when bigheaded carps spawn which would allow young individuals to exploit this seasonal resource. The small and large group discussions determined that the ability of northern pike to exploit small bigheaded carps as a food resource would overcome any potential declines cause by decreased availability of native forage fish or competition between larval northern pike and bigheaded carps for plankton. Therefore, bigheaded carps were estimated to have a negligible impact on northern pike at low or moderate densities, with moderate to very high certainty (Table 7-8; Table 7-9).

Alternatively, the groups estimated that bigheaded carps are likely to have a low to moderate impact on walleye, with moderate to low certainty. Walleye can reproduce later in the year than northern pike (5 - 16 ºC, Johnson 1961) and young walleye spawned later would likely still be planktivorous when bigheaded carps reproduce and so would be unable to feed on young bigheaded carps. Adult walleye could consume young bigheaded carps but only for a short window of time which the groups expect would lead to an overall negative impact on walleye (Table 7-10; Table 7-11). Uncertainty was high but could be improved with behavioral studies to determine if northern pike and walleye consume young bigheaded carps and if young bigheaded carps can displace native fishes from refuge habitats. Many of the positive or negative impacts that bigheaded carps could have on native game fish are dependent on bigheaded carps reproducing within the Sand Hill River. If bigheaded carp reproduction does not occur in the Sand Hill River, then both northern pike and walleye may show little effect as young bigheaded carps would not be available for consumption.

7.4.3.2 Final characterizations

Table 7-8. Sand Hill River Consequence for game fish (Northern Pike) – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)	G G_L, I_L				
	High certainty (+/- 30%)	K, L, I H_L, K_L, L_L				
	Moderate certainty (+/- 50%)	H				
	Low certainty (+/- 70%)					
	Very low certainty (+/- 90%)					

Table 7-9. Sand Hill River Consequence for game fish (Northern Pike) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)	2				
	High certainty (+/- 30%)	8				
	Moderate certainty (+/- 50%)	5				
	Low certainty (+/- 70%)	1	2			
	Very low certainty (+/- 90%)			1		

Table 7-10. Sand Hill River Consequence for game fish (Walleye) – Small group characterization, for moderate abundance only.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)		G, K, L,			
	Low certainty (+/- 70%)		I	H		
	Very low certainty (+/- 90%)					

Table 7-11. Sand Hill River Consequence for game fish (Walleye) – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)	1				
	Moderate certainty (+/- 50%)		7			
	Low certainty (+/- 70%)		7			
	Very low certainty (+/- 90%)		1	3		

7.4.4 Consequences for species diversity/ecosystem resilience

7.4.4.1 Justifications

Species diversity and resilience are important components of healthy ecosystems by maintaining ecosystem function when exposed to environmental changes. Ecosystem resilience may come from a redundancy (fish that may serve similar functions or fill similar ecological niches) in the roles of species in the ecosystems and it appears that there are redundant species in the Sand Hill River fish assemblage (MPCA 2014b). Therefore, even if a species is lost or declines due to invasion by bigheaded carps there are other species present which can maintain ecosystem function. Planktivores (e.g., bigmouth buffalo) that may directly compete with bigheaded carps are species most likely to be affected from an invasion by bigheaded carps (Irons et al. 2007; Sampson et al. 2009) but these species are not present in the Sand Hill River fish assemblage. Therefore, the small and large group discussions predict that the consequences of invasion by bigheaded carps on species diversity and ecosystem resilience would be low to moderate when bigheaded carps are present at a moderate abundance (Table 7-12; Table 7-13). It was also estimated that the effects of bigheaded carps on diversity and resilience would be low to negligible at low bigheaded carps density. Certainty around these estimates ranged from very low to moderate due to the difficulty involved in relating declines in diversity or resilience directly to bigheaded carps. There is also variability among sites and years in the survey data of fish assemblages (MPCA 2014b) which may make declines in diversity or resilience difficult to detect. Additional uncertainty was from the unknown effects that bigheaded carps may have on the benthic community, which constitutes a large portion of the Sand Hill River fish assemblage.

7.4.4.2 Final characterizations

Table 7-12. Sand Hill River Consequence for species diversity/ecosystem resilience – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)					
	Low certainty (+/- 70%)		K G_L, I_L	G, I		
	Very low certainty (+/- 90%)	K_L	H_L, L_L	H, L		

Table 7-13. Sand Hill River Consequence for species diversity/ecosystem resilience – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)					
	High certainty (+/- 30%)					
	Moderate certainty (+/- 50%)	1	4	2		
	Low certainty (+/- 70%)		2	5		
	Very low certainty (+/- 90%)		1	3		

7.4.5 Consequences for recreational boating and fishing from jumping silver carp hazard

7.4.5.1 Justifications

Most experts in the small and large group discussions felt that bigheaded carps in moderate abundance would have a low or moderate impact on recreational boating and fishing in the Sand Hill River, with most ranking their certainty as moderate or high. At low densities of bigheaded carps, recreators would be less likely to encounter them and so their effects on boating and angling would be negligible to low. Overall, many experts felt that recreational boating would show no change (Table 7-14; Table 7-15). There is very limited boating and fishing activity currently occurring on the Sand Hill River. Most of the angling pressure in the Sand Hill River comes from locals who would likely continue to fish due to the river's proximity, regardless of the abundance of bigheaded carps. However, boating and fishing activities may be negatively impacted if bigheaded carps were to invade lakes within the Sand Hill River watershed. Specifically, jumping silver carp may deter some recreators but it is unknown what abundances of bigheaded carps are needed to cause declines in recreational use. Additional information on abundances of bigheaded carps and declines in recreational activities from other river systems would help to refine estimated impacts.

7.4.5.2 Final characterizations

Table 7-14. Sand Hill River Consequence for recreational boating and fishing from jumping silver carp hazard – Small group characterizations.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)		H_L, L_L	G		
	High certainty (+/- 30%)	G_L, I_L	H, K K_L	L		
	Moderate certainty (+/- 50%)			I		
	Low certainty (+/- 70%)					
	Very low certainty (+/- 90%)					

Table 7-15. Sand Hill River Consequence for recreational boating and fishing from jumping silver carp hazard – Large group characterization for moderate abundance of bigheaded carps.

		Consequence				
		Negligible	Low	Moderate	High	Extreme
Certainty of assessment	Very high certainty (+/- 10%)		1			
	High certainty (+/- 30%)	2	4			
	Moderate certainty (+/- 50%)	3	2	1		
	Low certainty (+/- 70%)		5	1		
	Very low certainty (+/- 90%)					

7.4.6 Adverse effects: Research needs

Most studies on the impacts of bigheaded carps have focused on changes in native planktivores that may directly compete with the carp, and changes in zooplankton composition and abundance that may result from feeding by bigheaded carps. Because of the focus on zooplankton and competition, experts were fairly confident in assessing what changes are likely to occur in Sand Hill River plankton abundance. However, surveys of existing plankton abundance and composition in the Sand Hill River would help to further improve estimated impacts of bigheaded carps. Additionally, surveys would help to document changes in plankton that may occur following invasion by bigheaded carps. Because there is relatively little information available on the impacts of bigheaded carps on species they are not in direct competition with, further research is needed to determine how bigheaded carps may impact other native species, including the benthic community. Uncertainty around the estimated impacts of bigheaded carps on benthic oriented species, like golden redhorse, could be improved through evidence from river systems that have already been invaded including information related to changes in abundance or condition, potential physical displacement of native species, and impacts on recruitment through competition for planktonic resources. Additionally, information on the caloric and nutrient content of bigheaded carp feces will aid our understanding of how bigheaded carps may affect benthic communities.

There is also relatively little information on which predatory species consume bigheaded carps, contributing to uncertainty in how invasion by bigheaded carps will impact piscivorous species. Many predatory game fish like Northern Pike and Walleye may benefit from exploiting the high abundances of bigheaded carps that can occur following a successful spawning event. Feeding studies can help resolve uncertainty and determine what piscivorous species consume bigheaded carps and when. Piscivores are gape limited and bigheaded carps may rapidly outgrow the gape of many native predators. Therefore, modeling efforts to determine if bigheaded carps can spawn in the Sand Hill River would help determine if there will be young-of-year present for piscivorous fishes to consume, which could positively impact native piscivores. Bigheaded carps may negatively impact native prey that native piscivores typically exploit through competition for planktonic resources. Further research is needed to determine if bigheaded carps compete with native forage fish enough to cause a decline in abundance that could impact native game fishes.

The impacts of bigheaded carps on ecosystem function and resilience have not been examined in depth. Because bigheaded carps compete for resources, they could cause the loss or decline of some species. While reduced condition has been documented in some native species directly competing with bigheaded carps (e.g., Irons et al. 2007), the impacts of bigheaded carps on many other species had not been assessed. Bigheaded carps may also impact ecosystem functions including nutrient processing and cycling but these mechanisms remain unevaluated. Additional research is needed on the whole ecosystem impacts of bigheaded carps rather than focused studies on impacts on specific native species.

Further research is also needed to better evaluate the possible impacts of bigheaded carps of fishing and boating activities in the Sand Hill River. The small group believed that some information on the impacts of bigheaded carps on recreation likely already exist and a study released following group discussions shows that bigheaded carps negatively impact river use (Spacapan et al. 2016). Additionally, it may be informative to determine the densities of bigheaded carps that can cause a decline in boating or fishing activities. There may be a threshold abundance of bigheaded carps where lower abundances have no impact on recreation but high abundances decrease recreational use.

7.5 Overarching uncertainties, research needs & areas of disagreements

Much of the uncertainty surrounding this assessment of the impacts of bigheaded carps in the Sand Hill River results from ecological differences between this river and rivers in which bigheaded carps have been studied. Some portions of the Sand Hill River watershed are connected by shallow, small, or channelized habitats that are unlike areas where the movements and habitat use of bigheaded carps have been studied. Therefore, it is unclear whether bigheaded carps may use these habitats and whether or not they will readily move through them to reach other areas in the watershed. Further, in the James River basin in eastern South Dakota, a prairie stream that drains a predominantly agricultural landscape similar to the Sand Hill River basin, juvenile bigheaded carps were most abundant in low velocity, protected embayment formed by natural confluences with tributaries (Hayer 2014). In the Sand Hill River basin, few of these natural tributaries and confluences exist, so reproduction and recruitment in the Sand Hill River basin would, to our knowledge, be the first documented successful reproduction and recruitment in this type of habitat. Additionally, many fishes in the Sand Hill River are benthic and research on how bigheaded carps affect the benthic community (fish, invertebrates, microbes) would be invaluable. Minnesota Pollution Control Agency and Minnesota Department of Natural Resources currently conduct environmental surveys at multiple Sand Hill River locations and continued monitoring will be vital for detecting changes in the ecosystem if bigheaded carps do invade. Additionally, stakeholder surveys may help determine the current extent of boating and angling activities in the Sand Hill River to better assess recreational changes in the future.

8 Overall Risk Characterization

8.1 Overall establishment probabilities, resulting abundances, and potential adverse effect consequence levels

The overall characterizations of risk for each adverse effect in each watershed were arrived at by combining the overall predicted probability of establishment (Table 8-1) and the potential adverse effect characterizations (Table 8-3). The process used to arrive at these characterizations is described in the methodology (Section 2.3). The overall predicted probabilities of establishment are listed in Table 8-1. The Minnesota River – Mankato watershed had the highest overall predicted probability of establishment, at 70%, followed by the Lower St. Croix River at 45%, Nemadji River at 38%, and the Sand Hill River at 22%.

Table 8-1 Overall probability of establishment for each watershed.

Watershed	Overall Probability of Establishment
Minnesota River - Mankato	.70
Lower St. Croix River	.45
Nemadji River	.38
Sand Hill River	.22

The potential adverse effects were characterized for the most likely resulting abundance of bigheaded carps in each watershed, given establishment of bigheaded carps (Table 8-2). The potential adverse effects were also characterized for the second most likely resulting abundance level, but only in the small group. The directional shift in the small group adverse effect characterizations from the first to second most likely abundance level provides an indication of how the overall risk characterizations would change if the second most likely abundance level is realized (see Section 8-3).

The potential adverse effect consequence levels were characterized for each watershed for the most likely resulting abundance level (moderate) of bigheaded carps. These characterizations show the proportion of workshop participants who believed that a moderate abundance of bigheaded carps would result in each consequence level for each potential adverse effect (Table 8-3).

Table 8-2. Most likely and second most likely resulting abundance levels of bigheaded carps for each watershed.Included in parentheses are the percentages of participants who characterized the resulting abundance at each level.

Watershed	Most likely resulting abundance level	Second most likely resulting abundance level
Minnesota River - Mankato	Moderate (60%)	High (40%)
Lower St. Croix River	Moderate (62%)	Low (24%)
Nemadji River	Moderate (50%)	Low (40%)
Sand Hill River	Moderate (57%)	Low (24%)

Table 8-3. Summary of the consequence levels for the potential adverse effects. Percentages represent the proportion of workshop participants who characterized each potential adverse effect at a particular consequence level. For example, 52% of workshop participants thought that there would be a negligible impact on the Spotfin Shiner in the Minnesota River – Mankato watershed, if bigheaded carps establish in the watershed with a moderate abundance.

Potential Adverse Effect & Watershed	Consequence level				
	Negligible	Low	Moderate	High	Extreme
Non-Game Fish					
Minnesota: Spotfin Shiner	.52	.48			
Minnesota: Bigmouth Buffalo		.48	.52		
St. Croix: Gizzard Shad		.47	.53		
Nemadji: Common Shiner	.47	.37	.16		
Sand Hill: Golden Redhorse	.53	.42	.05		
Game Fish					
Minnesota: Channel Catfish	.79	.21			
St. Croix: Sauger	.06	.72	.22		
Nemadji: Black Crappie	.26	.63	.11		
Sand Hill: Norther Pike	.84	.11	.05		
Sand Hill: Walleye	.05	.79	.16		
Species diversity/ Ecosystem resilience					
Minnesota	.05	.05	.79	.11	
St. Croix		.11	.89		
Nemadji	.05	.32	.58	.05	
Sand Hill	.06	.39	.55		
Recreation Jumping Hazard					
Minnesota			.65	.35	
St. Croix		.05	.42	.48	.05
Nemadji		.37	.58	.05	
Sand Hill	.26	.63	.11		

8.2 Overall risk characterizations

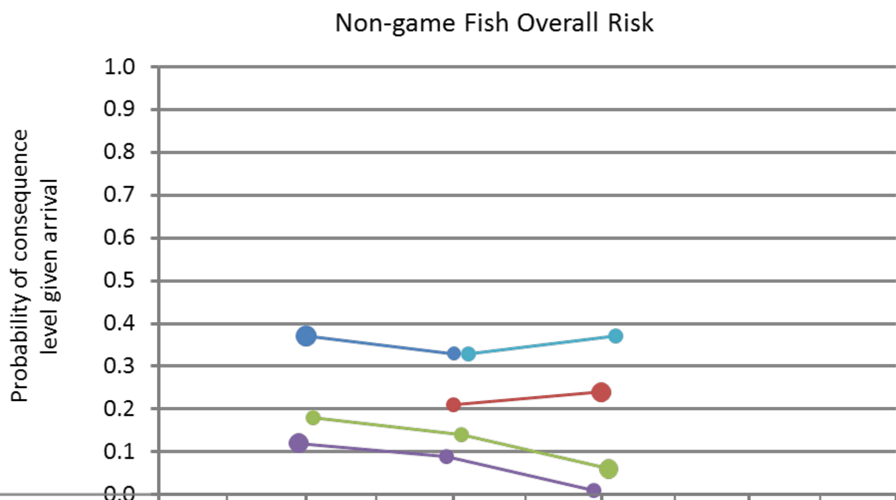
The overall risk characterizations, calculated as the probability of a specific consequence level given arrival of bigheaded carps to the watershed, are provided in Figures 8.1 – 8.4. As described in detail within the methodology (Section 2.3), the overall risk is a function of which consequence levels are expected given the likely resulting abundance of bigheaded carps and how likely those consequence levels are. How likely they are is dependent upon the overall establishment probability. As a result, watersheds with lower overall probabilities of establishment are more likely to have a lower overall risk. So the Sand Hill River watershed, for example, frequently has the lowest overall risk because of the fact that the overall likelihood of establishment was only 22%. The probabilities of all the consequence levels for a particular adverse effect and watershed sum to the overall probability of establishment for that watershed.

For the non-game fish (Figure 8-1), the overall risk varied between the consequence levels of negligible and moderate across all watersheds. The fish species and watershed combinations most likely to result in a moderate consequence level were the bigmouth buffalo in the Minnesota River (37%) and the gizzard shad in the St. Croix River (24%); both these fish species are planktivores. The other three fish species characterized for the non-game fish were not planktivores and were most likely to have a consequence level of negligible, followed by low. The certainty levels with these overall risk characterizations were either low or moderate.

The game fish overall risk (Figure 8-2) varied between the consequence levels of negligible and moderate for all watersheds. Unlike the non-game fish overall risk that had two watershed and fish species combinations most likely to result in a moderate consequence, all the watershed and fish species combinations for the game fish had the negligible or low consequence level as the most likely to occur. The most likely consequence level for the St. Croix River and sauger combination was low (33%) followed by moderate (10%) and negligible (2%). The most likely consequence level for the Nemadji River and black crappie combination was low (24%), followed by negligible (10%) and moderate (4%). The most likely consequence level for the Sand Hill River and walleye combination was also low (17%), followed by moderate (4%) and negligible (1%). For the Minnesota River and channel catfish, the most likely consequence level was negligible (55%), followed by low (15%), and for the Sand Hill River and northern pike, the most likely consequence level was negligible (19%), followed by low (2%) and moderate (1%). The certainty levels varied widely from high to very low. There were higher certainties for the lower consequence levels, with high certainty for three of the five negligible consequence levels and very low certainty for three of the four moderate consequence levels.

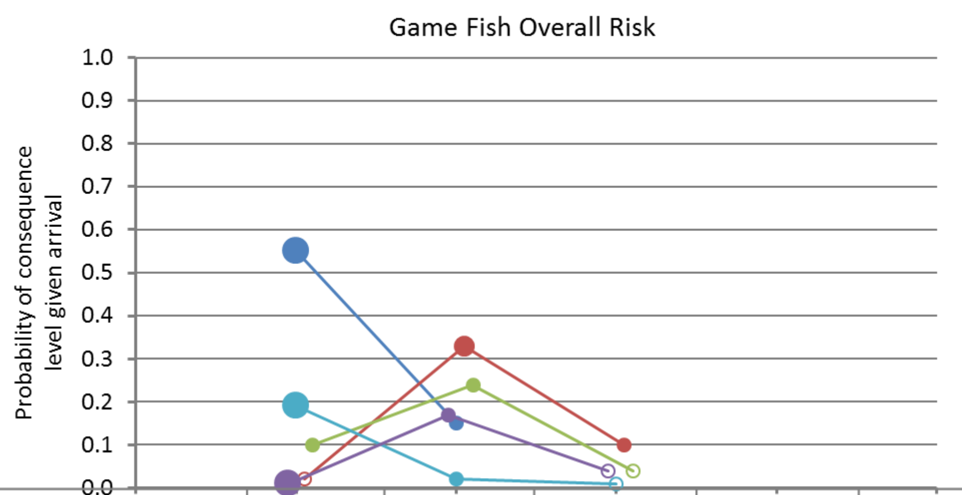
The species diversity/ecosystem resilience overall risk predictions (Figure 8-3) varied from negligible to high, and the moderate consequence level was the most likely for each of the watersheds. The Minnesota River watershed was the most likely watershed to result in the consequence levels of moderate (55%) and high (7%). The St. Croix River watershed was next most likely to result in a moderate consequence level (40%), followed by the Nemadji (22%) and the Sand Hill River (12%). For all watersheds except the Minnesota River, low was the second most likely consequence level after moderate. For the Minnesota River, high was the next most likely (7%). The only other watershed to have a high consequence level characterized was the Nemadji River at a 2% likelihood. The certainty levels for this overall risk varied from very low to moderate.

The jumping hazard overall risk (Figure 8-4) varied from negligible to extreme across all four watersheds. The Minnesota River watershed was the most likely of the 4 watersheds to result in a consequence level of high (24%), even though moderate was the Minnesota River's most likely consequence level (46%). The most likely consequence level for the St. Croix River was high (21%), followed closely by moderate (19%), with the smallest likelihoods being extreme (2%) and low (2%). The most likely consequence level for the Nemadji was moderate (22%), followed by low (14%) and high (2%), while the most likely consequence level for the Sand Hill River watershed was low (14%) followed by negligible (6%) and moderate (2%). The certainty levels for this jumping hazard overall risk ranged from low to high.



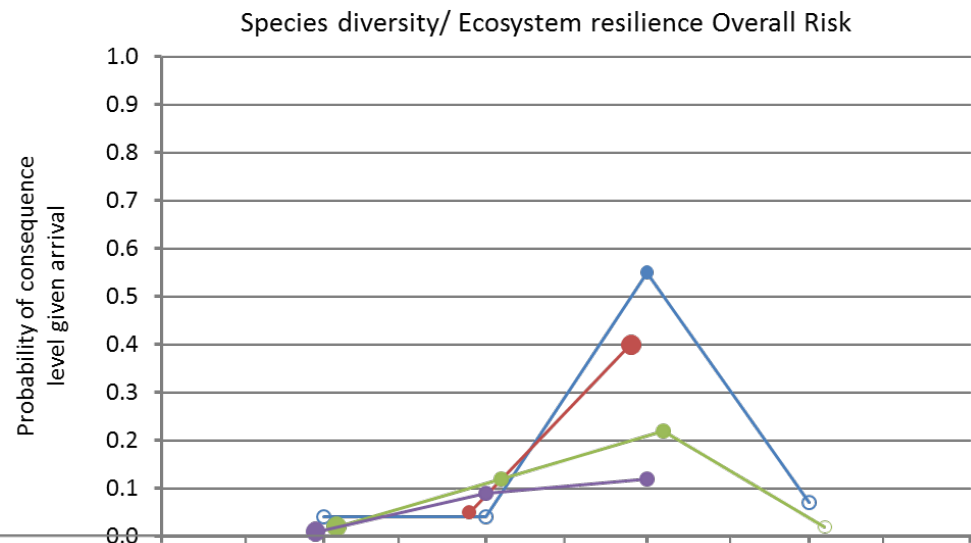
Consequence level :	Negligible	Low	Moderate	High	Extreme
MN: Bigmouth Buffalo		.33 (L)	.37 (L)		
MN: Spotfin Shiner	.37 (M)	.33 (L)			
St. Croix: Gizzard Shad		.21 (L)	.24 (M)		
Nemadji: Common Shiner	.18 (L)	.14 (L)	.06 (M)		
Sand Hill: Golden Redhorse	.12 (M)	.09 (L)	.01 (L)		

Figure 8-1. Non-game Fish Overall Risk. The x-axis lists the 5 possible consequence levels that workshop participants characterized, from least severe (Negligible) to most severe (Extreme). The y-axis displays the probability of each consequence level, given arrival of bigheaded carps. The probability that the bigheaded carps would not establish is not included here, but makes up the remainder of the probability of consequence. For example, for the St. Croix River watershed, the probability that bigheaded carps would NOT establish given arrival was estimated as .55 = 1 –.21 –.24. The certainty of the characterizations for each consequence level are represented in the table (VL=Very Low; L= Low; M=Moderate; H=High; VH = Very High) and by marker size (same 5 point scale with larger circles equaling greater certainty, and the hollow circle indicating Very Low).



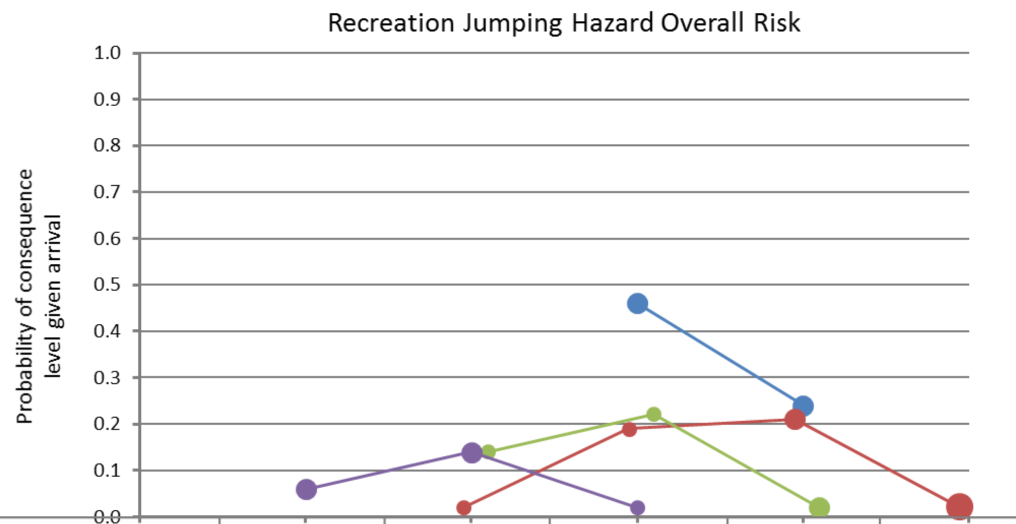
Consequence level :	Negligible	Low	Moderate	High	Extreme
Minnesota: Channel Catfish	.55 (H)	.15 (L)			
St. Croix: Sauger	.02 (VL)	.33 (M)	.10 (L)		
Nemadji: Black Crappie	.10 (L)	.24 (L)	.04 (VL)		
Sand Hill: Northern Pike	.19 (H)	.02 (L)	.01 (VL)		
Sand Hill: Walleye	.01 (H)	.17(L)	.04 (VL)		

Figure 8-2. Game Fish Overall Risk. The x-axis lists the 5 possible consequence levels that workshop participants characterized, from least severe (Negligible) to most severe (Extreme). The y-axis displays the probability of each consequence level, given arrival of bigheaded carps. The probability that the bigheaded carps would not establish is not included here, but makes up the remainder of the probability of consequence. For example, for the Minnesota River - Mankato watershed, the probability that bigheaded carps would NOT establish given arrival was estimated as .30 = 1 –.55 –.15. The certainty of the characterizations for each consequence level are represented in the table (VL=Very Low; L= Low; M=Moderate; H=High; VH = Very High) and by marker size (same 5 point scale with larger circles equaling greater certainty, and the hollow circle indicating Very Low).



Consequence level :	Negligible	Low	Moderate	High	Extreme
Minnesota	.04 (VL)	.04 (VL)	.55 (L)	.07 (VL)	
St. Croix		.05 (L)	.40 (M)		
Nemadji	.02 (M)	.12 (L)	.22 (L)	.02 (VL)	
Sand Hill	.01 (M)	.09 (L)	.12 (L)		

Figure 8-3. Species diversity/Ecosystem resilience Overall Risk. The x-axis lists the 5 possible consequence levels that workshop participants characterized, from least severe (Negligible) to most severe (Extreme). The y-axis displays the probability of each consequence level, given arrival of bigheaded carps. The probability that the bigheaded carps would not establish is not included here, but makes up the remainder of the probability of consequence. For example, for the St. Croix River watershed, the probability that bigheaded carps would NOT establish given arrival was estimated as .55 = 1 –.05 –.40. The certainty of the characterizations for each consequence level are represented in the table (VL=Very Low; L= Low; M=Moderate; H=High; VH = Very High) and by marker size (same 5 point scale with larger circles equaling greater certainty, and the hollow circle indicating Very Low).



Consequence level :	Negligible	Low	Moderate	High	Extreme
Minnesota			.46 (M)	.24 (M)	
St. Croix		.02 (L)	.19 (L)	.21 (M)	.02 (H)
Nemadji		.14 (L)	.22 (L)	.02 (M)	
Sand Hill	.06 (M)	.14 (M)	.02 (L)		

Figure 8-4. Recreation Jumping Hazard Overall Risk. The x-axis lists the 5 possible consequence levels that workshop participants characterized, from least severe (Negligible) to most severe (Extreme). The y-axis displays the probability of each consequence level, given arrival of bigheaded carps. The probability that the bigheaded carps would not establish is not included here, but makes up the remainder of the probability of consequence. For example, for the Minnesota River - Mankato watershed, the probability that bigheaded carps would NOT establish given arrival was estimated as .30 = 1 –.46 –.24. The certainty of the characterizations for each consequence level are represented in the table (VL=Very Low; L= Low; M=Moderate; H=High; VH = Very High) and by marker size (same 5 point scale with larger circles equaling greater certainty, and the hollow circle indicating Very Low).

8.3 Change in overall risk from second most likely resulting abundance

Small group adverse effect consequence characterizations for the second most likely resulting abundance of bigheaded carps provide an approximation of the direction and magnitude of change in the overall risk if such a resulting abundances were to be realized. The second most likely resulting abundance was high for the Minnesota River watershed and low for all other watersheds. Presented here are the direction and degree of change in consequence, and accompanying certainty, characterization for each small group member for each small group.

For the Minnesota River (Table 8-4), the high resulting abundance characterizations led to the following changes in relation to the moderate abundance: 1) an increase in certainty, 2) an increase in consequence level, 3) both an increase in certainty and consequence level, or 4) no change. The increase in consequence level was seen for the following potential adverse effects: non-game fish (bigmouth buffalo only), species diversity/ecosystem resilience, and recreation jumping hazard. The most significant shift came for the recreation jumping hazard, where most members (5/6) anticipated an increase in consequence level of one, and one member anticipated an increase of two. Such a shift would result in the overall risk for the recreation jumping hazard to range from high to extreme, instead of from moderate to high.

For the St. Croix River Watershed, the changes from the low resulting abundance varied, but were generally a decrease in consequence by one or sometimes two levels (Table 8-5). The change in certainty varied but was generally an increase in certainty. For the Nemadji River Watershed, the changes from the low resulting abundance ranged from no change to a decrease of one consequence level for non-game and game fish (Table 8-6). For the species diversity/ecosystem resilience and recreation jumping hazard potential adverse effects in the Nemadji River Watershed, small group members agreed that the low abundance would lead to a decrease in consequence by one level. There were generally no changes in certainty. The changes in consequence level for low resulting abundance in the Sand Hill River Watershed ranged from no change to a decrease in consequence by two levels (Table 8-7).

These changes in consequence level for the second most likely abundance provide a type of uncertainty analysis for the overall risk characterization. Specifically, they highlight how the uncertainty surrounding the resulting abundance of bigheaded carps may influence the overall risk characterizations. The most noteworthy finding from these changes is that for the Minnesota River there is either no change or an increase in the consequence level, and for the other watersheds there is either no change or a decrease in the consequence level. This means that for the second most likely abundance, the overall risk would increase or stay the same for the Minnesota River Watershed and would decrease or stay the same for the remaining watersheds.

Table 8-4. Changes in the MN River-Mankato Watershed consequence characterization for High resulting abundance. The table presents how small group members changed their consequence characterization for each potential adverse effect when considering the second most likely abundance level (High) compared to the most likely abundance level (Moderate). The number indicates the number of small group members. The middle square (shaded) indicates that the characterization of both consequence level and certainty was the same for both abundances.

MN River: Non-game fish; Spotfin Shiner						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1			(1)		
	No change			(4)		
	-1					
	-2					

MN River: Non-game fish; Bigmouth Buffalo						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1			(2)	(1)	
	No change				(2)	
	-1					
	-2					

MN River: Game fish; Channel Catfish						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1					
	No change			(5)		
	-1					
	-2					

MN River: Species diversity/Ecosystem resilience						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1			(2)	(1)	
	No change			(2)	(1)	
	-1					
	-2					

MN River: Recreation jumping hazard						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2				(1)	
	+1					
	No change				(4)	(1)
	-1					
	-2					

Table 8-5. Changes in the St. Croix River Watershed consequence characterization for Low resulting abundance. The table presents how small group members changed their consequence characterization for each potential adverse effect when considering the second most likely abundance level (Low) compared to the most likely abundance level (Moderate). The number indicates the number of small group members. The middle square (shaded) indicates that the characterization of both consequence level and certainty was the same for both abundances.

St. Croix: Non-game fish; Gizzard Shad						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2		(2)			
	+1		(1)			
	No change	(1)				
	-1					
	-2					

St. Croix River: Game fish; Sauger						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2		(1)			
	+1		(1)			
	No change		(2)			
	-1					
	-2					

St. Croix River: Species diversity/Ecosystem resilience						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1		(1)			
	No change	(1)	(1)			
	-1					
	-2			(1)		

St. Croix River: Recreation jumping hazard						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2			(1)		
	+1	(1)				
	No change		(2)			
	-1					
	-2					

Table 8-6. Changes in the Nemadji River Watershed consequence characterization for Low resulting abundance. The table presents how small group members changed their consequence characterization for each potential adverse effect when considering the second most likely abundance level (Low) compared to the most likely abundance level (Moderate). The number indicates the number of small group members. The middle square (shaded) indicates that the characterization of both consequence level and certainty was the same for both abundances.

Nemadji: Non-game fish; Common Shiner						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1					
	No change		(3)	(2)		
	-1					
	-2					

Nemadji River: Game fish; Black Crappie						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1		(1)			
	No change		(2)	(2)		
	-1					
	-2					

Nemadji River: Species diversity/Ecosystem resilience						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1					
	No change		(5)			
	-1					
	-2					

Nemadji River: Recreation jumping hazard						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1					
	No change		(5)			
	-1					
	-2					

Table 8-7. Changes in the Sand Hill River Watershed consequence characterization for Low resulting abundance. The table presents how small group members changed their consequence characterization for each potential adverse effect when considering the second most likely abundance level (Low) compared to the most likely abundance level (Moderate). The number indicates the number of small group members. The middle square (shaded) indicates that the characterization of both consequence level and certainty was the same for both abundances.

Sand Hill River: Non-game fish; Golden Redhorse						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2		(1)	(1)		
	+1		(2)	(1)		
	No change					
	-1					
	-2					
Sand Hill River: Game fish; Northern Pike						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1			(2)		
	No change			(3)		
	-1					
	-2					
Sand Hill River: Species diversity/Ecosystem resilience						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1					
	No change		(4)			
	-1		(1)			
	-2					
Sand Hill River: Recreation jumping hazard						
		Increase or decrease in severity of consequence				
		-2	-1	No change	+1	+2
Increase or decrease in certainty	+2					
	+1	(1)	(1)	(1)		
	No change			(1)		
	-1	(1)				
	-2					

9 Discussion

These risk assessment findings support the need for a reasoned and timely response to the threats posed by bigheaded carps. The findings show that the Minnesota River – Mankato and similar watersheds are at a higher risk, followed by the Lower St. Croix River and similar watersheds. Unfortunately, these two watersheds are found in the southern and eastern parts of the state, which are closest to the current invasion front. These findings support the need to prioritize management that can slow or prevent the spread into these areas, or that can lessen the consequence levels of any resulting adverse effects.

This section further discusses the key insights that emerged from this risk assessment, including: 1) the severity of risk varies across watersheds; 2) the severity of risk varies across potential adverse effects; 3) given the varying severity of risk, management decisions should consider the potential effects of bigheaded carps, of management action on bigheaded carps, and of management actions on native species; 4) research needs exist that could help improve the characterization of risk from bigheaded carps; and 5) this type of risk assessment process is well suited to inform decision making and societal discussions about invasive species.

9.1 Implications for management

9.1.1 The severity of risk varies across watersheds

This risk assessment reveals a gradient in the severity of overall risk across the watersheds we examined. The differences in overall risk across watersheds were a result of differing establishment probabilities and potential adverse effect consequence levels. First, the overall predicted probability of establishment for each watershed varied from a low of 22% (Sand Hill River) to a high of 70% (Minnesota River – Mankato), with 45% (Lower St. Croix River) and 38% (Nemadji River) in the middle. As described in Section 4 to Section 7, the biotic and abiotic factors influencing these differences included: spawning habitat, suitable temperature, suitable flow regimes, nursery habitat, food resources, potential predators, and adequate turbidity to avoid predation.

The other aspect of overall risk was the potential adverse effect characterizations (Table 8-3). These represent the estimated adverse effect consequence levels from bigheaded carps for each watershed, assuming bigheaded carps were to arrive, establish, and reach a moderate abundance (judged to be the most probable abundance level for all watersheds). The characterizations showed that when a moderate, high, or extreme consequence level was present for an adverse effect, it was always most probable in either the Minnesota River –

Mankato watershed or the Lower St. Croix River watershed. The consequence levels for the Nemadji River watershed largely ended up higher than the Sand Hill River and below the Minnesota River - Mankato and Lower St .Croix River.

For the non-game and game fish adverse effects, the higher consequence levels occurred for the planktivore fish species being considered (bigmouth buffalo for Minnesota River - Mankato and gizzard shad for the St. Croix River), because these species were seen as more likely to have dietary and habitat overlap with bigheaded carps. Other non-game and game fish species were deemed more likely to not have habitat and dietary overlap with bigheaded carps and to be able to find alternative prey if their primary prey were impacted by bigheaded carps.

One of the issues participants grappled with while characterizing the recreational jumping hazard potential adverse effect was the importance of risk perception. Participants expressed uncertainty concerning the degree to which a small number of jumping carp could have a large impact on recreation for a particular waterbody. Overall, for the severity of risk for the recreation jumping hazard, the differences across watersheds were attributed to differences in boating use and the density of bigheaded carps.

The overall risk, defined as the probability of consequence level given arrival, was determined by combining the establishment likelihood and the potential adverse effect consequence level (Figures 8-1 to 8-4). Higher consequence levels with larger probabilities represented higher levels of overall risk. The relative rankings of the overall risk, then, were: Minnesota > St. Croix > Nemadji > Sand Hill. There were a couple of places where this ranking did not hold true, including the game fish overall risk, where the Minnesota River was near the lowest risk, because the chosen game fish, channel catfish, was seen as having low dietary and habitat overlap with bigheaded carps.

For the resulting abundances of bigheaded carps, all watersheds had moderate for the most likely abundance and low for the second most likely abundance, except for the Minnesota River – Mankato watershed which had high as its second most likely abundance (Table 8-2). The result of this is that whereas the consequence levels of the potential adverse effects for the Sand Hill, St. Croix, and Nemadji watersheds would stay the same or decrease for the second most likely abundance level, the consequence levels for the Minnesota River potential adverse effects would increase or stay the same (see section 8.3). This provides further justification for the Minnesota River – Mankato watershed to have the highest overall risk.

The severity of the potential adverse effects are also likely to vary within a watershed with, for example, greater severity in the shallows and backwaters of rivers where bigheaded carps are more likely to reach higher densities and take part in jumping behavior.

9.1.2 The severity of risk varies across potential adverse effect

In addition to varying across watersheds, the severity of risk also varied across potential adverse effect. The overall risk posed to non-game fish, game fish, species diversity/ecosystem resilience, and recreation from the jumping hazard all varied notably. For example, the risks to non-planktivore non-game fish and all game fish were estimated as most likely to be negligible or low, with less than 10% of participants characterizing the consequence level as moderate (Figure 8-1; Figure 8-2). The risks to planktivorous non-game fish were slightly higher – most likely to be a moderate consequence level, followed by a low consequence level. Overall, then, workshop participants predicted that there would not be a high or very high consequence level for the non-game and game fish assessed in these watersheds, and believed the risk to these non-game and game fish species were lower than the risks posed to species diversity/ecosystem resilience and recreation from the jumping hazard.

The overall risk for the species diversity/ecosystem resilience potential adverse effect was notably higher than for the non-game and game fish species in consequence level, with moderate being considered the most likely consequence level for all watersheds. Two watersheds (Minnesota and Nemadji) had a small number of participants characterize the consequence level as high. Finally, the overall risk for the recreation jumping hazard saw the largest likelihoods of a high consequence level (24%, Minnesota and 21%, St. Croix), and the only example of an extreme consequence level (2%, St. Croix).

9.1.3 Management actions based on the variation of risk

The fact that there was not a uniform level of low risk across potential adverse effects and watersheds emphasizes the need to take reasoned action in the face of the threat posed by bigheaded carps. Given that the Minnesota River – Mankato and St. Croix River watersheds were at higher risk, it is important to take actions that can help reduce: 1) the likelihood that bigheaded carps will arrive in these watersheds, 2) the likelihood they will establish in these watersheds; and 3) the severity of the resulting adverse effects if they do establish. Possible management actions include, for example, species-selective deterrents, improving ecosystem resilience, restoring top native predators such as flathead catfish, and eliminating cross-watershed connections. Such management actions may take place in the watershed at risk, or, especially when trying to reduce spread, in an adjacent watershed or further downstream on the Mississippi River.

The fact that there was not a uniform level of high risk across potential adverse effects and watersheds is also important for management decision making. To ensure management actions do more good than harm, management decision making should consider: 1) the risks posed by bigheaded carps, 2) the effects of the management actions on bigheaded carps, and 3) the collateral damage effects of the management actions on native species (Kokotovich and Andow 2017; Buckley & Han 2014). Given the need to weigh these factors when considering management actions, the lack of a uniform high risk is consequential. It means that it is especially important to consider the possible collateral damage of management actions on native species, to ensure management actions do less harm than bigheaded carps are likely to.

This insight is especially significant in the context of potentially using species-selective deterrents or non-selective barriers as management actions, as they have the potential to have adverse consequences for native species. For example, the Granite Falls Dam in Minnesota provides an illustration of non-selective barrier effects on species richness and ecosystem resilience, with 40 of 97 native species in the watershed absent upstream of the dam (Aadland 2015). This is typical of 32 barrier dams evaluated across Minnesota with an average of more than 40 percent of native species found in the respective watersheds abruptly absent from the entire watershed upstream of these barriers. The conclusion that the barriers caused these species extirpations is validated by a rapid return of most of the absent species following dam removals (Aadland 2015). Sensitive species and species of greatest conservation need are most vulnerable to fragmentation while pollution-tolerant species are least effected. Extirpation and extinction of native fish and mussels resulting from dam construction and fragmentation has been well documented in the U.S. and globally (Rhinne et al. 2005; Haug 2009; Fu et al. 2003; Quinn and Kwak 2003). Therefore, if a primary intent of any proposed management action is to protect native species from bigheaded carps it should be considered that, based on data from existing non-selective barriers in Minnesota and elsewhere, the construction of non-selective barriers or non-selective deterrents may be counterproductive. Alternatively, species-selective deterrents, such as those using sound, provide the potential to slow the spread of bigheaded carps while not hurting native fish populations. While research is still advancing on such deterrents, this potential is promising. Other possible management actions that do not cause such harm natives include improving ecosystem resilience, restoring top native predators such as flathead catfish, and eliminating cross-watershed connections.

9.2 Implications for research

9.2.1 Research needs for an improved assessment of risk from bigheaded carps

The risk assessment process also helped identify a host of research needs. Many of these emerged during the small group sessions of the expert workshop. They are described in detail within the individual watershed sections (Section 4 through Section 7), but some key areas are summarized here. First, there is a need to study the impacts of bigheaded carps on watersheds similar to those in Minnesota. This includes better understanding the dynamics influencing establishment and the impact of bigheaded carps on the native species present in Minnesota. It also includes improving the understanding of how bigheaded carps effect waterbodies dissimilar to those they currently inhabit, such as the coldwater Nemadji River. A key part of this is ensuring there is adequate baseline information to detect changes. Second, there is a need for further research on how native fish species affect the population dynamics of bigheaded carps. For example, there is a need for more research exploring native fish species predation on and competition with bigheaded carps. Third, there is a need for further research on how bigheaded carps affect the benthic community and how that influences broader ecosystem dynamics.

Some overarching additional research needs include the need to look at the economic aspect of bigheaded carps, to explicitly consider the differences between rivers and lakes, to look at additional fish species, to extrapolate these findings to different watersheds in the state, and to regularly update these findings. First, looking explicitly at the economic aspects of the risks from bigheaded carps and of management actions would help inform decision making. While such an economic analysis fell outside the scope of this risk assessment, the risks characterized here would provide a good starting point for that effort. Second, although the scale of this risk assessment was at the level of the watershed, including both rivers and lakes, there was a focus on rivers because of their importance to the establishment and resulting abundance of bigheaded carps. There is a need, however, to explicitly study how the risks to lakes within a watershed may differ from the risks to rivers.

Third, there is a need to assess additional fish species within each watershed. The scope allowed for assessing one game and one non-game fish species in each watershed. Although this exposed important variations across fish species and watersheds, examining additional fish species would strengthen this assessment. Fourth, there is a need to build upon the approach to and findings from this risk assessment to assess the risks to other watersheds in Minnesota. The scope and findings of this risk assessment revealed some of the variation of risk that exists across watersheds and the implications for management, but looking at additional watersheds would further aid decision making. Finally, there is a need to regularly update these findings to keep up with the relevant scientific literatures. There was low certainty within the risk characterizations because of the limitations of current knowledge, the plasticity of bigheaded carps, and the differing and dynamic habitats within a watershed. Updating these findings as knowledge advances can help improve the certainty of the risk characterizations.

9.2.2 Using risk assessment to inform invasive species management

Whereas previous risk assessments for bigheaded carps have taken place at a broad scale (Cudmore et al. 2012; Kolar et al. 2007), this risk assessment's finer scale revealed decision-relevant information for the state of Minnesota and important nuances in the risks posed by bigheaded carps. Most significantly, the severity of risk varied across watersheds and potential adverse effects. This information can help determine and justify appropriate management actions and can help achieve more realistic expectations of the likely impacts from bigheaded carps. Another essential aspect of this risk assessment was how it started with an explicit values-based discussion about what aspects of the watershed were most valued and most important to protect from bigheaded carps. This ensured that the characterizations of risk were assessing the potential for harm and not just inconsequential change. It also helped ensure that the results were as useful as possible and specific to the current decision making context. Risk assessment, such as the approach utilized here, is well suited to inform invasive species management as it provides a set of tools that can synthesize scientific knowledge, necessary values-based judgments, and a specific environmental context.

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11 Appendix A: Workshop Participants and Report Authors

All workshop participants took part in the workshop meeting and were provided the opportunity to review this report. Workshop participants who participated in writing the report are starred. As discussed in section 2.4, project researchers (Adam Kokotovich & David Andow) assembled and revised the different sections of the report and wrote the Executive Summary, Methodology, Overall Risk Characterization, Discussion, and Appendices. The overall conclusions in this report are based on the findings that emerged from the risk assessment, but represent the views of the project researchers.

Table A.1: Workshop participant and report authors (starred).

Participant	Affiliation
Luther Aadland*	MNDNR
David Andow*	Project Researcher; University of Minnesota
Kelly Baerwaldt	US Fish and Wildlife Service
Katie Bertrand*	South Dakota State University
Duane Chapman	US Geological Survey
Alison Coulter*	Southern Illinois University
Ryan Doorenbos	MNDNR
Shannon Fisher	Minnesota State University - Mankato
Nick Frohnauer*	MNDNR
Seth Herbst	Michigan Department of Natural Resources
Michael Hoff	US Fish and Wildlife Service
John Hoxmeier*	MNDNR
Byron Karns	National Park Service
Adam Kokotovich*	Project Researcher; University of Minnesota
Matt O’Hara*	Illinois Department of Natural Resources
Brad Parsons	MNDNR
Keith Reeves*	MNDNR
Ed Rutherford*	National Oceanic and Atmospheric Administration
Tony Sindt	MNDNR
Peter Sorensen	University of Minnesota
Elliot Stefanik	US Army Corps of Engineers
John Waters	MNDNR
Mike Weber*	Iowa State University
Jamison Wendel	MNDNR
Dave Zentner	Stop Carp Coalition

12 Appendix B: Consequence Table

		Consequence description				
		1 – Negligible	2 – Low	3 – Moderate	4 – High	5 – Extreme
Adverse effect	**Non-game fish**	Undetectable changes	Small decrease in population	Moderate decrease in population, with detectable changes in structure of food web	Large decrease in population leading to many new food web connections	Severe decrease in, or extirpation of, non-game fish species, resulting in major changes in ecosystem
	Game fish	Undetectable changes	Small decrease in population leading to a minor reduction in angling quality	Moderate decrease in population, with a moderate reduction in angling quality	Large decrease in population, resulting in significant reduction in angling quality and in occasional closing of the fishing season for its protection	Severe decrease in, or extirpation of, game fish species - likely ending the natural fishery
	Species diversity / Ecosystem resilience	Undetectable changes in the structure or function of the ecosystem	Minimally detectable changes in the structure of the ecosystem, but small enough that it would have little effect on the ability to withstand external stressors	Detectable changes in the structure or function of the ecosystem and its ability to withstand external stressors	Significant changes to the structure or function of the ecosystem leading to significantly decreased ability to withstand external stressors	Restructuring of the ecosystem leading to very little ability to withstand external stressors
	Recreational opportunity – Jumping Hazard	Undetectable change – no sighting of jumping carp	Rare sightings of jumping carp, but does not cause changes in recreational boating and fishing	Occasional sightings of jumping carp, causing minor changes in recreational boating and fishing	Regular sightings of jumping carp and occasional collisions, causing changes in recreational boating and fishing	Severe and persistent recreational hazard from jumping carp, causing major changes to recreational boating and fishing

13 Appendix C: Findings and Implications Workshop

Overview

On March 15, 2017 a workshop entitled “Risk Based Management for Bigheaded Carps” was held at the University of Minnesota to discuss the findings and implications of this risk assessment. During this workshop, project researchers provided the March 15th, 2017 draft of the risk assessment report and provided presentations on the findings from the risk assessment. To discuss the risk assessment findings and their implications for management, and to provide feedback on the risk assessment report, workshop participants filled out a survey and took part in small and large group discussions. About 50 people attended the workshop including interested members of the public and individuals from: 5 federal agencies, the Minnesota Department of Natural Resources, non-governmental organizations, many local units of government, and academia. The feedback garnered from this workshop informed the final version of the risk assessment report.

Three aspects of this workshop are summarized here. First, the findings from the 10 question survey completed by workshop participants are provided. Second, a summary of the small group discussions is provided. Finally, this appendix concludes with a discussion of one of the important issues facing the management of bigheaded carps that emerged at the workshop – the conflicts concerning barriers and deterrents.

Summary of survey findings

Questions from the survey are presented, with bulleted summaries of the answers. When available, sample qualitative answers are provided.

Question #1: Which of the following best describes your affiliation?

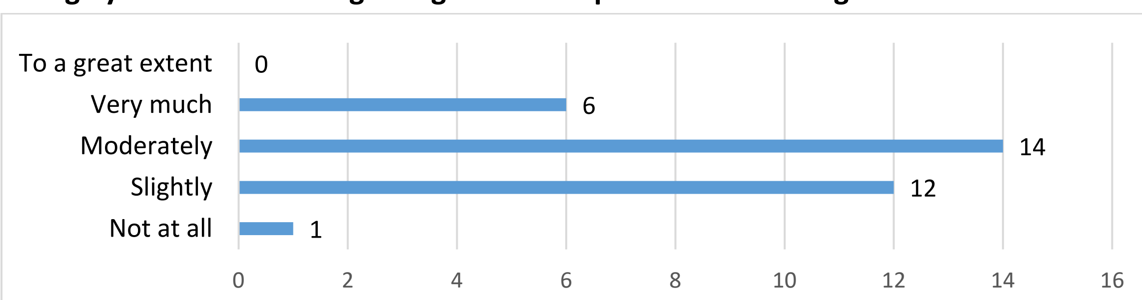
- Affiliations of respondents included: State agency (11); Federal agency (6); Academic institution (3); Stakeholder group (4); Interested individual (5); Local unit of government (4).

Question #2: What do you feel is the most important finding from the MN bigheaded carps risk assessment?

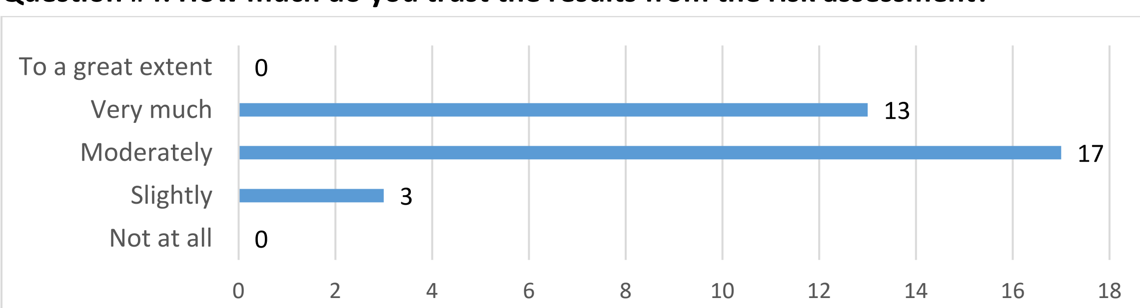
- Answers varied widely, but common themes included: 1) identifying the MN River-Mankato and Lower St. Croix River watersheds as higher risk; 2) recognizing the variation of risk across watersheds; 3) acknowledging the complexity and uncertainty present within these estimates; 4) acknowledging the importance of the potential for harm to native species from control measures.

Sample answers (each sentence comes from a different participant's response): The uncertainty and complexity impacting the findings. MN River and St. Croix River watersheds being at risk and need action soon. Understanding of the role of apathy and fear around the issue. No areas are the same nor should they be treated the same; Also our values differ and there is a need to be open and discuss in plain language. That a large group of people came together with varying perspectives to assess this, which is good. Acknowledging risk of control measures. There is still time, but establishment seems inevitable without action. The fish will not take over the entire state. Risk varies across watersheds and adverse effects. Understanding what is known and not known about Asian carp life history, especially as it applies to the waters of this state. Collaboration of experts and social science, brought up other aspects not usually considered by biological scientists. Damage to ecosystem resilience will likely be high, not so much for game fish. There is a lot of uncertainty and this uncertainty hampers our ability to make decisions and convince others to support these decisions.

Question #3: To what degree does the risk assessment and the discussions at this workshop change your understanding of bigheaded carps and their management?



Question #4: How much do you trust the results from the risk assessment?

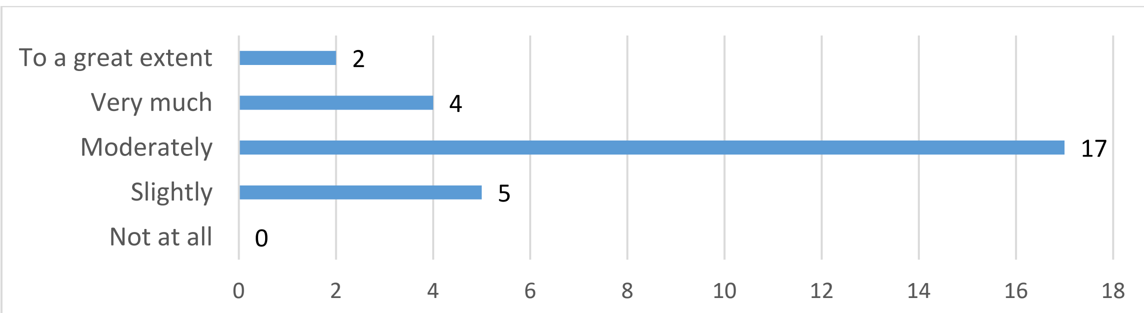


Question #5: How could they be more trustworthy?

- Answers largely identified the need to assess more fish species and watersheds, and to obtain more and better data.

Sample answers (each sentence comes from a different participant's response): More species of fish included, since only one game and non-game looked at per watershed. More workshops, more perspectives, more watersheds looked at. More data from similar systems. Replicate assessments with other experts. Better data. Have participants provide sources. More quantitative analyses. I think this is as strong as it can be for the diverse group of parties involved. Translation into plain language. Being more up front with limitations.

Question #6a: How useful do you think these findings will be to the current management context?

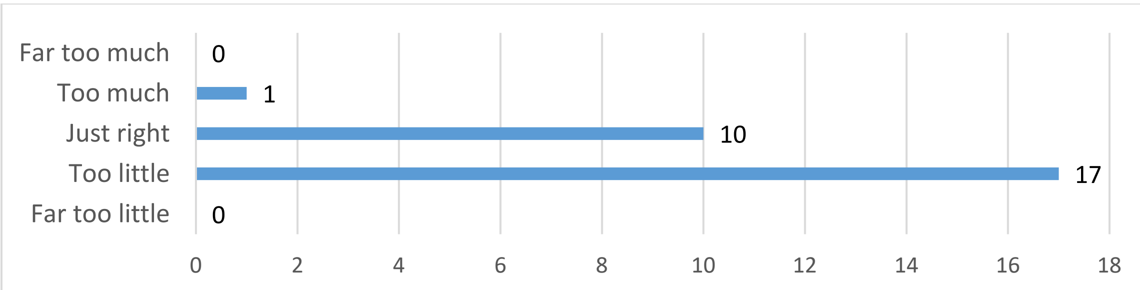


Question #6b: Why?

- Answers included justifications for why results would and would not be of use
- Justifications for why results would be of use included: 1) the importance of risk assessments for informing management decisions; 2) it is the first systematic analysis of risks for the state; 3) it provides justifications for continuing projects
- Justifications for why results would not be of use included: 1) the bureaucracy surrounding management will hamper its potential use; 2) the focus should be on prevention; 3) management comes down to resources

Samples answers (each sentence comes from a different participant's response): Need risk assessment before any management decisions. Emphasis should remain on prevention, since once established management options usually fail. Citizens want to know how this carp thing applies to them. I think these discussions have been occurring at the management level with similar understandings, much comes down to $ and staff numbers. Provides estimates of risk but lacks risk of management options, particularly barriers. It provides context but no real action items. More work needs to be done to flesh out the bureaucracy within management and how decisions are made; Current management still lacks true structured decision making. Best to know what you don't know. Because it's all we have to work with to date. I think it provides baseline data and justifications for continuing projects. They illuminate the need to act.

Question #7: Based on the risk assessment and discussions today, how would you characterize the current amount of management effort in Minnesota?



Question #8: What is the biggest remaining challenge facing the management of bigheaded carps?

- Answers emphasized: 1) scientific and political uncertainties; 2) the issues around barriers and deterrents, including whether they do more good than harm

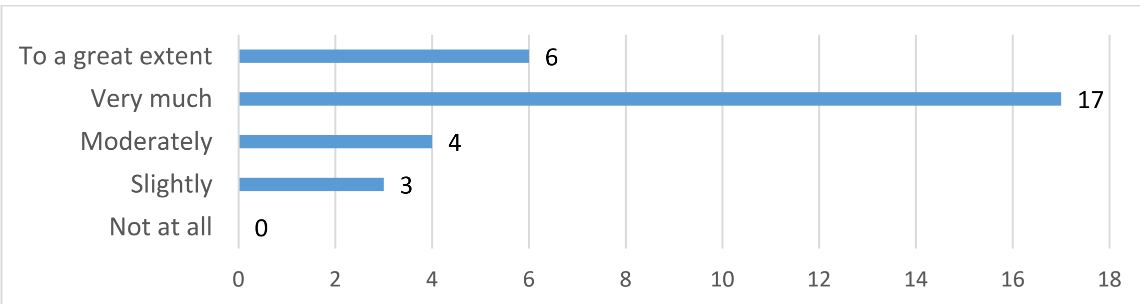
Sample answers (each sentence comes from a different participant's response): The debate between barriers and the resilience a diverse ecosystem needs to mitigate the threat. Uncertainty of everything: funding, research, food webs; Priorities of different organizations. Funding and quick response. Other AIS threats that grab the spotlight; Apathy. Getting other states on board. Funding strategies that don't damage ecosystems. Understanding and prioritizing management actions in and outside of MN based on collaborative approach. Funding and direction; what is our end game? Sharing information to bring results quicker. Data of how bigheaded carp will affect these basins. Implementing actions like barriers.

Question #9: What additional resources and/or information do we need to advance the management of bigheaded carps?

- Answers include a variety of research, politics, management, and society-related factors that could help advance the management of bigheaded carps

Sample answers (each sentence comes from a different participant's response): Database of research gathered together, to keep updating risk assessment. Sense of urgency. Resources for management actions. Well directed, cohesive management. Risk assessment on management options, including barriers. Research on river ecology, funding for temporary barriers to buy time. People, money, institutional support, and public support to continue adaptive and integrative management of Asian carp. Food web studies. Tag fish caught in Minnesota. More data. Identify most effective location for preventative actions. Zero in on end goals as managers. Quantitative estimation of potential impacts in watersheds.

Question #10: How important are meetings like these for the management of bigheaded carps?



Small group discussions

During the afternoon of the workshop small group sessions took place to discuss the implications of the risk assessment. Provided here is a summary of the key points that emerged during these discussions and that were not presented in the survey findings.

How do the risk assessment findings and this morning's events apply to your work, your organization and/or your views on bigheaded carps?

- Points raised in discussions emphasized how these findings can: 1) prioritize research activities and inform management, 2) help us understand what we do and do not know, 3) help provide better information to the public, and 4) help engage with the state legislature.

Based on the findings and this morning's events, what do we need to do going forward for the management of bigheaded carps? Are we on the right path or is an adjustment needed? What should be the focus of our management efforts?

- Points raised in the discussions concerning the needs going forward included: 1) scaling up the report to look at more species and watershed; 2) examining the effectiveness and non-target impacts of deterrents and barriers as management options; 3) continue to learn from other states; 4) better define management objectives, strategies, and priorities; 4) what is a realistic expectation for management instead of just 'we don't want them here'; 5) continue pursuing and evaluating deterrents at lock & dam #8 and #5;

What are the challenges going forward? Are additional information and resources needed? What is the largest challenge facing management?

- Points raised in the discussions concerning needs included: 1) communicating to public about what is being done; 2) leadership on the Mississippi River; 3) need to move faster and more definitively with management; 4) need to clarify uncertainty; 5) more data; 6) a local Asian carp task force; 7) a central hub for communication and information sharing, including funds to host it.
- Points raised in the discussions concerning challenges include: 1) educating the public; 2) the public's lack of faith in science; 3) how to communicate uncertainty in science; 4) sustained funding; 5) apathy & fear; 6) a lack of coordination between projects; 7) other environmental priorities; 8) the politicization of the issue; 9) conveying the need for impact and life history studies to funders.

Issues facing management: Barriers & deterrents

One of the remaining areas of conflict that became clear from the workshop survey and discussions concerned species-selective deterrents and non-selective barriers. First, there was miscommunication in terminology concerning the differences between species-selective deterrents and non-selective barriers, as some were using barrier to refer to both. Second, there were differing views about just how species-selective existing deterrent technology is, and of what level of efficacy (against bigheaded carps) and selectivity (so as not to hurt natives) is required before a deterrent technology should be put into use. Third, there were different views concerning what collateral damage on native species and ecosystem resilience from non-selective barriers or species-selective deterrents were acceptable when trying to reduce the likelihood of bigheaded carps spread. These two competing views can be seen in the following survey responses to the question asking about the biggest remaining challenge facing management:

> "So many unknowns, and fear pressuring action that is unnecessary and damaging to ecosystem health. Are known negative actions (i.e., dams, barriers) worth appeasing fears, when they are known to be more damaging than good? Explain to public that we are not even sure if they will have an impact or reach levels that might have a negative effect."

> “Knowing that acting in some capacity (even if barriers need refinement or all known effects on natives are incomplete) is better than inaction. Once they arrive in self-sustaining populations all the high level discussions that led up to the invasion/establishment will be for nothing. Finding a way to depoliticize this issue to free up state and regional and federal funding sources would be great”

These views indicate that there is a need for further study and deliberative discussions on these topics. The differences can be understood as conflicting types of risk profiles between two groups. Those who are skeptical of deterrents and barriers emphasized concerns about the likely impacts to native species that would occur if non-selective barriers or poorly working species-selective deterrents are used. This group also expressed concern that deterrents or barriers will not work as a permanent solution, and that if/when bigheaded carps make it past them, the deterrent or barrier damaged ecosystem will be more easily exploited. This group is most interested in management approaches based on strengthening ecosystem resilience and native predator populations.

Those supporting deterrents and barriers highlighted concerns about the likely impacts to native species from bigheaded carps, including the possibility that the impacts could be much worse than anticipated. This group expressed that the waterbodies in question are already impaired to the point where biotic resistance would not be an effective way to prevent establishment or lessen the severity of adverse effects. This group, then, asserted that species-selective deterrents (and potentially in some cases non-selective barriers) are the only real possible solution for avoiding the consequences from bigheaded carps, and that any effects on native species should be minimized as much as possible and then acknowledged as acceptable collateral damage.

The possible area of overlap between these two groups exists around species-selective deterrents. If there was truly a deterrent that was effective on bigheaded carps but had no impact on native species, this would likely be acceptable to all seeking to protect Minnesota’s waters from bigheaded carps. Research continues on deterrents, and a few questions are important for deterrent-related decision-making: What level of deterrent efficacy on bigheaded carps would successfully prevent establishment further upstream? What level of species-selectivity is adequate to protect native species? What level of resources are worthwhile to invest to improve the efficacy and selectivity of selective deterrents? What levels of effectiveness on bigheaded carps and species-selectivity on native species would make a deterrent worthwhile? Given the potential for species-selective deterrents to address this conflict and prevent adverse effects, this area of research is promising.

Other research questions that can help address this conflict include: 1) To what degree can biotic resistance (by, for example, increasing ecosystem resilience and native predators) lessen the likelihood of establishment and lessen the severity of any resulting adverse effects from bigheaded carps? 2) What are the impacts of different deterrents and barriers on native species and bigheaded carps? 3) How would species-selective deterrents and non-selective barriers impact native species and how would they make it easier for bigheaded carps to thrive if/when they get above them?

There is also clearly a need for people with differing views on this issue to better understand each other and to understand the common ground that does exist concerning the desire to protect native species from harm. More engagement on the intersecting science and values-based questions concerning deterrents and barriers is needed to help advance bigheaded carps management in Minnesota.

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Exploring tensions and conflicts in invasive species management: The case of Asian carp

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ABSTRACT

There is a growing recognition that scientific and social conflict pervades invasive species management, but there is a need for empirical work that can help better understand these conflicts and how they can be addressed. We examined the tensions and conflicts facing invasive Asian carp management in Minnesota by conducting 16 in-depth interviews with state and federal agency officials, academics, and stakeholders. Interviewees discussed the tensions and conflicts they saw impacting management, their implications, and what could be done to address them. We found three key areas of conflict and tension in Asian carp management: 1) scientific uncertainty concerning the impacts of Asian carp and the efficacy and non-target effects of possible management actions; 2) social uncertainty concerning both the lack of societal agreement on how to respond to Asian carp and the need to avoid acting from apathy and/or fear; and 3) the desired approach to research and management – whether it is informed by "political need" or "biological reality". Our study of these tensions and conflicts reveals their importance to Asian carp management and to invasive species management, more broadly. We conclude with a discussion of possible ways to address these areas of tension and conflict, including the potential of deliberative, participatory approaches to risk-related decision making and the need to productively engage with apathy and fear.

1. Introduction

As the fields of invasion biology and invasive species management continue to develop, there have been calls for them to become "more nuanced and less intellectually isolated" through a "growing recognition of complexity and ambiguity" (Davis, 2009, 10). This increasing appreciation for nuance, complexity, and ambiguity can be seen in different realms of invasive species scholarship. First, there is a growing appreciation that an invasive species can have both positive and negative effects on native species and ecosystems. Especially in altered landscapes, invasive species can serve as functional, structural, and compositional parts of transformed ecosystems, and can benefit certain native species – even while causing other types of harm (Tassin and Kull, 2015). Second, there is a more nuanced understanding of the effects of invasive species management, which can itself cause unintended harm to native species and ecosystems (Buckley and Han, 2014). Acknowledgment of this potential has increased the importance of assessing non-target impacts of management efforts (Lampert et al., 2014). Third, the simple narrative that native species are good and exotic species are bad has held little sway for some time in scientific discourse and is becoming more questioned in popular discussions about invasive species (Goode, 2016).

The scholarly literature on the social aspects of invasive species management, including the role of human values and political judgments, also shows considerable nuance. Much of this literature has focused on preventing human-mediated spread by seeking to understand how people engage in behavior that facilitates the spread of invasive species and how that behavior can be prevented (Clout and Williams, 2009). Recently, this focus has broadened by building on the idea that science alone is inadequate for determining what invasive species are of greatest concern and what management actions are desirable. One conclusion from this literature is that human values are essential to the judgment of whether the change caused by a particular invasive species is deemed harmful (Sagoff, 2009; Hattingh, 2011). Science can often be used to determine whether an invasive species is likely to have an impact on the environment, but it is fundamentally a value judgment whether that change is harmful. Such value judgments can be made explicitly and deliberately or in

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less transparent ways, but they are unavoidable in invasive species management. Second, conflict can exist over the value judgments in invasive species management, such as those concerning the desired state of nature, what constitutes harm from an non-native species, when management is worthwhile, or what non-target consequences of management actions are acceptable (Estévez et al., 2015; Buckley and Han, 2014; Larson et al., 2011). Some practices exist to avoid conflict over management (Larson et al., 2011), but there remains a need for further scholarship to explore the types of conflict that exist surrounding invasive species management and ways to address them (Estévez et al., 2015).

While existing literature points to the importance of exploring complexity and conflict in invasive species management, there remains a lack of work examining what form these issues take in empirical case studies. In addition, there is a need to better understand how scientific and social conflicts influence each other in invasive species management. Such case studies can improve understandings of the challenges facing invasive species management and explore possible ways to address these challenges. The research presented here explores the tensions and conflicts facing invasive species management via a case study of Asian carp management in Minnesota. Using in-depth interviews with managers, researchers, and stakeholders active with Asian carp management, we explore the tensions and conflicts that currently affect Asian carp management as well as possible ways to address these conflicts. These findings provide insights for Asian carp management and shed light on some of the broader challenges facing invasive species management.

1.1. Asian carp management

Silver, Bighead, Grass and Black carp, often referred to as "Asian carp", are four species of invasive fish that have been spreading to and affecting waterways across large portions of the United States. Asian carp were purposefully released into waterways of the United States in the mid-20th century for a variety of reasons including for their use in aquaculture. Silver carp (*Hypophthalmichthys molitrix*) and Bighead carp (*Hypophthalmichthys nobilis*), specifically, were promoted by state and federal agencies as a nonchemical and environmentally friendly way to improve water quality in retention ponds and sewage lagoons (Kelly et al., 2011). Subsequent unintentional release and large flood events are thought to have facilitated the escape of Asian carp into the Mississippi River system in the 1970s (Kelly et al., 2011). Since then they have been making their way upward and outward, with established populations in many river systems of the central and southern United States (Asian Carp Regional Coordinating Committee, 2014). Silver and Bighead carp have the ability to cause a variety of ecological and recreational impacts, from disrupting the aquatic food chain by consuming large amounts of plankton to, in the case of Silver carp, jumping up to 10 feet in the air when disturbed (Kolar et al., 2005).

As a result of the potential and realized threats posed by Asian carp, state and federal agencies have been actively managing invasive Asian carp across the central and southern United States (Conover et al., 2007). In Minnesota, a diversity of agencies work on Asian carp management including the Minnesota Department of Natural Resources, the US Fish and Wildlife Service, the US National Park Service, the US Geological Survey, the US Army Corps of Engineers. These agencies have different core responsibilities determined by their legal mandates, and must find ways to work across these differences when collaborating with other agencies. States can also have differing management priorities based on where they are located relative to the invasion front, which creates challenges for establishing basin-wide management priorities.

Of the four Asian carp species, Silver and Bighead are of particular concern in Minnesota because of the proximity of the self-sustaining breeding populations to the state and because of the negative effects they have caused in nearby areas where large populations are present. Individual Silver and Bighead carp have been captured in Minnesota each year since 2007, excluding 2010, and as far back as 1996, including 5 Bighead carp in the St. Croix river near Stillwater, MN in April 2015. The nearest reproducing population of Bighead and Silver carp, however, is thought to be in the Mississippi River in southern Iowa. State and federal agencies continue to conduct a variety of management and research efforts for Asian carp in Minnesota including, for example, monitoring, control measures, and deterrents to prevent spread. In 2015, the Upper Saint Anthony Falls Lock in Minneapolis was closed as the result of federal legislation to prevent Asian carp from being able to swim further north on the Mississippi River.

Asian carp management in Minnesota is a useful case study to examine the tensions and conflicts facing contemporary invasive species management. In addition to representing a complex contemporary invasive species management issue, our previous research (Kokotovich and Andow, 2015) and informational interviews revealed that although there is broad agreement on the management goal of minimizing the impacts from Asian carp while protecting native fish and ecosystems, there remain consequential tensions surrounding Asian carp management that warrant further study. Our goal for this research was to examine the tensions and conflicts that exist around Asian carp management in Minnesota to help better understand them, their implications, and how they can be addressed. After outlining the methodology, we present the findings from this research and conclude with a discussion of their implications and importance for invasive species management.

2. Methodology

To study these tensions, we conducted 16 in-depth interviews with individuals who have been actively involved with Asian carp management in Minnesota. We chose in-depth interviews because speaking individually with an interviewee helps provide the anonymity needed for interviewees to speak openly about the conflicts they perceive. In addition, in-depth interviews allow for follow-up questions and discussions that can help reveal key nuances. We used three main criteria to select interviewees who had been involved with Asian carp management in Minnesota. First, in order to obtain a breadth of views, we selected interviewees from the breadth of organizations involved with management, including state and federal agencies (e.g., Minnesota Department of Natural Resources, US National Park Service, US Army Corps of Engineers, US Fish and Wildlife Service, US Geological Survey), academia, and non-governmental organizations. Second, we selected individuals who had been most actively involved in management, as we judged through our attendance of state-level Asian carp meetings, such as the Invasion Carp Forum, and as identified by other interviewees. Third, we took steps to make sure we gathered the diversity of views present, by, for example, asking all interviewees for other important people to talk to and by continuing to conduct interviews until we reached a saturation point. After 16 interviews we reached a saturation point, both in terms of having talked to all key individuals mentioned by interviewees and in terms of no longer revealing novel understandings of the tensions and conflicts surrounding Asian carp management. Interviews lasted, on average, between 1 and 2 h each and were conducted in person and by phone. Interviews took place from March to May 2015.

A semi-structured interview process was followed where interviewees were all asked the same initial questions, but follow-up questions and conversations differed based on the specific responses of interviewees (Bernard, 2013). Interviewees were asked three main questions: 1) what are the tensions and conflicts you see as consequential for Asian carp management; 2) what are the implications of those tensions and conflicts; and 3) how could these tensions and conflicts be addressed or navigated? Follow-up questions sought to clarify the answers to each question and to explore the factors influencing them. The analysis of the interviews took place in two parts. First, notes were taken during the interviews to capture the main points articulated by interviewees, including basic descriptions of the tensions and conflicts, their implications, and what could be done to address them. These notes were used during the interviews to inform follow-up questions and discussions that ensured interviewees' views were comprehensively understood. Second, the interviews were transcribed and qualitatively analyzed using the qualitative analysis software Atlas.ti. This analysis involved thematic coding of the interviews to confirm the accuracy of the notes, apprehend additional nuance in interviewee responses, and identify quotations that were illustrative of key points. This analysis resulted in a set of described tensions and conflicts, including what contributed to them, their implications, how they related to one another, and how they could be addressed.

3. Findings

Our interviews with individuals involved with Asian carp management in Minnesota revealed three key areas of tension and conflict that provide insights on the challenges facing Asian carp management, and invasive species management more broadly: scientific uncertainty, social uncertainty, and the approach to research and management. Given our desire to understand the breadth of tensions and conflicts influencing management, we looked across all of the interviews to identify these areas of tension or conflict. This means that all three areas were not mentioned by every interviewee. However, all interviewees mentioned at least one area and all areas of tension and conflict were mentioned in each of the groups we interviewed: state agencies, federal agencies, academia, and NGOs. The awareness of these issues was shared across groups, even if interviewees differed in their exact articulations based on how they were situated in the management context. In these results, we first describe each area of tension or conflict in detail, including their implications and the factors that contribute to them. We conclude by discussing some of the ways that interviewees believed these tensions and conflicts could be addressed.

3.1. Scientific uncertainty

Two consequential scientific questions were frequently mentioned as being plagued by significant uncertainty: 1) what are the likely impacts from Asian carp in Minnesota? and 2) what are the likely impacts of management actions, such as deterrents, on both Asian carp and native fish species? Even though there are a variety of research efforts taking place – involving, for example, biobullets and pheromone attractants (Little et al., 2014) – there remain no definitive control solutions for Asian carp. Since there are currently no simple, straight forward solutions to Asian carp, and many interviewees stated that there are unlikely to be any in the future, a host of management and research efforts need to be considered. Interviewees believed that these two questions plagued by uncertainty are vital for determining a reasoned approach to decision making for a particular management action. Such a reasoned approach would need to weigh the following: 1) how will Asian carp likely harm Minnesota and how effective is the proposed management action at preventing harm from Asian carp? and 2) how does the proposed management action impact native species and how important is the health of native species for preventing harm from Asian carp? Without weighing these points it is impossible to determine if management is even warranted and if management actions do more good (in preventing adverse effects from Asian carp) than harm (in terms of non-target damage to native species).

First, interviewees stated that although there have been documented adverse effects of Asian carp in waterbodies further south of Minnesota, there remain questions about where and under what conditions such adverse effects could be experienced in Minnesota's waterways, if Asian carp were to establish. This is a result of both the diversity of waterways present in Minnesota and uncertainty about the conditions that are associated with and essential for the harmful impacts of Asian carp where they have already established. Without a good understanding of where and under what conditions adverse effects are likely to take place within the state, an important part of the decision making equation remains lacking.

Second, there is also significant uncertainty around the effectiveness and non-target impacts of management options. The effectiveness of certain deterrents, such as acoustic or bubble barriers, at slowing or stopping the spread of Asian carp remains poorly known, and although deterrent technology is already being used to try to slow or stop Asian carp spread, it is known to be less than 100% effective. In addition, even though management actions, such as the closing the Upper Saint Anthony Falls Locks, are expected to prevent Asian carp from swimming further north than Minneapolis on the Mississippi River (Lager, 2015), this will not stop the natural spread to areas downstream and will not stop human-mediated spread above the locks, such as through accidental transfer of juvenile Asian carp in bait. The use of deterrents, depending on how they are designed, can impede native fish passage and, as a result, cause harm to native fish populations. Many interviewees mentioned how such uncertainties can make it challenging to decide when and how a deterrent should be deployed.

Interviewees also articulated uncertainty about the extent that biotic resistance – the ability of ecological communities to resist negative impacts from Asian carp – could be enhanced by promoting healthy native fish populations. For example, could promoting healthy native fish communities serve as a way to increase predation on Asian carp and reduce the severity of the adverse effects they might cause? As interviewees stated, if that was the case, then it would be more important to look for ways to promote native fish health and to be wary of the negative impacts on native fish communities from deterrents. If, however, existing pollution and stresses on native fish communities make it unlikely that these communities could be restored to a level that would achieve effective biotic resistance, it could make more sense to pursue deterrents.

These scientific uncertainties have several implications for management efforts. First, they make it difficult to determine when and under what conditions deterrents should be used. There is a need to better understand the fundamental questions of where Asian carp are likely to cause adverse effects in Minnesota and under what conditions. And even if it is determined that Asian carp will likely cause adverse effects in a particular area, the uncertainties surrounding the impacts of deterrents on Asian carp and native species make it unclear whether they do more harm than good. The second way they complicate management efforts is through making it difficult to establish easy narratives about what needs to be done to address Asian carp. Interviewees expressed how it can be difficult to explain these uncertainties and their implications to politicians and the public.

3.2. Social uncertainty – Apathy/Fear

Social uncertainty emerged as a key area of tension and conflict in the interviews in two main ways: 1) the lack of agreement concerning the desired societal response to Asian carp, and 2) the tension created by trying to avoid undesirable societal responses based on apathy and fear. All interviewees believed that there was general societal agreement on the undesirable nature of Asian carp and their negative effects, in that nobody was arguing in favor of their introduction. Yet interviewees also believed that there was a lack of agreement about the appropriate societal response to Asian carp. The lack of agreement on the appropriate societal response was seen as making it more likely that the societal response would drift towards the extremes of apathy and fear.

In discussing this area of conflict, interviewees identified the problems associated with an apathy- or fear-based societal response to Asian carp and the difficulties of navigating between these extremes. Societal response, in this case, usually referred to the thinking and actions of people (e.g., the general public, individual stakeholders, politicians, state and federal agency personnel) as well as institutions (e.g., state and federal agencies, NGOs, and state and federal legislatures). In other words, apathy and fear were seen as ways of relating to Asian carp and Asian carp management that could be expressed and experienced at many organizational levels. None of our interviewees, those who have been actively involved in Asian carp management, believed that they themselves related to Asian carp from a place of apathy or fear; rather, it was a concern they had about others. Here we examine how interviewees conceived of the conflicts involving apathy and fear, and the relationship between the two.

Interviewees described an apathetic response to Asian carp as the general questioning of the need for any management, resulting from the belief that there is nothing that can be done, that even if something can be done it is not worth the resources, or that any impacts from Asian carp will not be significant. As one interviewee put it,

> *"Some people feel that invasive species are not that much of a threat or are the inevitable, so why fight it . . . there are people who say you are panicking, that it is a long ways off . . . It's just the sort of pulling the wool over your eyes, head in the sand, kind of attitude that you always run into when there is a crisis that is coming because there are always crises in place. To many minds, 'we have job issues, we have disparities issues, we have other [environmental] issues that are more important, so stop talking about carp.'"*

Interviewees believed that an apathetic response to Asian carp is undesirable because it leads to a lack of urgency or a feeling that management actions are unimportant. Whether impacting agency decision making or politicians, apathy was seen as a dangerous response because it leads to inaction. More often, interviewee concerns about apathy were aimed at the general public, who were seen as influencing politicians and agency decision makers. If the public cares and speaks out, then priorities are established and actions are taken. An apathetic response to Asian carp was often seen by interviewees as being the result of not knowing enough about Asian carp.

While an apathetic response was seen as undesirable, many interviewees also articulated how a fear-based response is also undesirable. They expressed concerns about addressing apathy by fueling fearful responses to Asian carp, especially given the uncertainty that exists around their likely impact in Minnesota. A fear-based response was seen as being based on the assumption that Asian carp will establish and lead to potentially catastrophic consequences and, as a result, it is of the utmost importance to prevent their establishment. One interviewee articulated such concerns in the following way,

> *"I think there is a mindset that we need to stop these things at all costs. That certainly is something that needs unpacking, in terms of what we are willing to do or give up to try to control them. The primary concern is that if we are willing to do anything, including poisons or barriers, then you have to think, well what is the underlying mission to what we are doing? Is it to protect native species from this invasive species or is it solely to keep this invasive species out?"*

A fear-based response was seen as having at least two unproductive implications. First, it leads to a strong desire for management irrespective of how likely significant adverse effects from Asian carp actually are. A fear-based response is grounded in the belief that Asian carp will cause significant consequences, regardless of how likely their establishment is and how likely consequential adverse effects would be even if they do establish. Those holding such a view are seen to be already convinced that it is extremely important to take action to keep Asian carp from establishing, no matter the evidence about where and under what conditions adverse effects are likely to occur. Second, this belief leads to a lack of concern about potential unintended and non-target consequences of management actions. A fear-based response is likely to align with the view that any negative impact on native species from management actions will pale in comparison to the catastrophic anticipated impacts of Asian carp, so the consequences from management actions become unimportant. In other words, if you think that Asian carp would decimate native fisheries and recreation, you will be more likely to support management actions regardless of their negative impacts and without considering where and under what conditions adverse effects from Asian carp are likely to occur.

Finally, many interviewees also discussed difficulties in navigating apathy and fear when working on Asian carp issues, especially with the public and politicians. In particular, interviewees expressed how difficult it was to avoid a societal reaction based on apathy or fear. One interviewee discussed this in the context of press releases for Asian carp captures in Minnesota,

> *"[Some] would like a press release on every single carp caught, every time. [Many in the DNR then ask], why is this newsworthy? We caught them before. If we put a press release every time we've caught one [it will lead to] oversaturation of the public which leads to apathy: 'they are here who cares, I've heard this before' . . . The flip side is, say maybe it's not oversaturation, but overemphasis on the issue, and people go down the road of Armageddon. We keep putting these out, so they must be horrible, so we must do something to stop them at any cost no matter what."*

The interviewee highlights how decisions about communication are informed by and have implications for how society responds to Asian carp. Frequent press releases on Asian carp findings could lead to either or both apathy and fear depending on how they are understood, making clear the nuance needed in communication efforts. As other interviewees discussed, however, avoiding press releases and societal discussion about Asian carp can also support an apathetic response to Asian carp, as it can keep the issue from emerging on the societal radar.

3.3. Management and research: "Political need vs. biological reality"

These two broad areas of uncertainty contributed to a third area of conflict that emerged from our interviews: the approach to management and research. Interviewees discussed the conflicts involving the direction of management and research in different ways, but one interviewee aptly summarized the main conflict as being between "political need" and "biological reality". Others elaborated that the conflict was about whether management and research priorities were chosen based on "political expediency" or "ecological soundness." In other words, many interviewees identified a disjuncture between what they thought should be achieved (identified as "ecological soundness" and being based on "biological reality") and what many decision makers and the public were willing and wanting to do (based on "political need" or "political expediency"). Interviewees generally thought that the "political need" approach was privileged more in the current context, and thought that ideas from the alternative "biological reality" approach needed to be promoted. The views of all interviewees did not necessarily fall neatly into one of these approaches. These approaches are a way of highlighting the key differences between two sets of logic interviewees saw influencing management and research. In this section we explore these two approaches to management and research, highlighting how they each relate differently to scientific uncertainty and social uncertainty.

3.3.1. Political need

Interviewees described the approach to management and research informed by "political need" as supporting quick fixes and easily justifiable, control-based management actions. This approach was seen as resulting from too much concern about social uncertainty, specifically apathy and fear, and from an underappreciation of scientific uncertainty. Although interviewees were most concerned with when politicians and decision makers – those making management and funding decisions – acted from a place of "political need", such ideas were seen as something that anyone, including the public or stakeholders, could support.

When informed by "political need," management and research were seen as responsive to the pressures of both apathy and fear. Responding to apathy required justifying the management and research taking place, and responding to fear required showing that something was being done. In both research and management, these factors were seen as leading to short-term, control-based management and research. Funders and politicians were also seen as likely to support short-term, quick-fixes that align with political and funding cycles. Yet this focus on doing something in a straightforward, short-term nature has its limitations, as one interviewee explained:

> *"So, I think there's this tension between science [which] takes time and people wanting direct outcomes. I could almost compare it to throwing criminals in jail versus trying to solve the problems in society that address why they became criminals. The easiest solution, the quickest solution is just to throw someone in jail, and it's cheaper than trying to get at all the background behind it. So, a quick-fix mentality really is in tension versus what's really required by science."*

So the sentiments expressed here are that the simple, short-term fix mentality prevents a discussion about what could be long-term, more foundational fixes – instead of trying to understand and address the causes of the problem, being happy to just address its symptoms.

Research that looks at more foundational issues and holistic fixes can be systematically excluded when funders and politicians desire short-term fixes. Instead of exploring the basic biology and ecology of Asian carp to help narrow in on a potential 'Achilles heel' to exploit in management, there is a focus solely on short-term, control-based research. Often, though, this control based research bears more explicit and predictable results than basic research or even high-risk, high-reward research. One interviewee shared how support for ecological or high-risk, high reward research can be difficult to sustain because "legislators want sure things. They want . . . fish killed." Many interviewees felt, however, that control-based management research can potentially be used to show the public and decision makers something is being done, even if it has no significant effects on Asian carp populations. One interviewee expressed these limitations in the context of management issues occurring in more southerly states with established Asian carp populations,

> *"It's like the commercial catch. It's nice to be able to see that there's fish on the deck and the public likes to see that, but does it actually have an impact on the population? It may not at all. Because you're not having an impact on the population you're really not doing anything. You're spending a lot of money to do nothing. What the public is seeing is; okay, you're doing something. The scientist is saying; wait a second, you're not really doing anything."*

An underappreciation of scientific uncertainty can also contribute to a short-term, quick-fix focus. Short-term, control-based management options emerge as neatly and clearly desirable only by downplaying the uncertainties concerning: where Asian carp will establish and with what effect, the efficacy of control-based efforts on Asian carp, and the consequences of control-based efforts for native fish species.

3.3.2. Biological reality

The approach to management and research that was placed in opposition to "political need" was identified by one interviewee as "biological reality". This direction for management and research was seen by interviewees as being based on a keen understanding of the biological reality of the scientific uncertainties surrounding Asian carp. In describing this approach to management and research, interviewees countered many of the problems they associated with the "political need" approach and focused on reducing uncertainty through research, pursuing biological, long-term management, and addressing rather than reacting to apathy and fear. The "biological reality" approach was seen as not currently influential, but as useful and needed for decision makers, politicians, and the public.

One key part of the "biological reality" approach is acknowledging and engaging productively with scientific uncertainty. First, this involves understanding the implications of scientific uncertainty for current management actions and determining research priorities that can help reduce scientific uncertainty to inform future management actions. This includes, for example, acknowledging when little is known about the potential non-target impacts of a management action, and recognizing the importance of this information for reasoned decision making. In addition to research on the non-target impacts of management actions, this approach calls for more biological and ecological research on Asian carp, such as research on Asian carp life history and the conditions under which they thrive. Instead of seeing biological research as less vital than research on control measures, this approach emphasizes how biological research could help inform control efforts. The strict division between biological and control research is challenged, and there is a recognition that a better understanding of life history and their interactions with other organisms could help inform and create new management actions.

The relationship to social uncertainty, and specifically apathy and fear also differed in the "biological reality" approach. Instead of reacting to apathy and fear, it sought to address social uncertainty and influence the societal reaction to Asian carp. That is, it sought to reduce the uncertainty around the societal reaction to Asian carp by reducing the uncertainty around scientific questions. By directing research toward understanding the likely impact of Asian carp in Minnesota and the efficacy and non-target impacts of management efforts, this approach seeks to develop insights that could make it easier to decide on the desired path for management.

Such an approach requires having research priorities based not on apathy or fear, but on addressing questions that are hampering management decision making. This approach assumes that more information about the likely effects of Asian carp and on the efficacy and non-target impacts of management efforts will make the desired path for management more obvious.

3.4. How to address tensions and conflicts – the right relationship to uncertainty

Interviewees also shared how they thought these conflicts and tensions could start to be addressed. One sentiment mentioned by some interviewees was the distinction between: 1) acknowledging and addressing scientific uncertainty and 2) wanting to eliminate uncertainty before pursuing management actions. There was an awareness of the need to prevent "paralysis by analysis;" that is, to avoid making a decision by continually saying that further analysis is needed. As one interviewee said, *"If we wait for the day when we are fully certain, all hell will break loose."* In other words, it may be too late to take meaningful action if no management actions are taken until there is full certainty about how Asian carp will impact Minnesota's waterways and how management actions will impact Asian carp and native species. This view points to the limits of only seeking to reduce scientific uncertainty, and highlights the need to take management actions in the face of uncertainty. Yet what counts as an acceptable level of uncertainty when making management decisions is both a scientific and values-based judgment.

Specific suggestions provided by interviewees for addressing these tensions and conflicts embraced a deliberative approach that fosters the right relationship to scientific and social uncertainty. One interviewee described how this approach would look,

> *"Yeah, well, it would really entail embracing the conflict, embracing the dialogue and different opinions so that there was this open exchange of views and empirical data so that everyone gets on the same page."*

Another echoed the call for dialogue, and articulated it in terms of managers and researchers,

> *"When you go to solve a problem you need managers and researchers in the same room. If you don't have that, researchers are going to run off and do their thing, and managers are going to run off and do their thing, and there is no consensus on what we need to be doing."*

These statements point to the need to better understand the complexities involving values-based ("views") and science-based ("empirical data") aspects of uncertainty, as well as how they intersect in determining research and management priorities. The goal, here, is not to eliminate scientific or social uncertainty, but to explicitly, deliberately, and justifiably make Asian carp research and management decisions in the context of that uncertainty, as we discuss further in the discussion. Such a process would acknowledge uncertainty, the potential importance of reducing uncertainty, and the potential need to act despite uncertainty. It also emphasizes the importance of providing researchers and managers an opportunity to deliberate at the intersection of the values-based and science-based aspects of the Asian carp issue.

4. Discussion

The findings from this study provide insights into the challenges facing Asian carp management and invasive species management, more broadly. The in-depth interviews revealed three consequential areas of conflict and tension that hinder Asian carp management: scientific uncertainty, social uncertainty, and the desired approach to management and research. We found that these three areas of tension and conflict influence and potentially reinforce each other. For example, when the likely impacts of Asian carp and management actions are not well known, it is more likely that people will diverge to extreme responses, including those based on apathy or fear. Similarly, neither an apathy- nor fear-based societal response to Asian carp will support efforts to reduce scientific uncertainty. An apathetic societal response is likely to lead to Asian carp being deemed inconsequential or unavoidable, thereby making it unimportant to support research to reduce scientific uncertainty concerning impacts of Asian carp or non-target impacts of management options. A fear-based societal response is likely to lead to the assumption that consequences from Asian carp will be severe and to increase demand for control-based management actions, such as deterrents, with little concern for their non-target impacts – also making it unimportant to reduce such scientific uncertainty. Finally, both scientific uncertainty and social uncertainty make determining the appropriate direction of research and management more difficult, and such lack of direction stalls efforts to address scientific and social uncertainty.

One possible way to address this challenging situation emerged in the discussion of the "biological reality" approach to management and research. This approach was based on reducing scientific and social uncertainty through research on pertinent questions – in this case, the likely impacts of Asian carp and management actions in Minnesota. Three points about the limitations of, and problems facing, this approach should be considered. First, what counts as a pertinent question is itself a value judgment, prone to disagreement (Nelson and Banker, 2007; Machamer and Wolters, 2004). As we discuss in more detail below, attention should be paid to the process used to arrive at these questions, and an explicit, inclusive, and deliberative process can help ensure that such decisions are substantively sound and trusted (Stern and Fineberg, 1996).

Second, although decreasing scientific uncertainty may reduce social uncertainty, it will not completely eliminate social uncertainty or the potential for social conflict around management (Sarewitz, 2004; Boertje et al., 2010). Even with perfect information about the impacts of Asian carp and the efficacy and non-target impacts of management options, there would still be the potential for values-based differences concerning management. One could imagine, for example, a variety of views concerning what amount of management is worthwhile to address a small established population of Asian carp that causes no significant ecological harm but that occasionally causes recreational hazards. The persistence of the potential for values-based differences means that there will always be a need to pursue deliberative engagement processes to productively address these values-based issues (Dietz and Stern, 2008).

Third, in describing the "biological reality" approach, interviewees did not often describe the role of the public, stakeholders, and politicians in supporting research. Even if this approach were to conduct research to address proactively social uncertainty, apathy, and fear, such research is at least partially dependent upon broader societal support. It would be difficult to continue with any research that is not supported by the public, stakeholders, or politicians (Clout and Williams, 2009). Here is where the nuance around the type of support becomes important. Without support the research is unlikely to be pursued. Yet if the public, stakeholders, or politicians give the kind of support that leans towards immediate control-based research and management, research on the key scientific uncertainties won't be fostered. So it is only with the right type of support that the desired form of research and management within the "biological reality" approach can advance.

4.1. Confronting contemporary invasive species management

The findings presented in this paper highlight some of the challenges facing contemporary invasive species management. We conclude by suggesting two areas of literature that may be helpful in addressing these challenges: the literature on risk-related decision making and the literature on apathy and fear. The first area of literature includes the well-established scholarship on using deliberative and participatory methods to inform risk-related decision making in the face of uncertainty (Jasanoff, 1993; Stern and Fineberg, 1996; Renn, 2008; Nelson et al., 2009). Risk assessment is recognized as an important tool to help synthesize science to inform invasive species management (Anderson et al., 2004), and the use of risk governance approaches that explicitly recognize the importance of value judgments and broad participation are particularly useful for the challenges revealed here.

First, explicitly recognizing value judgments is the first step in making sure that they are addressed in appropriate ways. There are many value judgments relevant to the tensions and conflicts discussed here, including: what type of change from an invasive species constitutes significant harm; how to evaluate and compare the benefits, costs, and non-target impacts of management actions; and what levels of certainty are necessary to move forward with management decisions. Recognizing the role of value judgments within these questions makes evident the need for involvement by a broad set of individuals (Stern and Fineberg, 1996; Hartley and Kokotovich In Press). Deliberative and inclusive participatory processes, then, can be used to help address these value judgements. Broad participation helps ensure that the assumptions and implications of value judgments are better comprehended, improving the basis for decision making (Stirling, 2008). Such involvement can also help: increase the local knowledge informing decisions, improve the participants' understanding of the decision making context, and increase the trust in decisions (Dietz and Stern, 2008). These insights could inform, as discussed in Section 3.4, a deliberative process with agency managers and researchers, and ideally stakeholders and academics, to identify key areas of uncertainty within the current management context and to deliberate on and decide what levels of uncertainty are acceptable for moving forward with decisions.

While our results indicate that reducing scientific uncertainty is one way to decrease apathy and fear, an over-emphasis on reducing scientific uncertainty can lead to undesirable outcomes such as policy stagnation or oversimplification of the problem (Pe'er et al., 2014). The second area of literature builds on the idea that apathy and fear can also be avoided by understanding their sources, their limitations, and how to address them. The use of fear can be an effective way of seizing the attention of the public or decision makers and can convey a sense of urgency (Gobster, 2005). It can also backfire, however, by overemphasizing the most immediate options. Especially in instances where people feel like they have little control over the situation, fear-based messages can cause people to react to the unpleasant feelings that come up through apathy, denial, or avoidance, thereby preventing a productive engagement with the issue (O'Neill and Nicholson-Cole, 2009). From our results we can add that fear may also lead to calling for immediate management action, regardless of its efficacy or collateral damage. Seeking to address apathy and fear should not involve attempting to remove emotion from invasive species management; rather, it should involve productively engaging with the emotions that are present in a particular context (Roeser and Pesch, 2016; Doherty and Clayton, 2011; Gobster, 2005). Trying to dismiss apathy or fear-based reactions as irrational or illegitimate without actually listening to what informs them will likely only reinforce them and make it even more difficult to have a broader discussion (Roeser and Pesch, 2016, 287). These insights can be used to design open and transparent conversations between stakeholders, the public, researchers, and managers that could at once: 1) seek to better understand, and not dismiss, existing views and emotions surrounding an invasive species management issue (including those based on apathy and fear) and the assumptions they are based, and 2) present, in a non-condescending or pressuring way, existing evidence about the invasive species and decision-making context that could help individuals reflect upon the assumptions behind their views and emotions.

This study contributes to the growing literature exploring the tensions and conflicts facing invasive species management. Our findings help better understand the challenges posed by the intersection of scientific uncertainty, social uncertainty, and invasive species research and management. These findings support the argument that value judgments are essential to invasive species management and need to be reflected on (Estévez et al., 2015). More broadly, they also contribute to efforts to more explicitly and productively engage with the role of values in environmental issues (Fernandez, 2016; Sarewitz, 2004).

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PLOS ONE

RESEARCH ARTICLE

Forecasting distributions of an aquatic invasive species (*Nitellopsis obtusa*) under future climate scenarios

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Abstract

Starry stonewort (*Nitellopsis obtusa*) is an alga that has emerged as an aquatic invasive species of concern in the United States. Where established, starry stonewort can interfere with recreational uses of water bodies and potentially have ecological impacts. Incipient invasion of starry stonewort in Minnesota provides an opportunity to predict future expansion in order to target early detection and strategic management. We used ecological niche models to identify suitable areas for starry stonewort in Minnesota based on global occurrence records and present-day and future climate conditions. We assessed sensitivity of forecasts to different parameters, using four emission scenarios (i.e., RCP 2.6, RCP 4.5, RCP 6, and RCP 8.5) from five future climate models (i.e., CCSM, GISS, IPSL, MIROC, and MRI). From our niche model analyses, we found that (i) occurrences from the entire range, instead of occurrences restricted to the invaded range, provide more informed models; (ii) default settings in Maxent did not provide the best model; (iii) the model calibration area and its background samples impact model performance; (iv) model projections to future climate conditions should be restricted to analogous environments; and (v) forecasts in future climate conditions should include different future climate models and model calibration areas to better capture uncertainty in forecasts. Under present climate, the most suitable areas for starry stonewort are predicted to be found in central and southeastern Minnesota. In the future, suitable areas for starry stonewort are predicted to shift in geographic range under some future climate models and to shrink under others, with most permutations indicating a net decrease of the species' suitable range. Our suitability maps can serve to design short-term plans for surveillance and education, while future climate models suggest a plausible reduction of starry stonewort spread in the long-term if the trends in climate warming remain.

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Introduction

Starry stonewort (*Nitellopsis obtusa*, Characeae) is a species of concern for both its endangered status (in parts of its native range in Europe and Asia) and its invasive status (in North America). The 'starry' of its common name comes from its characteristic star-shaped bulbils, starchy reproductive structures that enable spread via asexual reproduction [1]. In North America, female individuals of this species have not been detected to date [2]. It has a higher ecological plasticity than other charophytes [1,3]. For example, starry stonewort can flourish in hardwater (i.e., water with high mineral content) and habitats of varying depth, light availability, and sediment characteristics [4]. In addition, starry stonewort can grow densely, which may lead to displacement of native aquatic plant species and could have consequences for habitat quality [2]. Dense growth may also impair recreational activities such as swimming, fishing, and boating [1,3]. Although populations of starry stonewort in their native distribution in Europe and Japan have been declining [5–7], the species has shown great capacity to spread as an aquatic invasive species in North America [3,8,9].

In 1978, starry stonewort was first recorded in North America in the St. Lawrence River, where it was likely introduced through ballast water discharge from trans-Atlantic shipping [10]. Marine currents could have played a role in starry stonewort's dispersion, but this has been not explored. Five years later, starry stonewort was reported for the first time in Michigan, United States [1,10]. To date, starry stonewort has been reported in Indiana, New York, Pennsylvania, Wisconsin, Vermont, Ontario, and, in August 2015, in Minnesota [3,8,11,12]. The introduction of starry stonewort to inland lakes has been speculated to be associated with recreational boat activities from the movement of bulbils and alga fragments between different lakes [1,3].

In light of limited knowledge about the potential spread and impacts of starry stonewort in the Americas, improved knowledge of the species' invasion ecology is a priority. Among other efforts, identifying areas on the leading edge of the invasion range (e.g., Minnesota) with suitable conditions for starry stonewort is a priority for targeting surveillance and control. Ecological niche modeling can support these efforts. Ecological niche models correlate environmental conditions with species' occurrence records to identify suitable habitats where a species can persist and increase in population size without the need of further immigration [13]. This methodology has been used successfully with different taxa, scales, and ecosystems [13–15]. Furthermore, ecological niche models can be applied to forecast probable distributions of species over longer time periods, e.g., under future climate scenarios [16–20]. Predicting areas where starry stonewort could establish could inform surveillance efforts for early detection, raise local awareness, and prioritize allocation of resources for control [21].

Local conditions can influence occurrence of starry stonewort in North America. For example, in Lake Ontario, starry stonewort's distribution is associated with high conductivity, short distances to marinas, and low fetch [3]. In New York, Sleith et al. [1] found high pH and conductivity to be associated with starry stonewort. However, invasive species' occurrences are defined not only by local-scale characteristics, but also by larger scales of environmental factors that promote or limit spread over space and time [22]. Invasion of starry stonewort in the Americas is likely an ongoing process that has not reached equilibrium, and more water bodies are likely to be affected [8].

Recent reports of starry stonewort in Minnesota provide an opportunity to explore climatic factors that may influence future expansion. Here, we have constructed a series of ecological niche models to answer three main questions: (i) Which areas are vulnerable to starry stonewort invasion in Minnesota under present-day climate conditions? (ii) Which areas in Minnesota have suitable conditions for starry stonewort under future climate scenarios?, and (iii)

How do decisions regarding the geographic region used in model calibration influence predictions? We propose a protocol (Fig 1) to improve the workflow of ecological niche models for forecasting species invasions.

Methods

The ecological niche modeling approach employed was based on the **BAM** framework [23], which summarizes three components to define a species' spatial range. The first component is **B**, the presence of other organisms that promote (e.g., prey, symbionts) or restrict (e.g., depredators, parasites) the distribution of the species in a region. The second component corresponds to the set of abiotic environmental conditions, **A**, e.g., temperature, that are suitable for a species to persist without need of immigration. The final component, **M**, corresponds to the ability of the species to colonize biotically (**B**) and abiotically (**A**) suitable regions. Thus, the spatial distribution of a species is defined as $\mathbf{B} \cap \mathbf{A} \cap \mathbf{M}$ [23]. We focused on a broad-scale exploration of **A** and **M**, as a preliminary assessment of the invasion potential of starry stonewort in terms of abiotic suitability and dispersal potential. We estimated **A** based on the association of starry stonewort occurrences with bioclimatic variables across its range, and estimated **M** based on using three regions for model calibration (Fig 1).

Occurrences

Occurrence records of starry stonewort were published in Escobar et al. [8], which used data from digital repositories including the Global Biodiversity Information Facility (GBIF) [24] and the Global Invasive Species Information Network [25] using the keywords "*Nitellopsis obtusa*," "*Nitellopsis obtusa* var. *ulvoides*," and "*Chara obtusa*". Occurrences from invaded areas in the US were also derived from additional reports and publications [1,4,9,26]. Minnesota records were updated based on 2016 reports of new localities from the Minnesota Department of Natural Resources (MDNR, http://www.dnr.state.mn.us/invasives/ais/infested.html).

Occurrences were individually inspected to assure credibility and geospatial accuracy. All Minnesota, Wisconsin, and New York records have been confirmed by a Characeae expert (Ken Karol, New York Botanical Garden). Michigan has the most records and not all have been verified by experts. It is possible that reports from Michigan (and GBIF or other databases) include false records. Unfortunately, this is the best information that is available at this time. We chose to include all records based on the expectation that the error rate is relatively low and that the invaded region most likely to include false records (Michigan) is in the center of the species' invaded range, such that false occurrences would be unlikely to have a strong influence on niche estimation.

Oversampled areas, as a form of sampling bias, can generate model overfit [27]. To prevent this, we calibrated present-day models using occurrences filtered to one-per-cell according to the spatial resolution of cells in our environmental layers [28]. All the remaining occurrences were used for modeling. From the initial pool of 2,260 occurrences, 84 single occurrences (i.e., occupied pixel cells) remained in the entire species' range: 29 in the native range (34.5%; 2 in Japan, 27 in Europe) and 55 in the invaded range in the US (65.5%; Fig 2).

Model calibration region M

The selection of **M**, the model calibration region, has a strong influence on ecological niche model predictions [29]. For instance, considering only invasive populations can result in incomplete information about the environmental preferences of the species [13], or be insufficient to characterize environmental tolerances [30]. Explicitly testing different extents of the calibration region facilitates comparison of models and informs interpretation of results [31].

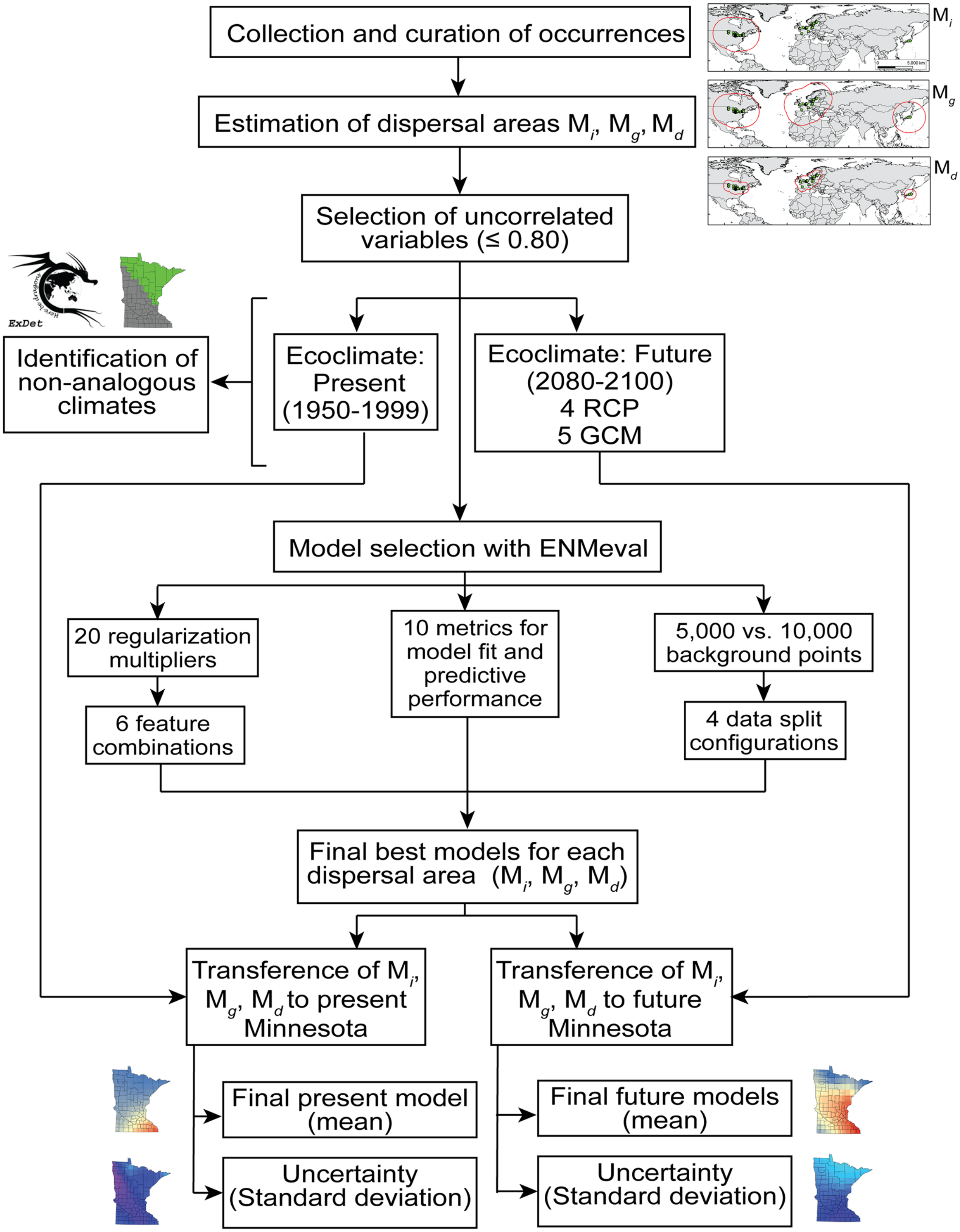

Fig 1. Workflow of the modeling process used in this study. Occurrences were collected, cleaned, and employed to estimate three model calibration regions (i.e., $\mathbf{M_i}$, $\mathbf{M_g}$, and $\mathbf{M_d}$). Present-day climatic variables were restricted to these model calibration regions and

compared to future climatic conditions in Minnesota. Models were parametrized using present-day climates in the three model calibration regions and the best models were projected to future climates in Minnesota using five climate models and four RCP scenarios.

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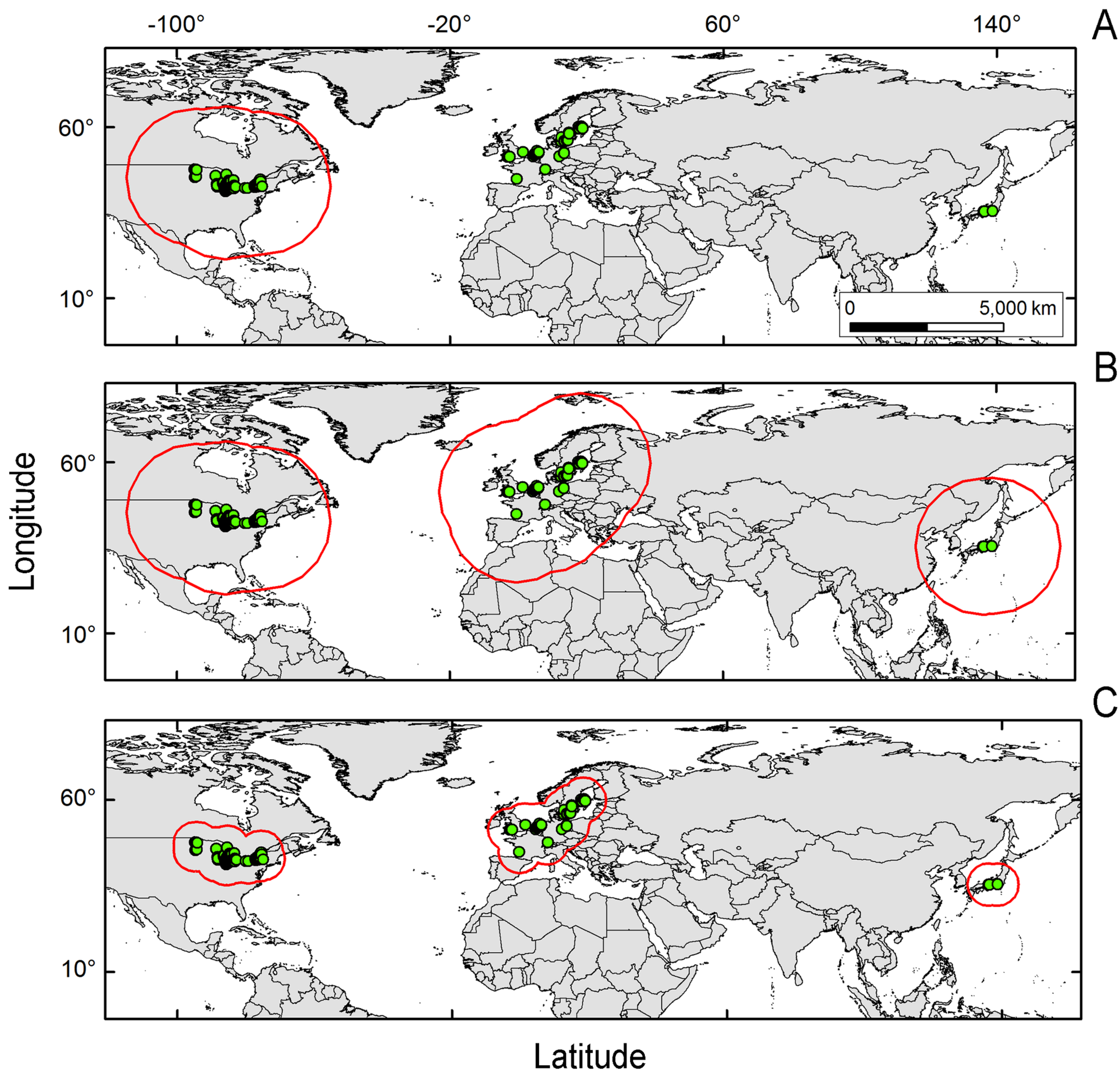

Fig 2. Model calibration region, M, explored in this study. Models were calibrated in three regions (red lines in A, B, and C) based on the distribution of starry stonewort populations (green points). **A.** Model calibration region based on an invasive population approach focused on starry stonewort populations in the invaded area of the United States and a high dispersal potential (i.e., 2,200 km), $\mathbf{M}_i$. **B.** Model calibration region considering the entire or global species' range in the United States, Europe, and Japan and a high dispersal potential (i.e., 2,200 km), $\mathbf{M}_g$. **C.** Model calibration region considering the entire or global species' range in the United States (left map), Europe (central map), and Japan (right map) and a reduced dispersal potential (i.e., 700 km), $\mathbf{M}_d$.

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Recent new records for starry stonewort in North America suggest that it may be expanding in North America from east to west and from south to north [8]. As a proxy of the dispersal potential of the species we used two distances for three **M** scenarios. First, we used the maximum distance between all known starry stonewort populations in the US (~2,200 km), as suggested by the data available (i.e., MDNR surveillance: http://www.dnr.state.mn.us/invasives/ais/infested.html) [23]. Considering that the species has been dispersing between distant lakes, we assumed that spatial barriers could be overcome in the model calibration regions. We used this distance as a buffer around starry stonewort occurrences to generate a model calibration region for the invaded range in the US ($\mathbf{M}_i$). This area corresponds to a model based on the invasive populations.

Furthermore, to account for starry stonewort environmental preferences across its entire range, we focused on two additional model calibration areas, including both native (Europe and Japan) and invasive populations (US). One of these calibration areas was based on the same maximum distance between all known starry stonewort populations in the US (~2,200 km; $\mathbf{M}_g$) and the other was a proxy of the maximum distance between closer neighbors populations in the US (~700 km; $\mathbf{M}_d$), which in our case corresponded to the distance between the last detection in Wisconsin and the first detection in Minnesota. We used these distances to generate a buffer around occurrences across the entire species' range (Fig 2). The $\mathbf{M}_i$ scenario encompasses inland and coastal regions of central and eastern Canada and all states in the continental US except those in the far west: California, Nevada, Oregon, Washington, and western portions of Arizona and Idaho. The $\mathbf{M}_g$ scenario encompasses all of those areas in addition to Europe, parts of northwestern Africa and Asia (Japan, North and South Korea, and parts of eastern China and Russia). The $\mathbf{M}_d$ scenario includes the Upper Midwest region in the US and southeastern Canada, portions of Southern, Northern, and Western Europe, and a small portion of Eastern Europe, and also Japan except by the Hokkaido island (Fig 2). All **M** scenarios included the area of interest for this study (Minnesota).

Environmental variables

As a proxy of **A**, we used the present-day Ecoclimate dataset (1950–1999) at 50-km spatial resolution [32]. Since starry stonewort occurs in both coastal and inland areas, we used climate variables covering both regions. This climate dataset is derived from the Coupled Model Intercomparison Project (CMIP5) and combines climatic patterns from multiple general circulation models from inland and marine ecosystems; thus, final climatic layers have global coverage. The role of oceanic dispersal in the invasion process of this species remains uncertain, however, we assumed that marine dispersal could play a role and include climate conditions in terrestrial and marine ecosytems in our model calibration regions. We used climatic variables likely to influence starry stonewort's macroscale distribution, selecting uncorrelated variables based on correlation coefficients $\leq$0.80 (Table A in S1 File). Specifically, we used annual mean temperature (˚C), mean diurnal temperature range (˚C), isothermality (%), temperature seasonality (˚C), maximum temperature of the warmest month (˚C), mean temperature of the wettest quarter (˚C), annual precipitation (mm/m^2), and precipitation seasonality (%) [32].

Climate models are considerably variable, thus, adding more scenarios of future climate would provide more information regarding the plausible variability in forecasts. Future climatic conditions for the end of the 21st century (2080–2100) were obtained from Ecoclimate, including four representative concentration pathways (RCPs; i.e., 2.6, 4.5, 6, and 8.5 W/m^2; here after numbers are shown without units) [32]. Each RCP scenario represents potential trajectories of greenhouse gas emissions projected to the future, ranging from the most optimistic (i.e., 2.6) to the worst-case scenario (i.e., 8.5) [32]. RCPs are the most updated climate scenarios from the Intergovernmental Panel on Climate Change (IPCC), Fifth Assessment Report (AR5), and replaced the SRES scenarios previously implemented by the IPCC AR4 [33]. The four RCP scenarios were estimated based on five different future general circulation models (GCM): CCSM, GISS, IPSL, MIROC, and MRI, allowing us to capture the variability in emissions (i.e., RCP scenarios) and climate simulations (e.g., CCSM vs. MRI).

Non-analogous climate evaluation

We explored areas with non-analogous (novel) climatic conditions between present-day climate in the calibration regions vs. future climate in the projection region of Minnesota. This resulted in a present vs. future comparison and calibration vs. projection regions. This analysis was done using the extrapolation detection (Exdet) tool developed by Mesgaran et al. [34]. Exdet identifies non-analogous environments between calibration and projection regions denoted as type I novelty [*sensu* 34]. Accounting for these non-analogous or novel environments enables a more confident interpretation of models [18,35,36].

Ecological niche models

Qiao et al. [37] proposed that multiple ecological niche modeling algorithms should be employed to identify the model that best fits with the available data, the study system, and the research question. We used Maxent to perform niche modeling because it enables the use of different variable transformations (features), i.e., linear (L), quadratic (Q), product (P), threshold (T), and hinge (H), and allows for different parameterizations (regularization values). In addition, Maxent allows automatic truncation in novel climates to avoid predictions in non-analogous environments.

Maxent is an occurrences-background algorithm, which estimates the most uniform probable distribution of the occurrences across a selected calibration region [13,38]. The background represents the summary of environmental conditions across the model calibration region. Because we explored two calibration regions (invaded range and two areas from the entire species' range) the available background varied. We developed models based on 5,000 and also 10,000 background samples.

Here, we tested 20 different regularization coefficient values ranging from 0.1 to 2. The regularization coefficients regulate the complexity of the model, higher values penalize for complexity and thus, produce simpler models (avoiding complex relationships between the data and the variables) that, in general, tend to have larger predictions [39]. Because assessing different configurations is recommended [39–41], we explored models based on six feature combinations reported in the literature: L, LQ, H, LQH, LQHP, and LQHPT [40].

We used raw values from Maxent to assess model fit according to Akaike's Information Criterion values corrected for small sample size (AICc), which ranks models based on their information content and complexity [42]; the model with the lowest AICc was selected (i.e, ΔAICc = 0) as best reconciling the goals of fitting occurrences with environmental input data and minimizing model complexity [41]. In addition, because low AICc does not represent the ability of the model to predict independent data, we also assessed predictive performance based on the full (AUC_{total}) and mean (AUC_{mean}) of the area under the curve of the receiver-operating characteristic (AUC) and the difference between training and testing AUC and its variability. These metrics assess if models can discriminate between occurrence and background points, with AUC values $\leq$0.5 consistent with randomly generated models unable to differentiate between backgrounds and occurrences. Because AUC has been questioned [43,44], we also used independent data to calculated mean omission rates (OR) from binary models based on using 100% ($OR_{100\%}$) and 90% ($OR_{90\%}$) of training occurrences as thresholds.

These metrics enable the proportion of independent occurrences predicted incorrectly to be quantified [40]. Evaluation of model predictions was performed using independent data obtained via dividing the occurrences in two sets, one for model calibration and one for evaluation. Calibration and evaluation data sets were developed based on four different data splitting configurations: (i) using one point at a time for model evaluation (i.e., Jackknife); (ii) apportioning the occurrences into four groups, each with an off-diagonal set for calibration and another for evaluation (i.e., block; as in [45]); (iii) selecting clusters of points and using half for calibration and the other half for evaluation (i.e., Checkerboard1 [40]), and (iv) partitioning the occurrences via cross-validation (*k*-fold; see [40]). Model evaluations were conducted using the R package ENMeval [40].

Model projection to Minnesota

Once the best regularization coefficient, feature configuration, and number of background points were determined for the calibration regions (Fig 2), the three selected models were projected to environmental conditions in Minnesota. Maxent allows strict model transference during model projection via 'extrapolation' and 'clamping' being deactivated [36,46]. This practice prevents unrealistic extrapolations of models into non-analogous (novel) environments that could be present in the projection region but absent from the calibration region [46].

In all, to identify the best model by calibration region ($\mathbf{M}_i$ vs. $\mathbf{M}_g$ vs. $\mathbf{M}_d$), we explored 120 parameter configurations (20 regularization coefficients × 6 feature combinations), and two background samples for each regions $\mathbf{M}_i$ and $\mathbf{M}_g$: 5,000 and 10,000; and 10,000 for $\mathbf{M}_d$ which was not explored due to the reduced extent of this calibration area (Table B in S1 File). The best models were projected to 20 future climate scenarios (4 RCP × 5 climate models). To inform interpretation of forecasts, we also estimated uncertainty of all final models. We parameterized final models based on our previous evaluations and generated surfaces of uncertainty using 80% of occurrences in Maxent and performed 25 bootstrap replications using random starting seeds. For final models, we selected the logistic output format in Maxent with clamping and extrapolation deactivated. We used the standard deviation of replicates as an indicator of uncertainty [38,47] (Fig 1) and developed a *t*-test ($\alpha = 0.05$) to compare the continuous suitability values of pixels among models in Minnesota.

Finally, we created an ensemble of models for different future climate scenarios in Minnesota. We averaged the final logistic models and calculated the standard deviations to identify areas where models were consistent (low SD) or diverged (high SD). There is debate about use of model ensembles, due to issues regarding interpretation of continuous units from different algorithms (e.g., general linear models vs. regression trees vs. Maxent) (see [13]). Here, we overcame such discrepancies by using the same suitability value (i.e., Maxent logistic), from the same parameterization so that differences only reflected differences in future climate models for Minnesota. We also estimated the number of lakes in Minnesota comprising the lowest and highest predictions of suitability using lake inventory data from the National Wetlands Inventory of the US Fish & Wildlife Service [48].

Results

Selected regularization coefficients differed by model calibration region: a regularization coefficient of 1.4 with LQHPT features provided the best fit (ΔAICc = 0) and good predictive performance (AUC_{total} = 0.98, AUC_{mean} = 0.96–0.97, $OR_{100\%}$ = 0.05–0.09, $OR_{90\%}$ = 0.14–0.16) for $\mathbf{M}_i$, 0.2 + LQ for $\mathbf{M}_g$ (ΔAICc = 0, AUC_{total} = 0.97, AUC_{mean} = 0.95–0.96, $OR_{100\%}$ = 0.01–0.04, $OR_{90\%}$ = 0.12–0.18), and 0.9 + LQ for $\mathbf{M}_d$ (ΔAICc = 0, AUC_{total} = 0.89, AUC_{mean} = 0.85–0.88,

$OR_{100\%}$ = 0.07–0.19, $OR_{90\%}$ = 0.01–0.02; Table B in S1 File). Our evaluations revealed that 10,000 background points provided good model fit and performance for the three model calibration regions explored. Logistic suitability values of starry stonewort models based on $\mathbf{M}_g$ (mean = 0.40, sd = 0.13) vs. $\mathbf{M}_i$ (mean = 0.13, sd = 0.07) were significantly different (t = 1098, df = 544500, $p < 0.001$), with higher suitability predicted when $\mathbf{M}_g$ was considered (Fig 3). Logistic suitability values of starry stonewort models based on $\mathbf{M}_d$ (mean = 0.30, sd = 0.13) vs. $\mathbf{M}_i$, and vs. $\mathbf{M}_g$ were also significantly different, with $\mathbf{M}_d$ showing higher suitability than $\mathbf{M}_i$ (t = 717.16, df = 551600, $p < 0.001$) but less than $\mathbf{M}_g$ (t = 315.76, df = 732220, $p < 0.001$; Fig 3). Model uncertainty was higher in the model calibrated in $\mathbf{M}_i$ ($\mathbf{M}_i$ vs. $\mathbf{M}_d$: t = 20.10, df = 592650, $p < 0.001$; sd $\mathbf{M}_i$ vs. $\mathbf{M}_g$: t = 79.35, df = 536950, $p < 0.001$; Fig 3). In present-day models, we found potential areas for starry stonewort distribution in southeast and central Minnesota and also in the Minneapolis-St. Paul metro region. The portion of Minnesota where starry stonewort has been confirmed to date was predicted to have high suitability for the model calibrated based on $\mathbf{M}_g$ and $\mathbf{M}_d$ (Fig 3).

The $\mathbf{M}_i$ model based on the invasive population in the US predicted only a small area of moderate suitability in central and southeastern Minnesota (Fig 3), while the model based on the entire species' range predicted a broad area of suitability across the state. Models from the global range $\mathbf{M}_g$ containing all the occurrences produced predictions with lower uncertainty. The $\mathbf{M}_d$ model calibrated based on the entire species range but with reduced dispersal potential predicted suitability resembling something between $\mathbf{M}_i$ and $\mathbf{M}_g$ (Fig 3). Prediction of starry stonewort suitability from $\mathbf{M}_d$ showed the highest uncertainty in western Minnesota.

Present-day climate across $\mathbf{M}_i$, $\mathbf{M}_g$, and $\mathbf{M}_d$ showed non-analogous environments across Minnesota under all RCP scenarios of the IPSL climatic model (Figs 4–6). All MRI emission scenarios showed Minnesota having analogous climates. Other climate models and emission scenarios showed different non-analogous climate configuration according to the **M** scenarios employed (Figs 4–6). For example, $\mathbf{M}_i$ under present-day climatic conditions overlapped with future climate conditions for all RCP scenarios in climate models GISS and MRI, RCP 2.6 and 4.5 in CCSM, and maintained environmental similarity in the northeastern part of Minnesota in the MIROC model (Fig 4). This pattern was similar for $\mathbf{M}_d$ (Fig 6) despite the lack of analogous environments in MIROC RCP 8.5. Models calibrated based on $\mathbf{M}_g$ included analogous environments except in the case of all RCP scenarios in the IPSL model and MIROC RCP 8.5, which showed non-analogous environments in a small region in southwestern Minnesota (Fig 5). According to Exdet, non-analogous conditions for the IPSL model were driven mainly by differences in mean diurnal range, while novel climates in the MIROC RCP 2.6, 4.5, and 8.5 and CCSM RCP 6 and 8.5 were driven by extreme values of maximum temperature of the warmest month (Figs 4–6). Novel climates in MIROC RCP 6 model were explained by the maximum temperature of the warmest month and by the mean temperature of wettest quarter.

Models calibrated based on $\mathbf{M}_i$ and $\mathbf{M}_d$ produced predictions with high uncertainties in Minnesota for all RCP scenarios (Figs 7 and 8). High suitability was predicted for $\mathbf{M}_i$ and $\mathbf{M}_d$ in scenarios CCSM RCP 2.6 and 4.5, MRI RCP 4.5, 6, and 8.5, and for $\mathbf{M}_d$ GISS RCP 6. Additionally, based on $\mathbf{M}_i$ and $\mathbf{M}_d$, models did not predict suitability under the IPSL climate model or predicted moderate suitability in small areas under the MIROC climate model (Figs 7 and 8), due to the absence of analogous environments (Figs 4 and 6).

The models from $\mathbf{M}_g$ transferred to future climate predicted an expansion of suitable areas under all GISS scenarios, with reduced suitability for future climate according to CCSM, IPSL, and MIROC (Fig 9). High variability was found for CCSM 2.6 and 8.5, GISS RCP 6, and all MRI scenarios. Some future climate scenarios indicated lack of suitability for starry stonewort throughout Minnesota (Fig 9). Suitability was not predicted for all IPSL scenarios due to non-

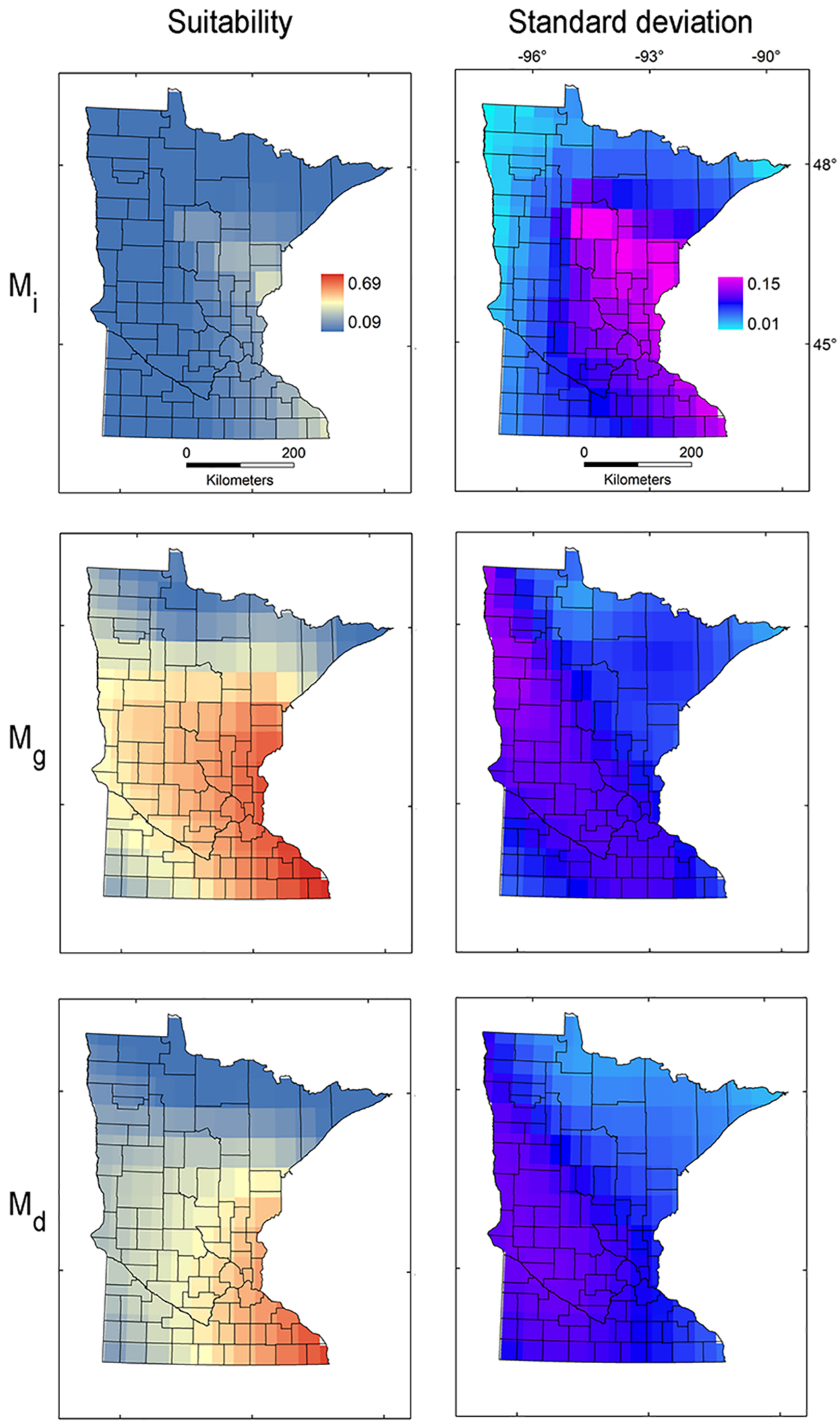

Fig 3. Ecological niche model transference to Minnesota under present-day climate. Ecological niche model predictions based on model calibration region in the invaded range with high dispersal ($\mathbf{M}_i$; top), entire species' range with high dispersal ($\mathbf{M}_g$; mid), and entire species' range with reduced dispersal ($\mathbf{M}_d$; bottom) projected to Minnesota to identify areas with high (red) or low (blue) environmental suitability (left) and high (pink) or low (light blue) model uncertainty (right).

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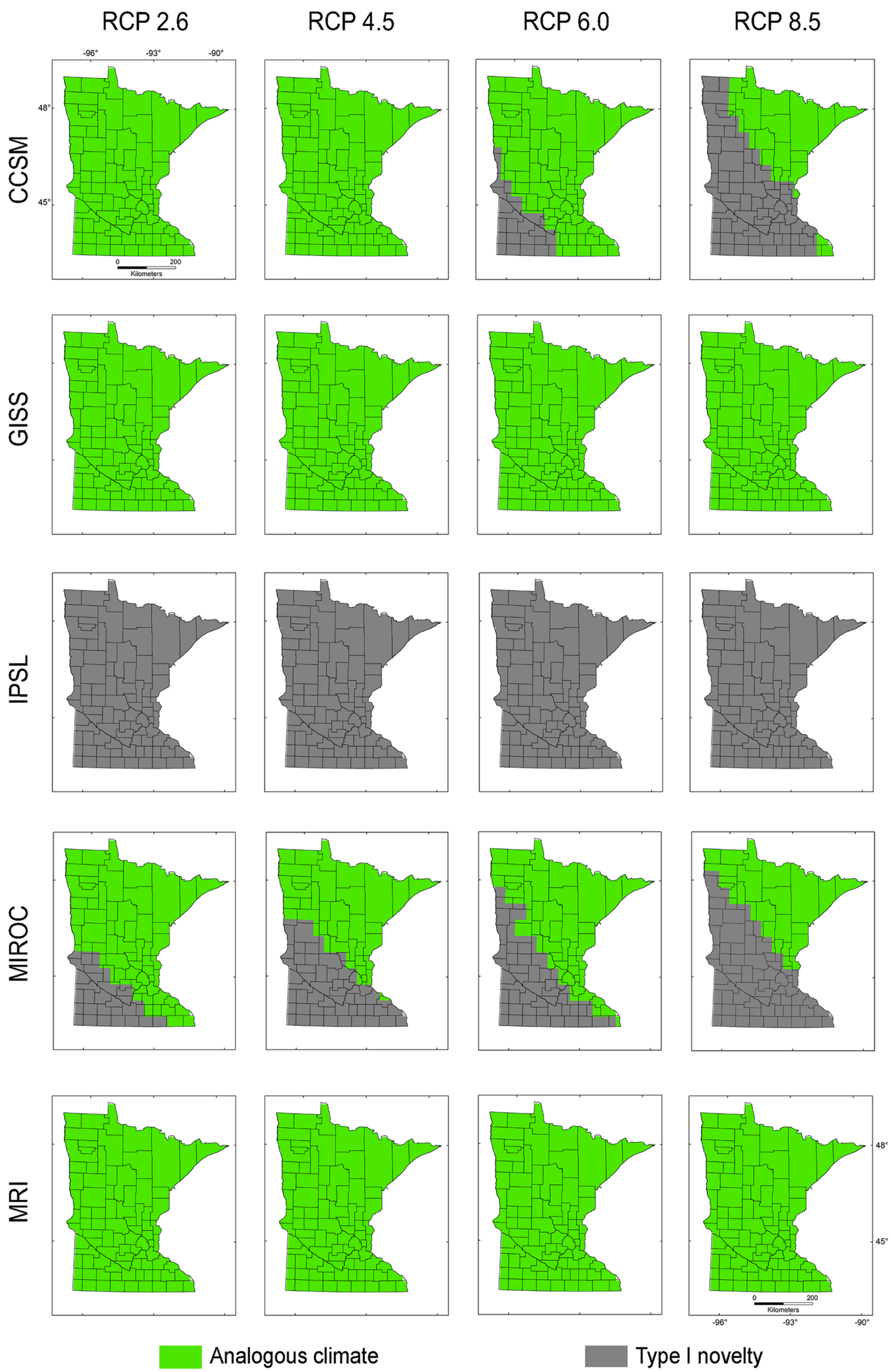

Fig 4. Environmental similarity comparison between the calibration M_i and the projection region of Minnesota. Exdet tool identified analogous climates between present-day climate in the calibration region from the

invaded range and future climate scenarios in the projection region of Minnesota. Areas with analogous (green) and non-analogous environments in Minnesota (grey) were identified for five future climate models (i.e., CCSM, GISS, IPSL, MIROC, MRI) and four RCP scenarios of CO_2 emissions (i.e., 2.6, 4.5, 6, and 8.5).

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analogous climates; while MIROC RCP 8.5 and CCSM RCP 8.5 showed unsuitability in analogous environmental conditions in all **M** scenarios. In general, climatic suitability is predicted to decrease under future climate conditions relative to present-day conditions (Fig 3 vs. Fig 10). The model ensemble showed a lack of agreement in predicted suitability among **M** calibration areas and RCP scenarios, with suitability values ranging from 0.01 to 0.12 for $\mathbf{M}_i$, 0.05 to 0.28 for $\mathbf{M}_g$, and from 0.06 to 0.30 for $\mathbf{M}_d$ (Fig 10). Areas with high values of suitability were also areas with high uncertainty in the model ensemble (Fig 10). In general, climatic suitability is predicted to decrease in the number of lakes of Minnesota under future climate conditions relative to present-day conditions except for the scenario RCP 2.6 from the climatic model CCSM and RCP 8.5 from MRI.

Discussion

Model predictions

We used a **BAM** ecological niche modeling framework to predict present-day and future climatic suitability throughout Minnesota for the aquatic invasive species starry stonewort. Under most future climate scenarios, the available range is predicted to shrink relative to present-day conditions. Based on the data available and the assumption of niche conservatism [49,50], all future climatic models under all RCP scenarios showed a decrease in suitable range relative to present-day conditions, with the exception of future climatic models: CCSM 2.6 and 4.5, and MRI RCP 4.5, 6, and 8.5 for $\mathbf{M}_i$, GISS RCP 6 for $\mathbf{M}_g$, and CCSM 2.6 and MRI 8.5 for $\mathbf{M}_d$, which showed increased areas of suitability with plausible range shifts. All these predictions, however, showed considerable uncertainty (Figs 7–9).

It is possible that our findings underestimate the potential invasiveness of starry stonewort by not capturing the full extent of its climatic tolerance [23]. Escobar et al. [8] recently described environmental tolerances of starry stonewort in its invaded and native ranges and found that invasion was associated with a shift in its realized niche, suggesting niche expansion, i.e., there were environmental conditions occupied by starry stonewort in the invaded range that lacked analogues in the native range [51]. This suggests that invasion potential may exceed what would be anticipated based on past performance alone, and starry stonewort may be able to expand into previously unoccupied environmental space [49,51]. Models could also be underestimating invasion due to overfitting from oversampled areas (i.e., sampling bias) and spatial autocorrelation in climatic variables; however, we minimized this risk by resampling occurrences to one per pixel and using coarse-resolution climatic variables, including data from remotely sensed imagery, to counter high spatial lag associated with data derived solely from climate stations [32,52,53].

The consensus areas of suitability across models (Fig 10) showed a pattern of reduced suitability across all **M** regions, suggesting a potential decline of the starry stonewort under warming climates in terms of the climates where the species is found to date. Model ensembles highlight areas of agreement across predictions, but their interpretation requires caution [17]. The lack of consensus of suitable areas for starry stonewort under future climate in Minnesota reflects the diversity of possible trajectories of future climate (Figs 7–9).

We note that our findings are based on estimated climatic tolerances and a proxy of establishment [23]. Numerous other factors, such as water chemistry, dispersal limitation, and

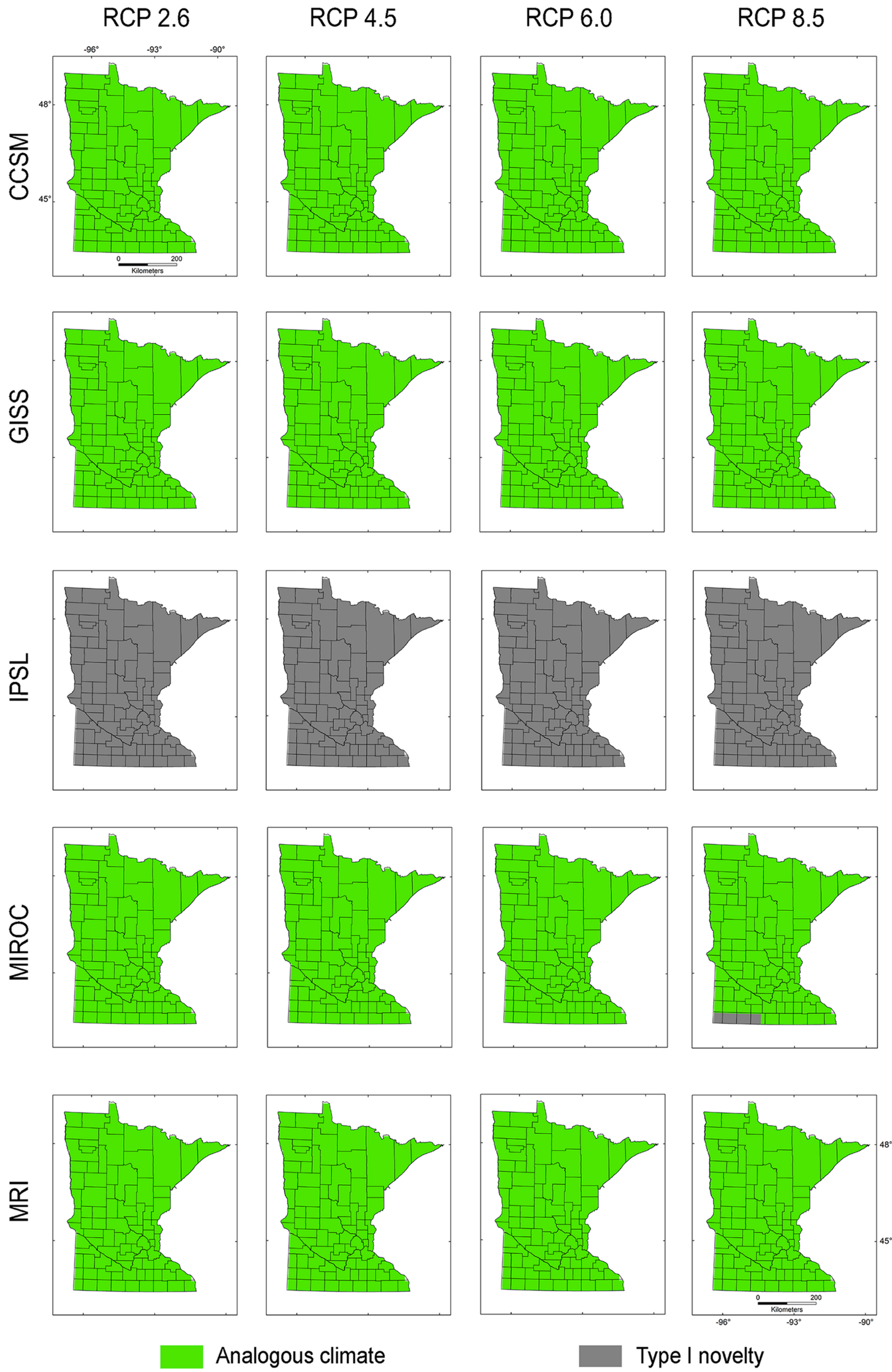

Fig 5. Environmental similarity comparison between the calibration M_g and the projection region of Minnesota. Legend as in Fig 3.

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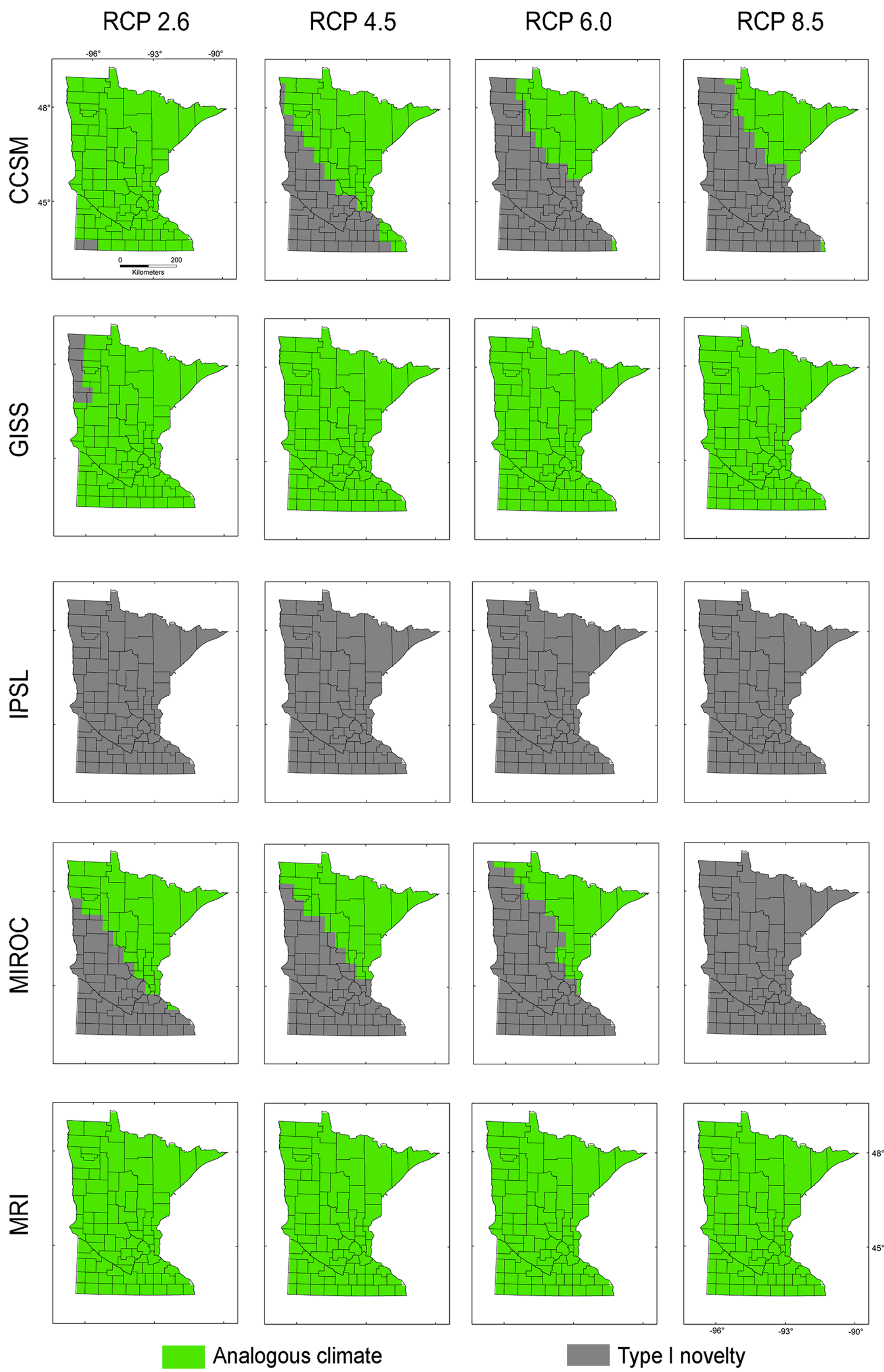

Fig 6. Environmental similarity comparison between the calibration M_d and the projection region of Minnesota. Legend as in Fig 3.

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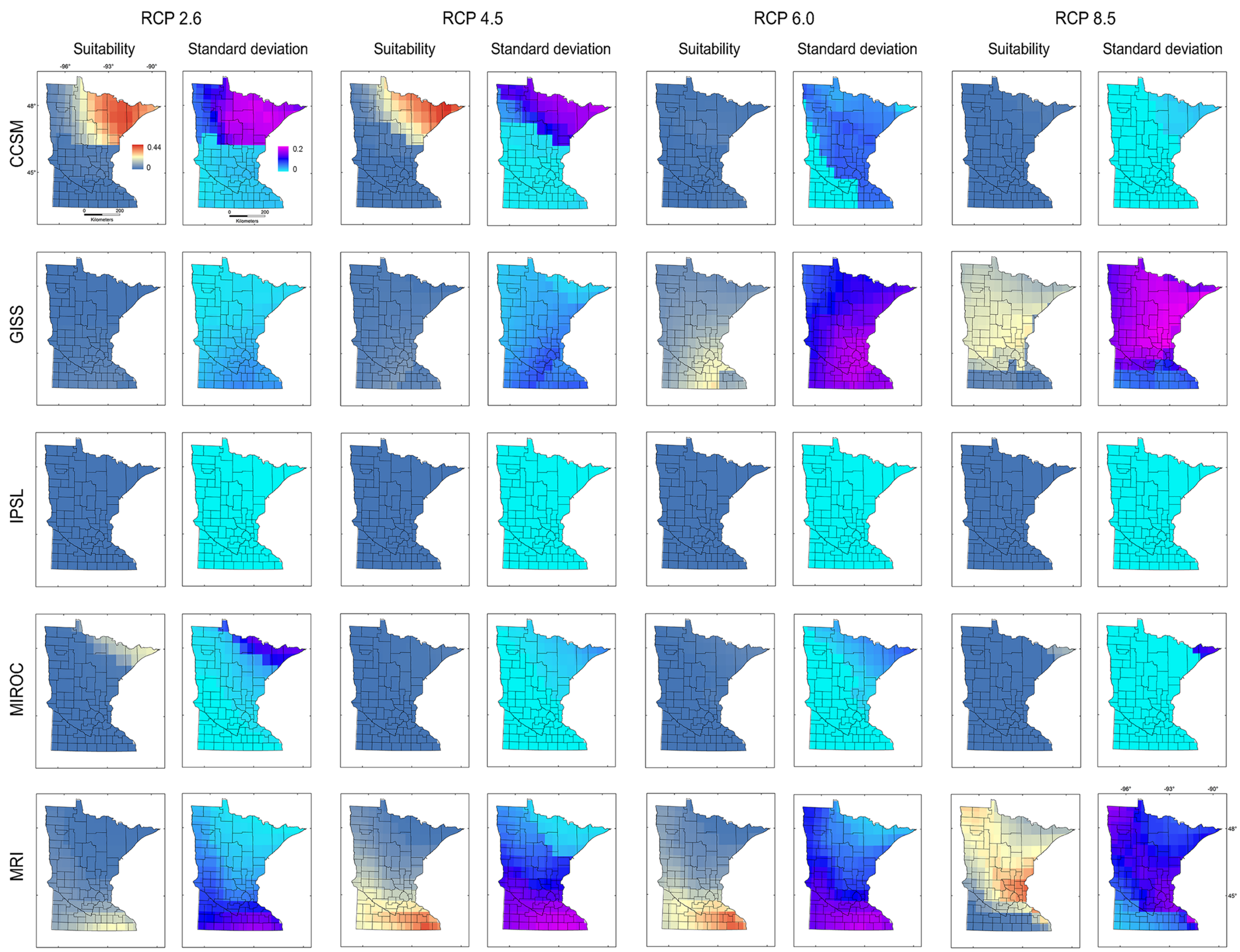

Fig 7. Ecological niche models of starry stonewort calibrated in M_i and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region M_i projected to Minnesota. Areas with high (red) or low (blue) environmental suitability (Suitability, left) and high (pink) or low (light blue) model uncertainty (Standard deviation, right) were identified for five future climate models (i.e., CCSM, GISS, IPSL, MIROC, MRI) and four RCP scenarios of CO_2 emissions (i.e., 2.6, 4.5, 6, and 8.5).

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agonistic interactions with resident biota, could limit starry stonewort expansion. However, a recent study of macrophyte communities in invaded lakes suggested plausible dominance of starry stonewort, with native species richness decreasing as starry stonewort increases in biomass [2]. These fine-scale, potentially complex and interacting factors cannot be accounted for in climate-based models, experiments would be needed to test the influence of these factors on starry stonewort population dynamics. Future research should assess how finer-scale abiotic variables (e.g., pH, conductivity, water clarity), biotic interactions, dispersal potential (via boater movement or natural water connectivity), and landscape factors (e.g., densities of roads and boat accesses) influence lake-level risk of starry stonewort invasion. Emergence of sexually reproductive populations could add new and longer-distance dispersal vectors due to small oospores that could potentially be spread by waterbirds or survive overland transport longer than bulbils [21].

Environmental variables

The environmental variables derived from the Ecoclimate repository are a promising alternative for modeling species distributed across inland and coastal/marine ecosystems [32], providing robust data on climatic variability needed for ecological niche models [54]. The 50-km spatial resolution of Ecoclimate variables mitigate the high spatial lag of finer-resolution climatic layers [52,53], which can produce flawed estimates due to high spatial autocorrelation from statistical downscaling [32,53]. We argue that during exploratory analyses, coarse-scale variables are useful for identifying plausible constraints for species establishment. Subsequent work can then incorporate finer-scale environmental variables (derived from remote sensing or habitat data) to complement climate-based models. Additionally, we developed analyses incorporating five future climate models: CCSM, GISS, IPSL, MIROC, and MRI, and four RCP emission scenarios: 2.6, 4.5, 6, 8.5. This allowed us to investigate a broader range of plausible climate scenarios. Ecological niche modeling of species invasions under future climates

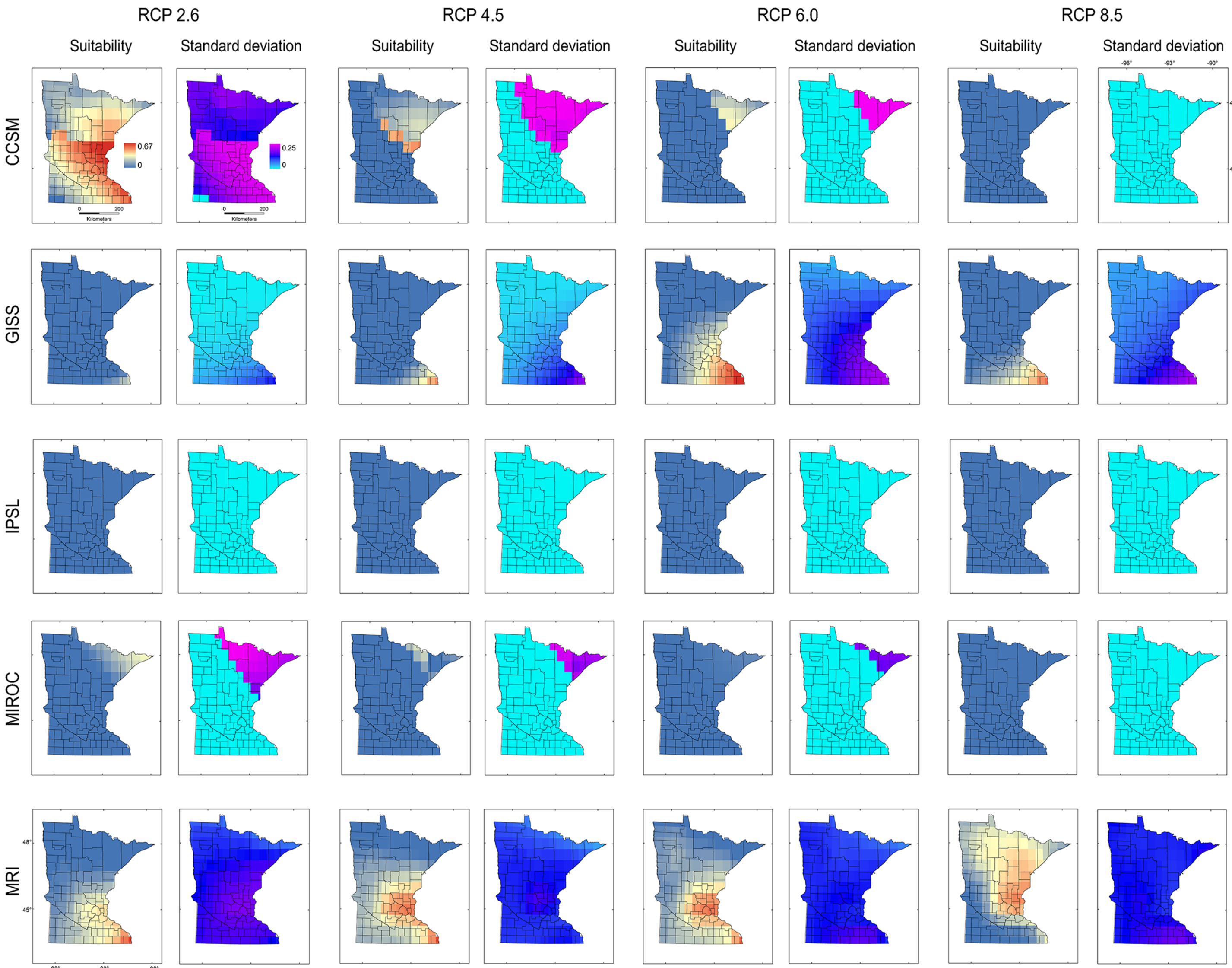

Fig 8. Ecological niche models of starry stonewort calibrated in M_d and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region $\mathbf{M_d}$ projected to Minnesota. Legend as in Fig 7.

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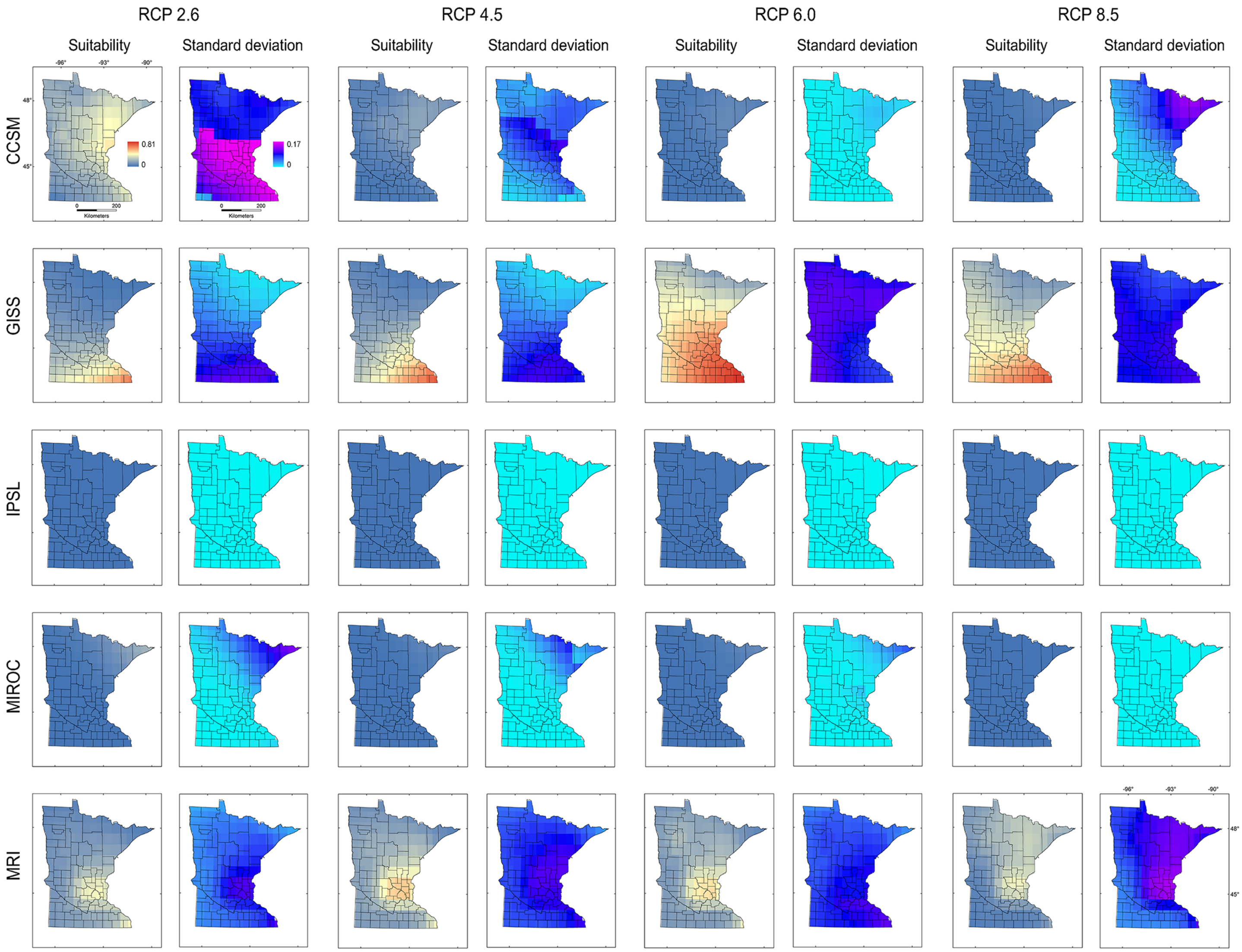

Fig 9. Ecological niche models of starry stonewort calibrated in $\mathbf{M_g}$ and projected to future climate scenarios in Minnesota. Ecological niche model predictions based on model calibration region $\mathbf{M_g}$ projected to Minnesota. Legend as in Fig 7.

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should incorporate alternative climate models and emission scenarios to reflect the uncertainty in future conditions.

The calibration region M

In agreement with previous studies using virtual species [29], our models based on empirical data suggest that a careless definition of the calibration region, **M**, may produce flawed results [23]. Restricting the model calibration region only to the invaded region, $\mathbf{M_i}$, in present-day climate (Fig 2), narrowed geographic predictions to southeastern Minnesota—all actual occurrences to date are outside of this region—as a result of the incomplete information provided to the algorithm (Fig 3). In contrast, considering the entire species' range for the two calibration regions $\mathbf{M_g}$ and $\mathbf{M_d}$ (Fig 2) included portions of central and central-north Minnesota where starry stonewort has known occurrences (Fig 3). We found that increasing the model calibration area generated an increase in AUC values, but from a practical perspective,

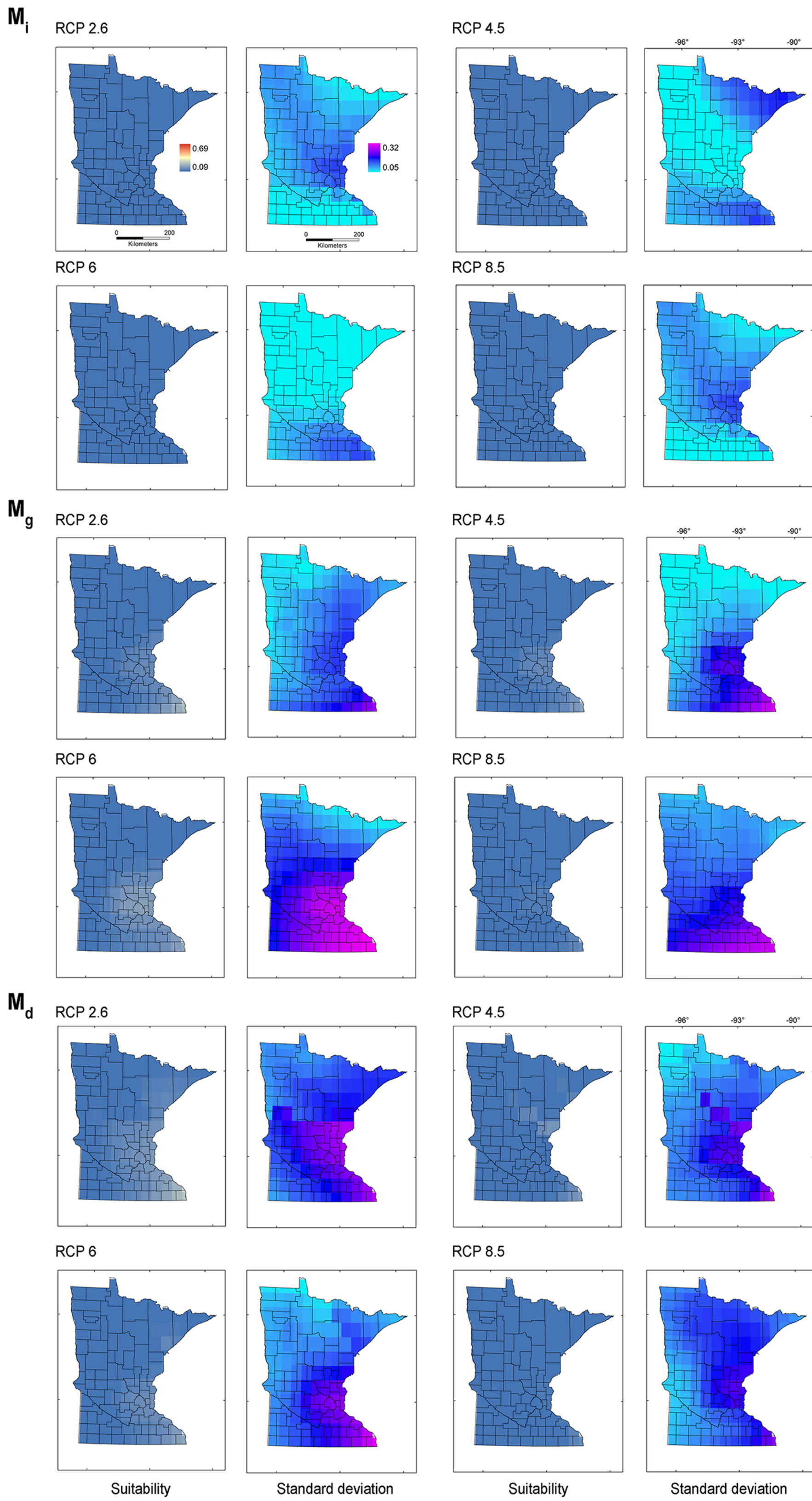

Fig 10. Starry stonewort future climate models ensemble. Model ensemble expressed as the average of continuous models in logistic format (left, 'Suitability'), showing areas with high (red) or low (blue) suitability

from all the RCP emission scenarios in comparison with the maximum range of suitability of climatic models projected to Minnesota in present environmental conditions (i.e., from the lowest [0.09] to the highest [0.69] suitability). Lack of agreement was estimated from the standard deviation of the final models (right, 'Standard deviation') and shows areas of high (pink) or low (light blue) disagreement among models. **Top**: Models calibrated in $\mathbf{M}_i$ and projected to future climate scenarios in Minnesota. **Mid**: Models calibrated in $\mathbf{M}_g$ and projected to future climate scenarios in Minnesota. **Bottom**: Models calibrated in $\mathbf{M}_d$ and projected to future climate scenarios in Minnesota.

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accounting for environmental conditions available in the entire range produced forecasts that were more reliable and more precautionary [30]; this suggests that AUC may not accurately reflect model performance due to high sensitivity of this metric to the extent of the model calibration region [29].

From a theoretical perspective, niche estimations should be guided by modern ecological niche theory [23]. According to Hutchinson [13,55], ecological niches occur in multidimensional environmental space, and species may not occupy all suitable abiotic environments (**A**) due solely to limiting biotic interactions (**B**; e.g., competition) (Fig 11 top). However, Soberón and Peterson [23] propose that Hutchinson's ideas were incomplete and that, in addition to **B**, a species can also be limited by its dispersal potential (**M**) (Fig 11 bottom). They propose that species rarely occupy their entire environmental potential and that the Hutchinsonian framework needs to be expanded. The **BAM** framework proposes that for a realistic **A** estimation for an invasive species, studies should include delimitations of **M** allowing a representative characterization of the dispersal potential of the species [23]. In other words, models aiming to estimate a good proxy of **A** should include all the areas where the species occurs, including the full native and invaded ranges. Thus, we stress that ecological niche modeling to forecast current and future biological invasions are dependent upon **M** (Fig 10 bottom). Ecological niche models calibrated in only a portion of the species' range or under a single **M** scenario may underestimate invasive potential (Fig 3). In this vein, our estimation of dispersal potential based on distance between populations in the invaded range may be confounded by search effort and may not reflect the actual directionality of spread. Genetic/genomic analyses could be used to reconstruct dispersal potential, invasion pathways, and directionality.

The extent of the calibration region was also crucial to establish the presence or absence of novel environments between calibration and projection regions, and between present-day and future climates [34,46]. Models $\mathbf{M}_i$ calibrated from the invaded range only, and models $\mathbf{M}_d$ calibrated based on a small dispersal potential (Fig 2), showed high levels of truncation of prediction in non-analogous novel climatic conditions across Minnesota, limiting our ability to project models to future scenarios (Figs 4 and 6). Conversely, $\mathbf{M}_g$ models from the entire species range with a hypothetical high dispersal identified suitable areas for starry stonewort in Minnesota under present-day and most future climate scenarios (Figs 5 and 9). This provides additional evidence that the calibration region extent plays a key role in ecological niche model projections for species invasions. Thus, model calibration regions should include the full distribution of the studied species under different **M** scenarios to capture the fullest possible set of environmental determinants of physiological tolerance of the organism, providing a firmer biological foundation for calibration region selection [13,31]. We urge researchers and reviewers to put special attention to the justification and biological support of the **M** area selected for model calibration in past and future ecological niche modeling studies.

Maxent and model evaluation

Current literature advocates Maxent for niche modeling due to its accessibility, user-friendly interface, and supporting literature [39]. However, the potential of Maxent to overestimate or

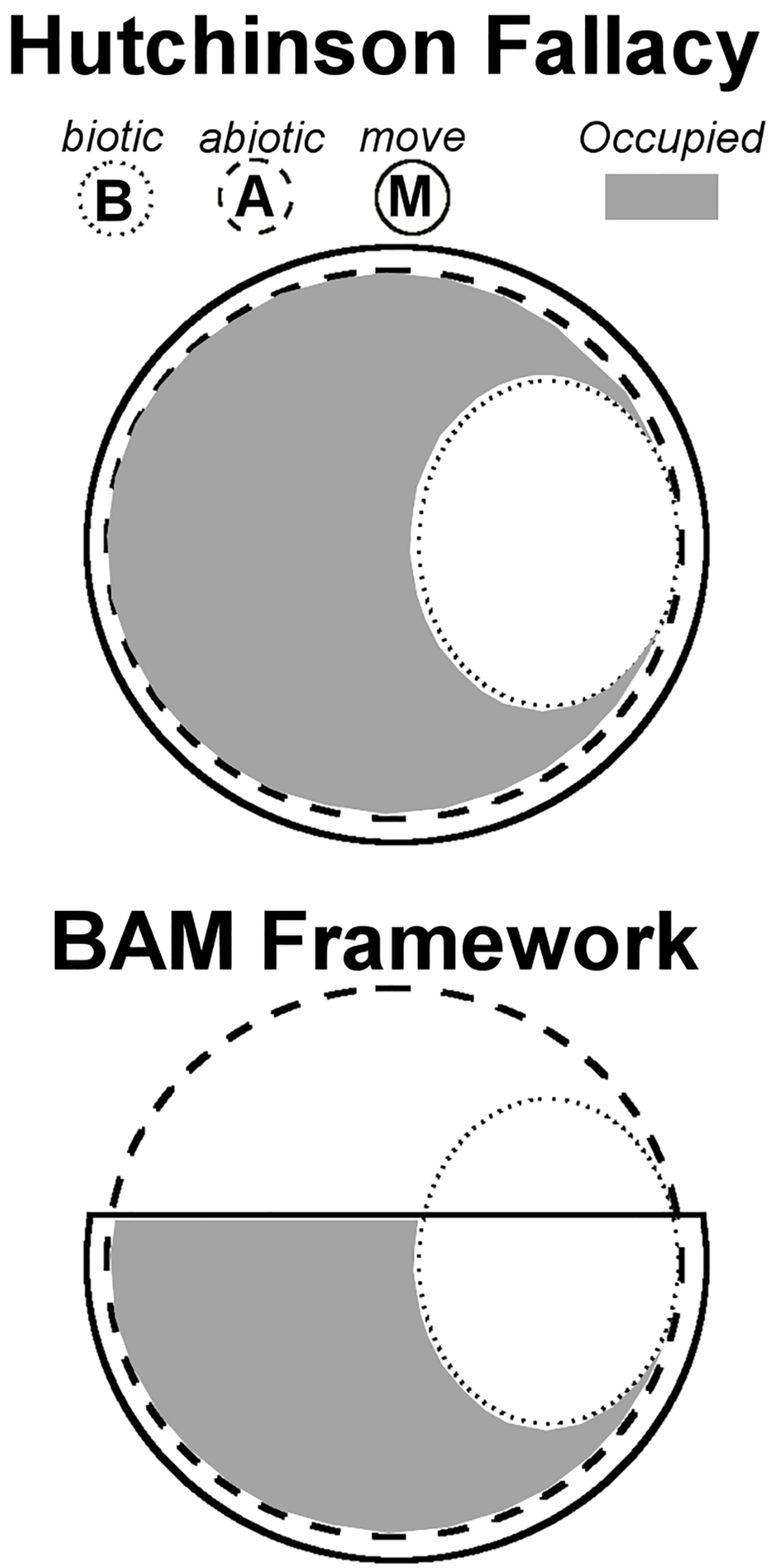

Fig 11. Conceptual framework used for interpretation of predictions. Top: The "Hutchinson Fallacy" expressed as the intersect of abiotic (**A**; dashed line) and biotic factors (**B**; dotted line) showing the

environments that a species can occupy (gray) or not (white area inside the dotted circle), based on biotic interactions solely (e.g., competitors). Note that under the Hutchinson's proposal, all the areas environmentally suitable can be reached by the species (i.e., entire circle), suggesting that the movement and dispersal potential of the species (**M**; solid line) is effective to occupy all the suitable conditions (i.e., **A** is contained in **M**). **Bottom**: The "BAM Framework" proposed by Soberón and Peterson [23] to explain that dispersal limitations (**M**) can also restrict the species to occupy (gray) only a portion of all the suitable environments (**A**). Note that in this example, the species can occupy a portion of the environmental conditions suitable due to the limited dispersal potential (i.e., half circle). **A** (abiotic) = environmental conditions suitable for the species; **B** (biotic) = interaction with other species; **M** (move) = movement or dispersal potential of the species.

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overfit predictions to the data available must be considered [18,27,38,39,41]. Maxent must be fitted for each study case considering the natural history of the species, the data available, and the assumptions involved. The results from our approach to control the extent of the calibration region, which included use of regularization coefficients, information-theory model selection, strict model evaluation, and strict model transference, support the contention that using the default parameterizations of Maxent, while convenient, is an inappropriate approach that can lead to inaccurate conclusions [29,41,46]. Thus, each modeling effort should include detailed individualized parameter selection, and model results should be critically assessed to determine if they are biologically sound, avoiding reliance on single model estimates [37].

Although predicted suitability from our present-day models ranged from minimal to broad across Minnesota (Fig 3), models with the two different calibration regions performed well in terms of omission rates and AUC values [40]. The heterogeneous suitability predicted under the two configurations reflects the sensitivity of ecological niche models to experimental design decisions (Fig 2) [13]; therefore, we propose that uncertainty estimation must be included as an essential component of ecological niche model estimations.

Conclusions

Starry stonewort is predicted to expand its current geographic range into novel areas across Minnesota under present-day climate conditions. Under future climate conditions, we estimate a reduction in suitability for the species. Our models are a step toward the development of management strategies to prevent and mitigate the spread of this species on the leading edge of its invasion. It is crucial to develop strategic interventions that target the role of human activities in starry stonewort spread. Further, our results suggest that sound forecasts require rigorous model design and evaluations to improve their reliability.

Supporting information

S1 File. Table A. Correlation matrix of environmental variables. **Table B.** Summary of model evaluations.
(DOCX)

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Supervision: Luis E. Escobar.

Writing – original draft: Daniel Romero-Alvarez, Luis E. Escobar, Sara Varela, Daniel J. Larkin, Nicholas B. D. Phelps.

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2013 Project Abstract
For the Period Ending July 31st, 2017

PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project #11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods – Phase 2: Risk Analysis
PROJECT MANAGER: Professor David Andow
AFFILIATION: University of Minnesota – Minnesota Aquatic Invasive Species Research Center
MAILING ADDRESS: 219 Hodson Hall, 1980 Folwell Avenue
CITY/STATE/ZIP: Saint Paul, MN 55108
PHONE: (612) 624-5323
E-MAIL: dandow@umn.edu
WEBSITE: http://www.maisrc.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $126,676

Overall Project Outcome and Results
Bighead and silver carps (bigheaded carps) pose a threat to Minnesota's waterways and there is a need to better understand their potential impacts to inform management actions. Towards this end, project researchers designed and conducted a risk assessment for bigheaded carps in Minnesota. Results from previous (Phase 1) research and a survey with risk assessment participants were used to focus the scope of the risk assessment on four potential adverse effects: impacts to game fish, non-game fish, species diversity/ecosystem resilience, and recreation (from the silver carp jumping hazard). Four watersheds were focused on, selected to be both geographically diverse and relevant to the current decision making context.

The risk assessment was conducted with the participation of twenty-three experts on bigheaded carps and Minnesota's waterways. A workshop was held to discuss the risk assessment findings and their implications for the management of bigheaded carps in Minnesota, and 50 people attended including stakeholders, researchers, managers, decision makers, and members of the public. Insights garnered from this workshop informed the final version of the risk assessment report, "Minnesota Bigheaded Carps Risk Assessment" which was released in May 2017.

This risk assessment represents the first systematic analysis of the risks posed to Minnesota from bigheaded carps and will both justify and inform future management efforts. Specific findings from this report include that the risk from bigheaded carps varies greatly depending on the watershed and potential adverse effect considered. The risk was higher for the species diversity/ecosystem resilience and recreation potential adverse effects and for the Minnesota River-Mankato and Lower St. Croix River watersheds. These findings emphasize the need for a timely management response to protect watersheds identified as most at risk, while ensuring that any collateral damage from management actions leads to less ecological harm than bigheaded carps are likely to cause.

Project Results Use and Dissemination
Project results were disseminated through conference presentations, presentations to stakeholders, media news stories, a journal article, and a project report. Professional conference presentations included: 1) The 2016 American Fisheries Society Meeting on August 24th, 2016; 2) The 2016 Upper Midwest Invasive Species Conference on October 18th, 2016; and 3) The 2016 Society for Risk Analysis meeting on December 13th, 2016. Project results

were also presented to academics and researchers at the November 22nd, 2016 Semi-annual All-MAISRC (Minnesota Aquatic Invasive Species Research Center) Meeting.

Presentations to stakeholders and members of the public included: 1) the Minnesota Invasive Carp Forum on March 10th, 2016; 2) the St. Croix River Association's AIS Group Meeting on June 8th, 2016; 3) the MAISRC Research Showcase on September 12th, 2016; 4) the "Risk Based Management for Bigheaded Carps" workshop held to discuss project findings and implications on March 15, 2017; and 5) the Minnesota Invasive Carp Forum on March 29th, 2017. Project outcomes and findings were also covered in a news update on Minnesota Public Radio on March 15, 2017.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 12: Characterizing spiny water flea impacts using sediment records
SUBPROJECT MANAGER: Donn Branstrator
AFFILIATION: University of Minnesota Duluth
MAILING ADDRESS: 1035 Kirby Drive
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PHONE: 218-726-8134
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WEBSITE: https://scse.d.umn.edu/biology-department/faculty-staff/dr-donn-branstrator
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $212,266
AMOUNT SPENT: $211,708
AMOUNT REMAINING: $558

Sound bite of Subproject Outcomes and Results
This project found that spiny waterflea have been present in Lake Mille Lacs and Lake Kabetogama since the 1930s, about 80 years before they were first detected. Evidence shows they were in low abundance until around the year 2000. This tells us that traditional detection methods may be inadequate.

Overall Subproject Outcome and Results
Although aquatic invasive species threaten Minnesota's environment, economy, and recreation, we still know little about the colonization histories and ecosystem impacts of some of the state's invaders such as spiny water flea. This project made large advances in understanding the colonization and impact of spiny water flea in Lake Mille Lacs, Lake Kabetogama, Lake Winnibigoshish, and Leech Lake through the collection and analysis of organism remains in lake bottom sediments over about a 120 year period from present (2017 or 2018) back to the year 1900. The results provide replicated evidence that spiny water flea was resident continuously in Lake Mille Lacs and Lake Kabetogama since the 1930s, or about 80 years before it was first detected in the open waters of either lake. Evidence demonstrates that spiny water flea had a prolonged history of low abundance in both lakes before about the year 2000 at which time it began to increase rapidly. Zooplankton that are prey and competitors of spiny water flea often declined in abundance after spiny water flea increased in abundance. There was no evidence of spiny water flea in the sediments of Lake Winnibigoshish. There was evidence of a small population of spiny water flea in the sediments of Leech Lake that dated to the year 2001, possibly representing a failed invasion. To date, Leech Lake has never been known to contain this organism. The data allow us to test hypotheses about the timing and impact of spiny water flea on the food webs of Minnesota lakes. The results re-cast our understanding of the timeline of spiny water flea invasion in Minnesota and underscore the value of lake sediments to study invasive species. The results suggest that traditional methods of spiny water flea detection with nets, as carried out by academic units and management agencies in Minnesota, may be inadequate to detect spiny water flea when it is low or transient in abundance.

Subproject Results Use and Dissemination
We have disseminated our project results at a variety of conferences and meetings as summarized below.

1) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 12, 2016)
2) MAISRC Research & Management Showcase (St. Paul, MN) – four laboratory presentations (September 12, 2016)

3) Coe College Wilderness Field Station (Ely, MN) – platform presentation (July 22, 2017)
4) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 13, 2017)
5) MAISRC All Members meeting (St. Paul, MN) – platform presentation (November 28, 2017)
6) MAISRC Science-In-Seconds competition (St. Paul, MN) – platform presentation (May 30, 2018)
7) MAISRC Research & Management Showcase (St. Paul, MN) – poster presentation (September 12, 2018)
8) Upper Midwest Invasive Species Conference (Rochester, MN) – poster presentation (October 15-18, 2018)
9) Association for the Sciences of Limnology and Oceanography Conference (San Juan, Puerto Rico) – poster presentation (Feb 23 – Mar 2, 2019)
10) Rainy-Lake of the Woods Watershed Forum Conference (International Falls, MN) – poster presentation (March 13-14, 2019)
11) Minnesota Department of Natural Resources meeting (St. Paul, MN) – skype presentation (May 14, 2019)

We have included images of two poster presentations that were displayed at science conferences.

M.L. 2013 Project Abstract
For the Period Ending June 30, 2018

SUBPROJECT TITLE: MAISRC Subproject 13: Eco-epidemiological Model to Assess Aquatic Invasive Species Management
SUBPROJECT MANAGER: Dr. Nicholas Phelps
AFFILIATION: University of Minnesota, Minnesota Aquatic Invasive Species Research Center
MAILING ADDRESS: 2003 Upper Bufford Circle, Skok Hall 135
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WEBSITE: www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $195,249
AMOUNT SPENT: $195,249
AMOUNT REMAINING: $0

Overall Subproject Outcome and Results

Aquatic invasive species (AIS) are spreading at an alarming rate in Minnesota, putting the urgent need for prevention at odds with limited budgets and capacity. To inform decision making, we have developed a series of integrated models that provide the cumulative risk of introduction and establishment of zebra mussels and starry stonewort in all Minnesota lakes. We first answered the question of 'can the species get there?' using network models to describe lake connections. The watercraft network was built with 1.6M MN DNR watercraft inspections from 2014-2017, with gaps and biases accounted for with a variety of statistical approaches. The water connectivity network was created at a finer resolution and larger geographic area than currently available using multiple sources of GIS data and satellite imagery. Next, we answered the question of 'will the species survive?' using advanced methods of ecological niche modeling. With current species distribution of the invaded and native ranges, paired with local environmental data, we projected suitability at the lake level. These three massive data sources fed into the development of an integrated model that quantified the risk of AIS invasion for each waterbody from 2018-2025. Not surprisingly the results suggest the number of infested waterbodies will increase in the years to come. However, with the integration of hypothetical management scenarios developed and incorporated during two project workshops, we demonstrated the value of this approach to assess management effectiveness by determining the number of new infestations averted. While the model is not perfect (no models are), the results are robust and provide useful information from which to make decisions. When considered across a watershed, county or state, the ability to rank waterbodies based on actual, not perceived, risk is a game changer for the prioritization of intervention strategies.

Subproject Results Use and Dissemination

The outcomes of this projects received considerable attention from AIS managers, lake associations and other researchers. We took full advantage of this opportunity and far exceed expectations to disseminate the results. We communicated to the scientific community with the publication of seven related manuscripts and have three more in preparation, and presentations at three scientific conferences. The project was presented to stakeholder audiences 11 times in formal settings and many informal settings. We worked closely with MAISRC to disseminate project updates through MAISRC's newsletter and social media. We have helped develop a project page on the MAISRC website (https://www.maisrc.umn.edu/modeling-ais) that has links to finalized risk ranking for each lake in Minnesota, project reports, and communications. In addition, all raw data and products generated as part of this project will be stored in the MAISRC-DRUM (Data Repository at UMN) for indefinite public access (web addressed TBD).

Reviews in Fisheries Science & Aquaculture

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Aquatic Invasive Species in the Great Lakes Region: An Overview

Luis E. Escobar, Sophie Mallez, Michael McCartney, Christine Lee, Daniel P. Zielinski, Ratna Ghosal, Przemyslaw G. Bajer, Carli Wagner, Becca Nash, Megan Tomamichel, Paul Venturelli, Prince P. Mathai, Adam Kokotovich, Joaquin Escobar-Dodero & Nicholas B. D. Phelps

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Aquatic Invasive Species in the Great Lakes Region: An Overview

Luis E. Escobar[a], Sophie Mallez[a], Michael McCartney[a], Christine Lee[a], Daniel P. Zielinski[a], Ratna Ghosal[a], Przemyslaw G. Bajer[a], Carli Wagner[a], Becca Nash[a], Megan Tomamichel[a], Paul Venturelli[a], Prince P. Mathai[b], Adam Kokotovich[a], Joaquin Escobar-Dodero[c], and Nicholas B. D. Phelps[a]

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ABSTRACT

Aquatic invasive species (AIS) are of concern in North America due to their devastating impacts on ecosystems and economies. The Great Lakes region is particularly vulnerable to AIS introduction and establishment with at least 184 nonindigenous species reported in this region from a large number of taxa including viruses, bacteria, diatoms, protozoa, arthropods, mollusks, fish, and plants. Representative species from these groups were explored, describing the features of their natural history and current efforts in prevention and control. Specifically, five AIS that are expected to spread to novel areas in the region are discussed: viral hemorrhagic septicemia virus and heterosporis (pathogens affecting fish), starry stonewort (an alga), zebra mussels (a bivalve), and carps (fishes). Novel strategies for AIS control include next-generation sequencing technologies, gene editing, mathematical modeling, risk assessment, microbiome studies for biological control, and human-dimension studies to address tensions related to AIS management. Currently, AIS research is evolving to adapt to known technologies and develop novel technologies to understand and prevent AIS spread. It was found that AIS control in this region requires a multidisciplinary approach focusing on the life history of the species (e.g., pheromones), adaptive management of anthropogenic structures (e.g., bubble curtains), and the integration of human dimensions to develop efficient management plans that integrate local citizens and management agencies.

KEYWORDS

Aquatic invasive species; Great Lakes; starry stonewort; heterosporis; zebra mussels

Introduction

Aquatic invasive species (AIS) have devastating effects on ecosystems as well as on local and national economies worldwide (Lovell et al., 2006). The Great Lakes region represents the largest freshwater body in the world, and the area is known for its rich biodiversity and economic importance (Mills et al., 1993). This region, however, has fragile ecosystems that have demonstrated a high vulnerability to AIS (Elsayed et al., 2006; Lumsden et al., 2007). At least 184 nonindigenous species have been reported within the Great Lakes region, across a vast range of taxonomic groups such as viruses, bacteria, diatoms, protozoa, arthropods, mollusks, fish, and plants (NOAA, 2016). Resulting AIS damage estimates can be up to $138 million per year; however, upon considering other side effects such as sport fishing losses, the negative impact of AIS in the Great Lakes may exceed $800 million annually (Rothlisberger et al., 2012). In this region, where aquatic ecosystems are an integral part of the economy and culture, tens of millions of dollars are spent annually on the prevention, control, and management of AIS (Rosaen et al., 2012; MNDNR, 2015).

Given the biological and economic impacts of AIS, this contribution presents an overview of the current knowledge, existing prevention and control research, and future steps in finding science-based solutions to AIS problems affecting the Great Lakes region of the U.S. Investigations across AIS taxa are key to improve detection, prevention, and control strategies. Fortunately, most invasive species share ecological features that promote their invasiveness and can in turn help us predict their spread, including ecological plasticity, high reproductive potential, habitat generalism, and favorable response to human-mediated dispersal and disturbance (Lockwood et al., 2006). Here, AIS research is explained for four main groups: microorganisms, plants, invertebrates and vertebrate animals. Representative species from different taxonomic groups were included with emphasis on AIS with ongoing expansion in the Great

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Lakes region. Viral hemorrhagic septicemia virus and heterosporis (microorganisms), starry stonewort (alga), zebra mussels (invertebrate animals), and carps (vertebrate animals) are described. This review aims to include critical information about the taxonomy, natural history, current research efforts, and future need for investigation for AIS that have potential to spread to non-infested areas in the Great Lakes region. This information may help fisheries biologists, environmental managers, and aquaculture professionals to be aware of the ongoing invasion process in this region. Finally, opportunities for future AIS research are discussed. Strategic research can be used to better inform management efforts and the allocation of limited resources among detection, prevention, and control activities.

Microorganism: Viral hemorrhagic septicemia virus

Outbreaks of viral hemorrhagic septicemia virus (VHSv; *Novirhabdovirus*) cause mortality in aquaculture facilities and in wild fish populations, especially in salmonids (Wolf, 1988; Kim and Faisal, 2011; Figure 1). Indeed, rainbow trout (*Oncorhynchus mykiss*) are considered the most important reservoir and propagator of the virus and are responsible for outbreaks in many countries around the world (Wolf, 1988; Smail and Snow, 2011). Furthermore, several other economically important fish species have experienced outbreaks in farm facilities (Ross et al., 1994; Garver et al., 2013). Based on the structural composition of the VHSv nucleoprotein and glycoprotein, four genotypes have been identified (genotype I–IV) (Einer-Jensen et al., 2004; Snow et al., 2004).

VHSv causes a disease that presents in both acute and chronic forms, with clinical and pathological signs depending on the stage of the disease (Wolf, 1988; Lovy et al., 2012). VHSv displays a variety of clinical and pathological alterations, including internal lesions; serous or sanguinolent edema; petechiae and hemorrhage in visceral organs, muscles, and brain; external lesions comprising ocular and skin hemorrhage exophthalmia, skin darkening, and pale gills. Also, some behavioral alterations appear, including anorexia, lethargy, and erratic swimming (Skall et al., 2005; Lovy et al., 2013; Cornwell et al., 2014; Munro et al., 2015).

Given the known risk factors and potential for catastrophic losses of farmed and wild fish populations, the management response in the Great Lakes region has

Figure 1. Fish kill in the Great Lakes region due to VHSv. Photo credit: Andy Noyes, Department of Environmental Conservation, State of New York.

largely focused on preventing overland spread (VHSv Expert Panel, 2010). This has included regulatory inspections prior to interstate movements of live fish or gametes as outlined by the U.S. Department of Agriculture – Animal and Plant Health Inspection Service's Federal Order (USDA-APHIS, 2008). The Federal Order was lifted in 2014; however, current state requirements within the Great Lakes region meet or exceed those standards and have been considered, at least in part, responsible for the slower than expected rate of invasion (Faisal et al., 2012). Additional precautions, such as egg disinfection (Groocock et al, 2013), disinfection of frozen baitfish (Phelps et al., 2013), and disease-free baitfish certifications (Vollmar et al., 2014) have been implemented to varying degrees to reduce risk of spread. Vaccines for VHSv have not been widely used in production facilities in the region and vaccine applications for wild fish populations has not been realistic.

Current efforts

Designing effective plans for VHSv prevention requires an accurate understanding of its distribution and abiotic and biotic preferences. Studying pathogen associations with their host and environment is essential to infectious disease prevention (Johnson et al., 2015). These relationships represent factors that shape the pathogen's distribution and may include the viral cycle, environmental features, host abundance, distribution (Figure 2), and susceptibility towards infection in the native and invaded range (Chow and Suttle, 2015). The factors involved in the organisms' presence within an environment are either *abiotic factors*, which include ecological variables related to physical phenomena (e.g., temperature, bathymetry, light, chemical compounds, among others) that limit the organism's distribution or *biotic factors*, which account for interspecies interactions (e.g., parasite–host dynamics, immunity, predation by phages, among others) that allow or limit virus development and transmission (Hurst, 2011).

The ecology of VHSv is constrained to the ecosystem used by the host and the host's internal environment (Hurst, 2011). For instance, its abiotic and biotic characteristics vary between free-living and parasitic phases. The virus cycle requires entry of the virus into susceptible cells of the host, viral replication using the cell's internal mechanisms, and exit from the cell or host (Nerland et al., 2011). This is accomplished by efficient vertical and horizontal transmission routes (Hurst, 2011) together with inherent capacity of the virus to survive in the aquatic environment (Nerland et al., 2011) and evade the host's immune system (Workenhe et al., 2010). Understanding the factors limiting or facilitating VHSv occurrence is crucial to anticipate and prevent its spread. A recent study explored the biogeography of VHSv across the Great Lakes region focusing on the abiotic components associated with VHSv occurrence, and found that temperature, bathymetry, and primary

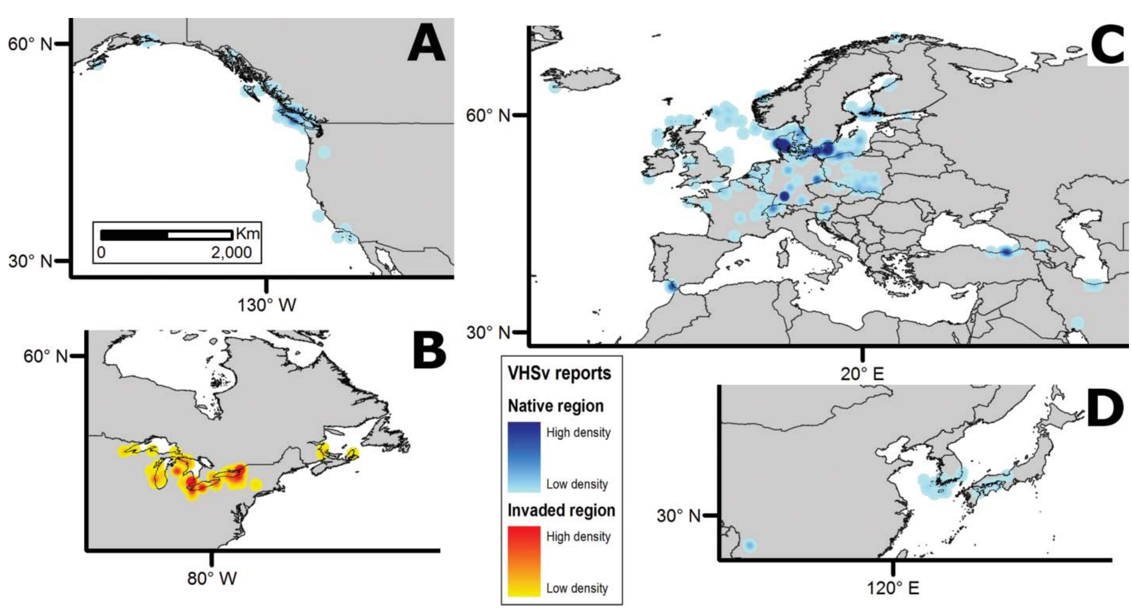

Figure 2. Hotspot areas of Viral Hemorrhagic Septicemia virus (VHSv) reports. Density of VHSv reports in its native (blue-white range) and invaded distributions (red-yellow range) across the west coast of North America (A), Europe (B), the Great Lakes Region of North America (C), and Asia (D). Continuous values estimated based on a Kernel Density Estimation from original VHSv reports in ArcGIS software version 10.3.1 (ESRI, Redlands CA) with one-degree bandwidth. Sources: http://www.fishpathogens.eu and http://gis.nacse.orgnfo/vhsv.

productivity can be associated with VHSv presence (Escobar et al., 2016). Studies focusing on VHSv tolerance to temperature have shown that it tends to maintain its biological cycle between 0 and 20°C *in vitro*, however the capacity to infect differs within this range: the optimal infective temperature is between 10 and 14°C (Estepa and Coll, 1997; Gaudin et al., 1999; Isshiki et al., 2002; Vo et al., 2015). At low temperatures (i.e., $\leq$5°C), infection occurred at a slower rate and at temperatures of approximately 25°C, infection did not occur (Isshiki et al., 2001; Vo et al., 2015). Temperature affects the virus' capacity to infect and use the host's cells by influencing the viral protein functionality, which is principally linked to fusion activity (Gaudin et al., 1999). These previous studies *in vitro* correlate with infection studies *in vivo* in which, depending on the genotype and species used, mortalities occurred between 8 and 25°C; suggesting a narrow temperature range to facilitate the disease. Optimal *in vivo* temperature for VHSv development is approximately 14°C in several species (Goodwin and Merry, 2011; Avunje et al., 2012; Goodwin et al., 2012), but some marine isolates exhibit greater mortalities between 8 and 10°C (Isshiki et al., 2002; Hershberger et al., 2013). This may be due to the fact that the infectivity of a virus strain may be enhanced by, and fish immunity compromised at, particular temperature ranges (Sano et al., 2009). These temperature–response differences seen between experimental designs may be explained partly by the greater biological complexity in experiments *in vivo*, principally related to immune response, in contrast to studies *in vitro*, which do not involve sophisticated immune components (Workenhe et al., 2010).

Future steps

Like other invasive species, a clear demarcation of the species range is critical to effective management. Although research and surveys have greatly informed the current status of VHSv in the Great Lakes region, many questions remain. At least 30 species have been found positive to VHSv in the Great Lakes region (Escobar et al., 2016), and it is necessary to identify further vulnerable fish species as well as transmission pathways, focusing on areas where susceptible species inhabit. Another important area of research is identifying which wild species are potential VHSv reservoirs. Surveys have detected key species in endemic areas (Mortensen et al., 1999; King et al., 2001; Skall et al., 2005; Frattini et al., 2006; Garver et al., 2013; Kim et al., 2013; Moreno et al., 2014; Ogut and Altuntas, 2014); however, additional effort is needed to identify important species in areas where VHSv has recently been detected or is still absent. This will allow researchers and managers to determine ideal "sentinel" fish species for long-term VHSv monitoring to inform early warning systems. Finally, a thorough evaluation of the >10 years of diagnostic testing history is needed to redefine the ongoing strategy for regulatory inspection and surveillance to ensure continued protection while minimizing costs (Gustafson et al., 2010).

Microorganism: Heterosporis

Heterosporis sutherlandae was initially detected by Sutherland et al. (2000) and D. Cloutman (personal communication) in the skeletal muscles of yellow perch (*Perca flavescens*) in the Great Lakes region. It is not clear if this microsporidian parasite is native or invasive but it has been reported in 45 waterbodies in the Great Lakes region, and has been identified as a disease of concern by the Great Lakes Fishery Commission (Phelps et al., 2015). Susceptible species include fishes important to aquaculture and sport fishing, such as walleye (*Sander vitreus*), rainbow trout (*Oncorhynchus mykiss*), and baitfish (Miller, 2009).

Members of the genus *Heterosporis* are spore-forming, unicellular, fish parasites that damage the skeletal muscle of susceptible fish hosts. Fish are exposed to the parasite by consuming infected fish or coming into contact with free-living spores in the water (Lom and Nilsen, 2003; Diamant et al., 2010; Al-Quraishy et al., 2012; Phelps et al., 2015). As the infection progresses, spores form intracellular sporphorous vesicles that rupture to release additional spores into the tissue (Figure 3). The result is a concave appearance of the fish, and a fillet that appears white or freezer-burned, has a soft and mushy texture, and is considered unfit for human consumption (Lom et al., 2000; Phelps et al., 2015). Spores are resistant

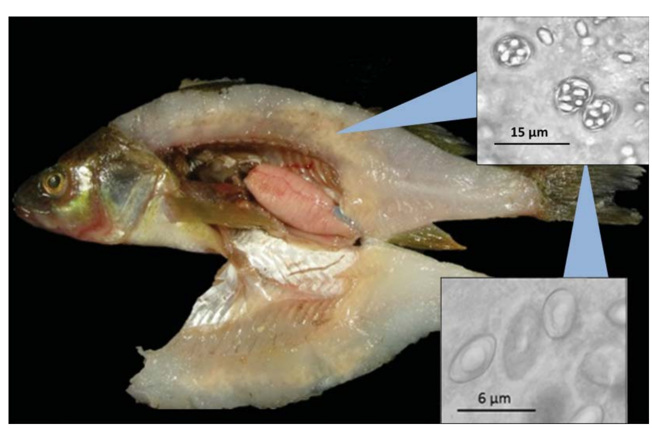

Figure 3. Heterosporis infection. Yellow perch (*Perca flavescens*) from Leech Lake with *H. sutherlandae* and characteristic muscle lesions; spores and sporophorous vesicles 400× (first insert) and spores at 1000× (second inset).

to standard laboratory disinfection procedures and can survive outside of a host for up to six months (Miller, 2009).

Current efforts

Current research has identified *H. sutherlandae* as unique with less than 96% rRNA gene sequence identity to other *Heterosporis* species, and has confirmed infection in yellow perch, northern pike (*Esox lucius*) and walleye (*Sander vitreus*) from inland lakes in Minnesota and Wisconsin (Phelps et al., 2015). Field collection is underway to identify host-specific factors (such as weight or age) or environmental factors (such as temperature) which may influence the spread or severity of *H. sutherlandae*. Concurrent laboratory infection trials are estimating pathogen transmission and virulence, and measuring physiological effects on the host of *H. sutherlandae* infection (M. Tomamichel, personal communication). A yield model is also in development using parameters estimated from experimental and field observations to predict the loss of harvest of yellow perch due to *H. sutherlandae* (P. Venturelli, personal communication).

Future steps

This parasite poses a threat to both farmed fish and wild populations. Because of the resistant nature of spores, *H. sutherlandae* could be difficult to eradicate in either a farm or natural environment. Once established, the pathogen could reduce harvest yield significantly. In addition, it would be difficult to prevent transfer to naïve populations by human or natural vectors. Therefore, the broad areas with potential to spread *H. sutherlandae* within fish populations in the region make it necessary to develop informed, evidence-based management and monitoring strategies (Escobar et al., 2017).

Alga: Starry stonewort

Starry stonewort (*Nitellopsis obtusa*; family Characeae) is a dioecious green alga that gets its namesake from the starchy, star-shaped bulbils that develop on its stem nodes and rhizoids for asexual reproduction (Bharathan, 1987; Lambert, 2009; Figure 4). Sexual reproduction via oospores is less prevalent in the dioecious taxa of Characeae, but starry stonewort has been documented to reproduce sexually under eutrophic conditions (Bharathan, 1983). Interestingly, only male specimens have been documented in starry stonewort's invaded range to date (Sleith et al., 2015). This suggests it is relying exclusively on the asexual growth of bulbils and fragments for its spread.

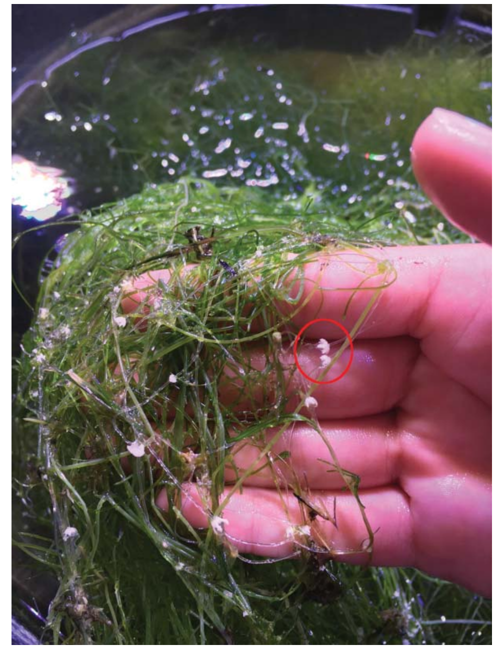

Figure 4. Starry stonewort (Nitellopsis obtusa). Note the bulbils (white structure inside red circle) attached to rhizoids (green structures). This image corresponds to a captive alga population under study at Minnesota Aquatic Invasive Species Research Center.

Starry stonewort is a charophyte, but it is similar to many invasive macrophytes in its ability to form monocultures and persist at nuisance growth levels in the littoral zone (Hackett et al., 2014).

Starry stonewort is native to Europe and Asia (Kato et al., 2014), and is established as an invasive species in many lakes of the Great Lakes basin (Escobar et al., 2016; Figure 5). The introduction of starry stonewort to North America is widely hypothesized to be from the ballast water of transatlantic ships (Hackett et al., 2014). Starry stonewort was first documented in New York in the St. Lawrence River system in 1978, and subsequent invasion have since been documented in Michigan (1983), Indiana (2008), Pennsylvania (2012), Wisconsin (2014), Vermont (2015), and Minnesota (2015) (Sleith et al., 2015; Escobar et al., 2016; Kippt et al., 2017).

Starry stonewort is used in many cytological studies due to its large cell size, but research on the ecology and biology of this species is severely underrepresented in the literature (Hackett et al., 2014). Initial detection of starry stonewort has often occurred inadvertently during routine plant surveys or from citizen reports to state agencies (Hackett et al., 2014; Kipp et al., 2014). For example, the first confirmed report of starry stonewort in Minnesota showed that growth of the alga spanned 53 acres of a lake, suggesting it may have persisted there for some time without being reported (MNDNR, 2015). Starry stonewort is very similar in appearance to native

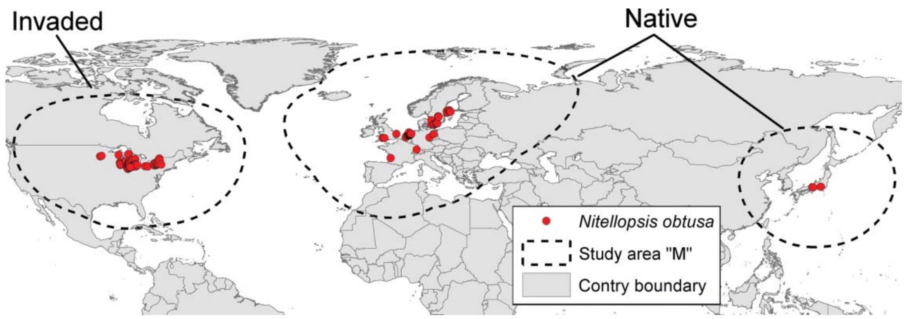

Figure 5. Native and invaded ranges of starry stonewort (Nitellopsis obtusa). Occurrences of starry stonewort (red points) resembling the global distribution of the species including the native and invade range of North America. Figure from Escobar et al. (2016) (Creative Commons Attribution 4.0 International License).

Muskgrasses (*Chara*; family Characeae), which further complicates identification and early detection efforts. Detection of starry stonewort on a case-by-case basis could limit the opportunity for early detection and rapid response management strategies. A coarse-scale ecological niche model of starry stonewort, based on climatic variables, has identified areas suitable for its establishment and further expansion across North America (Escobar et al., 2016).

Current efforts

Current research involves studying starry stonewort's ability to grow and spread, as well as assessing the efficacy of current chemical and mechanical management strategies used for its control. It is uncertain how long bulbils and fragments of starry stonewort can remain viable out of water; research to quantify these parameters is ongoing at the Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota and includes desiccation trials for bulbils and fragments, complemented with field experiments to determine the survival of the alga in boats and, in turn, spread by boater-assisted movement. Quantifying the desiccation tolerances of aquatic invasive plants is useful for characterizing expansion risk and preventing spread, and it has been investigated for other species including Eurasian watermilfoil (*Myriophyllum spicatum*) and curly-leaf pondweed (*Potamogeton crispus*) (Bruckerhoff et al., 2015). These experiments will inform management decisions regarding the placement and efficiency of boat launch monitoring personnel (Bruckerhoff et al., 2015). This information will be key to limiting and/or preventing further starry stonewort spread. Site characteristics of known starry stonewort occurrences are being aggregated to define the ecological parameters needed for its establishment. Because so little is known about starry stonewort, adaptive management is critical for ongoing treatments. The outcomes of starry stonewort treatments in Minnesota are being monitored in the field and in the lab. Starry stonewort was treated in Lake Koronis (Stearns County, MN, USA) during the summer of 2016 by mechanical harvest and algaecide applications. Bulbils from treated areas and an untreated control area were collected to asses sprouting, and field surveys were conducted to monitor biomass and bulbil density. Results of this research showed that although biomass was reduced following treatment, bulbils retained sprouting ability regardless of treatment (Glisson et al., in review).

Future steps

While there are many anecdotal observations regarding the impacts of starry stonewort, scientific conclusions backed by research and robust data are lacking. There are still major gaps of knowledge for this species, which hinders effective management. Applied ecological research is needed to understand starry stonewort's impacts on native plant communities, fish populations, and ecosystem functions. An effective control for this species, especially one that is capable of inducing bulbil mortality, is needed and collaborations between entities of invaded states may accelerate research leading to quicker management turnarounds.

Invertebrates: Zebra mussels

Benefiting from shipping traffic, commercial fishing, and the creation of canals connecting inland lakes, zebra mussels (*Dreissena polymorpha*) started to spread in Europe almost two centuries ago (Karatayev et al., 1998, 2003). The species was then introduced to North America, first into the Great Lakes in the mid-1980s in ballast water discharge of transatlantic boats (Hebert et al., 1989; Carlton 2008). By 2010, zebra mussels were found in more than 600 lakes and rivers across 26 U.S. states (Benson, 2014) and are one of the world's most economically and ecologically damaging aquatic invasive species. Costs associated with the control and management of zebra mussels in the hydropower industry and drinking water treatment plants (e.g., of mechanical and chemical treatments to remove mussels, training of personnel, reconstruction and retrofitting, lost production, among others) were estimated to be about $18 million per year from 1989 to 2005 throughout North America (Connelly et al., 2007; Chakraborti et al., 2014). Zebra mussels clog the water intake pipes of industrial facilities (Prescott 2010), compete with and smother native bivalve species (Karatayev et al., 1997; Lucy et al., 2014), and restructure aquatic food webs (Bootsma and Liao, 2014; Higgins and Vander Zanden, 2010; Mayer 2010). The dispersal ability and invasiveness of zebra mussels are due to their high fecundity, highly dispersive planktonic larval stage, attachment of adults to hard substrata via byssal threads, and from the ability of these mussels to reach such high densities that their total filtering capacity can remove 50% or more of the biomass of phytoplankton at the base of aquatic food webs (Hebert et al., 1989; Mackie, 1991; Higgins and Vander Zanden, 2010; Strayer, 2010).

Current efforts

Although the cumulative number of infested lakes has reached a plateau in several U.S. states in recent years (Figure 6), the number of infested lakes is increasing yearly in Minnesota (and perhaps Wisconsin) where zebra mussels continue to actively spread to new inland lakes. As a consequence, there is the potential to benefit greatly from targeted prevention in the Great Lakes region. Investigating the sources and pathways of zebra mussel spread is a key approach to prevent further introduction and is essential for effective measures to be taken (Estoup and Guillemaud, 2010).

To identify pathways of spread, population genetics is a powerful tool that has proven its ability to infer sources and routes of invasion in many cases of invasion worldwide, across diverse taxa. This is true despite recent arrival and short histories of many of the studied invasions (Lombaert et al., 2010; Ascunce et al., 2011; Rius et al., 2012; Perdereau et al., 2013). Traditional population genetic analyses (Weir and Cockerham, 1984; Saitou and Nei, 1987; Pritchard et al., 2000; Paetkau et al., 2004) coupled with approximate Bayesian computation analyses (Beaumont et al., 2002), a major step forward in the field (Miller et al., 2005; Pascual 2007), have allowed researchers to begin to draw inferences about the source waterbodies responsible for invasion outbreaks, and to evaluate useful contrasts of alternative invasion scenarios along with the statistical confidence in the scenarios preferred. In the Great Lakes region, these tools are now helping to identify waterbodies that serve as sources for spreading zebra mussels to new inland lakes, which may provide insight into important spread mechanisms or vectors (e.g., spread by veliger larvae in water moved from lake to lake by fishing boats, or by adult and juvenile mussels transported on boat lifts and other equipment). For instance, Mallez and McCartney (in review [Invasion population genetic model testing succeeds at small spatial scales: testing scenarios of spread for zebra mussels between Minnesota lakes]) have provided unexpected insights into the absence of large inland lakes (previously thought to be "super-spreaders") contributing to secondary spread and have started to examine the causes of the clustering of zebra mussel invasions – a pattern common in both European and North American invaded ranges (Kraft et al., 2002; Johnson et al., 2006).

Ongoing research aims also to develop rational approaches to population control in open waters, using molluscicides that are known to be highly toxic to zebra mussel adults and larvae, with relatively few nontarget effects if used responsibly (M. McCartney, personal communication). Across the United States, a handful of treatment attempts using both mechanical and chemical methods, and targeted at early stage invasions, have either successfully controlled (i.e., suppressed population growth and recruitment) or extirpated small infestations, thereby preventing explosive population growth (Wimbush, 2009; Fernald and Watson, 2014). These findings motivated a recent treatment attempt of a small isolated infestation in Christmas Lake in Minnesota (Lund et al., in press) that has not yet eradicated the zebra mussel population but has generated considerable new information about how to best conduct and evaluate the effectiveness of pesticide treatment efforts. Four ongoing open-water treatment attempts in Minnesota by the Minnesota Department of Natural Resources and MAISRC will provide more information to develop ways to best evaluate the outcomes and use them to improve zebra mussel management efforts. Just a few years ago, management was not considered to be an option for zebra mussel invasions, but attitudes may be slowly changing as this new research moves forward.

Future steps

The small geographic scale of the ongoing investigations into the population genetics of zebra mussel in the Great Lakes region makes inferences particularly challenging, as does the fast spread to inland lakes (post-2005). So far, analysis has focused on typical numbers of standard markers (i.e., nine microsatellite loci; S. Mallez and M. McCartney, personal communication). Studies conducted at MAISRC have turned to Single Nucleotide Polymorphisms (SNPs) and high-throughput genotyping

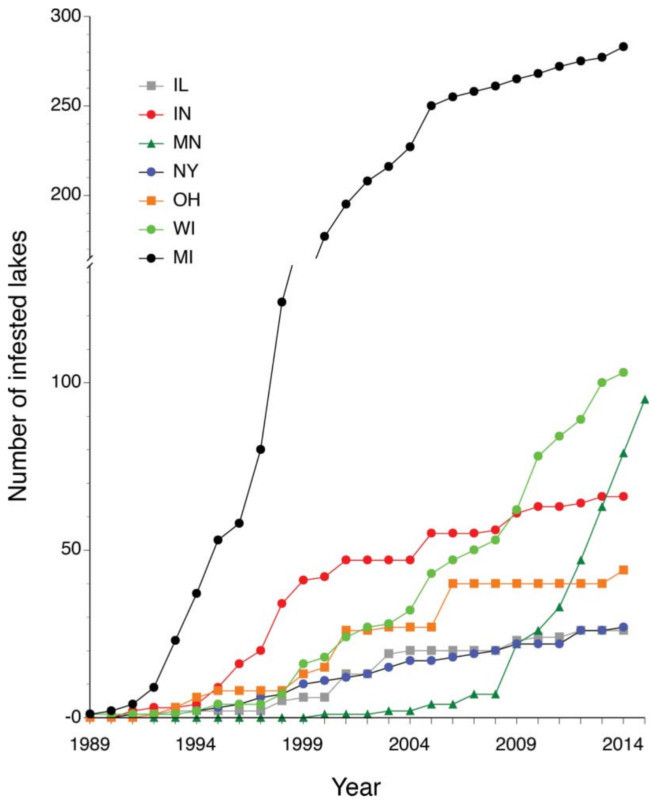

Figure 6. Pattern of spread of zebra mussels to U.S. inland lakes. The cumulative number of infested lakes is plotted against the year of infestation. The earliest date with confirmed presence of Zebra mussels was used as the date of first infestation. Only the U.S. states having more than 25 infested lakes are shown. They are: Illinois (IL), Indiana (IN), Minnesota (MN), New York (NY), Ohio (OH), Wisconsin (WI) and Michigan (MI). Data from Minnesota were obtained from the MN Department of Natural Resources. Data from other states were obtained from the US Geological Survey. From Mallez & McCartney, in review. The top trace shows the state with the greatest number of lakes infested (> 250, Michigan). This trace is shown with an axis break in order to be able to expand the scale for all the other states, with fewer lakes infested (< 100), so that their pattern of infestation can be viewed on the same figure panel.

by Next Generation Sequencing technologies known as Sequence Based Genotyping (Andrews et al., 2016), which is capable of generating large numbers of SNPs covering the entire genome. SNPs can detect finer genetic structure than typical genetic markers (e.g., microsatellite, Jeffries et al., 2016), and when several to hundreds of thousands of SNPs are assayed, these markers can provide geographic resolution at a scale similar to that of a U.S. state (Elhaik et al., 2014). The genomic resources from ongoing studies, including the sequence of the zebra mussel reference genome (M. McCartney and S. Mallez, personal communication), will create other opportunities such as identifying genes that control processes that could be targeted with gene-editing technologies (Gantz and Bier, 2015).

Vertebrates: Common carp

The common carp or (*Cyprinus carpio*) is one of the most invasive and ecologically destructive fishes in the world (Vilizzi et al., 2015). It is one of nine species of fish included among the world's 100 worst invaders (http://www.issg.orgnfo/worst100_species.html). Native to Eurasia, common carp have been introduced to all continents except Antarctica (Balon, 1995). Although the common carp is ubiquitous in many regions of the world, it is especially widespread and abundant in North America and Australia, where its biomass commonly exceeds 400 kg/ha (Bajer et al., 2009; Matsuzaki et al., 2009). Common carp feed in benthic sediments sorting out edible items (insect larvae, plant seeds, etc.) using a specialized sensory organ (palatal organ) and cross-current filtration. By aggressively feeding in the lake bottom, common carp uproot aquatic vegetation, increase turbidity, and increase transport of nutrients from the sediments into the water column (Zambrano et al., 2001; Bajer et al., 2009). Excessively abundant (>100 kg/ha), common carp can "flip" shallow lakes from a clear water state with submerged aquatic vegetation into turbid systems that lack aquatic vegetation and are dominated by algae and cyanobacteria (Zambrano et al., 2001). This leads to reduced numbers of waterfowl, amphibians (often through predation on tadpoles), insects, and possibly also fish. Lakes that lack aquatic vegetation also have reduced capacity to store or transform nutrients, thus carp contribute to excessive nutrient export out of watersheds they invade. It has been estimated that common carp are a major factor of degradation of 70% of lakes within the Great Plains Ecoregion in North America.

Current efforts

In North America, common carp are managed primarily by physical removal, treating lakes with nonspecific fish toxin (i.e., rotenone), and water draw-downs. Winter seining is the most effective form of removal because common carp form dense winter aggregations that can be located using telemetry and removed using large seine nets (Bajer et al., 2011; Figure 7). This strategy can be very effective and selective but is limited to lakes in which common carp only infrequently produce young due to native fish predation (Lechelt and Bajer, 2016). The use of rotenone and draw-downs are applied less often as they kill all fish in lakes and particularly for the draw-downs, are possible only in lakes with engineered outlets. In Australia, where physical removal is not effective due to large, connected river systems and high rates of recruitment, common carp control has focused on

Figure 7. Winter seining for carp in a lake in Minnesota after baiting using corn in a square drilled through the ice.

developing genetic technologies (e.g., daughterless, female-lethality) and the use of pathogens (Cyprinid herpesvirus-3, KHV) both of which remain in developmental stages (McColl et al., 2014; Thresher et al., 2014). Overall, aside from lakes in which common carp can be controlled using winter removal or those that can be dewatered/poisoned, there are no sustainable common carp control strategies.

Future steps

New understanding of the life history and cognitive abilities of common carp offers new control possibilities. Biocontrol appears to be a viable strategy in many lakes in the Great Lakes region and across other temperate regions of North America. Indeed, studies of common carp recruitment showed that in some lakes in Minnesota, common carp are unable to produce young because native predatory fishes, such as the bluegill sunfish (*Lepomis macrochirus*) that consumes common carp eggs and larvae. As common carp evolved spawning migrations that access predator-free habitats, such as shallow marshes prone to winter hypoxia, predator escape was achieved (Bajer and Sorensen, 2010; Bajer et al., 2012, 2015). This allows for several possible control strategies. First, some marshes can be aerated to stabilize native predators. Second, migratory routes can be exploited to remove adults that move to marshes or block juveniles that migrate from marshes to lakes. New autonomous transport systems developed for salmonids in the western United States are currently being developed at MAISRC to remove carp. Perhaps even more exciting is the possibility that cognitive aspects of common carp's foraging behavior could also be exploited to develop selective toxin delivery systems. Common carp are known to consume grain-based products, such as corn, that are not consumed by most species native to North America. They can be conditioned to aggregate in specific areas of lakes by systematic application of such baits (Bajer et al., 2011). There is an opportunity to condition (train) common carp to consume baits that are selective to them and then switch the baits with ones that contain a toxin (such as Antimycin A; Marking, 1992). This effort is also currently being pursued at MAISRC. Finally, new genetic technologies are being developed to make male common carp sterile (P. Bajer, personal communication). Integrated strategies that employ various tools that target specific weaknesses in life history and behavior offer the most promises, and their effectiveness has already been demonstrated in model systems of lakes.

Novel opportunities for AIS management

Most of the current approaches for the management of the invasive vertebrate species like the common carp include physical removal and whole lake poisoning. These approaches are nonspecific, impacting both the invasive and the native fish, and are also relatively inefficient in regions with low density of invading individuals (e.g., the invasion front; ACRCC, 2014). Approaches including pheromones, environmental DNA, or eDNA, and sound application are highly species-specific, and will greatly enhance the chances of early detection of few individuals at the invasion front.

Early detection research-pheromones

Measuring the presence/absence of a species in a natural environment is a key factor in assessing the spread of invasive species (ACRCC, 2014), and therefore, our ability to target prevention and control efforts. This is particularly important in regions where the invaders occur at low densities. Recent studies have recognized the potential of measuring chemical signals, like pheromones, that can be used to detect the presence of a species in a natural environment (Xi et al., 2011; Stewart and Sorensen, 2015; Sorensen and Johnson, 2016). Released by animals, pheromones are chemical signals which readily disperse in the natural environment (Wyatt, 2014). Fish heavily rely on pheromones for the purpose of shoaling with conspecifics, upstream migration, and to select mates during the spawning period (Sorensen and Johnson, 2016). Several techniques are being developed to measure fish pheromones and proof-of-concept studies have had some success (Fine and Sorensen, 2005; Xi et al., 2011). However, measuring pheromones in natural environments has been mostly limited to the invasive sea lampreys (*Petromyzon marinus*; Xi et al., 2011). Nonetheless, with the progress being made in this field, i.e., identification of sex pheromones in different fish species, measurements in natural waters could become a reliable tool to inform the presence and sexual maturity of species of interest.

Most of the traditional methods (netting and electrofishing) used in locating the invasive fish are non-species specific (Sorensen and Johnson, 2016), expensive, (Bajer et al., 2011), and less efficient in low-density areas (ACRCC, 2014). Recent trends to measure biochemical and molecular markers in natural environments are appealing due to the species-specific nature of these markers and the relative ease in collecting water samples. For instance, eDNA that is released by fishes, can now be measured with extreme sensitivity (Jerde et al., 2011; Eichmiller et al., 2014; Gingera et al., 2016) and has been widely used to detect the presence of invasive carp (Jerde et al., 2013). Like eDNA, measuring pheromone concentrations could provide valuable information on the presence or absence of fish while adding new information on reproductive condition. It is important to note, however, that multiple pheromone candidates and eDNA markers need to be targeted to detect multi-species assemblages.

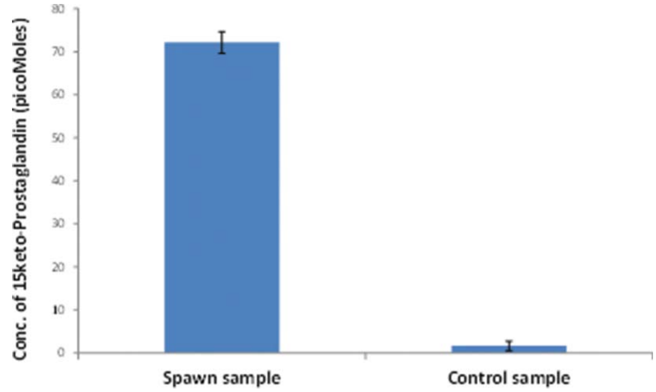

Figure 8. Concentration of 15-Keto-ProstaglandinF2α measured in lake water samples. Samples collected for hormone measurements from spawning aggregation (spawn sample) and 100 meters away from the aggregation (control) in Wasserman Lake, Minnesota. Spawning aggregations typically consisted of 3–4 males and 1–2 females. Source: Modified from Sorensen and Johnson (2016).

Of special interest to analysis is prostaglandin F2α (PGF2α) and its metabolite, 15-keto PGF2α (Figure 8), which drive ovulation and sexual behavior in all female carp and serve as female pherormone in both common and bigheaded carp (*Hypophthalmichthys nobilis*) (Stacey, 2003). These species release PGF2α and its metabolites, but in species-specific ratios (Sorensen and Johnson, 2016). Thus, measuring a combination of products will help determine the presence of a particular species. Further, to develop pheromone measurements as a biomarker to indicate the distribution and abundance of invasive fish, research should be directed towards determining the degradation rates and dilution effects of these compounds in natural environments.

Prevention research-bubble curtains

Abundance of common carp in Midwestern North America appears to be attributable to the tendency of adults to leave lakes and use wetland habitats for spawning, where predator densities are low, and of juveniles to return to the lakes (Bajer and Sorensen, 2010). Disrupting this bi-directional movement could provide significant gains towards long-term carp control. Although upstream movement of adults can be prevented by temporary physical screens (Chizinski et al., 2016) or electrical barriers (Verrill and Berry, 1995), such technologies are ill-suited to stop small downstream-moving juveniles because fine screens clog and fish can drift past an electric field. Behavioral deterrents (i.e. light and sound) could provide a safe and inexpensive solution for such applications (Noatch and Suski, 2012). In

Figure 9. Bubble curtain. Upstream view of bubble curtain tested in Zielinski and Sorensen, (2015) in Kohlman Creek, Minnesota (45°01′36″ N 93°02′48″ W). Upstream of the bubble curtain is a wetland used by carp for spawning; a chain of lakes is downstream. The bubble curtain blocked up to 60% of juvenile common carp moving downstream.

particular, sound has special promises since common carp have well-developed hearing abilities (Ladich and Fay, 2013) that are superior to many native fish in the Great Lakes region.

A bubble curtain is one behavioral deterrent that produces acoustic and hydrodynamic stimuli which could be deployed inexpensively in small streams that connect lakes and wetlands, which are common in the Great Lakes region. In the laboratory, juvenile common carp movement through a circular channel was reduced by 75–80% with a bubble curtain when air flow and bubble size were optimized for sound production (Zielinski et al., 2014). The same system blocked up to 60% of downstream swimming juvenile carp in a stream connecting a wetland and a lake (Zielinski and Sorensen, 2015; Figure 9). Avoidance responses to bubble curtains appear to be species specific as both walleye and muskellunge were shown to be minimally deterred by bubble curtain systems (Flammang et al., 2014; Stewart et al., 2014). Ultimately, bubble curtains are an inexpensive tool for sites where reductions in common carp movement, not total elimination, is the goal.

Bubble curtain efficacy could be improved by combining them with additional stimuli like sound from underwater speakers or strobe lights (Perry et al., 2014). Using bubble curtains to deflect rather than block movement can reduce air flow requirements (Zielinski and Sorensen, 2016) or facilitate the use of traps to remove carp. A similar electric deflection screen and trap system was found to be effective in sea lamprey control (Johnson et al., 2016). Future studies should continue to examine nontarget species responses and how moderate reductions in passage could be integrated into common carp management schemes.

AIS control research-human-dimensions

The social aspects of invasive species management are receiving renewed attention (Tassin and Kull, 2015). Although social aspects of invasive species are classically thought of in terms of how humans mediate the spread of invaders and how that can be reduced (Clout and Williams, 2009), there is a growing realization of the importance of values-based judgments within many different aspects of invasive species management. Many invasive species management decisions contain values-based judgments and have the potential for conflict, from defining what species are invasive, to determining when a particular management action is worth the required resources and what degree of nontarget effects of management actions are acceptable (Carballo-Cardenas, 2015). For example, although scientific studies can identify how an invasive species may impact native species, it is fundamentally a values-based choice to determine when inconsequential change becomes significant harm and what resources should be expended to address or prevent that harm (Sagoff, 2009). There have been calls to more explicitly identify and reflect upon the values-based nature of invasive species management (Larson and Kueffer, 2013).

Two key ways to address the values-laden nature of invasive species management are ecological risk assessment and qualitative inquiry into problematic invasive species management issues. First, ecological risk assessment provides a way to inform management priorities by characterizing risk in a way that synthesizes existing scientific knowledge with transparent values-based judgments about the management context (U.S. EPA, 1998). For example, risk assessment makes explicit key values-based judgments, such as what ecological entities are most valued and important to assess, what is considered harmful to those entities, and what spatial and temporal scale is being considered. Risk assessment for invasive species can take place at a variety of scales, degrees of formality, and levels of participation, but it is generally concerned with determining the likelihood of introduction, establishment, or spread of a species, and the resulting probability and severity of economic, ecological, or human health consequences (Anderson et al., 2004). Risk assessments for AIS have been conducted at national and regional scales to help inform invasive species management (Kolar et al., 2007; Cudmore et al., 2012). To support efforts to inclusively and reflexively arrive at necessary values-based judgments within invasive species risk assessment, the risk assessment process can be opened to a deliberative process with a broad range of participants (e.g., academic and agency researchers, state and federal managers, and stakeholders; Stern and Fineberg, 1996). Focus groups, surveys, and expert workshops can all be used during a risk assessment process to arrive at key values-based judgments informing risk assessment and to characterize the risk itself. A participatory risk assessment that drew upon these methods was used to help better understand the impacts from, and prioritize management for silver (*Hypophthalmichthys molitrix*) and bighead carps in the Great Lakes region (Kokotovich and Andow, 2017).

Another way to address the values-laden nature of AIS management, and the conflicts that may result, is to study conflicting case studies using qualitative methods (Carballo-Cardenas, 2015). In-depth interviews or focus groups with people involved with a particular management issue can help identify, reflect upon, and address key conflicts that hamper management. For example, in-depth interviews were used to study the tensions and conflicts impacting carp (*H. molitrix, H. nobilis, Ctenopharyngodon idelle, Mylopharyngodon piceus*) management in Minnesota (Kokotovich and Andow, 2017). These interviews revealed how scientific uncertainty (concerning the effects of carp and the efficacy and collateral damage of management actions) and social uncertainty (concerning the lack of societal agreement on how to respond to carp and the need to avoid acting out of apathy and/or fear) combine to complicate efforts to determine the desired path for carp research and management. These findings emphasized the need to reflect on questions such as: what level of certainty is required to act, what is the acceptable level of collateral damage from potential management actions, and how can we avoid apathy- and fear- based responses to Asian carp? Qualitative research like this is well-suited to explore the values-related challenges facing invasive species management with the necessary detail and nuance.

The need to deal with the values-laden nature of invasive species management will grow with the emergence of complex and conflictual invasive species issues. The need to incorporate social science expertise in invasive species management is apparent (Larson and Kueffer, 2013) from designing and conducting risk assessments that deal with values-based judgments to helping disentangle conflict-ridden management issues. Although the door has opened for this type of work, there is a need for it to grow and to be recognized as essential to productive AIS management.

AIS control research-microbe-mediated approaches

Interactions between AIS and microbes are potential targets for biological control and management strategies. Microbes, such as bacteria and fungi, could interact with AIS via several mechanisms, which could be pathogenic or mutualistic in nature (Kowalski et al., 2015). For example, the introduced AIS might initially encounter fewer pathogens in its new habitat, although it is likely that the number of novel pathogens would increase over time. In addition, the AIS might colonize a new habitat with or without their native mutualist, or with a novel mutualist that could enhance its competitive ability and invasiveness.

Several steps have been proposed to develop microbe-mediated AIS management approaches (Kowalski et al, 2015). The first step is characterizing the microbial communities associated with AIS and native species, across time and space. The advent of high-throughput sequencing technologies has enabled an in-depth understanding of host-associated microbial communities when compared to traditional culture-based methods. Recently, several studies utilized this approach to elucidate microbes associated with invasive carp species, including common carp, bighead carp, silver carp, and grass carp (*Ctenopharyngodon idella*; van Kessel et al., 2011; Wu et al., 2012; Li et al., 2014, 2015; Ni et al., 2014; Ye et al., 2014; Eichmiller et al., 2016). Similar studies must be performed on other high priority AIS such as Eurasian watermilfoil, curly-leaf pondweed, hydrilla (*Hydrilla verticillata*), zebra mussels, and quagga mussels (*Dreissena bugensis*). Second, elucidating the functional contribution of specific microbes towards the fitness and competitive ability of AIS. Third, targeting key AIS–microbe interactions for control or enhancement. Finally, evaluating the efficacy and feasibility of each control method under field conditions. These studies would help to better inform the use of microorganisms for AIS control, reducing our current dependency on chemicals and manual removal.

Final remarks

The Great Lakes region of the United States is of great ecological and economic importance. Unfortunately, these sometimes-fragile ecosystems have a high vulnerability to aquatic invasive species, which can result in both expensive and irreversible damages. Invasive species that pose threats to this region range from enormous fish such as bighead carp to microscopic pathogens such as VHSv. This vast diversity of species and taxa among AIS that are present in the Great Lakes region adds great complexity to control and management issues. Therefore, a dedicated response to these issues across many levels – from research to implementation – is crucial. Finding solutions to AIS will require not only scientific advancement, but also personal responsibility, adaptation of norms, informed policy, and effective agency management. Additional research from partners across the region and the globe will be critical. As illustrated, there are numerous ongoing studies to address AIS. By developing an in-depth understanding of the biology and ecology of AIS, we can discover weaknesses in their life cycles that can be targeted for control. Indeed, targeting the vulnerability of an invader's biology has worked (e.g., sea lamprey in the Great Lakes; http://www.seagrant.umn.edunfo/aisnfo/sealamprey_battle), and it can work for other AIS as well. Aquatic invasive species are a vexing problem and managers must be equipped with robust information and effective tools to mobilize citizens who care about the quality and integrity of inland waters.

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A Probability Co-Kriging Model to Account for Reporting Bias and Recognize Areas at High Risk for Zebra Mussels and Eurasian Watermilfoil Invasions in Minnesota

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Zebra mussels (ZMs) (*Dreissena polymorpha*) and Eurasian watermilfoil (EWM) (*Myriophyllum spicatum*) are aggressive aquatic invasive species posing a conservation burden on Minnesota. Recognizing areas at high risk for invasion is a prerequisite for the implementation of risk-based prevention and mitigation management strategies. The early detection of invasion has been challenging, due in part to the imperfect observation process of invasions including the absence of a surveillance program, reliance on public reporting, and limited resource availability, which results in reporting bias. To predict the areas at high risk for invasions, while accounting for underreporting, we combined network analysis and probability co-kriging to estimate the risk of ZM and EWM invasions. We used network analysis to generate a waterbody-specific variable representing boater traffic, a known high risk activity for human-mediated transportation of invasive species. In addition, co-kriging was used to estimate the probability of species introduction, using waterbody-specific variables. A co-kriging model containing distance to the nearest ZM infested location, boater traffic, and road access was used to recognize the areas at high risk for ZM invasions (AUC = 0.78). The EWM co-kriging model included distance to the nearest EWM infested location, boater traffic, and connectivity to infested waterbodies (AUC = 0.76). Results suggested that, by 2015, nearly 20% of the waterbodies in Minnesota were at high risk of ZM (12.45%) or EWM (12.43%) invasions, whereas only 125/18,411 (0.67%) and 304/18,411 (1.65%) are currently infested, respectively. Prediction methods presented here can support decisions related to solving the problems of imperfect detection, which subsequently improve the early detection of biological invasions.

Keywords: risk assessment, spatial modeling, geostatistics, early detection, surveillance, reporting, observation bias

INTRODUCTION

Aquatic invasive species (AIS) have the potential to affect animal, environmental, and public health (1, 2). The state of Minnesota in the United States has experienced numerous AIS incursions and spend over 10 million dollars each year on activities intended to prevent, control, or manage AIS (3, 4).

Zebra mussels (ZMs) (*Dreissena polymorpha*) and Eurasian watermilfoil (EWM) (*Myriophyllum spicatum*) are AIS of concern for Minnesota and have been reported in Minnesota since 1989 and 1987, respectively (5). The first introduction of ZMs into North America is attributable to ballast water from transatlantic ships (6). ZMs are rapidly propagating bivalves that disrupt the stability of the food web in aquatic ecosystems affecting both pelagic and benthic species (7). Removal of ZMs colonizing public water supply pipes and pipes of industrial facilities has cost nearly $267 million in the ZM affected region in North America between 1989 to 2004 period (8). Similarly, EWM, an invasive aquatic macrophyte, was likely introduced into North America through aquarium trade (6). EWM proliferates rapidly impeding the effective removal or control strategies upon establishment in a waterbody (9). Dense vegetation of EWM outcompetes native macrophytes and interrupts recreational activities (9). An intensive hand harvesting project to control EWM, conducted in the upper Saranac Lake in New York, reported a labor cost of $351,748/year in that one lake alone (10).

Aggressive and costly programs have been implemented in Minnesota to control AIS (3). For example, since 2014, $10 million per year has been allocated by the Minnesota legislature to provide resources for county-based AIS prevention activities, such as education, surveys, and watercraft inspections (4). However, because the risk of AIS invasion had not been previously quantified, the resources were distributed proportionally to the share of boat ramps and trailer parking spaces in each county (4). The funds are invested on prevention of the introduction or limitation of the spread of AIS within the county (3, 4). Because of the high economic and conservation burden posed by the invasions, forecasting of the areas at high risk for invasions is an urgent research priority (2).

The two AIS have been invading Minnesota waters for approximately 30 years; therefore, the measurement of propagule pressure, i.e., the "introduction effort," needs to be focused at the local scale such as at individual waterbody (11). As a solution, previous studies have suggested using surrogate variables such as the number of boat ramps and distance to the major roads in the absence of waterbody-specific data when measuring the propagule pressure (12). One of the most challenging waterbody-specific variables is the measurement of human-mediated dispersal (9, 12, 13). Use of human population density as a proxy for the human-mediated dispersal may serve as a solution. However, densely populated areas may also tend to report the invasions more frequently, compared to less populated areas (14),[1] which may also lead to reporting bias and underreporting.

The objective of this study was to estimate the potential range expansion of ZMs and EWM in Minnesota, using a combination of network analysis and co-kriging, a spatial interpolation technique to account for underreporting. The advantage of using co-kriging is that the technique enables the prediction of values for the locations without observed data, using other correlated and highly sampled variables (15, 16). Co-kriging is commonly used in gold mining and lake and reservoir studies, and has rarely been used in veterinary epidemiological and public health studies as well (17–20). Environmental conservation studies, such as the controlling the spread of invasions, often suffer from lack of data and reporting bias because of the financial constraints on surveillance (1). In Minnesota, invasions are often reported by volunteers and the presence of the AIS may be missed in some waterbodies due to insufficient coverage, which decreases the sensitivity of the reporting. The specificity of the reporting system, instead, may be considered acceptable, given that false positive cases are unexpected. False positives are unlikely because, the Minnesota Department of Natural Resources (MNDNR) confirms newly reported invasions prior to adding them to the official online database of infested waters (5). Consequently, the limitation of this passive surveillance system is the potential underreporting of the conditions. Co-kriging may also compensate for the reporting bias and underreporting by augmenting the predictive power of one variable with the support of other correlated and highly sampled variables.

Recognition of areas at high risk may act as an early warning system and help the prioritization of waterbodies for a targeted and efficient allocation of limited resources to improve both defensive and offensive management strategies (21, 22). Such risk targeted approaches certainly represent improvements over the random selection of waterbodies for surveillance and management purposes (23, 24). For example, current guidelines for conducting AIS early detection and baseline monitoring in lakes of Minnesota suggest that volunteers select waterbodies based on factors such as public water access, boater traffic, tourist activity, etc. (25). However, selecting waterbodies based on multiple criteria is challenging and we propose that a method which take all the most relevant risk factors into account and provide a risk rank would be a better fit to guide the volunteers. Study results may inform risk-based surveillance and management of invasions (21, 23), a process defined as making decisions for identifying, evaluating, selecting, prioritizing, and implementing control measures (26). This work demonstrates the use of analytical models to estimate risk while accounting for reporting bias, with the ultimate objective of evaluating and modifying the policies and practices on biological invasions (23).

MATERIALS AND METHODS

Study Area and AIS Presence Data

A total of 18,411 point locations representing waterbodies of Minnesota were considered as the study population in this study. Waterbodies were mainly lakes and ponds ($n = 18,263$) and were represented by the centroids of each waterbody. In addition to the lakes, several riverine locations ($n = 148$) from major rivers

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were included in the analysis. Riverine locations were identified at the rivers' midpoint within each county. The locational data for the waterbodies were extracted from the GIS layer referred to as "MNDNR Hydrography," which is available from the Minnesota GIS Commons (27). Presence data for confirmed AIS locations were collected from the MNDNR database (5). By the end of 2015, there were 125/18,411 (0.67%) ZMs and 304/18,411 (1.65%) EWM infested waterbodies in Minnesota (5, see text footnote 1). The confirmed presence of the AIS was used in the study regardless of the magnitude of infestation, because assessments on the magnitude of infestation are not available.

Waterbody-Specific Variables

Waterbody-specific variables ($n = 6$), were used as predictors in the co-kriging models. The six waterbody-specific variables included (1) ZMs or (2) EWM invaded waterbody, (3) connectivity to another ZM and (4) EWM invaded waterbody *via* a stream or a river, (5) boater traffic between waterbodies, and (6) inverse of the Euclidean distance to the nearest major road. Status of the invasions, i.e., confirmed presence of invasion was the primary variable for each AIS (variables 1 and 2). For the validation purposes, models were fit for years 2010 and 2015; therefore, two sets of each variable were calculated. The number of waterbodies from which each variable is available varied over the time (**Table 1**). However, the same boater traffic variable was used in both 2010 and 2015 model fits because boater traffic was calculated based on a survey conducted in 2013, as described below. The Euclidean distance to the nearest major road variable was the same for both 2010 and 2015 assuming the major roads remained unchanged.

Proximity and connectivity to infested waterbodies have been recognized as key risk factors leading to ZM and EWM invasions (9, 28). Because of the pairwise distance calculation for the semi-variance of candidate variables in the model, the kriging process includes the distance between locations as an integral part of the algorithm (15). Therefore, when AIS presence/absence is the primary variable, the spatial dependence, i.e., the distance to the nearest infested location is inherently included in the co-kriging model.

TABLE 1 | Number of waterbodies with the characteristic of each variable by 2010 and 2015.

		Number of waterbodies by 2010	Number of waterbodies by 2015
1	ZM invasion status[a]	57	125
2	EWM invasion status[a]	251	304
3	Connectivity to another ZM invaded waterbody *via* a river or a stream[b]	2,392	3,658
4	Connectivity to another EWM invaded waterbody *via* a river or a stream[b]	3,129	3,715
5	Eigenvector centrality of the boater traffic network	1,376	1,376
6	Inverse of the Euclidean distance to the nearest major road	18,411	18,411

[a]*Presence only.*
[b]*Connected waterbodies only.*

Surface water connectivity between waterbodies *via* a stream or a river was obtained by intersecting the map of the river and streamlines features with the polygon features representing lakes, ponds, and reservoirs using ArcGIS version 10.3.1 (29). River and streamline feature data were obtained from the "Stream Routes with Kittle Numbers and Mile Measures" GIS layer available from the Minnesota GIS Commons (30). Several published studies identified the potential for downstream (e.g., *via* downstream drift) and upstream (e.g., *via* watercraft) spread of ZMs and EWM (28, 31, 32). However, the distance measures denoting the extent of the spread upstream or downstream were either not studied or varied among the published literature. Therefore, for simplicity, an invasion was assumed to occur both up and down stream regardless of the flow direction. Invaded locations that were not directly intersecting a river or streamline were given a buffer distance of 100 m around the point location, and the closest river or stream feature was assigned as connected because the proximity to the infested location poses the risk of invasion (7, 9). Rivers and streams were represented by a unique identification number referred to as "Kittle Numbers" assigned by the MNDNR (30, 33). Kittle numbers consisted of an alphabetical letter, followed by a string of digits (33). For example, if an invaded waterbody was connected to kittle number #H026, then any waterbody connected to #H026 was assigned as connected to an invaded waterbody. Connectivity networks were generated separately for ZMs and EWM.

Boater traffic between waterbodies may lead to human-mediated dispersal of AIS (9, 13). Here, boater traffic was measured using data collected by the MNDNR Watercraft Inspection Program, a survey conducted since 1992 as a conservation measure to protect state waters (34). The Watercraft Inspection Program survey is conducted at selected waterbodies. Priority for data collection is given to those that are invaded, located near an invaded waterbody, highly used, or located close to popular travel destinations (34). The boaters who visit the waterbodies were interviewed regarding the previous waterbody visited and the waterbody they plan to visit next. In 2013, the Watercraft Inspection Program surveys were conducted at 240 locations, and 119 (49.6%) of those locations were invaded by either ZMs or EWM. Because of the miscellaneous reporting errors, only 21% of the surveys were eligible to be used in the final Watercraft Movement Network. Based on the survey, boater traffic data were available from 1,376 unique waterbodies (7.5% of the total waterbodies). Because the analysis was focused on predicting the current risk of invasions rather than understanding the impact of boater traffic on past invasions, it was assumed that movements recorded in 2013 were representative of movement patterns observed between 1987 and 2015.

Network analysis, which provides a framework to identify units that are frequently or intensely connected within the network and identify contact patterns (35), was applied to the Watercraft Inspection Program data from 2013. A total of 187,074 surveys were conducted between April 25, 2013 and November 30, 2013. Recreational boater movement data are not collected during the winter season (34). In the analysis, network "nodes" were the waterbodies and visits between waterbodies served as "edges." Each completed survey accounted for two edges, representing the following links: (1) between the previously visited location and the surveyed location, and (2) between surveyed location and the next stated location that the watercraft would visit. Three centrality measures, namely, the Eigenvector, Betweenness, and Degree were calculated for the network. The centrality measure that highly correlates with the status of the invasions by ZM and EWM was chosen, upon calculating the Pearson correlation analysis. Eigenvector centrality was chosen as the network parameter representing the connectivity of each waterbody within the watercraft movement network. Eigenvector centrality is a representation of the relative importance of a node regarding its position and connectivity to other highly connected nodes in the network (35). It was assumed that highly connected nodes could play a major role in distributing AIS.

Distance to the nearest major road represents the convenience of accessibility to a waterbody. Boater traffic data are collected from limited waterbodies; however, an indirect measure of the potential visitations is the calculation of road accessibility (12, 36). Therefore, distance to the nearest major road from the waterbodies was calculated using the major roads map of 2012, available through the Minnesota Geospatial Commons and originated from the Department of Transportation (37). As defined in the metadata of the spatial layer, road classes including interstate highways, freeways, arterials, and major collectors were considered as major roads in the analysis (37). The inverse of the Euclidean distance was used as the variable when fitting the models.

Data Analysis: Co-Kriging to Estimate the Probability of Introduction

Probability co-kriging was used to estimate the probability of ZM or EWM introduction into the waterbodies, conditional to the distance between locations and other waterbody-specific variables. Co-kriging is a linear weighted averaging method in which weights are selected to minimize the variance of the estimation error by accounting for the spatial correlation between the waterbody-specific variables; weights are dependent on the distance between sampled locations (15). In this study, multiple correlated waterbody-specific variables were used to estimate the spatial distribution of the dependent variable in the non-sampled locations (15). The primary variable subjected to co-kriging is the invasion status of ZMs or EWM. Therefore, the "sampled locations" were those confirmed to be infested, whereas "not sampled locations" were those that without infestation reports. The cross correlation between variables is used to improve the predictions because the predictions are derived from both primary and secondary variables (15). A complete description of the application of co-kriging is available elsewhere (15, 19).

Pearson correlation coefficient was calculated to determine the correlation between the six waterbody-specific variables. Variables with a correlation coefficient ≥ 0.1 were selected to be included in the co-kriging models. Multiple co-kriging models were fit for both ZMs and EWM separately. Each model included the primary variable, i.e., the status of the invasion and two correlated variables. All possible two-way combinations were fit. Considering the potential mutualism between ZM and EWM suggested by multiple studies (38, 39), the variable pairing also included the use of invasion status of ZMs as a correlated variable used in co-kriging model to predict Eurasian milfoil and *vice versa*. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC), a plot of model sensitivity (true positives) and 1 − specificity (i.e., false positives) (40). AUC values lower than 0.7 are considered relatively inaccurate because the proportion of false and true positive results is not substantially different, whereas AUC values greater than 0.7 are generally considered appropriate (40). Models with AUC value greater than 0.7 were considered accurate in this study.

The variables contributing to the co-kriging model with highest AUC were chosen. Hence, final models consisted of the primary variable representing the invasion status of each AIS and two other waterbody-specific variables. AUC values were calculated for each of the co-kriging models by true validation, which was done by fitting models to the invasions by 2010 and validating using the invasions reported between 2011 and 2015. Results of the co-kriging analysis were the probability of finding an AIS invaded waterbody conditional to the presence of an invaded location in the proximity and the waterbody-specific variables incorporated into the model. Small lag sizes (e.g., 0.04 km) and few lags (e.g., $n = 12$) were used in the computation of the co-kriging semivariogram. The use of small lag size and few lags was intended to reduce the exponential increase in the influence of an infested location to the nearby cells, i.e., to reduce the effect of high spatial autocorrelation (15). The choice of the parameter values for the co-kriging attributes such as the anisotropy factor and the angle were based on the spatial cluster analysis and directionality tests for the data (see text footnote 1). The parameter values are summarized in Table S1 in Supplementary Material.

The performance of the final co-kriging models for ZMs and EWM was estimated based on the predictive powers of the candidate models. The predictive powers were measured estimating the sensitivity and specificity, and the AUC of the candidate models. In the context here, sensitivity and specificity reflect the ability of the model to predict invaded and not invaded waterbodies, respectively. Because the goal of the model was to predict potential infestations, high sensitivity, rather than high specificity, was targeted when optimizing the models. In addition to the true validation, the co-kriging models were cross validated using k fold cross validation ($k = 5$). Cross validation is a process in which a set of AIS infested locations were left out from the model fitting, and the fitted model output was used to estimate the probability of finding an AIS invasion at those left out locations (41). Eighty percent of the cases were used for the model training, and testing was done using the 20% of the withheld cases for each validation. To maintain the consistency, the co-kriging parameters recognized during the true validation were used when fitting the models for the cross validation.

Interpretation of the Co-Kriging Outputs

Predicted probabilities were extracted for each of the waterbodies from the probability output of the co-kriging models, for ZMs and EWM separately. The outputs were ranked into five "risk rank" categories based on the quantiles of the output probability values. The risk ranks 1 through 5 were defined as follows: (5) very high, (4) high, (3) intermediate, (2) low, and (1) negligible risk of AIS introduction. The co-kriging risk rank resulting with highest sensitivity and specificity was considered the threshold for each model. The calculated probabilities of AIS invasion using co-kriging represent current risk status. In the absence of effective eradication measures to remove AIS from invaded waterbodies, the waterbodies that are currently recognized to be at risk will remain in the same status while the intensity of the risk of invasion may increase when newly AIS invasions are reported (**Figures 1** and **2**).

RESULTS

The Pearson correlation coefficients for each variable pair are summarized in **Table 2**. The variable pair with the highest AUC value for the true validation of the ZM model was the Eigenvector centrality of the watercraft movement network and the distance to the nearest major road (AUC = 0.78), whereas EWM was best predicted by the Eigenvector centrality of the watercraft movement network and the surface water connectivity to infested waterbodies (AUC = 0.76). The AUC values, sensitivity, and specificity at the threshold risk rank = 3 for the cross validations and true validation of co-kriging models are summarized in **Table 3**. The final model included the variables that were correlated with the invasion status and highly sampled.

Output maps for both ZM and EWM co-kriging and the number of waterbodies classified under each risk rank are seen in **Figures 1** and **2**. **Figure 1** illustrates the risk maps for the models fitted for the invasions by 2010, whereas **Figure 2** shows the risk based on the invasions by 2015. Therefore, by 2015, at the risk rank = 5, a total of 2,293 (12.45%) and 2,289 (12.43%) waterbodies were at very high risk of invasion by ZMs and EWM, respectively. Among the waterbodies at very high risk at risk rank

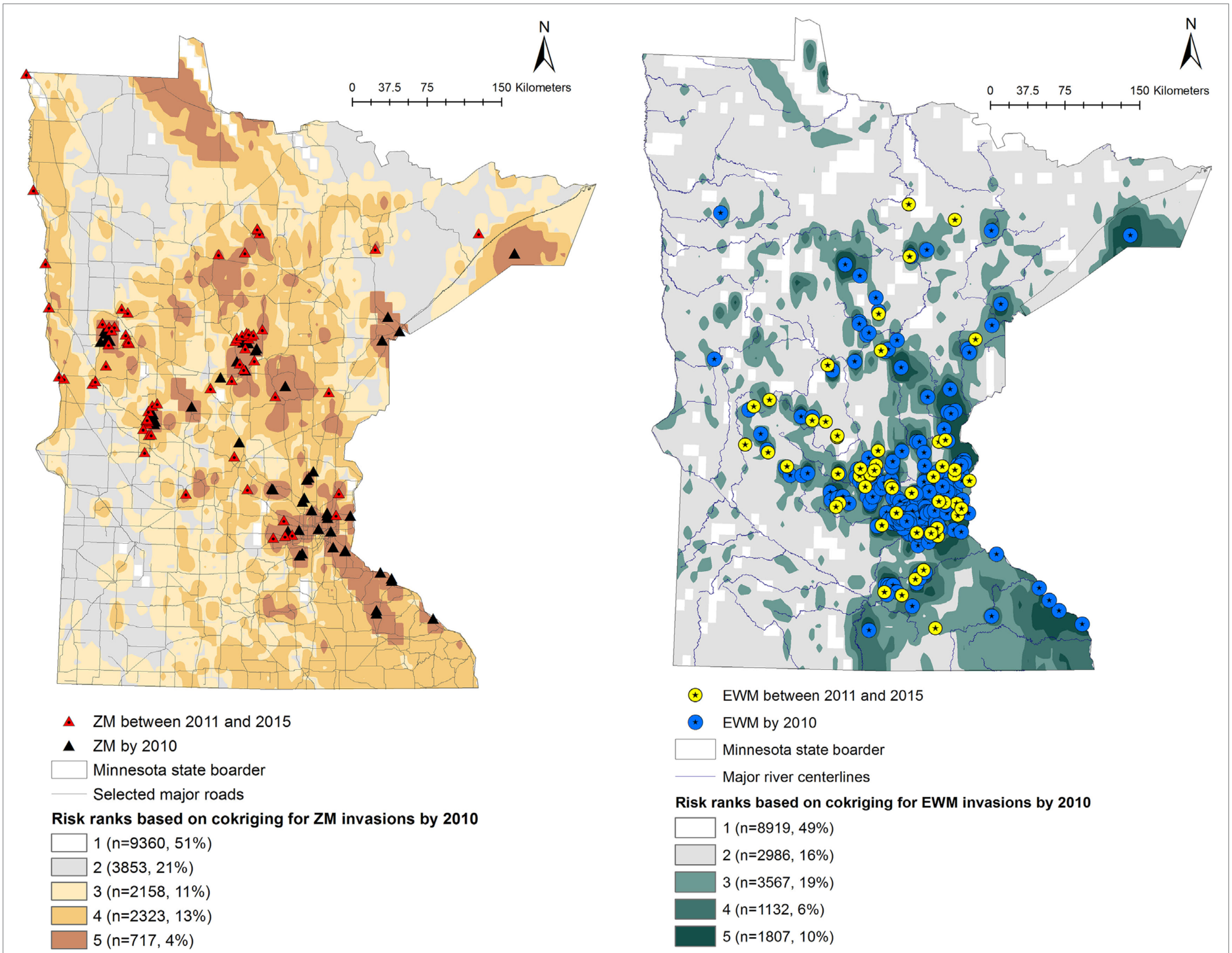

FIGURE 1 | Co-kriging model outputs illustrating the probability of introduction of zebra mussels (ZMs) and Eurasian watermilfoil (EWM) to Minnesota waterbodies, for the invasions as of 2010. The risk classes 1 through 5 indicate the intensity of the probability of introduction, where class 5 represents a high probability of ZM or EWM introduction. The number of waterbodies under each category and as a percentage of the total waterbodies (*n* = 18,411) is listed.

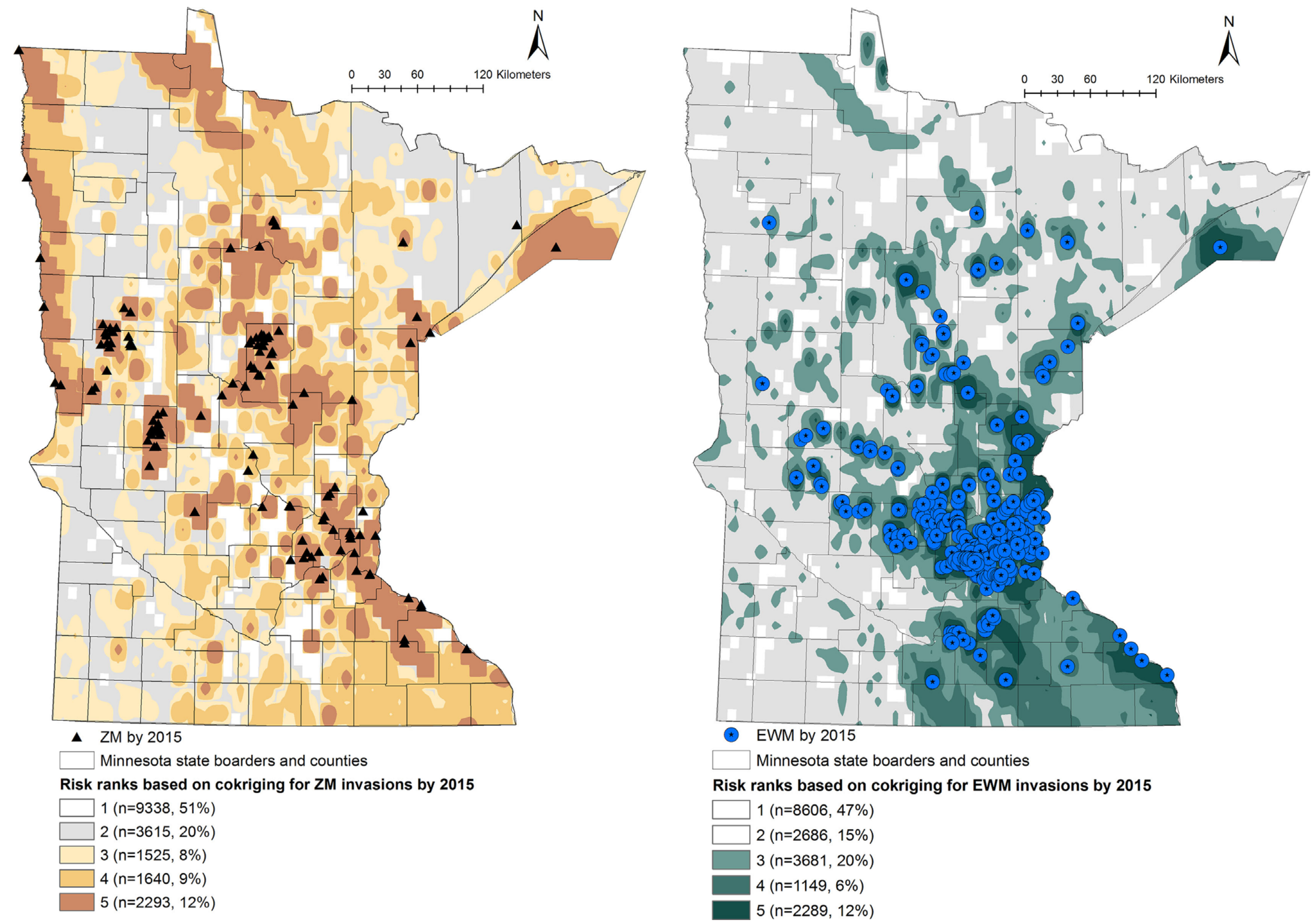

FIGURE 2 | Co-kriging model outputs illustrating the probability of introduction of zebra mussels (ZMs) and Eurasian watermilfoil (EWM) to Minnesota waterbodies, for the invasion status of 2015. The risk classes 1 through 5 indicate the intensity of the probability of introduction, where class 5 represents a high probability of ZM or EWM introduction. The number of waterbodies under each category and as a percentage of the total waterbodies (n = 18,411) is listed.

TABLE 2 | Pearson correlation coefficient for the six waterbody-specific variables used in the study.

		ZM invasion status (primary variable)	EWM invasion status (primary variable)
1	ZM invasion status	1.00	0.10
2	EWM invasion status	0.10	1.00
3	Connectivity to another ZM invaded waterbody *via* a river or a stream	0.12	0.04
4	Connectivity to another EWM invaded waterbody *via* a river or a stream	0.09	0.10
5	Eigenvector centrality of the boater traffic network	0.28	0.34
6	Inverse of the Euclidean distance to the nearest major road	0.21	0.09

ZM, zebra mussels; EWM, Eurasian watermilfoil.

5 for both the AIS, 755 waterbodies were in common. Therefore, a total of 3,827 (20.78%) waterbodies were at high risk for either ZM or EWM invasions.

TABLE 3 | Summary of co-kriging model validations for the probability of zebra mussel (ZM) and Eurasian watermilfoil (EWM) introductions in Minnesota.

		AUC	Sensitivity at risk rank 3	Specificity at risk rank 3
Cross validation	ZMs	0.73	0.70	0.63
	EWM	0.79	0.82	0.74
True validation	ZMs	0.78	0.78	0.72
	EWM	0.76	0.83	0.61

Cross validation was done using the k fold test (k = 5). True validation was done by fitting models for invasions as of 2010 and validating using the invasions reported between 2011 and 2015. Area under the receiver operating characteristic curve (AUC), sensitivity, and specificity at the threshold risk are summarized.

DISCUSSION

This study was aimed at predicting the risk of ZMs and EWM invasions in Minnesota using network analysis and co-kriging, a geostatistical modeling technique. Recognizing areas at high risk for invasion may facilitate early detection and efficient control through risk-based management. This study emphasized the use of co-kriging on observed data affected by underreporting and other reporting biases by augmenting the predictive power of one variable with the support of other correlated and highly sampled variables. In the absence of active surveillance, invasions are recorded based on public reporting and subsequent confirmation by the MNDNR. Therefore, presence of the AIS may be missed in some waterbodies due to insufficient coverage, resulting in underreporting. Results suggested that, by 2015, nearly 20% of the waterbodies in Minnesota were at high risk of invasions by either or both AIS. This included 2,293/18,411; 12.45% waterbodies at risk of ZM invasions and 2,289/18,411; 12.43% waterbodies at risk of EWM invasions, whereas only 125/18,411 (0.67%) and 304/18,411 (1.65%) confirmed the invasions, respectively. Recognition of areas at high risk may act as an early warning system and help prioritization of water bodies for risk-based surveillance and management.

The key predictors of the best fitted co-kriging models, for both ZMs and EWM, were the distance to the nearest infested location and the boater traffic, i.e., Eigenvector centrality of the boater traffic network. This result emphasizes the proximity between waterbodies and human-mediated dispersal as useful predictors of potential invasions (7, 9). The strong relationship between hitchhiking ZM larvae along with the residual water, boat equipment, and recreational gear is a known risk factor for invasions (13). Affirmatively, the secondary variables in the final co-kriging model for ZMs were both indicators of human-mediated dispersal of the AIS, the boater traffic and the distance to the nearest major road which represents the convenience for frequent accessibility. The final co-kriging model for EWM suggests that their distribution is attributable to the proximity between waterbodies as determined by the invasion status of EWM, the natural dispersal *via* connecting surface water such as rivers and the human-mediated transportation (i.e., variables 2, 4, and 5). The predictive power of the boater traffic using the Eigenvector centrality measure is augmented with the use of the inverse distance to the nearest major road as a secondary predictor, which adjusted for the potential underreporting. The Pearson correlation between ZM invasions and the inverse of the distance to the nearest major road was 0.21 (**Table 2**), which was stronger than other variables. Distance to the nearest major road represents the convenience of frequent accessibility to the waterbody.

In the absence of active surveillance, AIS invasions are recorded based on public reporting and subsequent confirmation by the MNDNR (5). Therefore, densely human populated areas are likely to be reported with invasions more frequently than less populated areas, where underreporting is possible (14; see text footnote 1). Considering the commonalities between waterbodies with currently reported invasions and searching for waterbodies with similar characteristics using waterbody-specific variables may be one of the solutions to correct for underreporting (25). However, selecting waterbodies based on multiple criteria such as public water access, boater traffic, and tourist activity. is challenging and through this study we provide a method which take the most correlated variables into account and produce risk maps and risk ranks for each waterbody, which may offer a better guidance to volunteers who search for potential invasions. This approach of risk-based and targeted surveillance would provide more opportunities to reduce the problem of underreporting.

An important strength of the present study is that the boater traffic was calculated at the waterbody level. This is more informative compared to the representation of boater movement by county centroids, such as the studies by Stewart-Koster et al. (22) and Buchan and Padilla (12). Representation of the boater traffic by county leads to either overestimation or underestimation of the importance of individual waterbodies (22).

Areas at high risk for AIS infestations may be identified using a variety of modeling techniques. Species distribution modeling (42), diffusion models (43), gravity models (44), regression models (12), machine learning techniques (45), risk models (46), and model combinations (22) are approaches commonly used for the estimation of AIS distribution risk. Some of the abovementioned computationally complex modeling techniques are powerful when determining the risk of invasions; however, the complexity of these models can make the translation of the model output into practice a difficult task. Compared to above modeling techniques, co-kriging is a less complicated analysis. When translating the science to policy, the concept of using correlated and highly sampled variables to estimate unknown variables is rather simple and straightforward. Therefore, the use of co-kriging as an introductory tool to assess the risk and introducing the method to the decision-makers perhaps is a step further into translating science into practice.

One limitation of our approach is that co-kriging interpolation assumes that the probability of AIS introduction is a continuous variable across geographical space (15). However, the probability of AIS introduction is waterbody specific and not a continuous variable. In this study, the assumption of continuous probability may be justified because Minnesota is a water rich state with over 19% of the state is consisting of lakes, ponds, rivers, and wetlands (27). This assumption of continuous probability is also supported by the density and complexity of the overland boater traffic (Figures S1 and S2 in Supplementary Material). Although this simplification of continuous probability is held commonly in spatial modeling (20), the invasions only occur at the susceptible locations, i.e., the waterbodies. In co-kriging, probability is computed for cells and, here, we assumed the probability of infection to be 0 for those cells in which no waterbody was found, whereas the probability of AIS introduction was computed for cells that was occupied, at least in part, by a waterbody. Presentation of co-kriging models in the format of isopleth maps with a continuous probability surface is common in the spatial modeling (20). As mentioned in the methods, magnitude and the duration of the infestation would have been ideal to be included in the analysis because it is a measure of the risk an infested waterbody pose on susceptible waterbodies (9). However, magnitude of invasions was not readily available because the collection of magnitude of invasions is a costly and labor-intensive process (47, 48) and the distribution of AIS within waterbodies is patchy based on the substrate compositions (48, 49). Similarly, the assignment of surface water connectivity both upstream as well as downstream, without limiting the distances, may lead to potential overestimation of the risk of invasion. However, assignment of distance limits of upstream and downstream transmission was subjective as described by multiple studies (28, 31, 32). Another limitation is the lack of AIS distribution data in the states adjacent to Minnesota, which is important for effective cross-boundary control and preventive measures. For example, waterbodies in east central Minnesota are affected by both ZMs and EWM. However, the study described by Stewart-Koster et al. (22) indicated low risk of the ZM and EWM invasion across the border in northeastern Wisconsin (22). Our study does not account for ZMs and EWM invasions in the adjacent states either, which indicates the risk of invasion may have been underestimated. Being confined within the political boundaries often results in reducing the model accuracies (50). The geographical area for the analysis was not expanded to the Midwest or great lakes because some of the required data, such as boater movement, was not available from all the locations.

As seen in **Figure 2**, a total of 5,458 (29.64%) of the waterbodies were recognized to be equal or above the threshold risk rank 3 for ZM invasions. Similarly, 7,119 (38.66%) of the waterbodies were predicted to be above the risk rank 3 for EWM invasions. From a management stand point, these numbers of waterbodies are still too high to plan a cost-effective risk-based surveillance or develop targeted management plans. Therefore, risk-based management using limited resources requires prioritizing the waterbodies at high risk for screening (21, 24). This inherent difficulty of recommending sample sizes to be collected from risk regions is also discussed by another study where co-kriging was used to conduct a *post hoc* comparison of the association between highly pathogenic avian influenza (H5N1) incidences and intensity of surveillance activities of sampling wild birds by administrative region (20). Resource availability, degree of risk awareness, and participation in reporting by the region were recognized as key factors defining the extent of surveillance efforts (20). We suggest focusing on the waterbodies of biological and recreational importance. This can be a value-based judgment and should include a variety of stakeholders and agreed upon criteria. Prioritization of the waterbodies could also be done by conducting a risk-based survey by subdividing the counties into smaller polygons or using township areas. One such approach is the hexagonal tiling method, which is commonly used in ecological studies (51). The risk rank generated from this study may also be useful to improve the MNDNR's Watercraft Inspection Program by recruiting watercraft inspectors at areas recognized to be at high risk for invasions and not currently inspected.

Risk-based management is not a novel concept (21, 26). However, the attempt to incorporate spatial models in invasion risk assessment to inform the decision and policy-making process may improve the efficiency and effectiveness of the AIS control programs, through targeted and risk-based sampling schemes (23, 24). As demonstrated here, co-kriging enables predicting values for locations without complete data, using correlated and highly sampled variables, which can be used as a solution to the underreporting in ecological and epidemiological studies. This work seeks to encourage the use of scientifically supported quantitative procedures such as network analysis and co-kriging to solve the problem of imperfect detections, which subsequently improve the early detection of biological invasions.

AUTHOR CONTRIBUTIONS

KK conducted the data mining, data analysis, and the manuscript writing. MA edited the manuscript. NP contributed in obtaining data, interpretation of the results, and manuscript editing. AP consulted the data analysis, troubleshooting of the method, and manuscript editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fvets.2017.00231/full#supplementary-material.

FIGURE S1 | The boater traffic between waterbodies based on the Watercraft Inspection Program conducted by Minnesota Department of Natural Resources. The data from year 2013 are illustrated. Panel **(A)** represents the movement of boaters from previously visited waterbody-to-waterbody where the survey data were collected. Panel **(B)** represents the movement of boaters from waterbody where the survey data were collected-to-the waterbody where they plan to visit next.

FIGURE S2 | An illustration of the Eigenvector centrality for the waterbodies in the boater traffic network created using the surveys of Watercraft Inspection Program conducted by Minnesota Department of Natural Resources. The data from year 2013 are illustrated.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 14: Cost-effective monitoring of lakes newly infested with zebra mussels
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $266,500
AMOUNT SPENT: $225,553
AMOUNT REMAINING: $40,947

Sound bite of Subproject Outcomes and Results
We evaluated five survey designs for estimating zebra mussel density. Double-observer surveys that allow for imperfect detection are optimal for lakes with low density; quadrat counts that assume perfect detection are optimal at higher densities. A training video, data collection worksheets, and an analysis tutorial were made available online.

Overall Subproject Outcome and Results
The current lack of standardized methods for surveying zebra mussels during their earliest stages of lake colonization limits our ability to track changes in density over time or to evaluate effectiveness of treatment programs (e.g., as required by DNR permits). We evaluated 5 different survey designs for estimating zebra mussel density (2 designs in 2017 and 3 designs in 2018), employing methods that utilize counts by two divers to estimate the probability of detecting mussels in the surveyed area. We also compared survey designs in terms of their density estimates, associated measures of uncertainty, and sampling efficiencies (time required to complete a survey), using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data. In 2017 in Lake Burgan, we estimated that a diver could detect between 5% and 41% of the mussels present in the surveyed area, depending on the specific diver and on whether the lake bottom was vegetated, with vegetation having the larger effect on detection. Accounting for low detectability of zebra mussels led to an estimate of density over three times higher than the observed density. Thus, for every zebra mussel detected by our divers, approximately two were missed. Using the data collected in 2018 and further simulation and analytical work, we found that double-observer survey designs that allow for imperfect detection are optimal when surveying lakes at low density, whereas quadrat counts that assume perfect detection are optimal at higher densities. We developed a training video, data collection worksheets, and an analysis tutorial so that others may implement our proposed survey designs in newly infested lakes. These tools benefit Minnesotan's by providing better ways to monitor lakes infested with zebra mussels and to evaluate the effects of treatment options on zebra mussel density.

Subproject Results Use and Dissemination
We have developed several resources to facilitate uptake of our survey methods, including a website describing the project (https://zebramusselsurveys.netlify.com/), an instructional video demonstrating the survey methods (https://www.youtube.com/watch?v=E3ui8SVeBC0&feature=youtu.be), data sheets and google forms for data entry (https://zebramusselsurveys.netlify.com/forms), and an analysis vignette or tutorial using open-source software to analyze data collected from our survey designs (https://zebramusselsurveys.netlify.com/tutorial).

We have submitted a paper to Freshwater Science describing the survey methods we used in our first field season, along with estimates of density in Lake Burgan in 2017; we received a favorable review, and it has been forwarded to the editor for final consideration. We are currently working on an additional manuscript comparing the different survey methods in terms of their sampling efficiency (time required to complete a survey) and the resulting density estimates and associated measures of uncertainty using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data.

We have presented our research results via oral and poster presentations at professional conferences (Upper Midwest Invasive Species Conference, Hawaii Conservation Conference), MAISRC Research & Management Showcase events (oral presentations and a “hands on” demonstration of our survey designs), and a MAISRC outreach event sponsored by the Pelican River Watershed District. In the fall of 2019, we plan to offer a MAISRC-sponsored webinar to discuss our work, allowing us to reach a broad audience of scientists and managers interested in zebra mussel monitoring and control efforts.

Estimating densities of zebra mussels (*Dreissena polymorpha*) in early invasions using distance sampling

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27 May, 2019

Abstract

Estimating the density and distribution of invasive populations is critical for management and control efforts, but can be a challenge in nascent infestations when densities of populations are low. Statistically valid sampling designs that account for imperfect detection of individuals are needed to estimate densities across time and space reliably. Survey methods that yield reliable estimates allow managers to determine how invader biomass impacts ecosystem services and evaluate population trends and effectiveness of control measures. We investigated the use of distance sampling by SCUBA divers to determine densities of invasive zebra mussels (*Dreissena polymorpha*) in two recently invaded lakes in central Minnesota. This framework allows divers to cover the large areas necessary in low-density, recent infestations. We estimated that a diver could detect between 5% and 41% of the mussels present in the surveyed area, depending on the specific diver and on whether the lake bottom was vegetated. We also found that a key assumption of conventional distance sampling (e.g., perfect detection on the transect line) was not met. Therefore, accurate density estimates required a double-observer approach. These results highlight the importance of accounting for detectability when comparing estimates over time or across lakes, particularly when different observers conduct surveys. Further evaluation is needed to determine if changes in field sampling techniques can meet the assumptions behind conventional distance sampling for freshwater mussels. Furthermore, we suggest that the efficiency of distance sampling should be compared to alternatives such as quadrat sampling across a range of mussel densities.

Introduction

Native to a small region of southern Russia and the Ukraine (Stepian et al. 2013), zebra mussels (*Dreissena polymorpha* Pallas 1771) have spread throughout Europe (A. Y. Karatayev, Burlakova, and Padilla 1997; A. Y. Karatayev, Padilla, and Johnson 2003) and North America (Benson 2013) to become one of the world's most widespread and damaging aquatic invasive species (A. Y. Karatayev et al. 2007). The economic costs of these invaders in the United States is estimated to be in the hundreds of millions of US dollars per year with impacts including the fouling of water treatment and power plant intake pipes, hydropower facilities, as well as impacts to recreation, tourism, and lakefront property (O'Neill, Jr. 2008; Bossenbroek et al. 2009; Limburg et al. 2010). Ecological impacts arise from the ability of zebra mussels to reach high population densities, smothering and outcompeting native species. High densities of these suspension feeders lead to the removal of high volumes of planktonic organisms from lakes and rivers, resulting in population declines and local extinctions of native mussels and other invertebrates (A. Y. Karatayev, Burlakova, and Padilla 1997; Ward and Ricciardi 2013), damage to fish populations (D. L. Strayer, Hattala, and Kahnle 2004; McNickle, Rennie, and Sprules 2006; Lucy et al. 2013; David L. Strayer and Malcom 2018), and the restructuring of aquatic food webs (Higgins and Vander Zanden 2010; C. Mayer et al. 2013; Bootsma and Liao 2013).

Ecological impacts scale with zebra mussel density and biomass, but quantitative data on zebra mussel populations are only available for a few water bodies (Higgins and Vander Zanden 2010). Control efforts using chemical treatments and physical removal (e.g., Wimbush et al. 2009; Lund et al. 2018), have to date focused on newly invaded water bodies with low-density, localized infestations. In these water bodies, mussels are more challenging to locate, and even intensive underwater surveys can fail to detect mussels that remain after treatment (Lund et al. 2018). To determine how well treatments reduce densities and how environmental conditions influence treatment efficacy, efficient and reproducible survey designs are needed to facilitate comparisons across space and time—as is the case for surveys of native clams and other freshwater mollusks (Dorazio 1999).

In the North American Great Lakes, ship-based surveys using Ponar grabs and sled dredges have typically been used to survey zebra mussel populations (Marsden 1992; Nalepa, Fanslow, and Pothoven 2010; David L. Strayer and Malcom 2018). Surveys of inland lakes occur over a much smaller areas and are often conducted with a self-contained underwater breathing apparatus (hereafter, SCUBA) (e.g., Kumar, Varkey, and Pitcher 2016), which may offer more reliable assessments of distribution and density. SCUBA-based methods often apply quadrat surveys (D. L. Strayer and Smith 2003).

However, quadrats may be suboptimal when attempting to survey large portions of a water body due to the effort required to move between distant sites (e.g., Giudice et al. 2010; Ferguson et al. 2014). Line transects, which sample along a continuous path, are an attractive alternative to quadrat surveys because they minimize the time spent moving between sampling locations.

To estimate changes in relative densities of populations separated in time or space, we often need to account for changes in the detectability of individuals (Mackenzie and Kendall 2002). Techniques such as capture-recapture methods (Huggins 1991), removal estimators (Nichols et al. 2000), or distance sampling (Buckland et al. 2001) are commonly used to account for variation in detectability that occurs due to changing environmental conditions or due to different observers. A common issue with line transects is that the probability of detecting individuals can decline with distance from the transect line. This effect can be modeled with distance sampling, where the surveyor measures the perpendicular distance of each detected individual (or cluster of individuals) from the transect line. This additional information is then used to model how detection changes as a function of distance, and thus, to correct for imperfect detection (Buckland et al. 2015). An important assumption of conventional distance sampling is that all individuals on or near the line are detected. Double-observer designs relax this assumption by estimating the probability that both observers detect a mussel through sight-resight methods (Borchers et al. 2006).

Here, we apply single- and double-observer distance sampling to estimate population densities of zebra mussels in two recently invaded lakes in central Minnesota. We tested whether the underlying assumptions of conventional distance sampling were met and illustrate how to analyze the data using existing tools. Furthermore, we show how to extend standard approaches to account for unimodal detection functions and covariates that affect both mussel detection and density.

Methods

Study area

We surveyed for zebra mussels in Lake Sylvia in Stearns County, MN and Lake Burgan in Douglas County, MN (Figure 1). Lake Sylvia covers an area of 34 hectares and has a maximum depth of 15 meters (m) while Lake Burgan covers an area of 74 hectares and has a maximum depth of 13 m. Zebra mussels were first verified in Lake Sylvia in 2015 (personal communication Christine Jurek, Caleb Silgjord Minnesota Department of Natural Resources) and Lake Burgan in 2017 (personal communication Lucas Raitz, Michael Bolinksi Minnesota Department of Natural Resources).

Survey design

Lake Sylvia

We allocated survey effort using a stratified systematic sampling design (Pooler and Smith 2005). First, we surveyed eight transects in the area in which zebra mussels were initially discovered and reported to the Minnesota Department of Natural Resources. We concentrated effort this way because areas where mussels are first discovered—assumed "infestation zones"—are typically the sites targeted for SCUBA surveys. Transects in the infestation zone were each 30 m long and spaced 3 m apart, though transects were stopped short of 30 m if divers ran into the thermocline, where visibility was found to drop precipitously. We then surveyed two peripheral clusters of 3 transects each, located 150 m to either side of the infestation zone. The transects in these clusters were 3 m apart. Finally, we conducted ten outlying transects dispersed evenly along the remaining shoreline (Figure 1A). Survey points were determined using a bathymetry shapefile in ArcMap provided by the Minnesota Department of Natural Resources. The start of a transect was placed in a depth of 3 to 8 m and oriented perpendicular to the shoreline to cover a range of depths. We located the start point of the transect using a GPS unit (Garmin GPSMAP 64s).

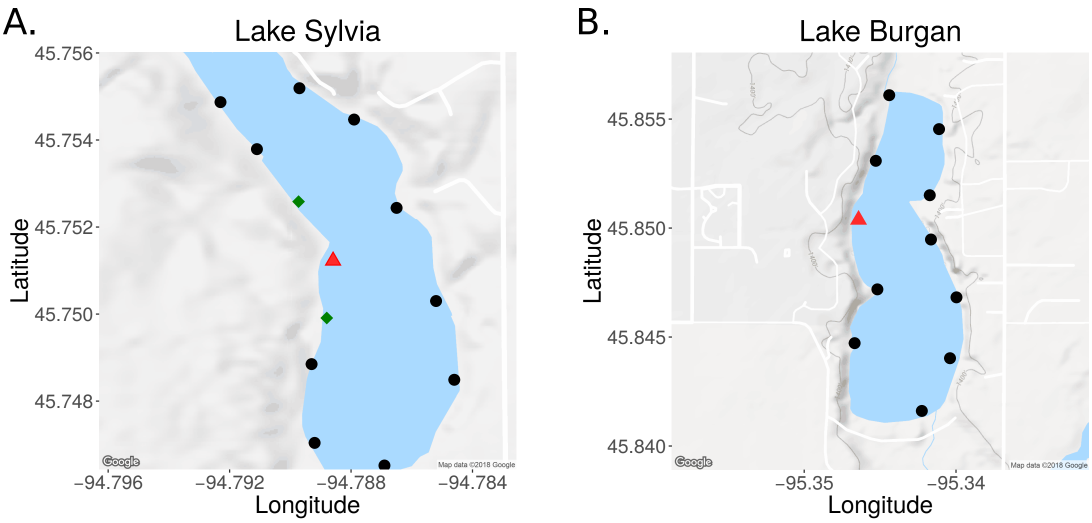

Figure 1: Transects for zebra mussel surveys conducted in Lake Sylvia (panel A) and Lake Burgan (panel B) in the summer of 2017. Transects in normal-effort strata are given as black dots. Red triangles indicate transects in the high-effort strata, where we conducted 8 transects, green diamonds represent the peripheral clusters, where we conducted 3 transects at each location.

Lake Burgan

In Lake Burgan, we did not know the initial location of the zebra mussel report so we used a modification of the above survey design. We initially surveyed eleven transects evenly spaced along the perimeter of the lake, with the first transect chosen near the boat launch (Figure 1B). After sampling these initial eleven transects, we sampled an additional seven transects, spaced 3 m apart, in the area with the highest observed density. We treated the eight transects taken in this region as a high-effort stratum. The remaining ten transects were allocated into a second, normal-effort stratum.

Data Collection

Lake Sylvia

We surveyed Lake Sylvia using a single dive team consisting of two people. The first (primary) diver was responsible for detecting zebra mussels. Whenever the primary diver detected a zebra mussel (or cluster of mussels), she recorded the number of mussels in the cluster and the distance from the transect start to the point where we made the detection (hereafter transect distance), approximated to the nearest 0.25 m. The diver also measured the perpendicular distance from the location of the detection to the transect line (hereafter detection distance) using a meter tape measured to the nearest quarter centimeter. The primary diver also classified and recorded the substrate that the zebra mussel was found on (hereafter "fine-scale substrate") using one or more of the following categories: mud, sand, gravel, pebble, rock, vegetation, wood, native mussel, metal, or other substrate. These substrate determinations were made qualitatively by the dive team.

To determine how detection and density varied due to environmental conditions, the second diver collected habitat and environmental data along each transect. The second diver classified the dominant substrate types in the current segment. Substrate classifications included mud, silt, sand, gravel, pebble, rock, and other. The diver recorded multiple substrate types when there was no clear dominant substrate type or when habitats were interspersed. In addition, the diver recorded the presence or absence of plant cover. Whenever there was a change in the substrate type or plant presence, she recorded the new substrate, plant presence, depth, and the transect distance where the change occurred. The segments formed by these changes were later used to model spatial variability in zebra mussel densities.

Lake Burgan

In Lake Burgan we collected data using the same methods as described for Lake Sylvia, except that each transect was surveyed independently by two dive teams, each team consisting of two members. We alternated which team went first on each transect, with the second dive team beginning their survey after the first team finished so that each team collected data independently.

Study data were entered into a REDCap (Research Electronic Data Capture) database hosted at the University of Minnesota (Harris et al. 2009). REDCap is a secure, web-based application designed to support reliable data capture for research studies by providing quality control of data entry, and auditing trails for data manipulation and export.

Statistical analyses

Although we present data on our survey design and data collection for both Lake Sylvia and Lake Burgan, we did not try to estimate detection probabilities or densities in Lake Sylvia because a critical assumption of conventional distance sampling, namely perfect detection near the transect line, was not met (Figure 2). This assumption can be relaxed using double-observer surveys as implemented in Lake Burgan. Therefore, the statistical methods described in the following sections only apply to the data collected in Lake Burgan.

We estimated zebra mussel density using a two-stage approach, also called density surface modeling (following D. L. Miller et al. 2013 as illustrated in Figure 3). In the first stage, we fit a detection function using the observed distances, including the use of the sight-resight data collected by our observers to estimate the maximum detection probability. This allowed us to determine whether detection is perfect near the transect line, an important assumption of conventional distance sampling (Buckland et al. 2001). In the second stage, we estimated density by fitting a model to the segment-level counts corrected for the surveyed area and estimated detectability in each segment (Hedley and Buckland 2004). A critical assumption of this analysis and other distance sampling methods is that the density of animals does not vary with distance from the transect line. We considered this assumption to hold in our study since: 1) we used a systematic-random sampling design to determine transect locations; and 2) our transects were narrow and placed in relatively homogeneous habitat.

We present two, parallel analyses of the Lake Burgan data. The first approach, which we refer to as the *simple density estimator*, uses existing statistical tools to estimate density assuming a single detection function applies to both observers and all transects. The second approach, which we refer to as the *covariate-modified density estimator*, accounts for strata, unimodal detection functions, and covariates that affect both zebra mussel detection and density. Although this approach requires a more customized analysis, it is appealing because it provides a framework for investigating the effects of covariates on detection and density. In the following sections, we describe the steps for these analyses in more detail.

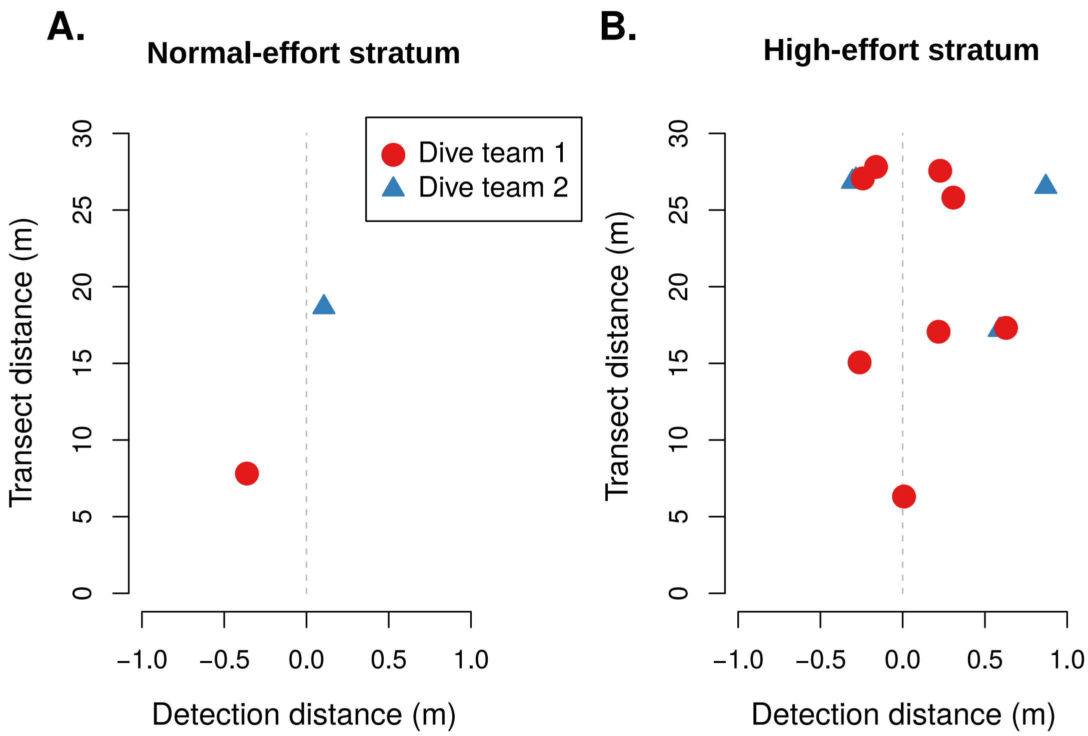

Figure 2: Detections of mussels along two transects in Lake Burgan by two dive teams. The dotted gray line denotes the transect line and each point denotes the recorded position of a detected zebra mussel. Panel A illustrates a transect in the normal-effort stratum, panel B illustrates a transect in the high-effort stratum. All distances are given in meters.

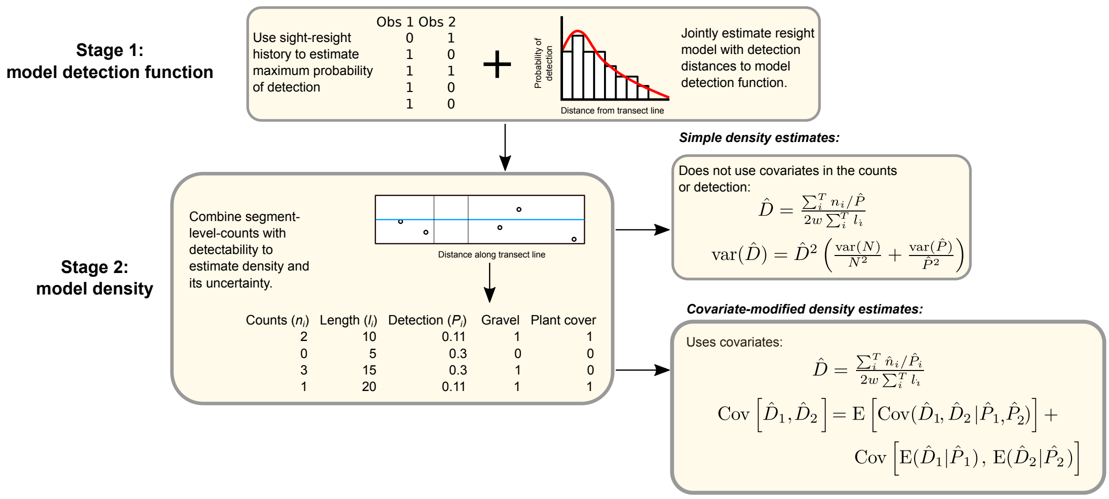

Figure 3: Work flow of the two-stage modeling approach. Estimation of animal density requires a count of observed individuals in each transect (n_i) where the total counts over T transects is N, the length (l_i) and width (w) of the transect, and the detectability of animals in the transect (P_i). The density of the sample is denoted as D.

Detection estimation

We applied sight-resight distance sampling in Lake Burgan to determine whether the assumption of perfect detection near the transect line, as required by conventional distance sampling, was met. Before we could implement this approach, we needed to decide which mussels were seen by both dive teams and which were seen by only the first or second dive team. We did not mark individuals detected by the first dive team because marks could have affected their detectability by the second team. Therefore, we used the proximity of the detections to each other to classify whether a pair of zebra mussel detections were a resight of a single zebra mussel (Figure 2).

We classified two detection events as the same zebra mussel when the difference in the detection distances for the pair was less than 0.2 m, and the difference in transect distances between the pair was less than or equal to 0.25 m. We determined these thresholds after visualizing nearest neighbor distances, but note our analyses were extremely robust to changes in these classification distances (Appendix 2). The thresholds we used here are reasonable because at these low densities it was apparent when the two dive teams detected the same mussel (e.g., Figure 2). At higher densities, there would have been much more uncertainty about whether two detections at similar locations corresponded to the same zebra mussel or not. In such cases, it would be appropriate to mark mussels and use dependent double-observer methods. Alternatively, more formal approaches to incorporating measurement error into distance sampling could be applied (Conn and Alisauskas 2018).

Simple detection estimates Histograms of the detection distances (Figure 4) suggested that the maximum detection probability might have occurred off the transect line. To ensure that standard, monotonic distance functions could be applied, we left-truncated the detection distance at 0.2 m. Truncation removed the potential effects of the hump and allowed us to use the standard distance functions without any modifications.

We modeled detection probabilities using two model subcomponents. The first subcomponent, $g(y)$, describes how distance (y) leads to changes in the probability of detection and is determined by modeling the distribution of detection distances. We applied the half-normal distance function, defined as $g(y) = e^{-(y-0.2)^2/2\sigma^2}$, where $y - 0.2$ is the

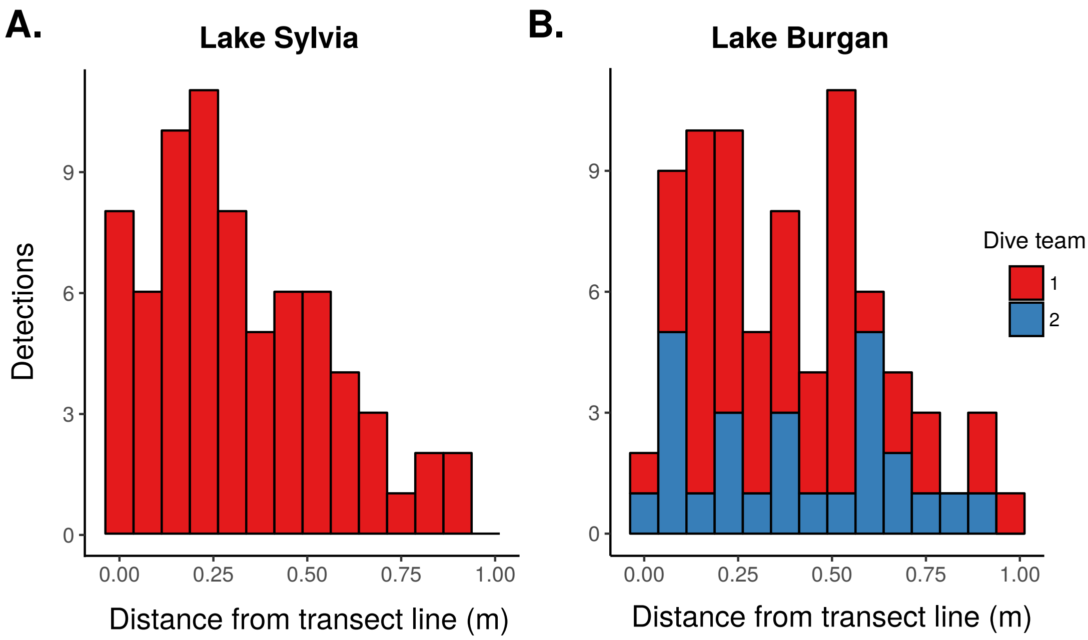

Figure 4: Stacked histogram showing the total number zebra mussel detections made by dive team 1 and dive team 2 in the summer of 2017. Panel A gives the total counts in Lake Sylvia from 24 transects and panel B gives the total counts in Lake Burgan from 18 transects. Distance bin widths are 0.075 m.

detection distance, accounting for the 0.2 m truncation distance, and σ controls the scale of the detection function (Buckland et al. 2015). All estimates for this detection model were made using the mrds (mark-recapture distance sampling) package in R (J. Laake et al. 2018).

We used a second subcomponent of the detection function to scale the distance function by the maximum probability of detection, estimated from the sight-resight data. This second piece of the detection function used a sight-resight model to estimate the detection probability at 0.2 m. The probability of detection by either observer at the truncation distance is $\pi(0.2) = \pi_1(0.2) + \pi_2(0.2) - \pi_1(0.2)\pi_2(0.2)$, where $\pi_k(0.2)$, for $k = 1, 2$, is the probability that the k^{th} dive team detects a mussel at the detection distance of 0.2 m.

For the simple density estimator, we assumed the dive teams had the same detection function and estimated $\pi(0.2)$ using the mrds (mark-recapture distance sampling) package in R (J. Laake et al. 2018). We then combined the two model components to determine the probability of detecting a zebra mussel cluster within our transect by integrating the distance function over the transect width to give the probability of detecting a mussel in the transect, $P = \pi(0.2) \int_{0.2}^{1} g(y)\, dy$.

The sight-resight model used the point independence assumption described by Borchers et al. (2006), which accounts for the effects of unmodeled covariates that can induce unexpected correlations between observers. This can occur if, for example, both dive teams find it easier to detect larger mussels and mussel size is not included in the model. Under these conditions the observers' detections may be correlated even though dive teams act independently. Point independence addresses this issue by modeling the detection probability at a single detection distance, usually specified to be where detection is maximized (here, at 0.2 m).

Covariate-modified detection estimates Next, we explored estimators of detection and density that relaxed some of the assumptions of the simple density estimator. In particular, we fit a unimodal detection function and included covariates that were thought to influence detection probabilities.

Our detection distances illustrated in Figure 4 indicated that the detection function may be unimodal, with the maximum detection probability occurring off the transect line. We tested two alternative models describing how detection changed with distance. The first model we fit was the half-normal detection function, which assumes detection is maximized on the transect line. This detection function was defined as $g(y) = e^{-y^2/2\sigma^2}$ over the width of the transect ($0 \leq y \leq 1$). Second, we fit the unimodal function of Becker, Christ, and Reed (2015), which uses two truncated half-normal distributions that share a common mode, μ_k (where $k = 1$ or 2 for each of the observers). The unimodal detection function for observer k was defined as $g(y) = e^{-(y-\mu_k)^2/2\sigma_l^2}$ for $0 \leq y \leq \mu_k$ and $g(y) = e^{-(y-\mu_k)^2/2\sigma_g^2}$ for $\mu_k < y \leq 1$. In this model, σ_l served as the scale parameter for distances less than the mode and σ_g served as the scale parameter for distances greater than the mode. We assumed that the detection peak was the same for both observers ($\mu_1 = \mu_2$) and estimated parameters by maximizing the log-likelihood of $g(y)$ using the nloptr package in R (Ypma 2015). We selected the best detection model in each lake using AIC, an estimate of the Kullback-Liebler divergence, which measured the relative discrepancy between each model and reality. The AIC is a popular approach for measuring model parsimony, representing a trade-off between model fit and complexity with the goal of achieving optimal predictive ability (Taper and Ponciano 2016).

In the unimodal model, the probability of detection by either observer at the mode, μ, was modeled as a logit-linear function of the observed covariates: plant presence, water clarity, and observer. Thus, the detection probability at the mode for observer k in segment j was modeled as $\text{logit}(\pi_{k,j}(\mu_{k,j})) = \beta_0 + \beta_1\text{Plant}_j + \beta_2\text{Clarity}_j + \beta_3\text{Observer}_k$, where Clarity was a continuous variable, Plant was an indicator variable that was 0 when plants were absent and 1 when present, and Observer was an indicator variable that was 0 for dive team 1 ($k = 1$) and 1 for dive team 2 ($k = 2$). All estimates of $\pi(\mu)$ were made using the mrds (mark-recapture distance sampling) package in R (J. Laake et al. 2018).

Density estimation

We estimated densities in Lake Burgan following the two-stage approach described in Hedley and Buckland (2004). As in the detection models described above, we present two parallel analyses of the Lake Burgan data. The first analysis applied existing statistical tools to the truncated data. We then showed how to extend this analysis to account for strata and covariates that affect zebra mussel density.

Simple density estimator Denote the counts for the i^{th} transect as n_i, the total counts in the lake over T total transects as $N = \sum_i^T n_i$, the length of each transect as l_i, the total length of all transects as $L = \sum_i^T l_i$, and the estimated detection probability as $\hat{P}$. The estimated density was then $\hat{D} = \frac{\sum_i^T n_i/\hat{P}}{2w\sum_i^T l_i}$ (Buckland et al. 2001). The variance in the estimated density was

$$\text{var}(\hat{D}) = \hat{D}^2\left(\frac{\text{var}(N)}{N^2} + \frac{\text{var}(\hat{P})}{\hat{P}^2}\right). \tag{1}$$

The first term in equation 1, $\text{var}(N)$, was the variance in the total counts over all segments ($N = \sum_i n_i$), while the second piece was the variance in the detectability, $\text{var}(\hat{P})$. We used the design-based estimator for the variance in the total counts, $\text{var}(N) = \left(L\sum_i^T l_i(n_i/l_i - N/L)^2\right)/(T-1)$, where the contribution of each segment to the total variance was weighted by the segment length. The R package mrds estimates $\hat{P}$ using maximum likelihood and computes the variance in detectability from the Hessian matrix (J. Laake et al. 2018).

Covariate-modified density estimates We modeled the total zebra mussel counts at the segment-level, using covariates to explain variation in density. Segments were defined based on changes in habitat characteristics along the transect as described in the data collection section. We assumed, conditional on environmental covariates, that abundance within each segment followed a Negative Binomial distribution. We used the log of the segment survey area multiplied by the estimated average probability of detection in the segment as an offset in the model to control for survey effort and detectability. This transformed the observed counts into zebra mussel densities. We used a log-link to model the effects of plant presence (classified as presence/absence), depth, and gravel substrate (classified as presence/absence) as covariates of zebra mussel density. Although we recorded multiple substrate types, gravel was the only type that had enough variation to be considered as a predictor variable. We used AIC to test whether a smoothing spline of segment location was needed to smooth the spatial variation in density that was not explained by the environmental covariates. Density models were fit using maximum likelihood estimation implemented in the R package mgcv (Wood 2006).

We estimated the density in the j^{th} stratum using the estimator, $\hat{D}_j = \sum_{i=1}^{T_j} \left(\hat{n}_i/\hat{P}_i\right) /2w \sum_{i=1}^{T_j} l_i$, where the summation runs over all T_j segments in the stratum. The terms in the sum are, $\hat{n}_i$, the estimated count in the i^{th} segment in stratum j, $\hat{P}_i$, the estimated detection probability in the i^{th} segment of stratum j, and l_i, the length of segment i in stratum j. The detection probabilities were estimated using the methods described in the previous section, and the counts, $\hat{n}_i$, were modeled in the second stage of the density surface model. The overall population size was determined by weighting the estimates from each stratum in proportion to the amount of area in the lake they represented, $\hat{D} = w_{\text{high}}\hat{D}_{\text{high}} + w_{\text{low}}\hat{D}_{\text{low}}$, where the stratification weight for high-effort strata was $w_{\text{high}} = 1/11$ and for normal-effort strata was $w_{\text{low}} = 10/11$.

We applied the conditional covariance formula (Bain and Engelhardt 2000) to derive a variance expression that propagated the uncertainty from the detection model through to the uncertainty estimate for zebra mussel density (derivation given in Appendix 1). The total variation in density was calculated by summing the variances and covariances across all segments, with the covariance terms used to account for correlation resulting from using a common detection model to adjust counts in all segments (J. Fieberg and Giudice

2007). The resulting covariance between the density estimates has two terms, analogous to the covariate independent case in equation 1. Below we indicate the covariance for segment 1 in stratum j and segment 2 in stratum j' (D_1 and D_2):

$$\mathrm{Cov}\left[\hat{D}_1, \hat{D}_2\right] = \mathrm{E}\left[\mathrm{Cov}(\hat{D}_1, \hat{D}_2|\hat{P}_1, \hat{P}_2)\right] + \mathrm{Cov}\left[\mathrm{E}(\hat{D}_1|\hat{P}_1),\, \mathrm{E}(\hat{D}_2|\hat{P}_2)\right]. \tag{2}$$

The first term in equation 2 accounts for uncertainty in the counts, given the estimated detection model parameters, while the second term accounts for uncertainty in the detection parameters.

We determined the covariance estimates using a parametric bootstrap (Hedley and Buckland 2004). For the first term in equation 2, we simulated 10^4 sets of parameters obtained from segment-level count model using a multivariate normal distribution with mean given by the maximum likelihood estimates of the density model and covariance matrix approximated by the inverse of the estimated Hessian matrix (Bain and Engelhardt 2000). We used the simulated parameters to predict the counts for each segment, and then scaled these counts by the estimated segment-level detection probabilities ($\hat{P}_i$) and the amount of area surveyed in each segment. The covariance of these scaled counts was then plugged into the first term of equation 2.

We estimated the second term in equation 2, the covariance matrix of the detectability correction estimates, by simulating 10^4 sets of detectability parameters from a multivariate normal distribution with mean given by the maximum likelihood estimates of the detectability function and covariance matrix approximated by the inverse of the estimated Hessian matrix (Bain and Engelhardt 2000). We used the simulated detection parameters to estimate the segment-level detection probabilities, $\hat{P}_i$. Lastly, we calculated the covariance between the segment-level detectability corrections, scaled by the estimated segment-level count densities, and plugged the result into the second term of equation 2.

Finally, we the calculated the total variance in the density estimate by using the stratification weights to account for the proportion of lake area surveyed in each strata. We scaled the full density covariance matrix, Σ, by the vector of weights (W) where the i^{th} entry of the vector was w_{high} or w_{low}, depending whether transect i was in the high- or normal-effort stratum. The total variance in density was then given by $W^{\mathrm{T}}\Sigma W$.

Results

Substrate in the Lake Sylvia segments was predominately sand and silt (Table 1). We also had a few segments with gravel, pebbles, and rocks. We found that zebra mussels were always found in segments with silt and often in segments with sand, broadly consistent with the available substrate frequencies. The fine-scale substrates that we found zebra mussels predominately attached to in Lake Sylvia, in order of frequency, were wood, rocks, and gravel.

Substrate in the Lake Burgan segments was predominately silt and sand (Table 1), followed by gravel, and rocks. We found zebra mussels in habitats at rates similar to availability with detections occurring primarily in sand and silt, followed by gravel and rocks. Zebra mussels in Lake Burgan were found attached to gravel, rocks, and wood. We also detected one mussel attached to a native mussel, one mussel attached to scrap metal, and two detections were on other materials such as fabric and unidentified mollusks.

Table 1: The frequency of available substrate types in segments and substrate types in segments where zebra mussel detections occurred (potentially classified with multiple types so proportions do not sum to 1), and the type of substrate zebra mussels were attached to (proportions sum to 1).

	Sand	Silt	Gravel	Pebbles	Rocks	Wood	Native mussel	Other
Lake Sylvia								
Available coarse spatial scale substrate	0.73	0.70	0.05	0.02	0.02	0.00	0.00	0.00
Coarse spatial scale substrate with mussel detections	0.53	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Fine-scale substrate with mussel attachment	0.00	0.00	0.18	0.00	0.35	0.41	0.05	0.01
Lake Burgan								
Available coarse spatial scale substrate	0.88	0.90	0.55	0.00	0.04	0.00	0.00	0.00
Coarse spatial scale substrate with mussel detections	0.91	0.87	0.65	0.00	0.04	0.00	0.00	0.00
Fine-scale substrate with mussel attachment	0.00	0.00	0.46	0.00	0.40	0.06	0.02	0.06

In the left-truncated detection data set from Lake Burgan, the first dive team made 35 detections, and the second dive team made 19 detections, with 6 detections being shared by both teams for a total of 48 unique zebra mussel detections. In the full detection data set, the first dive team made 49 detections while the second dive team made 26 detections; 9 of the detections were made by both teams for a total of 66 unique zebra mussel detections. Of these 66 unique detections, 64 were of single zebra mussels and 2 were of clusters of size 2.

Detection estimation

Simple detection estimates In the left-truncated detection data, set we estimated the scale parameter, $\hat{\sigma}$, of the detection function to be 0.43 (SE = 0.07). The estimated probability of detecting a zebra mussel, $\hat{P}$, was 0.24 (SE = 0.08).

Covariate-modified detection estimates In our analysis of the full detection data set, the unimodal detection function was more parsimonious than the half-normal model (ΔAIC = 0.23). This small difference means we were unable to reliably distinguish between these two models.

We estimated the location of peak detection in the unimodal detection function, μ, at 0.15 (SE = 0.08) m. The scale coefficient for distances less than μ was estimated as $\sigma_l = 0.11$ (SE = 0.09) m and for distances greater than μ was $\sigma_g = 0.45$ (SE = 0.07) m. The detection functions for different observers and with plants present and absent are illustrated graphically in Figure 5.

The sight-resight model coefficients suggested that the second dive team had lower detection probabilities than the first team and plant presence decreased the probability of detecting zebra mussels (Table S1). The positive clarity coefficient suggested that detectability increased with water clarity as expected. However, the estimated confidence intervals of the clarity effect were very wide and overlapped 0 (Table S1). Therefore, we also ran a reduced model with the clarity covariate removed. The model without clarity had a lower AIC (Table 2), and reduced the standard error in density due to detectability (the second term in equation 1) from 0.05 to 0.008; removing clarity had minimal impact on the other regression parameter estimates. Thus, moving forward, we

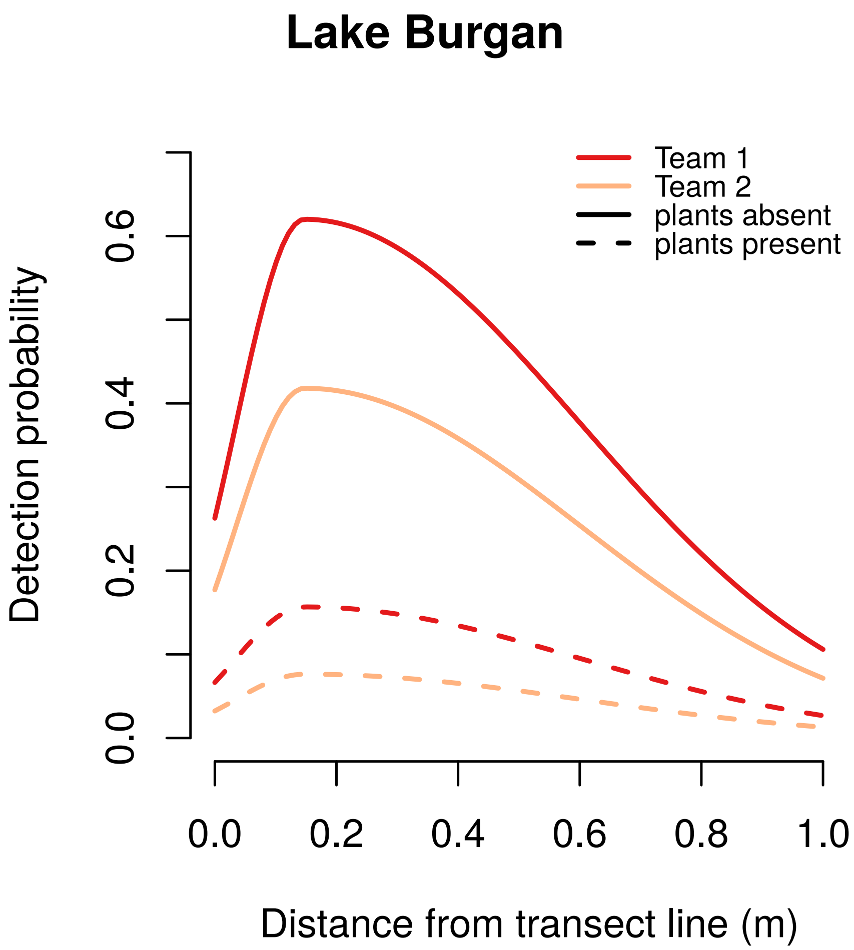

Figure 5: Estimated detection functions in Lake Burgan from the unimodal detection model. We used a double-observer survey to estimate the detection probabilities for each team in the presence or absence of plants.

Table 2: Covariate selection tables for the Lake Burgan analysis. The spatial regression spline is written as s(Easting, Northing).

	log-likelihood	k	AIC	ΔAIC
Detection model				
Observer + Plants + Clarity	-50.25	5	110.50	0.55
Observer + Plants	-50.98	4	109.96	0.00
Density model				
Depth + Plants + Gravel + s(Easting, Northing)	-45.50	6	143.91	2.85
Depth + Plants + Gravel	-46.65	4	141.06	0.00

Table 3: Estimated probability of detecting a zebra mussel in Lake Burgan under different conditions using the reduced detection model (without the water clarity covariate).

	Observer 1		Observer 2	
	Estimate	Standard error	Estimate	Standard error
No plant cover	0.41	0.08	0.28	0.08
Plant cover present	0.1	0.07	0.05	0.04

only present results using the reduced detection model. The estimated probability of detecting a zebra mussel in Lake Burgan for each of the dive teams was low, even under favorable conditions, and ranged from 0.08 (dive team 2 with plant cover present) to 0.62 (dive team 1 with no plant cover present) (Table 3).

Density estimation

We constructed 49 different survey segments from the original 18 transects in Lake Burgan. Segments were based on observed habitat transitions as described in the methods and varied in length from 1 to 30 m. The observed density of zebra mussels in Lake Burgan, uncorrected for detection, was 0.08 mussels per square-meter (m^{-2}).

Simple density estimates In the left-truncated data set, we estimated the overall density, corrected for detection, in Lake Burgan to be 0.24 (SE = 0.1) mussels m^{-2} with 67% of this error arising due to uncertainty in the detection parameters.

Covariate-modified density estimates Using the unimodal detection function, environmental covariates, and strata, we estimated the overall density, corrected for detection, in our transects to be 0.25 (SE = 0.09) mussels m^{-2} with 10% of this error arising due

Table 4: Estimates of covariate effects in the count and detection models of Lake Burgan.

Variable	Parameter estimate	Standard error	95% confidence interval
Detection model			
observer	-0.86	0.38	(-1.61, -0.1)
plants	-2.37	0.41	(-3.18, -1.57)
Density model			
plants	-0.43	0.54	(-1.5, 0.63)
depth	-0.05	0.06	(-0.16, 0.06)
gravel	0.12	0.38	(-0.62, 0.86)

to uncertainty in the detection parameters. This estimate was consistent with the simple density estimate obtained above, and both estimators led to a three-fold increase in the estimated density relative to the observed density.

In the normal-effort stratum, we estimated densities of 0.28 (SE = 0.11) mussels m^{-2}, and in the high-effort stratum we estimated density to be 0.25 (SE = 0.09) mussels m^{-2}. Interestingly, the normal- and high-effort strata had nearly the same estimated densities. We attribute this result to defining strata in the field using observed densities and not testing for statistical differences among transects.

Our estimate of the scale parameter in the negative binomial distribution was 1.477, indicating overdispersion relative to the Poisson distribution. The model without any spatial structure was more parsimonious than the model with the spatial smooth term (Table 2). Parameter estimates from the generalized linear model indicated that zebra mussel densities tended to be lower in shallower areas and in areas with plant cover, whereas gravel had a small positive effect on density (Table 4). However, all of these covariate estimates had high uncertainty with confidence intervals that included zero.

Discussion

We have demonstrated that line transects with double-observer surveys can be suitable for estimating invasive zebra mussel densities in newly infested lakes. This method allows researchers to cover more area compared to quadrat surveys, at the cost of imperfect detection. Importantly, we found that accounting for the low detectability of zebra mussels led to estimates of density over three times higher than the observed densities. Our estimates were robust, with both the simple and covariate-modified estimators giving similar answers. Nonetheless, the double-observer survey in Lake Burgan highlighted the difficulty that our dive teams had in detecting zebra mussels even near the transect line. Thus, we conclude that single-observer methods are generally not appropriate for estimating zebra mussel densities.

Detection data from both Lake Sylvia and Lake Burgan exhibited a peak near 0.2 m from the transect line, suggesting that detection probabilities may have been highest just off the transect line (Figure 4). We were surprised to find this peak in our dive surveys, though similar patterns are known to occur in many aerial surveys (Quang and Lanctot 1991). Although we demonstrated methods that provide a solution to this phenomenon, we emphasize that the statistical evidence favoring the unimodal detection function that we used is still equivocal and more samples will be needed to determine whether this effect is real or an artifact of sampling variation. Alternatively, density can be estimated after first truncating the data to remove this peak. Truncation eases the analysis by allowing the application of standard detection functions that can be implemented in existing R packages such as mrds (J. Laake et al. 2018).

It is worth considering the potential causes of a unimodal detection function in dive surveys to determine whether it can be eliminated by improvements in study design. In aerial trials that display unimodal detection, low detection near the transect line arises due to the fact that animals close to the transect appear to pass by more quickly than animals further away (Becker and Quang 2009). One suggestion to address this effect is to have observers focus their eyes more on areas near the transect line (Buckland et al. 2015). We emphasized the importance of detecting all mussels on or near the transect line to our divers, but perhaps additional training in this area would be helpful. We also know of at least one case when our lead diver missed a zebra mussel near the transect because she returned to the transect line ahead of where she left to measure the detection distance. Finally, laying down the transect line may kick up silt and cover nearby mussels. This effect could be eliminated by having divers start their search a small distance away from the transect line.

A complication in our preparation of the field data for analysis was determining whether detections made by the first observer were also made by the second observer. Error in the distance measurements made classifying redetections more difficult than anticipated.

Alternatives, such as the removal design (Moran 1951; Otis et al. 1978), remove individuals from the population once they are detected. This ensures that the second observer always detects new individuals. The cost of this design is that the second observer's detection history is conditional on the record of the first observer. Under this constraint, we have less information for estimation and must assume that the two observers have the same detection function, an assumption that could be problematic based on the differences between observers found here. This assumption can be made more tenable by rotating the role of primary and secondary observers as we did in our surveys (Cook and Jacobson 1979).

Previous studies have found that sediment grain size affects the ability of zebra mussels to attach to lake bottoms (Berkman et al. 1995). We found no evidence that the density of zebra mussels was preferentially linked to certain substrate types, though our study was not specifically designed to detect these effects as it was not balanced across substrate types. Further, our classification of substrate types was qualitative, so we were not able to distinguish fine-scale changes in the spatial distribution of sediment size. Also, the lakes we studied were at very low densities of infestation; substrate associations may emerge as populations reach higher densities. We did find evidence that the detection of zebra mussels was linked to habitat, with detection being significantly lower in segments with plant cover. This effect on detection can make defining sampling strata post-hoc problematic when not accounting for detectability.

We see several available options for obtaining more precise distance survey estimates under the constraint of limited survey effort. It may be possible to combine transect surveys with remote-sensing technologies (e.g., acoustic surveys). SCUBA-surveys could be used to calibrate more extensive, but less accurate counts via a double-sampling approach (Thompson 2004). Alternatively, remote sensing data could be used for stratification, allowing for increased survey effort in areas where mussels are most likely to be detected. Finally, an increase in the number of transects surveyed would lead to reduced variability in the counts. Thus, it may be better to survey faster at the cost of lower detection if this allows divers to incorporate additional transects.

Several studies have used surveys of freshwater mussels to examine the trade-offs between survey efficiency, coverage, and the probability of discovering low-density mussel populations (e.g., Green and Young 1993; Metcalfe-Smith et al. 2000; Smith 2006). Understanding how these trade-offs constrain our ability to estimate population density and distribution is essential for optimizing effort and may have important implications for our ability to evaluate control measures on invasive species such as zebra mussels. A major limiting factor that prevents the broad application of optimal survey theory is that the trade-off function, describing how changes in search efficiency affects coverage and detectability, is generally unknown (Giudice et al. 2010).

We are aware of one previous study that compared distance- and quadrat-based surveys of freshwater mussels (briefly described in D. L. Strayer and Smith 2003). In that study, survey methods were implemented in equal-sized areas. Quadrats generally provided more precise estimates of density though differences between the two methods decreased as densities increased. We expect that, relative to quadrat counts, distance surveys should be able to cover a larger area in an equal amount of time. To compare survey efficiencies, it would be necessary to control survey time (or cost) rather than survey area. Future data collection efforts should attempt to capture information on survey effort, which would allow for comparisons among the efficiencies of survey methods. Comparisons of survey efficiencies are especially relevant to efforts to monitor recently invaded lakes where densities need to be estimated over large areas of lake bottom to determine the extent of the invasion.

Author Contributions

JF and MM obtained funding for the study; JF and MM designed the study with input from JMF, NSB, and LS; NSB and LS collected the data; JMF analyzed the data with input from JF; JMF led the writing of the paper and all authors contributed critically to the drafts and gave final approval for publication.

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M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 16: Sustaining walleye populations: assessing impacts of AIS
SUBPROJECT MANAGER: Gretchen Hansen
AFFILIATION: University of Minnesota
Organization: University of Minnesota
Mailing Address: 2003 Upper Buford Circle, 135 Skok Hall
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Email Address: ghansen@umn.edu
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $ 198,700
AMOUNT SPENT: $ 197,569
AMOUNT REMAINING: $1,130

Sound bite of Subproject Outcomes and Results
We evaluated the impacts of zebra mussels and spiny waterflea on walleye and yellow perch. Age-0 walleye were >10% smaller at the end of summer following invasion by either AIS, but age-0 yellow perch growth was not consistently affected. Food resources supporting walleye and yellow perch varied among lakes.

Overall Subproject Outcome and Results
Minnesota lakes experience ecosystem-level changes following the introduction of aquatic invasive species (AIS), specifically zebra mussels and spiny water fleas. However, the effects of these AIS on fish are poorly understood and vary among lakes. We evaluated the impacts of zebra mussels and spiny water fleas on walleye and yellow perch in Minnesota's nine largest walleye lakes. We compared age-0 walleye and yellow perch growth over 35 years, including pre- and post-invasion. Age-0 walleye were >10% smaller at the end of summer following invasion by either AIS. Age-0 yellow perch growth decreased following zebra mussel invasion, although this effect was not statistically significant. Smaller length at the end of the growing season was associated with decreased survival to later life stages for walleye in 7 of the 9 study lakes.

We used stable isotope analyses to understand which habitats and food resources support walleye and other fish and to assess their position in the food web in each lake. We documented a high degree of variability in the resources supporting all life stages of walleye. In general, juvenile walleye relied on offshore prey resources in invaded lakes. Combined with reduced growth rates, these results suggest that as zooplankton food resources decline following invasion, young walleye are not sufficiently accessing alternative prey resources to maintain pre-invasion growth rates. Variability in walleye diets among lakes may reflect differences in lake productivity or morphology, not necessarily the presence of AIS.

Our results demonstrate that zebra mussels and spiny water flea influence the growth rates of age-0 walleye and that a wide range of food resources and habitats support walleye in these lakes. Declines in growth rates of young walleye are an early signal of potential negative effects on walleye. This information can guide managers on the most effective and sustainable walleye harvest and stocking strategies in invaded lakes.

Subproject Results Use and Dissemination

- A manuscript documenting the results of our historical growth analysis has been submitted to the peer-reviewed journal Biological Invasions (submitted draft attached).
- We have delivered several presentations at scientific conferences, meetings with managers, and to the public:
    - Bethke, B. September 2017. From little bugs to big fish: beginning to understand how AIS disrupt sport fisheries. Minnesota Aquatic Invasive Species Research Center Showcase, St. Paul, MN
    - Hansen, GJA. June 2017. Sustaining walleye populations: assessing impacts of AIS on food webs. Minnesota DNR Large Lakes meeting. Isle, MN.
    - Hansen, GJA. January 2018. Systems change in Midwestern lakes. Minnesota DNR Roundtable meeting. Bloomington, MN.
    - Ahrenstorff, T, B. Bethke, H. Rantala, and G. Hansen. June 2018. Sustaining walleye populations: assessing impacts of AIS on food webs. Minnesota DNR Research meeting. Glenwood, MN.
    - Hansen, GJA. March 2018. Ecosystem changes and effects on Walleye management. Lake of the Woods Fisheries Input group. Baudette, MN.
    - Hansen, GJA. February 2018. Systems change in Midwestern lakes. Minnesota DNR Fisheries Academy. Camp Ripley, MN.
    - Hansen, G. J. A**.,** T. Ahrenstorff, B. Bethke, V. Brady, J. Dumke, W. French, J. Hirsch, K. Kovalenko, R. Maki, H. Rantala. 2018. Effects of zebra mussels and spiny water flea on sport fish in Minnesota's nine largest walleye lakes. Upper Midwest Invasive Species Conference. Rochester, MN.
    - Hansen, G. J. A**.,** B. Bethke, T. Ahrenstorff, V. Brady, J. Dumke, W. French, J. Hirsch, K. Kovalenko, R. Maki, H. Rantala. 2018. You are what you eat! Beginning to understand how AIS disrupt sport fisheries. Minnesota Aquatic Invasive Species Research Center Annual Showcase. St. Paul, MN.
    - Bethke, B.J. 2018.From little bugs to big fish: beginning to understand how AIS impact sport fisheries. Emily Lakes Association Meeting. Cross Lake, MN.
    - Ahrenstorff, T. G.J.A. Hansen, B. J. Bethke, T. Ahrenstorff, W. French, J. Hirsch, H. Rantala, K. Kovalenko, J. Dumke, V. Brady, R. Maki, T. Wagner. 2019. Walleye and yellow perch first year growth changes with zebra mussel and spiny water flea invasion in Minnesota's large lakes. Minnesota and Dakota Chapters of the American Fishery Society Annual Meeting, Fargo, ND.
    - Hansen, G.J.A., B. J. Bethke, T. Ahrenstorff, W. French, J. Hirsch, H. Rantala, K. Kovalenko, J. Dumke, V. Brady, R. Maki, J. LeDuc. 2019. Effects of zebra mussel and spiny water flea on sport fish in Minnesota's large walleye lakes. Minnesota and Dakota Chapters of the American Fishery Society Annual Meeting, Fargo, ND.
    - Bethke, B.J. G.J.A. Hansen, T. Ahrenstorff, H. Rantala, H. Kelly, W. French, J. Hirsch, K. Kovalenko, R. Maki, J. Dumke, V. Brady. 2019. Fisheries food web effects of zebra mussels and spiny water flea in large north temperate lakes. Society for Freshwater Science Annual Meeting, Salt Lake City, UT.
    - Hansen, G.J.A., B. J. Bethke, T. Ahrenstorff, W. French, J. Hirsch, H. Rantala, K. Kovalenko, J. Dumke, V. Brady, R. Maki. 2019. Effects of zebra mussel and spiny water flea on sport fish in Minnesota's nine largest walleye lakes. Minnesota Department of Natural Resources Large Lakes Meeting, Walker, MN.
- Our work has been covered in the popular press and University media:

- *DNR Launches high-tech study of food webs in Minnesota's largest walleye lakes*. Tony Kennedy, **Star Tribune**. 19 August 2017 http://www.startribune.com/dnr-launches-high-tech-study-of-food-webs-in-minnesota-s-largest-walleye-lakes/441088893/
- Minnesota scientists dive deep to learn why walleye are stressed. Dan Gunderson, **Minnesota Public Radio**. 18 July 2017 https://www.mprnews.org/story/2017/07/18/scientists-digging-deeper-to-understand-factors-affecting-walleye
- Are lake invaders affecting walleye? June Breneman, **NRRI news**. 27 July 2017 https://www.nrri.umn.edu/natural-resources-research-institute/news/ais-walleye

- We worked with MAISRC communications staff to develop a project fact sheet (Attached), which we distributed to interested citizens and to DNR offices.
- We have maintained an active social media presence (on Twitter) describing our ongoing research. The MNDNR and NRRI public information staff are in contact with the MAISRC communications coordinator to facilitate posting of information to social media posts of all three organizations.
- We worked with MAISRC staff to develop a video describing our work, viewable here: https://www.maisrc.umn.edu/news/walleye-video

Attachment 1: growth manuscript in review
Attachment 2: Fact sheet

From little bugs to big fish:

beginning to understand how AIS disrupt sport fisheries

Sustaining Walleye Populations: Assessing the Impacts of AIS

A collaborative project seeking to understand links between invertebrate invasion and sport fish populations in Minnesota's largest walleye lakes

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Young sport fish, like walleye, can be negatively affected by zebra mussels and spiny waterfleas.

Zebra mussels:

- Found in 344 water bodies in Minnesota
- Become very abundant in lakes
- Remove nutrients from the water that would otherwise support micro-organisms (zooplankton), which small fish eat

Spiny waterfleas:

- Found in 66 water bodies in Minnesota
- Are large zooplankton that eat smaller zooplankton
- They replace the small zooplankton, but are difficult to eat because of their large spine, reducing the amount of food for small fish

Want to learn more?

Contact the Minnesota Aquatic Invasive Species Research Center at maisrc@umn.edu or www.maisrc.umn.edu, or reach out to a member of the research team:

Bethany Bethke
bethany.bethke@state.mn.us
(218) 302-3271

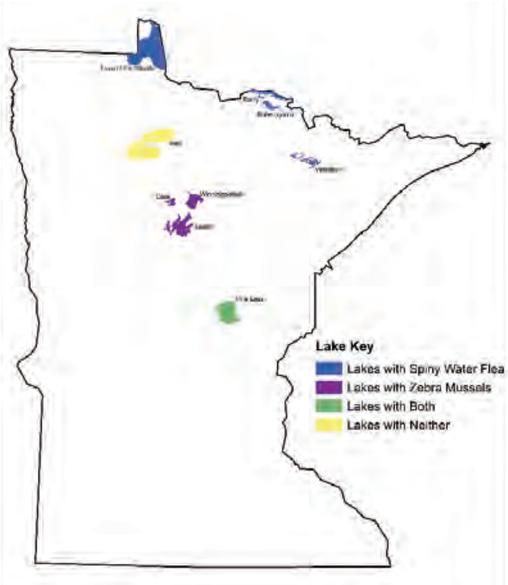
Spiny waterflea photo: Green Lake Association

The research

Project goals: In lakes with and without zebra mussels and/or spiny waterflea, compare fish food habits and compare fish growth and catch rates over time.

Sampling:

- The DNR samples these lakes annually, in the summer and the fall
- We're working with existing sampling to get more large and small fish and invertebrates
- Sampling in Leech Lake, Red Lake, and Lake Mille Lacs is complete
- This summer, researchers will be sampling at Cass Lake, Lake Winnibigoshish, Lake of the Woods, and Lake Vermilion
- Data will be analyzed over the winter, with results expected in 2019



M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 17: Building scientific and management capacity to respond to invasive *Phragmites* (common reed) in Minnesota
SUBPROJECT MANAGER: Daniel Larkin
AFFILIATION: University of Minnesota
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WEBSITE: http://larkinlab.cfans.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $283,568
AMOUNT SPENT: $269,773
AMOUNT REMAINING: $13,795

Sound bite of Subproject Outcomes and Results

We mapped the distribution of invasive *Phragmites*, investigated its spread potential, and developed strategies for coordinated response in collaboration with agency staff and other resource managers. Published an action plan outlining how spread could be stopped and reversed; including management recommendations, cost estimates, and region-specific response guidance. Created mnphrag.org.

Overall Subproject Outcome and Results

MnPhrag is an early detection and response effort targeting invasive *Phragmites australis* (common reed) (www.mnphrag.org), with the goal of supporting landscape-scale, strategic management throughout Minnesota. We mapped the distribution of invasive *Phragmites*, investigated its spread potential, and developed strategies for coordinated response in collaboration with agency staff and other resource managers. We engaged professionals and citizen scientists in reporting suspected populations; conducted intensive search efforts in under-sampled regions; and revisited unverified reports from a web-based invasive species reporting system. Over 70 active observers helped us identify 435 invasive *Phragmites* populations statewide, and we showed that non-experts can reliably distinguish invasive from native *Phragmites* using an identification guide we developed (www.maisrc.umn.edu/identifying-phragmites). The value of this “crowdsourcing” approach to surveillance is reflected in most invasive stands we identified being small populations (90% are <0.25 acres), for which effective control is much more feasible. Invasive *Phragmites* is producing viable seed in Minnesota, which increases spread risk; however, the extent of seed production varies across populations, and there is still time to prevent further spread through sound, sustained control efforts. We are working closely with diverse stakeholders to support coordinated response efforts. Our work has also brought state agencies together to address crosscutting issues related to invasive *Phragmites*’ regulatory status, including its use in some wastewater treatment facilities in “reed beds” for removing water from biosolids. We recently published an action plan outlining how *Phragmites* spread could be stopped and reversed in Minnesota; this assessment includes management recommendations, cost estimates, and region-specific response guidance (www.maisrc.umn.edu/reversing-spread). Our findings reveal a window of opportunity to slow and reverse spread of invasive *Phragmites*, which would benefit Minnesotans by protecting vital natural resources. This approach to statewide surveillance, and framework for a coordinated, landscape-scale response, are strategies that could be applied to other invasive species issues in Minnesota.

Subproject Results Use and Dissemination

Information from this project has been disseminated through 19 invited talks, 6 contributed presentations, 1 webinar, 1 radio interview, and reports and resources published on our website (www.mnphrag.org). Our *Phragmites* Identification Guide and the report "An assessment to support strategic, coordinated response to invasive *Phragmites australis* in Minnesota" are included as attachments. Project findings are being used by the Minnesota Noxious Weed Advisory Committee, the Minnesota Department of Natural Resources, the Minnesota Department of Agriculture, and the Minnesota Pollution Control Agency to assess risk of *Phragmites* invasion in Minnesota and review relevant regulations, permitting, and policy.

An assessment to support strategic, coordinated response to invasive *Phragmites australis* in Minnesota

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University of Minnesota

Department of Fisheries, Wildlife, and Conservation Biology

Minnesota Aquatic Invasive Species Research Center

May 2019

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Control cost information was provided by Dan Shaw and Carol Strojny (BWSR); Bill Bartodziej (Natural Shore Technologies, Inc.); Mike O'Connell (Lake Management, Inc.); Tory Christensen and Patrick Kelly (Landbridge Ecological); Patrick Selter (PLM Lake and Land Management Corp.); Lee Shambeau (4 Control); Mike Hiltner (Prairie Restorations, Inc.); Doug Mensing (Applied Ecological Services, Inc.); Dale Sutherland (Nutrien Ag Solutions); Keegan Lund, Ray Norrgard, and Ricky Lien (MNDNR); and Dave Hanson (MNDOT).

Information related to the use of invasive *Phragmites* at wastewater treatment facilities and alternative approaches was provided by Sheryl Bock and Randy Thorson (MPCA); Eric DeVenecia and Frederick Hegeman (Wisconsin DNR); Miles Falck (GLIFWC); and Chad Abel and Gabrielle VanBergen (Treaty Natural Resources Division of the Red Cliff Band of Lake Superior Chippewa).

Reviewers who provided helpful suggestions on earlier versions of this assessment include Wendy Crowell, Allison Gamble, Keegan Lund, and Laura Van Riper (MNDNR); Monika Chandler (MDA); Sheryl Bock and Randy Thorson (MPCA); Mary Jo Youngbauer (Chisago County SWCD); and Brandon Van Tassel (Community Action Duluth).

We also extend our thanks to the many individuals who have reported, submitted samples, and continue to scout for invasive *Phragmites* populations throughout the state.

Assessment summary

Invasive *Phragmites* (*Phragmites australis* subsp. *australis*) has spread across the wetlands of many regions of North America, and is well-documented to have detrimental effects on wildlife, fish, native plants, water supply, and recreational uses. This tall, fast-growing, non-native wetland grass spreads to lakeshores, wetlands, roadside ditches, and other wet habitats, sometimes after intentional introduction, as occurred in Minnesota. Numerous reports over the past ten years had suggested that the invasion of this species was progressing in Minnesota and that the window of time might be closing to efficiently respond and prevent widespread damage to the state's wetlands. Over the last two years, our research has verified 389 invasive *Phragmites* populations in Minnesota. Many populations are producing viable seed and so have high capacity for further spread. However, these numerous populations currently add up to an area of approximately 50 acres. In light of these findings, a coordinated, statewide control effort with the aim of eliminating all established populations is still feasible, if pursued without delay. Invasive *Phragmites* has the capacity to quickly spread and overtake areas; partial or uncoordinated responses are unlikely to be beneficial or cost-effective. This assessment suggests strategies for collaboration, coordination, and implementation of control efforts; provides control cost estimations; details core competencies for participating entities; identifies potential funding sources; and addresses possible challenges associated with such a response.

We present invasive *Phragmites* status information and possible response strategies tailored to 12 regions of the state. This regionalized approach is intended to highlight differences in distribution and the social and environmental contexts in which invasive *Phragmites* occurs across Minnesota, and to empower regional and local organizations to quickly mobilize and initiate response efforts. Some regions include many populations with various sizes, habitats, and property ownerships, while others include only a few populations under similar invasion contexts. Each regional section contains a description of the regional status of invasive *Phragmites*, potential partner organizations and funding options, estimated control costs, and training and capacity needs.

Review of the scientific literature shows the most effective approach for controlling invasive *Phragmites* to be end-of-summer herbicide treatment, supplemented by winter or late summer mowing to remove dead stems. It is likely that this management schedule will need to be repeated for three years to eliminate the plant from most sites. While burning, cutting, and water-level management have also been employed in invasive *Phragmites* management, these approaches have either been shown to be ineffective or come with important caveats. The type of equipment required to conduct control (e.g., backpack sprayer, boat, etc.) will need to be varied depending on characteristics of the targeted site. Only equipment that can be sufficiently decontaminated of plant propagules should be used in conducting control to avoid contributing to invasive *Phragmites* spread.

In addition to wild invasive *Phragmites* populations, there are 16 wastewater treatment facilities in Minnesota that use invasive *Phragmites* in their operations. While the invasive *Phragmites* at these facilities are potential sources of spread, they also support wastewater treatment operations by dewatering biosolids following sewage treatment. Ultimately, a plan for transitioning these facilities to effective, alternative dewatering methods would be needed for a truly comprehensive response to invasive *Phragmites* in Minnesota. While potential alternatives are being evaluated, best management practices to minimize spread risk should be developed for facilities' dewatering operations and materials disposal.

An effective statewide response to invasive *Phragmites* is only possible with local to state level partners and partnerships. To varying degrees, invasive *Phragmites* falls under the jurisdiction of multiple state agencies, including the Minnesota Department of Natural Resources, Minnesota Department of Transportation, Minnesota Department of Agriculture, and Minnesota Pollution Control Agency. Response efforts could be coordinated by state agency staff – either by managing control contracts directly or by administering funds to regional and local entities – or by regional and local organizations implementing private or grant-funded projects from non-agency sources. Cooperation with private and commercial landowners will be essential. Regardless of the level at which control efforts are organized, a truly statewide response will require significant coordination, which could potentially be centralized and designed to work across jurisdictions. We do not identify "priority" populations for control in this assessment because a partial approach is inconsistent with the well-understood biology of this species—that all seed-producing populations have high capacity to trigger broader spread.

Participants in invasive *Phragmites* response should be trained in several core competencies to ensure effective and responsible management. Individuals conducting surveillance for new populations must know how to report their findings and distinguish invasive *Phragmites* from the native subspecies (*Phragmites australis* subsp. *americanus*) or how to collect and submit specimens to an expert for identification. Those implementing control will need to acquire the appropriate permits, follow applicable herbicide-use regulations, and determine the control approaches and equipment needs specific to each site. Adequate reporting and evaluation of control efforts will be needed to support comprehensive response and to facilitate adaptive management.

Responding to invasive *Phragmites* statewide will require substantial financial investment at the outset. Several potential sources of funding to support invasive *Phragmites* response are identified in this assessment. We have estimated costs for three years of herbicide treatment and mowing of all verified wild populations at $818,500-2,019,000. These costs are comparable to costs of invasive *Phragmites* control efforts conducted in other states, though Minnesota is unique in that this level of investment can be deployed at a time when reversal of spread remains feasible. Should potential partners choose to wait to implement response efforts, control costs will increase as invasive *Phragmites* becomes more widespread and difficult to manage, requiring more complicated equipment and more labor. It is critically important to recognize that choosing not to respond is choosing to allow invasive *Phragmites* spread to escalate, and this choice will severely limit the feasibility of control within the not-too-distant future.

Mobilizing a strategic, coordinated response to invasive *Phragmites* statewide is clearly an ambitious undertaking that will come with many challenges. Lack of support from state, regional, and local entities; private landowners; or grant programs would hinder efforts. Depending on the rate of invasive *Phragmites*' spread, the potentially short window of opportunity for effective response requires mounting efforts both quickly and responsibly. Coordinators will need to ensure that control efforts are of sufficient quality and include adequate follow-up and equipment decontamination. Potential pathways for reinvasion will need to be addressed and ongoing monitoring will be needed to support early response to newly detected populations. While the challenges are real, they are not insurmountable, and overcoming them will yield significant benefits for the state.

Acronyms and abbreviations

Abbreviation	Meaning
AIS	Aquatic invasive species
AISPA	Aquatic Invasive Species Prevention Aid
BNSF	BNSF Railway Company
BWSR	Minnesota Board of Soil and Water Resources
CPL	Conservation Partners Legacy Grant Program
CWMA	Cooperative Weed Management Area
DNR	Department of Natural Resources
EDDMapS	Early Detection and Distribution Mapping System
GLRI	Great Lakes Restoration Initiative
LCCMR	Legislative-Citizen Commission on Minnesota Resources
LSOHC	Lessard-Sams Outdoor Heritage Council
MAISRC	Minnesota Aquatic Invasive Species Research Center
MDA	Minnesota Department of Agriculture
MNDNR	Minnesota Department of Natural Resources
MNDOT	Minnesota Department of Transportation
MPCA	Minnesota Pollution Control Agency
NFWF	National Fish and Wildlife Foundation
SWCD	Soil and Water Conservation District
UMN	University of Minnesota
USFWS	United States Fish and Wildlife Service
UTV	Utility vehicle

Contents

Introduction

A highly invasive European lineage of common reed (*Phragmites australis* subsp. *australis*), a wetland grass, has been introduced to multiple locations in Minnesota and appears to be spreading. While native *Phragmites* (*P. australis* subsp. *americanus*) is an important component of Minnesota's wetland flora, invasive *Phragmites* can have strong negative impacts on biological diversity, wildlife, habitat quality, and recreation (Meyerson et al. 2016). Invasive *Phragmites* tends to grow very tall and dense, creating unsuitable shelter and food for wildlife and fish, and displacing native flora that would otherwise provide those benefits (Able and Hagan 2000, Minchinton et al. 2006, Meyer et al. 2010). The native subspecies has been largely displaced by the invasive along the New England to mid-Atlantic coast (Saltonstall 2002, 2011). Invasive *Phragmites* has also been shown to invade shoreline areas and can block views of and access to water, thereby impeding recreation (see also About invasive *Phragmites*). Several U.S. states have exceedingly large invasive *Phragmites* populations, and some are forced to fund expensive annual control projects just to prevent further spread and provide localized relief of negative ecological and recreational effects (Figure 1).

Recent research at the University of Minnesota has documented the distribution of invasive *Phragmites* and assessed its ability to reproduce and spread by seed within Minnesota (hereafter, referred to as the "MNPhrag" project). The following points summarize key findings:

- Over the past 2 years, 389 individual invasive *Phragmites* populations have been verified throughout Minnesota using a combination of crowdsourcing and targeted surveillance.

- Reporters are able to accurately identify invasive *Phragmites* 95% of the time, based on comparison of reporters' morphological identifications to genetic tests.

- A map of the statewide distribution of invasive *Phragmites* shows it to be most common in the Twin Cities metropolitan region, Chisago and Wright counties, and in and around the city of Duluth (Figure 2).

- In addition to the 389 verified wild invasive *Phragmites* populations, there are 16 wastewater treatment facilities in Minnesota that use invasive *Phragmites* in their operations.

- While invasive *Phragmites* has long been known to be capable of spreading through accidental transport of vegetative structures (e.g., rhizomes and stolons), it was previously thought that invasive *Phragmites* had little capacity for sexual reproduction and spread by seed. However, invasive *Phragmites* is now broadly understood to produce viable seed (Kettenring and Whigham 2009), and MNPhrag research has confirmed that, even under Minnesota's climate, invasive *Phragmites* populations in the state are producing viable seed.

Figure 1.

A) European common reed (*Phragmites australis* subsp. *australis*) is an invasive wetland grass.

B) Secretive marshbirds like the least bittern nest more frequently in marsh meadow habitats than invasive *Phragmites* stands. Invasive *Phragmites* can also negatively affect fish populations, as has been shown in mummichogs on the East Coast (Able and Hagan 2000).

C) It is capable of invading a wide variety of wetland habitats, including lakeshores, marshes, and roadside ditches.

D) An extensive invasive *Phragmites* monoculture (light green) in Wisconsin along Lake Michigan; similar conditions are found in New England, Michigan, and Nebraska, necessitating control efforts to reduce abundance.

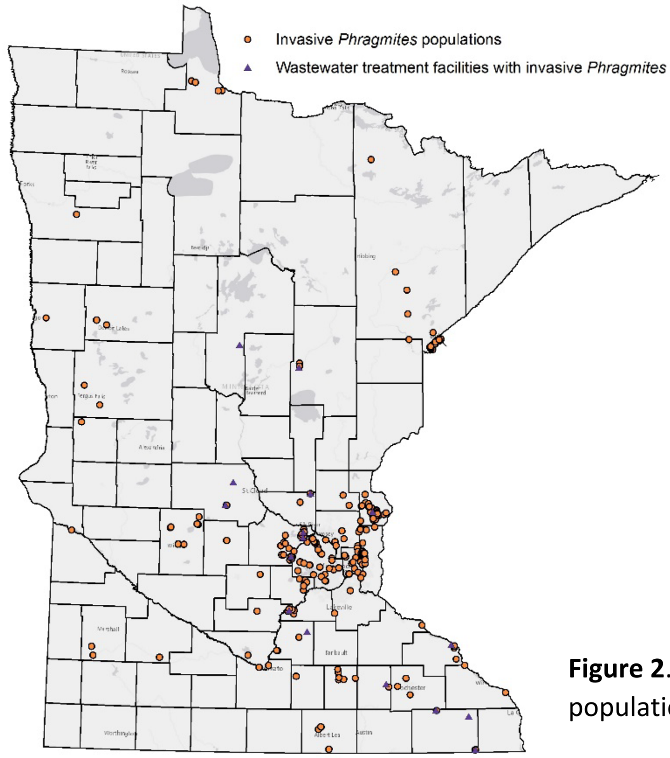


Figure 2. Verified invasive *Phragmites* populations throughout Minnesota.

The window of opportunity to limit invasive *Phragmites* invasion in Minnesota is now. With less than 400 verified populations, the state has relatively low invasive *Phragmites* abundance. Neighboring states and provinces are not large sources of invasive *Phragmites*. Wisconsin regulates invasive *Phragmites* as a prohibited species in its western half and is systematically controlling invasive *Phragmites* populations there, reducing potential for further introductions from across Minnesota's eastern border. There have been few reports of invasive *Phragmites* in North Dakota, South Dakota, and Iowa. However, invasive *Phragmites* populations have been spreading through southern Ontario and into Manitoba (ISCM 2019, Ontario 2019). Proactive, coordinated control and monitoring could minimize negative impacts of invasive *Phragmites* and reverse its spread. Delaying response to invasive *Phragmites* invasion will increase the costs of control activities and reduce their effectiveness, as controlling large populations is difficult (Quirion et al. 2018, Rohal et al. 2019). Based on the distribution of invasive *Phragmites* populations in Minnesota, likelihood of further spread, and resources in place for management of non-crop invasive plants, the capacity for coordinated control of invasive *Phragmites* varies regionally across Minnesota.

Invasive *Phragmites* is a shared problem, as it inhabits roadsides, lakeshores, wetlands, and other habitats on both publicly and privately owned lands, and is used in some municipal wastewater treatment facilities. Successful response will hinge upon commitments by regional and local organizations, the support and collaboration of state agencies, and cooperation by individual landowners (Epanchin-Niell et al. 2010). In addition, ongoing surveillance will require "eyes on the ground" at the local level. The intention of this document is to support a comprehensive statewide response to invasive *Phragmites*. For each of 12 regions of Minnesota, we characterize the various environmental and social contexts in which invasive *Phragmites* has been found, identify potential partner organizations, and propose strategies that could be implemented to control invasive *Phragmites* populations. We also address regional and statewide coordination and training needs, current and future actions to prevent spread from wastewater treatment facilities, potential funding sources, and likely challenges, and estimate control costs to support effective response.

A proposed goal for invasive *Phragmites* response

With the limited distribution of invasive *Phragmites* in Minnesota, a well-designed and coordinated landscape-scale response, along with continuing surveillance, could effectively eliminate it from the state. Invasive species practitioners know that management is most effective in the early stages of invasion, when the invasive is not yet widely abundant and distributed across the landscape (Simberloff et al. 2013). Despite 389 populations of invasive *Phragmites* having been verified across Minnesota, these populations comprise an area of approximately 50 acres, as opposed to hundreds or thousands of acres in other states across the country. Invasive species control efforts often aim to meet site-specific goals, which can be challenging to meet since species' dispersal is not bound by political or property boundaries. Effective control approaches are well understood and documented for invasive *Phragmites*. A coordinated, landscape-scale effort aimed at eliminating it from Minnesota would at least delay and could realistically reverse its spread in the state. Additional pioneer populations would continue to arise from various sources, but ongoing surveillance and rapid response would allow maintenance of very low abundance statewide. The costs of the initial control effort, followed by management of intermittent new invasions, would likely be far lower than the costs of allowing invasive *Phragmites* to continue to spread—i.e., the costs associated with perpetual nuisance control and asset preservation, and the costs resulting from degradation of wetlands, lakeshores, and other habitats and the ecosystem services they provide.

Because functionally eliminating invasive *Phragmites* from the state appears to be attainable, we did not attempt to prioritize populations for control. At this stage, all populations must be given priority, as this is fundamental to a successful response at the landscape-scale given the biology of the species. Depending on management outcomes, prioritization could later be considered following an initial, concerted response effort.

How to use this document

The intended audience for this document is federal to local agencies and organizations who may be involved in invasive *Phragmites* response efforts. Part I of this assessment provides stakeholders with an overview of regional complexity, capacity, and potential strategies. Regional and local partners may not need to read the regional sections outside their area, while we encourage those coordinating at the statewide level to read the document fully. It is recommended that partners read Parts II-IV as well as the regional section that applies to them, as Parts II-IV expand on the information provided in Part I, with critical considerations for effective and appropriate response efforts. Those reading the document fully will find some redundancies in the information presented across the regional sections, which are intended for regional and local partners interested in a particular region. The appendices describe important caveats regarding how information was compiled. We urge entities participating in invasive *Phragmites* response efforts to read Parts II-IV and the appendices, particularly for important considerations regarding recommended use of regional control cost estimates, property ownership determinations, and recommendations and requirements for control implementation.

This assessment is intended to support landscape-scale invasive *Phragmites* response efforts by characterizing capacity, identifying needs, and posing potential strategies for implementation. We hope that the information presented in this document will aid development of plans, identification of partners and resources, and carrying out organized and thoughtful control and monitoring.

Part 1:
Regional assessments of invasive *Phragmites* response needs

Invasive *Phragmites* response regions

This assessment takes a regional approach to account for the various invasion scenarios (i.e., characteristics of invasive *Phragmites* populations and the environmental and social context in which they occur) and organizational capacities specific to different parts of the state. It assumes coordination and support at the statewide level is integral to a successful, comprehensive response.

The 12 regions in this assessment were defined largely based on the distribution of verified invasive *Phragmites* populations, county boundaries, active invasive *Phragmites* control efforts, tribal boundaries, and the presence of cooperative weed management areas (CWMAs) and other entities with an interest in invasive plant management. Environmental characteristics and boundaries, watershed boundaries, land use, and the operating units of state agencies were also considered. With the configuration defined here, each region has at least one CWMA and at least one verified invasive *Phragmites* population (with the exception of the Northeast Region; Figure 3). Partner organizations involved in invasive *Phragmites* response may find adjustments to this regional configuration necessary to more efficiently plan for implementation.

The region-specific sections that follow describe invasive *Phragmites* abundance, population characteristics, response capacity and strategies, and estimated control costs. These sections, as well as the reference sections, can be used by participating organizations in communications and coordination of invasive *Phragmites* response efforts. The regions are ordered from highest-to-lowest number of verified invasive *Phragmites* populations. Please see the Methods appendix for a description of how costs were estimated, land ownership was determined, strategies and restoration sites were identified, and capacity was evaluated, along with associated caveats.

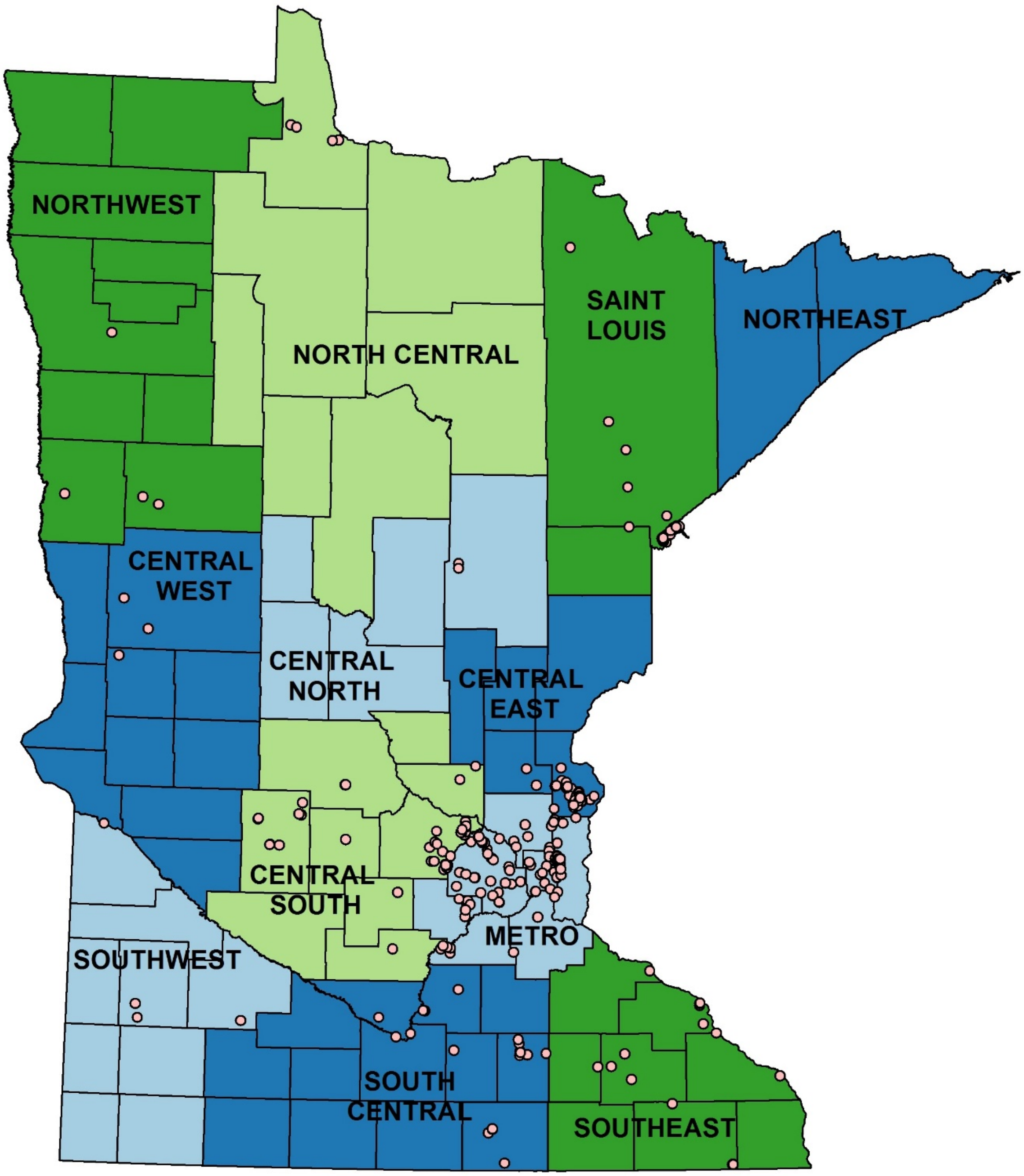


Figure 3. The 12 response regions under which invasive *Phragmites* status, response capacity, and strategies are described in this assessment.

Metro region

Counties

- Anoka
- Carver
- Dakota
- Hennepin
- Ramsey
- Scott
- Washington

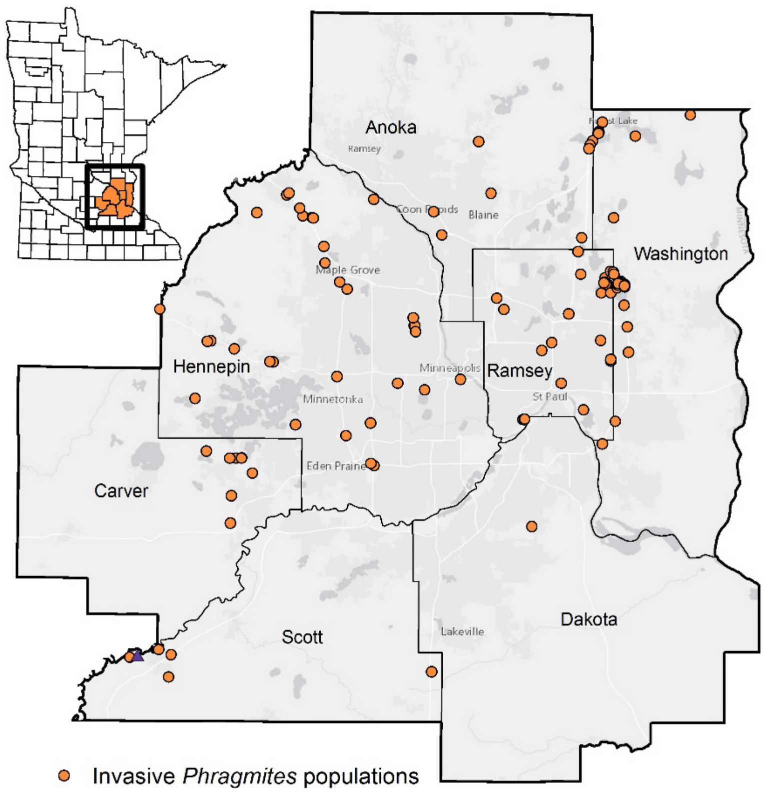
Invasive *Phragmites* status

The seven-county Metro Region has 108 verified invasive *Phragmites* populations to date. Thirty-seven of these are along rights-of-way managed by the Minnesota Department of Transportation (MNDOT). There are another 22 lake and shoreline populations in White Bear Lake. Most populations (68%) are 1,000 sq. ft. or less in size. The largest population is approximately 1 acre and is located in a wetland extending across properties owned by the Minnesota Vikings and other commercial entities. Other relatively large populations (0.7-0.85 acres) have been verified in Maplewood's Priory Neighborhood Preserve and in the city of Saint Louis Park along a railway right-of-way and the Cedar Lakes Trail. Populations estimated at less than ½ acre occupy a variety of habitats, with many along roadsides, in White Bear Lake, in county and municipal parks, and on commercially owned property. There is also a wastewater treatment facility in Scott County using invasive *Phragmites* as part of their operations.

Invasive species response capacity

While a large proportion of invasive *Phragmites* populations in Minnesota occur in the Metro Region, this region has significant invasive species response capacity. The region is within a single MNDOT district (the Metro District), through which state and federal roadside maintenance is coordinated. White Bear Lake has an active conservation district and active restoration and homeowners' associations. Additionally, there are CWMAs in Anoka, Washington, Ramsey, Dakota, and Scott counties. Minnesota Department of Natural Resources (MNDNR) has aquatic invasive species specialists and wildlife managers operating in this area out of their Central Region. Some of the populations are on land owned by the BNSF and Soo Line railroad companies, which may have their own rail maintenance personnel or be willing to allow access to their property for control activities. Other private entities may be willing to contribute funds toward invasive *Phragmites* control on their properties.

Invasive *Phragmites* has been verified within the boundaries of 18 of the 34 watershed districts and management organizations in the Metro Region. The Shakopee Mdewakanton Sioux Community is also located in this region. There are County Agricultural Inspectors and Soil and Water Conservation Districts (SWCDs) in every county; these oversee noxious weed law and implement natural resources programs, respectively.



Invasive *Phragmites* populations
Wastewater treatment facilities with invasive *Phragmites*

Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 108*

Coverage area	Number of populations	Habitat types invaded	Number of populations	Property ownership	Number of populations
≤500 sq. ft.	57	Roadside	38	Private	17
>500 sq. ft. – .25 acre	41	Lakeshore	30	Municipal	13
>.25 – 1 acre	9	Wetland	21	County	7
>1 – 2 acres		Mixed	8	Lake	22
>2 acres		Stormwater pond	6	State	3
Unknown	1	Industrial		MNDOT	37
		Riverine	5	Federal	
		Other		Mixed	9

*This total does not include an invasive *Phragmites* population in use in the operations of a wastewater treatment facility in Scott County.

Invasive *Phragmites* response options

Because of the high density of populations, elimination of invasive *Phragmites* from the Twin Cities Metro Region will be challenging. It should be possible, however, with substantial funding, control coordination, and collaboration among participating organizations. Cooperation with MNDOT will be particularly important for controlling the large number of roadside populations. While most populations are relatively small, controlling the largest populations will require collaboration with city parks departments and commercial entities. In some cases, coordinators will need cooperation from landowners to access private properties. Coordinated monitoring and reporting from partner organizations will support early detection and comprehensive response. Collaboration with the Scott County wastewater treatment facility using invasive *Phragmites* will also be needed for efforts to be comprehensive.

A truck, utility vehicle (UTV), or other vehicle with a mounted herbicide tank and hose could be used to treat many of the roadside, wetland, and lakeshore populations in this region. Some of the populations in White Bear Lake will only be accessible by boat, while shoreline populations may be treatable from shore using an ATV or backpack sprayer. Five to 10 populations may warrant the use of a wetland-adapted vehicle.

Mowing dead invasive *Phragmites* stems (while not recommended as a control strategy alone) increases the effectiveness of subsequent herbicide treatments. Most populations in the Metro Region could be knocked down or cut using a flail mower, forestry mower, or similar equipment, though larger wetland-adapted vehicles may be needed in some cases. A few populations are small enough that they could be cut by hand using a brush saw.

Estimated control cost for region: $175,000-$301,500 over three years

Cost estimation notes

Values presented include three-year costs of control (herbicide application and mowing) only; costs of restoration, project administration by contractees, surveillance, purchasing equipment, and other expenses are not included. The largest populations, near the Minnesota Vikings property, White Bear Lake, Priory Neighborhood Preserve, and the Cedar Lakes Trail may likely require more than three years of control. These values also do not include costs of transitioning to alternative methods for the wastewater treatment facility (see the Invasive *Phragmites* at wastewater treatment facilities section). Only minimal coordination across partner organizations and with ongoing plant management efforts (e.g., state or county highway maintenance) was assumed; further collaboration among coordinators could reduce control costs. For more information about how costs were estimated, see the Methods appendix.

Over three years, we estimated that roadside populations under MNDOT or other state ownership throughout the region could be controlled for $41,000-112,000. Populations under private, county, and municipal ownership could be controlled in Hennepin County for $60,500-74,500; Ramsey County for $29,000-40,500; Carver County for $6,500-12,500; Anoka County for $5,500-9,000; and Washington County for $2,500-5,000. Some of the populations in Hennepin and Ramsey counties may require employing a Marsh Master® or other appropriate wetland-adapted vehicle, which would significantly increase costs. Populations in and around White Bear Lake and Otter Lake in Ramsey and Washington Counties would best be managed under one contract and could be controlled for $28,000-45,000. The small population at Lebanon Hills Regional Park could be controlled for $2,000-3,000, with most of these costs being associated with labor and mobilization (e.g., transportation, equipment movement, etc.).

Possible funding structure

Private entities may be interested and able to support invasive *Phragmites* control efforts in this region. Populations on MNDOT and other state-owned properties could be managed along with other roadside maintenance activities. The Costs and funding sources section describes dedicated funding for maintenance of parks and trails. Control of populations under private, municipal, or county ownership could also be supported by many of the programs described in that section. As described in Coordination and networking strategies, funding could be awarded through a state-administered grant program or by regional or local entities directly.

Several populations along highways in the Metro Region are being treated by MNDOT.

Training and capacity needs

Identification, reporting, equipment decontamination, and an understanding of permitting and herbicide use requirements are core competencies for organizations and individuals participating in invasive *Phragmites* response. Participants in surveillance must be capable of distinguishing native and invasive *Phragmites* (or submitting samples to an expert for identification) and know how to report suspected new invasive populations. Management methods should be determined appropriate to a given site and will require access to necessary equipment. If particular equipment cannot be adequately decontaminated, an alternative approach should be used. MNDNR invasive aquatic plant management permits will be needed for control activities in most aquatic environments, and only herbicide formulations approved for aquatic use can be used in those scenarios. Only Commercial Pesticide Applicators licensed through the Minnesota Department of Agriculture (MDA) can be contracted to apply herbicides. Control activities should be reported and evaluated to support effective response across regions and the state.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Central East region

Counties

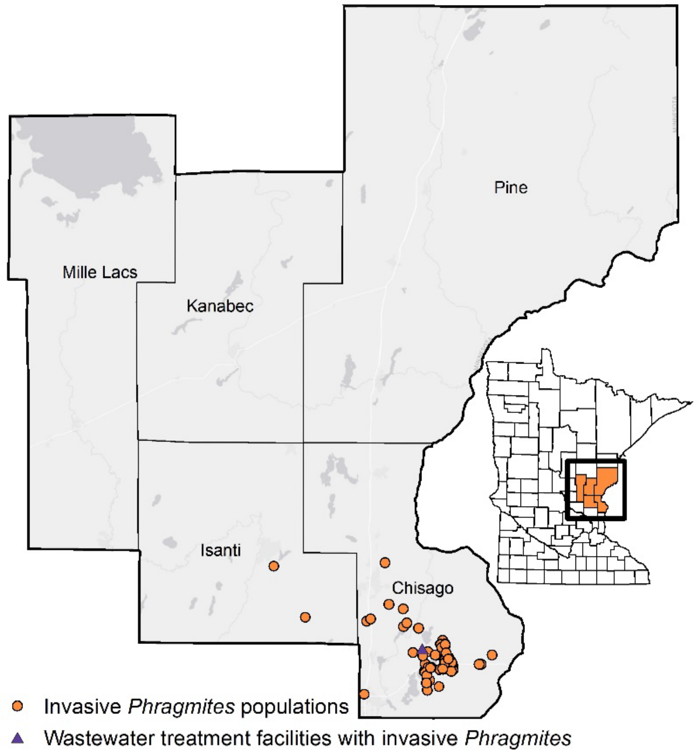
- Chisago
- Isanti
- Kanabec
- Mille Lacs
- Pine

Invasive *Phragmites* status

Nearly 80% of the 92 invasive *Phragmites* populations verified in the Central East Region occur along the shores of North Center, South Center, Chisago, South Lindstrom, and North Lindstrom lakes in Chisago County. All but three lakeshore populations are less than ¼ acre in size, with the largest (estimated at approximately 0.7 acres) occupying private land and the remaining two extending onto county- and state-owned properties. 74% of lakeshore populations cover areas ≤1,000 sq. ft. Most of these extend onto private residential or agricultural properties while some occur along municipal, county, or MNDOT-managed roadsides. The remaining, non-lakeshore populations are along county- and MNDOT-managed roadsides (some of which appear to extend into private properties), in municipal stormwater ponds, and state- and privately owned wetlands. All are ≤¼ acre. There is also a wastewater treatment facility in Chisago County that uses invasive *Phragmites* in their operations.

Invasive species response capacity

The Chisago-Lindstrom Lakes Association and the Center Lakes Association are committed to the management of invasive species and protecting the interests of lakeshore owners. They have already initiated invasive *Phragmites* education and control efforts, in collaboration with the Chisago Lakes Improvement District, Center City Public Works, Comfort Lake-Forest Lake Watershed District, Isanti County, and the Minnesota DNR and DOT. MNDNR aquatic invasive species specialists and wildlife managers operate out of MNDNR's Central and Northeast regions. State and federal highway maintenance in this region is coordinated under three MNDOT districts (Districts 1, 3, and Metro). Kanabec County has the only CWMA. The Mille Lacs Band of Ojibwe is also in this region. There are SWCDs and County Agricultural Inspectors in every county, which implement natural resource programs and oversee noxious weed law, respectively.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 92*

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.	57		Roadside	15		Private	6
>500 sq. ft. – .25 acre	33		Lakeshore	70		Municipal	5
>.25 – 1 acre	2		Wetland	3		County	8
>1 – 2 acres			Mixed	2		Lake	
>2 acres			Stormwater pond	2		State	3
Unknown			Industrial			MNDOT	5
			Riverine			Federal	
			Other			Mixed	65

*This total does not include an invasive *Phragmites* population in use in the operations of a wastewater treatment facility in Chisago County.

Invasive *Phragmites* response options

With 92 verified invasive *Phragmites* populations, the Central East Region is fortunate to have lake associations that are already planning response and surveillance efforts. Continued coordination and engagement with partners, and substantial funding, will be needed to eliminate invasive *Phragmites*. Because of shared property ownerships, private landowners, cities, counties, and the state will need to be engaged in lakeshore control activities. Coordination with state and county highway maintenance departments will be needed to control roadside populations. Early detection of populations and comprehensive response would be supported by coordinated surveillance and reporting. Collaboration with the wastewater treatment facility using invasive *Phragmites* in its operations will also be needed to support comprehensive response.

Depending on the habitat invaded, herbicide treatments could be conducted using a boat, truck, UTV, or other vehicle with a mounted tank and hose. The lakeshore populations could be treated using a boat, or in some cases from land via a backpack sprayer or ATV. A truck, tractor, or UTV could be used for the roadside populations. A vehicle adapted for use in wetland environments may be needed for a few populations.

A flail mower or similar equipment could be used to mow or knock down standing dead invasive *Phragmites*, which has been shown to improve the efficacy of herbicide treatments. Knocking down stems may be more feasible for lakeshore and wetland populations, while mowing could be used along roadsides. For some lakeshore populations, mowing or knockdown may be difficult.

Estimated control cost for region: $45,000-$145,500 over three years

Cost estimation notes

Estimates include herbicide application and mowing costs over the course of three years of management; surveillance, restoration, project administration by contractees, equipment, and other related expenses are not included. The largest lakeshore populations may likely require more than three years of control. Implementing an alternative dewatering method at the wastewater treatment facility also is not included (see the Invasive *Phragmites* at wastewater treatment facilities section). Coordination among organizations or with other vegetation management efforts (e.g., state and county highway maintenance activities) could reduce control costs, as we assumed only minimal coordination in developing estimated costs. The Methods appendix further describes how cost estimates were developed.

We estimated that all the lakeshore populations in the Central East Region could be controlled over the course of three years for $26,000-99,000. Populations on Chisago County private and county-owned properties could be controlled for $12,000-36,500. An estimated $2,500-4,000 would cover control activities for the invasive *Phragmites* populations on MNDOT-owned sites. Populations in the other two state-owned sites could be controlled for $2,500-3,000, and the populations in Isanti County could be controlled for $2,000-3,000.

Possible funding structure

The funding programs described in the Costs and funding sources section could support control of many of the invasive *Phragmites* populations in the Central East Region. Funding could be applied for by regional or local entities or awarded through a state-administered grant program, as described in Coordination and networking strategies. Alternatively, private entities or regional and local organizations could fund control efforts. Control of populations on state and MNDOT-owned lands could also be funded by the programs described in Costs and funding sources or by integrating invasive *Phragmites* control with their previously planned maintenance activities.

Training and capacity needs

Partners involved in invasive *Phragmites* response will need to be able to identify invasive *Phragmites*, report and evaluate actions, decontaminate equipment, and follow permitting and herbicide use requirements. Those involved in surveillance must be able to differentiate between invasive and native *Phragmites* (or submit samples to an expert for identification) and know how to report suspected new populations. Those involved in control activities will need to be able to determine the appropriate management approach. Necessary equipment may need to be acquired and only equipment that can be sufficiently decontaminated should be used. The use of aquatic-approved herbicide formulations and acquisition of invasive aquatic plant management permits from MNDNR will be essential for work in aquatic environments. Only MDA-licensed Commercial Pesticide Applicators can be contracted for these activities. Reporting and evaluation of the results of control activities should be conducted to support effective response.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Some populations found in Chisago County lakes are well established, while other populations are still small with sparse stems.

Saint Louis region

Counties

- Carlton
- Saint Louis

Invasive *Phragmites* status

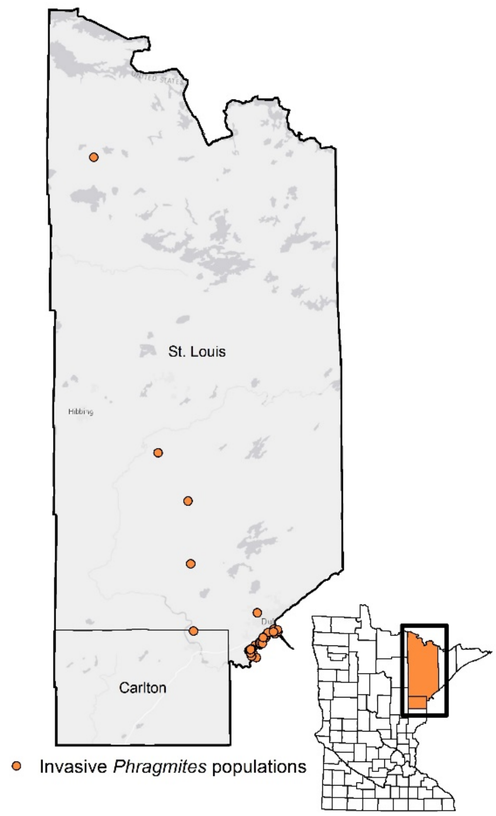
Thirty-three of the 67 invasive *Phragmites* populations verified in the Saint Louis Region are lakeshore (37%) and wetland (12%) populations in and around the port of Duluth. These tend to have mixed ownership, spanning from private, commercial or railway properties to areas owned or managed by the city of Duluth and the Duluth Port Authority. Many have been estimated to be approximately ¼ acre in size. The largest population has been estimated at approximately 2.5 acres. There are several large populations near Grassy Point, Rice's Point, and Spirit Lake Marina, including a 1.5-acre population on state-owned property. There are also several ¼-acre populations in stormwater ponds in Duluth's Oneota neighborhood.

Outside Duluth, two populations have been verified along Highway 53, estimated at ¼ acre and 1 acre. The single population in Carlton County is estimated at ¼ acre and is along Highway 33.

Invasive species response capacity

Significant invasive *Phragmites* control efforts are already being conducted and coordinated by a partnership including the Saint Louis River Alliance, Community Action Duluth, the Great Lakes Indian Fish and Wildlife Commission, and the 1854 Treaty Authority. The Duluth Port Authority and the BNSF and Soo Line railroad companies may be able to provide property access. The railway companies may also be able to use their own maintenance staff for invasive *Phragmites* control. Other private entities may be willing to contribute some of their own funds towards invasive *Phragmites* control on their properties. MNDNR aquatic invasive species specialists and wildlife managers work out of MNDNR's Northeast region. MNDOT-managed roadways are maintained through MNDOT District 1.

There are CWMAs in both Carlton and Saint Louis counties. Lands of the Fond du Lac Band of Lake Superior Chippewa and a small portion of the lands of Bois Forte Band of Chippewa are also within this region. North and South SWCDs in Saint Louis County and SWCD in Carlton County implement natural resource programs. Each county has a County Agricultural Inspector that oversees noxious weed law.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 67

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.	4		Roadside	4		Private	31
>500 sq. ft. – .25 acre	48		Lakeshore	25		Municipal	8
>.25 – 1 acre	8		Wetland	8		County	1
>1 – 2 acres	1		Mixed	19		Lake	
>2 acres	1		Stormwater pond	6		State	3
Unknown	5		Industrial	5		MNDOT	3
			Riverine			Federal	
			Other			Mixed	21

Invasive *Phragmites* response options

Of all the regions, the Saint Louis Region has the largest estimated cost for eliminating invasive *Phragmites*. With complex property ownership scenarios and an abundance of relatively large populations, persistent efforts and substantial funding will be needed. Continued collaboration and coordination among public and private entities are essential. Coordinated surveillance and reporting from partners will support early detection and comprehensive response.

Depending on site characteristics, most herbicide treatments in this region could be conducted using a truck, UTV, or boat with a mounted tank and hose reel. Some large wetland populations may require employing a wetland-adapted vehicle.

While mowing alone is not an effective invasive *Phragmites* control method, it can improve the effectiveness of subsequent herbicide treatments. Most sites could probably be mowed using a knockdown via vehicle or other equipment, while a few may warrant a flail mower or similar equipment. A few of the smaller populations could alternatively be cut with a brush saw. Some of the lakeshore and wetland sites may only be accessible for mowing during the winter.

We identified several populations in this region that could benefit from native habitat restoration to prevent reinvasion following elimination of invasive *Phragmites*. These include the large population at Grassy Point, the ¼ acre populations near US Steel Creek, and the small population near Duluth Haines Road and Highway 53. These five were noted in particular for restoration due to their size and close proximity to sites with high ecological value and the St. Louis River Estuary.

Estimated control cost for region: $309,500-$842,000 over three years

Cost estimation notes

Values presented include three-year estimates of invasive *Phragmites* control (herbicide application and mowing) only; costs of restoration, project administration by contractees, surveillance, equipment, and other expenses are not included. The largest populations in this region may likely require more than three years of control. Coordination with planned vegetation management activities (e.g., state or county highway maintenance) or among organizations could reduce control costs, as only minimal coordination was assumed in developing estimates. The Methods appendix describes our process for estimating costs.

Control of populations under private, county, municipal, and mixed ownership in Saint Louis County make up the bulk of the cost, estimated at $259,500-712,000 over three years. Populations on MNDOT-owned properties could be controlled for $25,000-62,000. Invasive *Phragmites* on other state-owned sites could be controlled for $25,000-68,000.

Possible funding structure

The majority of populations in this region could be controlled with the support of one or more funding sources described in the Costs and funding sources section. With many populations within the Great Lakes Basin, the Great Lakes Restoration Initiative may be a particularly useful source. Funding could be awarded through a state-administered grant program or to regional and local entities directly (see Coordination and networking strategies). Those sources could also fund control on state-owned lands, or agencies could integrate invasive *Phragmites* control with previously planned vegetation management efforts. The rail companies may also be able to integrate invasive *Phragmites* control with their existing maintenance activities.

Training and capacity needs

Partners in invasive *Phragmites* response efforts should be capable of identifying and reporting invasive *Phragmites* and decontaminating equipment, and be aware of herbicide-use and permitting requirements. MNDNR invasive aquatic plant management permits are typically needed for control at lake and wetland sites, and herbicides applied at wet sites must be approved for use in aquatic environments. Additional permissions may also be needed for work done in the Saint Louis River Estuary and Duluth-Superior harbor. Additionally, only MDA-licensed Commercial Pesticide Applicators can be hired to conduct treatments. Control and restoration activities should be specific to each site and necessary equipment may need to be acquired. Only equipment that can be sufficiently decontaminated should be used. Evaluation and reporting of control activities will support effective management. Individuals and organizations participating in invasive *Phragmites* response will need to be able to distinguish between native and invasive *Phragmites* and report populations or know where to submit samples for verification.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Treatment of invasive Phragmites populations in the Duluth port area is well underway thanks to coordination by members of the St. Louis River Alliance.

Central South region

Counties

- Benton
- Kandiyohi
- Meeker
- McLeod
- Sherburne
- Sibley
- Stearns
- Renville
- Wright

Invasive *Phragmites* status

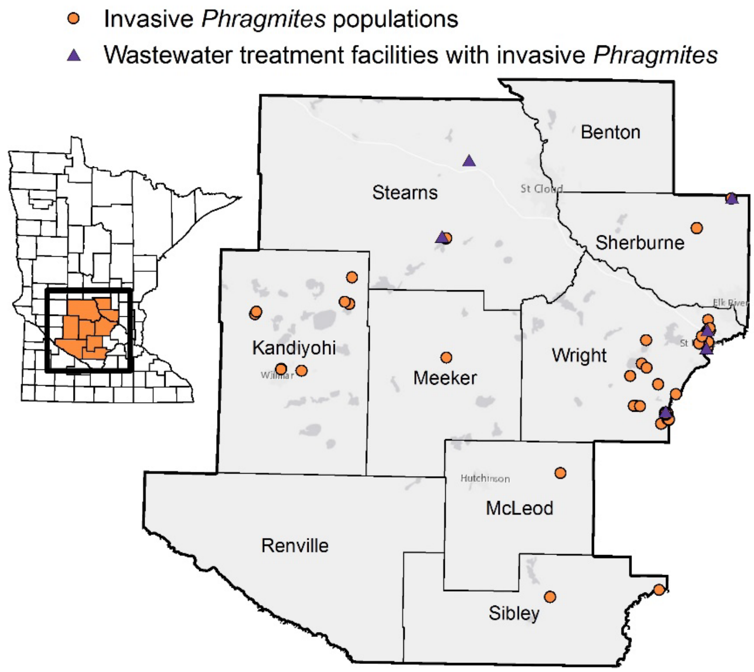
The Central South Region has 64 wild (i.e., non-wastewater treatment) invasive *Phragmites* populations, as well as 6 of Minnesota's 16 wastewater treatment facilities that use or have used invasive *Phragmites* in their operations. Three of these facilities are in Wright County, and many of the wild invasive *Phragmites* populations in the region are situated near them. There are also two invasive *Phragmites*-using wastewater treatment facilities in Stearns County and one in Sherburne County. The majority of populations in this region are along roadsides, in wetlands, and in stormwater ponds with private, state, county, and municipal ownership.

Most populations in this region are <10,000 sq. ft., though the largest population has been estimated to cover approximately 4 acres, making it the largest population in the state; this population is in Kandiyohi County along County Road 40 and extends onto a privately owned wetland. Other relatively large populations in Kandiyohi County include a 1-acre wetland population near Swenson Lake and a ½-acre population along the Glacial Lakes State Trail. Meeker County has a roadside population estimated at approximately 1.5 acres that extends into private land. There are also ½-acre populations in Wright County along Highway 12, including two wetlands under private and municipal ownership and a third wetland near the Princeton wastewater treatment facility in Sherburne County. Kandiyohi County also has a lakeshore population estimated at 10,000 sq. ft. on commercial property near Foot Lake Radio Station.

There are several populations estimated to cover <10,000 sq. ft. There is a single, small population in McLeod County, along Highway 7 near Clouster Lake Wildlife Management Area, extending onto private property. Sherburne County has a 2,400 sq. ft. lakeshore population in Sherburne National Wildlife Refuge. Finally, there are two populations at a cement plant in Stearns County.

Invasive species response capacity

There are CWMAs in Kandiyohi, Meeker, Stearns, and Wright counties. At MNDNR, wildlife managers and aquatic invasive species specialists work out of MNDNR's Central and Southern regions. MNDOT Districts 3, 7, and 8 coordinate state and federal roadside maintenance in this region. Watershed districts also cover much of the Central South Region; including the Buffalo Creek, Clearwater River, High Island Creek, Middle Fork Crow River, North Fork Crow River, and Sauk River Watershed Districts. There are SWCDs and County Agricultural Inspectors in every county, which implement natural resources programs and oversee noxious weed law, respectively. Other, private entities may be willing to contribute some of their own funds towards invasive *Phragmites* control on their properties.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 64*

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.	17		Roadside	16		Private	22
>500 sq. ft. – .25 acre	37		Lakeshore	3		Municipal	17
>.25 – 1 acre	5		Wetland	17		County	5
>1 – 2 acres	1		Mixed	13		Lake	
>2 acres	1		Stormwater pond	10		State	3
Unknown	3		Industrial	2		MNDOT	11
			Riverine			Federal	1
			Other	3		Mixed	5

*This total does not include 6 invasive *Phragmites* populations in use in the operations of wastewater treatment facilities in Wright, Stearns, and Sherburne counties.

Invasive *Phragmites* response options

Invasive *Phragmites* populations in the Central South Region encompass the full range of habitats, sizes, and property ownerships. With several large populations and wastewater treatment facilities using invasive *Phragmites*, successful response will hinge upon continuous collaboration, control coordination, and substantial funding and support. Because invasive *Phragmites* has been found on lands varying across public and private ownership, engaging partners in control activities will be important. Partner participation will also be needed to support coordinated surveillance and reporting for early detection and comprehensive response. Collaboration with the wastewater treatment facilities is also needed.

Most populations could be treated using a truck, UTV, or other vehicle with a mounted tank and hose. A few populations could be treated with a backpack sprayer. The large wetland populations are likely to require a wetland-adapted vehicle, such as a Marsh Master® or similar equipment.

For mowing, which can make subsequent herbicide treatments more effective, most populations could be knocked down using a vehicle or other equipment or cut with a Brush Hog®, flail or forestry mower, or similar machine. A few populations may be small and sparse enough to use a brush saw to cut by hand. Some of the larger wetland populations may require larger equipment, such as a Marsh Master® with an amphibious cutter, for mowing.

Due to the high ecological value of the surrounding site, restoration of the population at Sherburne National Wildlife Refuge should be considered following elimination of invasive *Phragmites* to prevent reestablishment.

Estimated control cost for region: $171,000-$454,000 over three years

Cost estimation notes

All estimates include three-year costs of herbicide application and mowing; costs of surveillance, restoration, project administration by contractees, equipment purchase, and other related expenses are not included. The largest wetland and roadside populations may likely require more than three years of control. Also excluded are costs of implementing alternative dewatering methods in the wastewater treatment facilities (see the Invasive *Phragmites* at wastewater treatment facilities section). Further coordination among organizations or with plant management efforts already being conducted by a given public or private entity (e.g., state or county highway maintenance activities) could reduce costs below these estimates, as only minimal coordination was assumed in cost estimation. The Methods appendix further describes how control costs were estimated.

Populations on private, county, and municipally owned lands could be controlled for: $94,000-255,000 in Kandiyohi County; $30,000-80,000 in Wright County; $13,500-35,500 in Meeker County; $4,500-13,500 in Sibley County; $4,000-12,500 in Sherburne County; and $2,500-3,500 in Stearns County. Populations on MNDOT-owned properties could be controlled for $13,500-40,500 over three years and on the other four state-owned properties for $6,500-10,000. The population in Sherburne National Wildlife Refuge could be controlled for $2,500-3,500.

Possible funding structure

One or more of the funding sources described in the Costs and funding sources section could support control of invasive *Phragmites* populations in this region. Funding could be awarded to regional and local organizations or administered at the state level through grants. Control of populations on federal, MNDOT, or other state-owned lands could be included with populations funded through grants, or by integrating invasive *Phragmites* control with previously planned agency plant management efforts. Some commercial entities in this region may also be willing and able to contribute funds.

Training and capacity needs

Core competencies for invasive *Phragmites* response include the ability to identify the plant, report and evaluate activities, decontaminate equipment, and follow permitting and herbicide use requirements. Entities involved in surveillance must be able to identify invasive *Phragmites* subspecies and report their findings or submit samples for verification. Aquatic approved herbicide formulations will be required for populations in aquatic environments, as will invasive aquatic plant management permits from MNDNR. Contracted herbicide applications can only be conducted by an MDA-licensed Commercial Pesticide Applicator. Partners coordinating and conducting control and restoration activities must be able to determine and implement actions specific to each invasive *Phragmites* site, and support effective response through evaluation and reporting of the results. Specialized equipment may need to be acquired in some cases and only equipment that can be adequately decontaminated should be used.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

The Delano WWTF is one of three WWTFs in Wright County that uses invasive Phragmites.

Southeast region

Counties

- Dodge
- Fillmore
- Goodhue
- Houston
- Mower
- Olmstead
- Wabasha
- Winona

Invasive *Phragmites* status

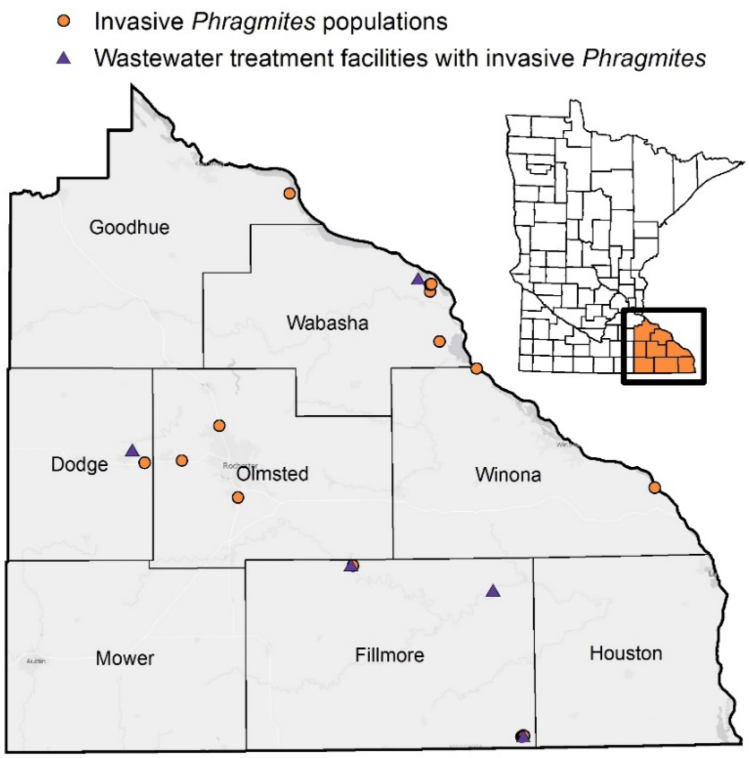
The Southeast Region has 23 verified wild (non-wastewater treatment) invasive *Phragmites* populations and five wastewater treatment facilities using invasive *Phragmites* in their operations: one in Dodge County, one in Wabasha County, and three in Fillmore County. Many of the wild populations are located in wetlands or stormwater ponds or along roadsides near the facilities. While numerous populations in close proximity to wastewater treatment plants are on municipal or county properties, some populations appear to extend onto private properties. The largest population in this region has been estimated at 6,400 sq. ft.; all others are ≤2,500 sq. ft.

Roadside populations identified in this region are along MNDOT-managed highway rights-of-way. There is a small population that extends between MNDOT-managed lands, McCarthy Wildlife Management Area, and lands owned by the Soo Line Railroad Company. Another small population is in a retention pond at the intersection of County Highway 117 and Highway 63. Finally, there is a small population in Frontenac State Park in Goodhue County, which has been treated for the last 2-3 years and will require ongoing monitoring.

Invasive species response capacity

State and federal highway maintenance in this region is coordinated under MNDOT District 6. MNDNR wildlife managers and aquatic invasive species specialists operate out of MNDNR's Southern and Central regions.

There are CWMAs in the Southeast Region in Wabasha, Winona, and Houston counties. This region also contains the following watershed districts: Crooked Creek, Turtle Creek, Bear Valley, Cedar River, Belle Creek, Stockton-Rollingstone-Minnesota City. The Prairie Island Indian Community is also in this region. Every county has a County Agricultural Inspector, who oversees noxious weed laws, and an SWCD, which focuses on natural resources.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 23*

Coverage area	**Number of populations**		**Habitat types invaded**	**Number of populations**		**Property ownership**	**Number of populations**
≤500 sq. ft.	12		Roadside	6		Private	7
>500 sq. ft. – .25 acre	11		Lakeshore			Municipal	6
>.25 – 1 acre			Wetland	13		County	2
>1 – 2 acres			Mixed	1		Lake	
>2 acres			Stormwater pond	2		State	1
Unknown			Industrial			MNDOT	6
			Riverine			Federal	
			Other	1**		Mixed	1

*This total does not include five invasive *Phragmites* populations in use in the operations of wastewater treatment facilities in Fillmore, Dodge, and Wabasha counties.
**This is one of the wetland populations on municipal property in Newburg Township near the wastewater treatment facility, though it is on the far side of a ditch outside the dike.

Invasive *Phragmites* response options

Invasive *Phragmites* populations in the Southeast Region span a variety of habitat types and property ownerships. Adequate funding and coordination among partnering organizations will be critical to controlling the 23 small-to-moderately sized wild populations that have been verified. A few of the sites will be challenging to manage because they have steep slopes or will require navigating deep, wet ditches. Participation from MNDNR and MNDOT for populations on their properties, as well as cooperation from private landowners, will be important. Collaboration with wastewater treatment facilities that have invasive *Phragmites* beds will also be essential for supporting comprehensive efforts. Coordinated surveillance and reporting by partners would support comprehensive response and early detection of new populations.

Most populations could be treated using a tank and hose reel extending from a truck, tractor, or UTV. A few of the larger populations may require the use of a wetland-adapted vehicle. A few populations are small enough that hand wicking could be used to avoid non-target plants.

For this region, knockdown using wetland-adapted equipment would be sufficient to prepare most sites for herbicide treatment. A brush saw could be used for small sites. There are a small number of sites where a flail or other mower or a Marsh Master® may be needed. Knockdown or mowing should not be used alone for control, but can increase the effectiveness of subsequent herbicide treatments.

Due to their proximity to sites with high ecological value, the wetland populations south of N County Road 24 could benefit from restoration following elimination of invasive *Phragmites* to prevent reinvasion.

Estimated control cost for region: $21,000-$42,500 over three years

Cost estimation notes

All estimated costs presented include three years of herbicide treatment and mowing; estimates do not include costs of restoration, project administration by contractees, surveillance, equipment, or other expenses. The costs of converting to alternative dewatering technologies at wastewater treatment facilities are also not included (see the Invasive *Phragmites* at wastewater treatment facilities section). Only minimal coordination among organizations was assumed. Further coordination among partners and/or with concurrent plant management efforts (e.g., state and county highway maintenance) could reduce control costs. More information about how cost estimates were developed can be found in the Methods appendix.

Invasive *Phragmites* populations on private and municipal properties in Fillmore County could be controlled for $7,500-16,500. Wabasha County populations in private and county-owned wetlands could be controlled for $7,000-13,000. Controlling invasive *Phragmites* along MNDOT-managed roadsides is estimated to cost $2,500-6,000. The remaining populations, in Frontenac State Park and a retention pond in Olmsted County, could be controlled for approximately $2,000-3,500 each.

Possible funding structure

The programs described in the Costs and funding sources section could fund invasive *Phragmites* control in this region. Funds could be awarded directly to regional and local entities or administered through a state-level grant program (see Coordination and networking strategies). Management of populations on MNDOT and state-owned lands could be included with others managed through grants, or alternatively controlled in combination with MNDOT's previously planned maintenance efforts. Some private or commercial entities, such as the rail company, may be willing to contribute funds or integrate invasive *Phragmites* control with their own maintenance activities.

Training and capacity needs

There are core competencies for individuals involved in invasive *Phragmites* response, including ability to identify the plant, report and evaluate activities, decontaminate equipment, and follow permitting and herbicide use requirements. Partners will need to be able to distinguish between native and invasive *Phragmites* (or submit samples for confirmation) and report their findings. Control and restoration strategies should be site-specific and specialized equipment may need to be acquired in some cases. Only equipment that can be sufficiently decontaminated should be used. With the majority of populations being located in wetlands, control activities will require permits from MNDNR and managers will need to use herbicide formulations approved for aquatic use. Only an MDA-licensed Commercial Pesticide Applicator can be contracted to conduct herbicide applications. Managers should evaluate and report on control activities to support effective response.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

The Peterson WWTF has four beds containing invasive Phragmites to serve this rural municipality.

South Central region

Counties

- Blue Earth
- Brown
- Cottonwood
- Faribault
- Freeborn
- Jackson
- Le Sueur
- Martin
- Nicollet
- Rice
- Steele
- Watonwan
- Waseca

Invasive *Phragmites* status

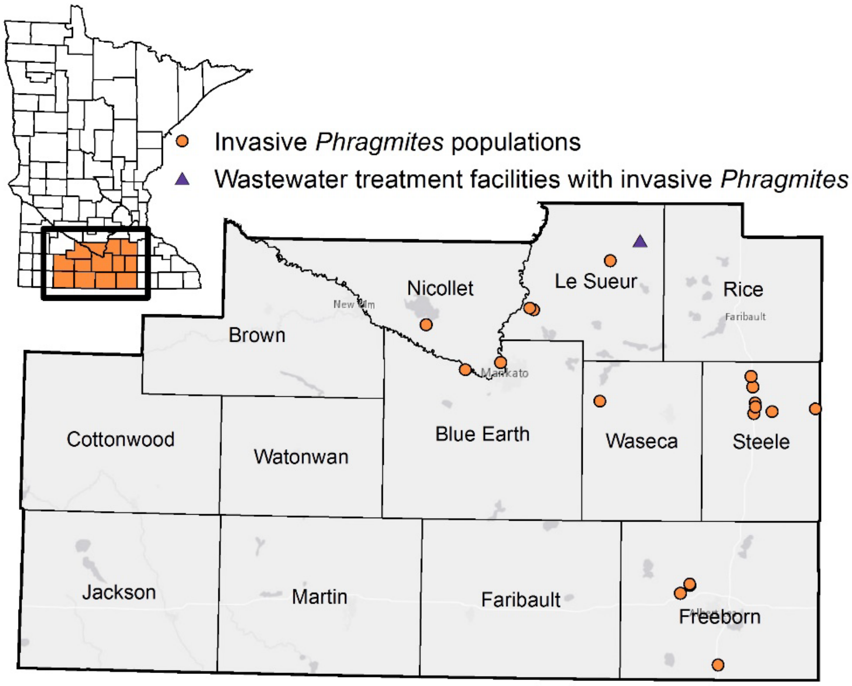
All but a few of the 18 invasive *Phragmites* populations verified in the South Central Region are along roadsides. Most of these roadside populations are on MNDOT-managed highway rights-of-way and a few are along county roads. They range from 120 sq. ft. to 0.4 acres in estimated area. Some populations appear to extend onto private agricultural, residential, and commercial properties. The two largest populations are along Highway 13 and at the Highway 14 and I-169 intersection. One small population borders Swan Lake Wildlife Management Area.

Non-roadside populations are in the wetlands and along the shores of Lake Emily. The larger of the lakeshore populations is estimated to be about one acre and appears to be on private, residential property. The other population is on Ludwig Island in Lake Emily, which is county-owned land. Additionally, a wastewater treatment facility in Le Sueur County uses invasive *Phragmites* in its operations.

Invasive species response capacity

The South Central Region includes MNDOT Districts 6 and 7, through which state and federal highway maintenance is coordinated. Each county also has a roadside maintenance department. MNDNR wildlife managers and aquatic invasive species specialists operate out of MNDNR's Southern region.

This region has several CWMAs, including single-county CWMAs in Rice and Steele counties and a multi-county CWMA encompassing Blue Earth, Brown, Cottonwood, Faribault, Freeborn, Jackson, Le Sueur, Martin, Watonwan, and Waseca counties. There are also several watershed districts, including the Cedar River, Heron Lake, North Cannon River, Shell Rock River, and Turtle Creek watershed districts. Counties also have SWCDs managing natural resources and County Agricultural Inspectors who oversee noxious weed laws.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 18*

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.	6		Roadside	13		Private	8
>500 sq. ft. – .25 acre	10		Lakeshore	1		Municipal	
>.25 – 1 acre	2		Wetland			County	1
>1 – 2 acres			Mixed	3		Lake	
>2 acres			Stormwater pond			State	1
Unknown			Industrial			MNDOT	7
			Riverine			Federal	
			Other	1		Mixed	1

* This total does not include an invasive *Phragmites* population in use in the operations of a wastewater treatment facility in Le Sueur County.

Invasive *Phragmites* response options

With more than half of populations covering relatively moderate to large areas, substantial funding and persistent effort from partners will be needed to eliminate invasive *Phragmites* from the South Central Region. Participation from state and county highway departments will be important for coordinating or allowing control activities, as the majority of populations are along roadsides. The multi-county CWMA could be valuable for surveillance and outreach activities, as well as coordination of control efforts for the lakeshore and wetland populations. Cooperation with the wastewater treatment facility will be needed for comprehensive invasive *Phragmites* response. Participation in coordinated surveillance and reporting from partner organizations would support early detection of new populations and effective response. Entities coordinating control will need permission to access areas where invasive *Phragmites* has extended onto private properties.

Most invasive *Phragmites* populations in this region could be treated using a truck or UTV with a mounted herbicide tank and hose reel. A wetland-adapted vehicle may only be needed for the largest population. A boat is necessary to reach the population on Ludwig Island for both herbicide treatment and mowing.

Mowing could be done for the majority of populations using a flail mower or other mower; knockdown may be sufficient for some of these. The largest population may require larger equipment such as a Marsh Master®. Two populations are small enough that they could be cut using a brush saw. Mowing alone is not effective for controlling invasive *Phragmites* in the long-term but has been shown to make subsequent herbicide treatments more effective.

Estimated control cost for region:
$31,000-$78,000 over three years

Cost estimation notes

Detailed information about how costs were estimated can be found in the Methods appendix. All values presented are three-year estimates of control (herbicide application and mowing) costs, which do not include restoration, project administration by contractees, equipment, surveillance, or other expenses. The largest lakeshore population may likely require more than three years of control. The cost of installing an alternative method for dewatering at the wastewater treatment facility is also not included (see the Invasive *Phragmites* at wastewater treatment facilities section). We assumed minimal coordination among organizations and with other vegetation management efforts (e.g., state and county highway maintenance). Further coordination could reduce control costs.

We estimate $7,000-22,000 would cover three years of herbicide application and mowing of roadside populations under MNDOT ownership. Remaining populations within the boundaries of the multi-county CWMA could be controlled for $19,000-46,000. Private and county-owned sites in Steele County could be controlled for $3,000-7,000 and the population at Rice Lake State Park could be controlled for $2,000-3,000.

Possible funding structure

Invasive *Phragmites* control in this region could be funded through one or more of the programs described in the Costs and funding sources section, through state-administered grants or to regional and local entities directly (see Coordination and networking strategies). Integration with ongoing agency plant management activities being performed at state-owned sites could cover management of invasive *Phragmites*.

Training and capacity needs

Invasive *Phragmites* identification, reporting and evaluation, equipment decontamination, and compliance with permitting and herbicide-use requirements are core competencies for partners involved in response. Those involved in surveillance must be able to identify *Phragmites* subspecies (or submit samples for verification) and report findings. Control approaches should be tailored to each site and specialized equipment may be needed in some cases. Only equipment that can be sufficiently decontaminated should be employed. For wet sites, such as the lakeshore and wetland locations, aquatic-approved herbicide formulations must be used and invasive aquatic plant management permits from MNDNR may be needed. Contracted herbicide applications can only be conducted by an MDA-licensed Commercial Pesticide Applicator. Management activities should be reported and their results evaluated to monitor progress and effectiveness.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Some large populations are likely to have been established for several years.

Southwest region

Counties

- Lac qui Parle
- Lincoln
- Lyon
- Murray
- Nobles
- Pipestone
- Redwood
- Rock
- Yellow Medicine

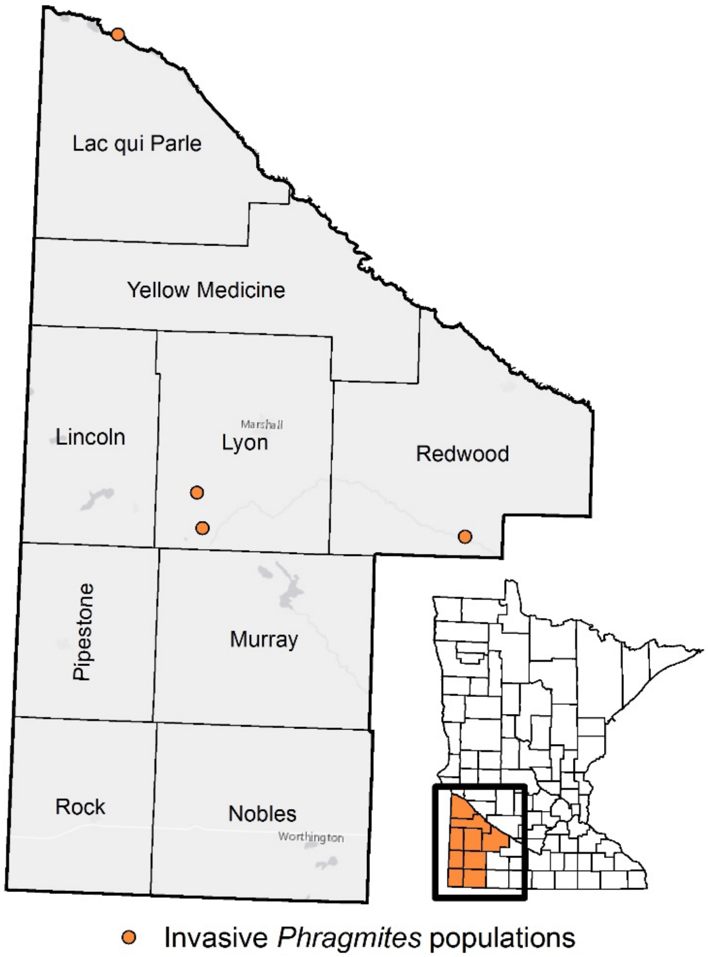
Invasive *Phragmites* status

The Southwest Region has four verified invasive *Phragmites* populations along roadsides and into adjacent wetlands. The largest population is estimated to cover ½ acre in a wetland area in Lac Qui Parle Wildlife Management Area. The second-largest population, estimated at 4,000 sq. ft., is along Highway 23 in Lyon County and may extend between properties owned by BNSF Railway and MNDOT. There is a 3,000 sq. ft. population along Highway 14 in Redwood County, near Lamberton Wildlife Management Area and extending onto private property. This population spans lands with different ownership types (private agricultural land, MNDOT, and MNDNR). The last population, in Lyon County, is estimated to cover 1,600 sq. ft. and is located in a wetland near Highway 14.

Invasive species response capacity

MNDNR wildlife managers and aquatic invasive species specialists operate out of MNDNR's Southern Region. Highway maintenance in the Southwest Region is coordinated under MNDOT Districts 7 and 8. BNSF Railway may have maintenance personnel who manage weeds near their tracks, or who could allow access for such purposes.

There is a single CWMA in this region in Redwood County. In addition, the boundaries of several watershed districts (Heron Lake, Kanaranzi-Little Rock, Lac Qui Parle-Yellow Bank, Okabena-Ocheda, Upper Minnesota River, Yellow Medicine River) cover much of this region. The Upper Sioux Community and Lower Sioux Community are also in this region. County Agricultural Inspectors and SWCDs in each county address noxious weeds and natural resource issues, respectively.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 4

Coverage area	Number of populations	Habitat types invaded	Number of populations	Property ownership	Number of populations
≤500 sq. ft.		Roadside		Private	
>500 sq. ft. – .25 acre	3	Lakeshore		Municipal	
>.25 – 1 acre	1	Wetland	3	County	
>1 – 2 acres		Mixed	1	Lake	
>2 acres		Stormwater pond		State	1
Unknown		Industrial		MNDOT	2
		Riverine		Federal	
		Other		Mixed	1

Invasive *Phragmites* response options

The four populations in this region will require dedicated control efforts to eliminate invasive *Phragmites*. MNDNR staff could coordinate control of the populations in or near state wildlife management areas. They could collaborate with MNDOT and the private landowner for the population adjacent to Lamberton Wildlife Management Area along Highway 14. Collaboration with or permission to access property from BNSF Railway will also be needed. All of the entities listed above may be able to assist with coordinated surveillance and reporting to support early detection and comprehensive invasive *Phragmites* response.

The variability in size and wetness of the sites will warrant different types of equipment. The ½ acre population in Lac Qui Parle Wildlife Management Area could be treated with herbicide using a wetland-adapted vehicle. The Lamberton Wildlife Management Area population may be accessible using a truck or UTV with a tank and hose. Both populations could also be mowed or knocked down using a wetland-adapted vehicle. The remaining populations, located along state-managed roadsides, could be treated from a truck or other vehicle with a tank and hose for herbicide application. Mowing or knockdown could be done with a flail or other type of mower to increase the effectiveness of subsequent herbicide treatments.

Due to the high ecological value of Lamberton Wildlife Management Area and the adjoining property, it would be beneficial to restore the nearby site following elimination of invasive *Phragmites* to prevent reinvasion.

Estimated control cost for region:
$13,000-$28,000 over three years

Cost estimation notes

The populations at Lamberton and Lac Qui Parle Wildlife Management Areas could be controlled for $11,000-21,500 over the course of three years. An estimated $2,500-6,500 would be needed for invasive *Phragmites* control on MNDOT-owned sites in Lyon County. Estimates include three-year costs of herbicide application and mowing only; restoration, project administration by contractees, surveillance, equipment, and other costs are not included. The large population near Lac Qui Parle Wildlife Management Area may likely require more than three years of control. Estimates assume minimal coordination among organizations or with planned vegetation management activities (e.g., state and county highway maintenance); control costs could likely be reduced with further coordination. The Methods appendix further describes how costs were estimated.

Possible funding structure

Control of the invasive *Phragmites* populations on state-owned lands could be funded through integration with planned agency maintenance activities. BNSF Railway may have funding or staff to contribute for the population extending onto their property. Alternatively, organizations could apply for funding through one of the programs described in the Costs and funding sources section. These funds could be awarded through a state-administered grant program or directly to regional and local groups (as described in Coordination and networking strategies. The Minnesota Board of Soil and Water Resources (BWSR) CWMA Grant Program could help increase regional capacity.

Training and capacity needs

Effective response will rely on partners' ability to identify invasive *Phragmites*, evaluate and report response actions, decontaminate equipment, and comply with herbicide use and permitting requirements. Partners involved in surveillance must be able to identify invasive *Phragmites* and report their findings or submit specimens for verification. Wet sites should only be treated with herbicide formulations approved for aquatic use and control activities may require a permit from MNDNR. Contracted herbicide applications may only be conducted by an MDA-licensed Commercial Pesticide Applicator. The use of control approaches and equipment specific to each site (and only equipment that can be sufficiently decontaminated following use), as well as reporting and evaluation of activities, will be needed for effective management.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

The extent of invasive Phragmites appears to be very limited in southwestern Minnesota.

North Central region

Counties

- Beltrami
- Cass
- Clearwater
- Hubbard
- Itasca
- Koochiching
- Lake of the Woods

Invasive *Phragmites* status

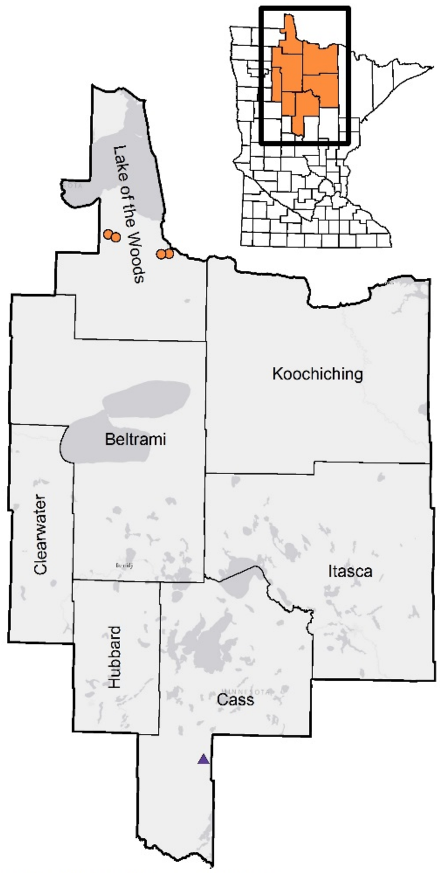
The North Central Region has four verified invasive *Phragmites* populations along Highway 11 and a stretch of railroad in Lake of the Woods County. The largest population is estimated to cover 1,200 sq. ft. There is also a wastewater treatment facility using invasive *Phragmites* in their operations in Cass County.

Railroad corridors appear to facilitate the spread of invasive Phragmites.

Invasive species response capacity

Three MNDOT districts cover this region (Districts 1-3) and the verified invasive *Phragmites* populations are all within District 2. Canadian National Railway may have staff who maintain and remove weeds from the tracks, or could allow access to their property for these purposes.

Itasca County has the only CWMA in this region. There are four watershed districts that work on water-related issues; the boundaries of the Red Lake Watershed District encompass much of Beltrami County and a portion of the Warroad, Wild Rice, and Roseau River watershed districts extend into the western edge of this region. MNDNR aquatic invasive species specialists and wildlife managers operate out of MNDNR's Northwest and Northeast regions. The Bois Forte Band of Chippewa, Leech Lake Band of Ojibwe, and Red Lake Nation have much or all of their lands in this region. The northwestern part of the lands of the White Earth Nation are also in this region. Each county has a County Agricultural Inspector who oversees noxious weed laws and an SWCD that works on natural resources.



Invasive *Phragmites* populations
Wastewater treatment facilities with invasive *Phragmites*

Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 4*

Coverage area	Number of populations	Habitat types invaded	Number of populations	Property ownership	Number of populations
≤500 sq. ft.	2	Roadside		Private	4
>500 sq. ft. – .25 acre	2	Lakeshore		Municipal	
>.25 – 1 acre		Wetland		County	
>1 – 2 acres		Mixed	4	Lake	
>2 acres		Stormwater pond		State	
Unknown		Industrial		MNDOT	
		Riverine		Federal	
		Other		Mixed	

* This total does not include the invasive *Phragmites* population in use at the wastewater treatment facility in Cass County.

Invasive *Phragmites* response options

With collaboration, coordination, and landowner permissions, invasive *Phragmites* could be eliminated from this region with modest effort and funds. The four, relatively small populations identified in Lake of the Woods County could be controlled over the course of a few years. Cooperation with the wastewater treatment facility will be needed as well. Partner organizations could assist with coordinated surveillance and reporting efforts to support early detection and response to new populations. Necessary equipment for control may include a truck or other vehicle mounted with a tank for herbicide and a flail mower or other type of mower to prepare the site for subsequent spraying.

Estimated control cost for region:
$2,000-$3,000 over three years

Cost estimation notes

We assumed herbicide application and mowing would be contracted for all four populations together. Because only minimal coordination was assumed in our estimates, combining invasive *Phragmites* control efforts with other plant management activities, either by the railroad company or MNDOT, could reduce control costs. Values include costs associated with herbicide application and mowing only; costs of surveillance, restoration, project administration by contractors, equipment, and other expenses are not included. Costs of transitioning to alternative dewatering strategies at the wastewater treatment facility are also not included (see the Invasive *Phragmites* at wastewater treatment facilities section). For more information about how costs were estimated, see the Methods appendix.

Potential funding sources

Canadian National Railway or MNDOT could integrate control of the invasive *Phragmites* populations in this region with routine maintenance activities. Alternatively, the programs described in Costs and funding sources could be approached for financial support. The BWSR CWMA Grant Program could help bring additional capacity to this region.

Training and capacity needs

Identification, reporting and evaluation, equipment decontamination, and compliance with herbicide use and permitting requirements are core competencies for invasive *Phragmites* response partners. Those participating in surveillance must be able to identify and report invasive *Phragmites* or submit samples for verification. Managers should be able to determine site-specific control approaches. Only equipment that can be sufficiently decontaminated should be used. Those participating in response efforts should be aware of how to report and evaluate control actions to support response effectiveness. They should also know to use aquatic approved herbicides and acquire permits for work in aquatic environments, and that only MDA-licensed Commercial Pesticide Applicators can be contracted to conduct herbicide treatments.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Northwest region

Counties

- Becker
- Clay
- Kittson
- Mahnomen
- Marshall
- Norman
- Pennington
- Polk
- Roseau
- Red Lake

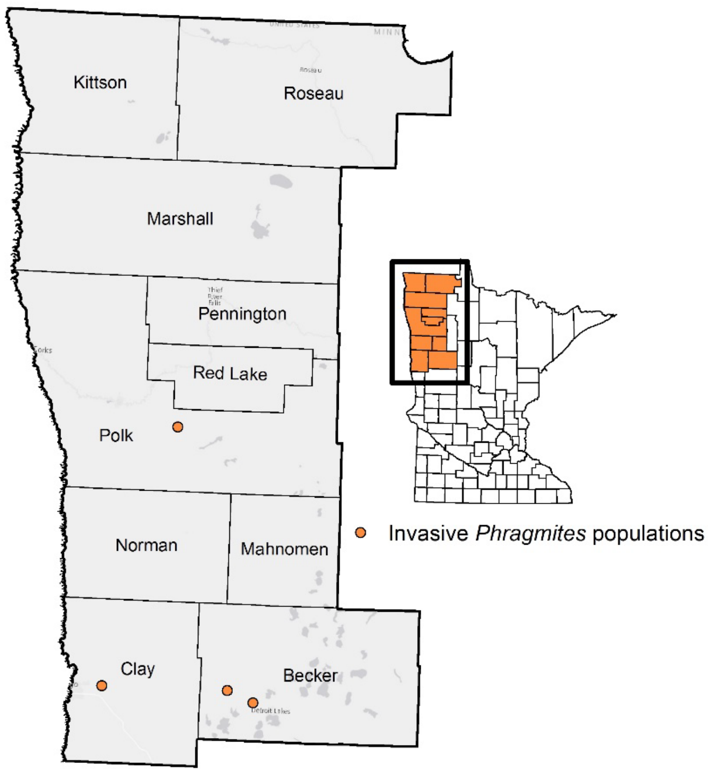
Invasive *Phragmites* status

There are four verified invasive *Phragmites* populations in the Northwest Region. There is a population in Becker County along a MNDOT-owned right-of-way that has been estimated to cover approximately 2 acres—one of the larger populations in the state. A second, small population in Becker County is on private land bordering Highway 10 and Boyer Lake. The third population is also along Highway 10 in Clay County. The last population is within Glacial Ridge National Wildlife Refuge, running linearly along County Road 45 and a BNSF railroad corridor; this is a small population, approximately 200 sq. ft. in size, mixed with native *Phragmites*.

Invasive species response capacity

State and federal highway maintenance is coordinated under MNDOT Districts 2 and 4. The population in Glacial Ridge National Wildlife Refuge involves multiple property ownerships; control will require coordination between the U.S. Fish and Wildlife Service (USFWS), Polk County Maintenance Department, and BNSF Railway. The USFWS has staff dedicated to management of the refuge. The Polk County Maintenance Department conducts vegetation control on their roadside rights-of-way and BNSF Railway may also have staff who work to remove weeds along their railroad corridors, or who would be able to provide property access for control activities.

This region has CWMAs in Becker, Mahnomen, Marshall, Norman, Red Lake, and Roseau counties, as well as the eastern half of Polk County. There are also several watershed districts in the region, including the Buffalo-Red River, Cormorant Lakes, Joe River, Middle-Snake-Tamarac Rivers, Pelican River, Red Lake, Roseau River, Sand Hill River, Two Rivers, Warroad, and Wild Rice watershed districts. MNDNR aquatic invasive species specialists and wildlife managers operate out of MNDNR's Northwest Region. The majority of the White Earth Nation's land is within this region. All counties have an SWCD (Polk County has two, East and West) and County Agricultural Inspector, which work on natural resources and noxious weeds, respectively.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 4

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.	1		Roadside	3		Private	1
>500 sq. ft. – .25 acre	2		Lakeshore			Municipal	
>.25 – 1 acre			Wetland			County	
>1 – 2 acres	1		Mixed	1		Lake	
>2 acres			Stormwater pond			State	
Unknown			Industrial			MNDOT	2
			Riverine			Federal	
			Other			Mixed	1

Invasive *Phragmites* response options

With few populations, elimination of invasive *Phragmites* from the region should be possible with adequate funding, surveillance, and coordination among public and private entities. This region has significant capacity for coordinated surveillance and reporting, through which a broader group of partners than those coordinating control could be involved.

Herbicide treatment of the large population could be conducted using a roadside vehicle with a mounted tank and hose for covering large stands. A flail mower or other equipment could be used to mow or knock down dead stems (mowing can facilitate subsequent herbicide treatments but is not an effective control approached when used alone). Part of this population is located on a steep slope, which could present challenges depending on equipment availability.

The smaller populations are highly manageable and do not yet require sophisticated equipment. Herbicide treatment could be done using a backpack sprayer (or hand wick to avoid native *Phragmites* within the targeted area), and a brush saw could be used in winter to remove dead biomass.

Estimated control cost for region: $33,000-$84,000 over three years

Cost estimation notes

The populations in Becker and Clay Counties are in close proximity along Highway 10 and could be managed under the same contract for approximately $31,000-81,000. The population in Glacial Ridge could be controlled for around $2,000-3,000. Values presented include three-year costs of herbicide treatment and mowing only; costs associated with restoration, surveillance, project administration by contractees, equipment, or other expenses are not included. The largest population may likely require more than three years of control. As only minimal coordination was assumed in developing cost estimates, control costs could be reduced with further coordination among partners or by integrating with concurrent vegetation management efforts, e.g., by BNSF Railway, USFWS, and/or MNDOT staff. More information about how costs were estimated can be found in the Methods appendix.

Possible funding structure

The invasive *Phragmites* populations on federal and state sites could be controlled as part of ongoing plant management activities by agencies, or by BNSF for the population that extends onto their property. Alternatively, control on federal and state sites, as well as privately owned sites, could be funded through one of the programs described in the Costs and funding sources section. Funding could be awarded either through state-administered grants or directly to regional or local organizations (as described in Coordination and networking strategies).

Training and capacity needs

Core competencies for partners involved in response efforts include being capable of identifying invasive *Phragmites*, reporting and evaluation, decontaminating equipment, and awareness of herbicide use and permitting requirements. Individuals capable of distinguishing and reporting native and invasive *Phragmites*, or submitting samples for identification will be needed. Those conducting control should have sufficient expertise to apply site-specific approaches. Specialized equipment may be needed in some cases and only equipment that can be sufficiently decontaminated following use should be employed. Only MDA-licensed Commercial Pesticide Applicators can be contracted to apply herbicides. Partners should also be aware of permitting and herbicide use requirements for activities at wet sites. Effective response can be supported by reporting and evaluation of management activities.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Most populations of invasive Phragmites are identifiable by their dense inflorescences well into winter.

Central West region

Counties

- Big Stone
- Chippewa
- Douglas
- Grant
- Otter Tail
- Pope
- Stevens
- Swift
- Traverse
- Wilkin

Invasive *Phragmites* status

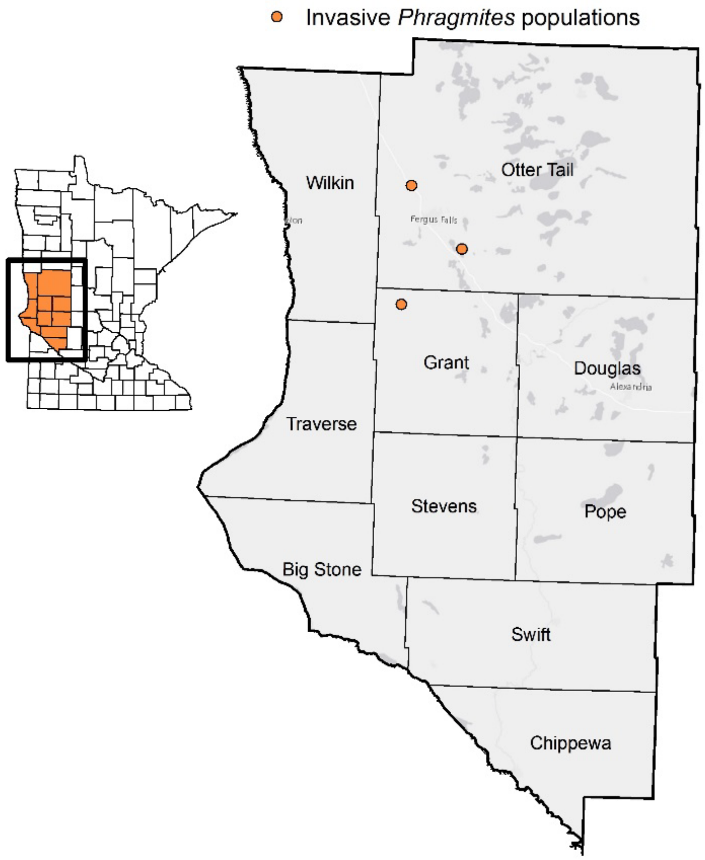
Three invasive *Phragmites* populations have been verified in the Central West Region. Otter Tail County has two populations: a 6,000 sq. ft. population along I-94 and a small roadside population bordering the Central Lakes Trail in the town of Dane Prairie. The last population is in a state-owned wetland in Grant County and is of unknown size.

Invasive species response capacity

State and federal roadside management is coordinated under MNDOT Districts 4 and 8. MNDNR wildlife managers and aquatic invasive species specialists operate out of MNDNR's Northwest and Southern regions.

The Central West Region has several CWMAs, which coordinate with partner organizations to respond to invasive species. There is a single-county CWMA in northeastern Otter Tail County and two multi-county CWMAs in Pope/Swift and Traverse/Big Stone Counties. This region also has a well-developed network of watershed districts, including the Bois De Sioux, Buffalo-Red River, Middle Fork Crow River, North Fork Crow River, Pelican River, Sauk River, and Upper Minnesota River watershed districts. In addition, every county has a County Agricultural Inspector who oversees noxious weed laws, as well as an SWCD, which directs natural resource programs.

The invasive Phragmites population along the Central Lakes State Trail is encroaching on a pocket of remnant prairie which is host to several interesting plant species, including grass of Parnassus.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 3

Coverage area	**Number of populations**		**Habitat types invaded**	**Number of populations**		**Property ownership**	**Number of populations**
≤500 sq. ft.			Roadside	1		Private	
>500 sq. ft. – .25 acre	2		Lakeshore			Municipal	
>.25 – 1 acre			Wetland	1		County	
>1 – 2 acres			Mixed			Lake	
>2 acres			Stormwater pond			State	1
Unknown	1		Industrial			MNDOT	2
			Riverine			Federal	
			Other	1		Mixed	

Invasive *Phragmites* response options

Invasive *Phragmites* could likely be eliminated from this region with relatively modest coordination and funding. Coordinated surveillance and reporting would support early detection and response to prevent further spread.

For all verified populations, herbicide treatment could be conducted using a truck, tractor, or UTV with a mounted tank and hose reel. Mowing or knockdown could be done using a flail mower or other equipment. While mowing alone is not sufficient for control, it can improve the efficacy of subsequent herbicide treatments.

Estimated control cost for region: $6,500-$16,500 over three years

Cost estimation notes

All three populations, which are relatively close to one another and are under shared public ownership (state lands), could be managed for an estimated $6,500-16,500. The largest populations may likely require more than three years of control. The population of unknown size was assumed to be ¼ acre in size for cost estimation purposes. Cost estimates are for three years of herbicide application and mowing activities only and do not account for restoration, project administration by contractees, surveillance, equipment, or other expenses. Increased coordination could reduce control costs, as minimal coordination among organizations and with planned vegetation management activities (e.g., state and county highway maintenance) was assumed in our estimates. The Methods appendix includes further information on how cost estimates were developed.

Possible funding structure

With the three populations being on state-owned lands, control could be integrated into existing state-level plant management activities. Alternatively, the programs described in Costs and funding sources could provide support.

Training and capacity needs

There are some core competencies for response partners, including ability to identify invasive *Phragmites*, report on and evaluate efforts, decontaminate equipment, and comply with herbicide use and permitting requirements. Surveyors must be able to identify and report invasive *Phragmites* or submit samples for verification. Managers must be able to determine control actions appropriate to each site. Only equipment that can be sufficiently decontaminated should be employed. Some of the sites are expected to be wet, requiring the use of herbicide formulations approved for aquatic environments and control permits from MNDNR (though there are exceptions for control activities by MNDNR staff on MNDNR lands). Any herbicide applications for hire may only be conducted by MDA-licensed Commercial Pesticide Applicators. Control actions should be reported and evaluated to support effective response.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Central North region

Counties

- Aitkin
- Crow Wing
- Morrison
- Todd
- Wadena

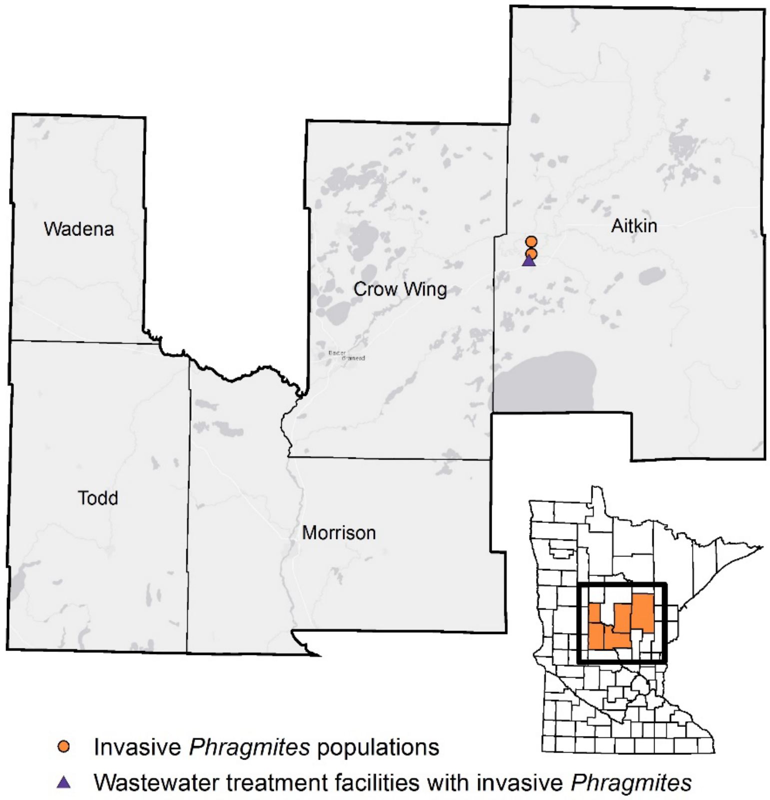
Invasive *Phragmites* status

Two populations have been verified in the Central North Region, both of which are along county roads. The largest of these populations is in Aitkin County, has been estimated to be an acre in size, and appears to extend along County Road 1 onto private agricultural land. Another population in Aitkin County is an estimated 600 sq. ft. in size. There was a wastewater treatment facility using invasive *Phragmites* in their operations in Aitkin County, though the operator at this facility reported that the plant was removed from the operation in 2010.

Invasive species response capacity

County highway departments work to control weeds and conduct other maintenance activities along county-owned roadsides. There is one CWMA in this region in Wadena County that works to control weeds. MNDNR aquatic invasive species specialists and wildlife managers operate out of three regions (Northeast, Northwest, and Central). Highway maintenance in the Central North Region is coordinated under MNDOT Districts 1 and 3. A portion of the Sauk River Watershed District is in the southwest corner of this region. Every county has an SWCD and County Agricultural Inspector, which work on natural resource issues and oversee noxious weed laws, respectively.

Patches of invasive Phragmites occur along nearly 2.5 miles of Cty Rd 1 from the Mississippi River north to 390th St.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 2

Coverage area	**Number of populations**	**Habitat types invaded**	**Number of populations**	**Property ownership**	**Number of populations**
≤500 sq. ft.		Roadside	2	Private	
>500 sq. ft. – .25 acre		Lakeshore		Municipal	
>.25 – 1 acre	1	Wetland		County	1
>1 – 2 acres	1	Mixed		Lake	
>2 acres		Stormwater pond		State	
Unknown		Industrial		MNDOT	
		Riverine		Federal	
		Other		Mixed	1

* This total does not include the invasive *Phragmites* population that was in use in the operation of a wastewater treatment facility in Aitkin County. From conversations with the operator at the Aitkin facility, their invasive *Phragmites* plants were removed in 2010.

Invasive *Phragmites* response options

The two invasive *Phragmites* populations could be eliminated from this region through dedicated control and monitoring efforts, with the larger population expected to require more control effort. Coordinated surveillance and reporting efforts by the entities listed above and others would support early detection and response to new populations.

Suitable equipment for controlling verified populations could include a flail or other mower and a truck or UTV equipped with a tank for herbicide application. Mowing can increase the effectiveness of subsequent herbicide treatments but will not result in long-term control if used alone. Permission to access private property will be needed, at least for the larger of the two populations.

Estimated control cost for region: $11,000-$24,000 over three years

Cost estimation notes

We assumed management of the two populations in Aitkin County would be coordinated under the same contract, estimating a combined cost of $11,000-24,000. As only minimal coordination was assumed, further coordination among partner entities or with county highway maintenance activities could likely reduce control costs. These estimates do not include the cost of implementing alternative dewatering methods at the wastewater treatment facility, should they be needed to remove any residual invasive *Phragmites* propagules (see the [Invasive *Phragmites* at wastewater treatment facilities] section). Values presented include three-year costs of control only; costs of restoration, project administration by contractees, surveillance, equipment, and other expenses are not included. The largest population may likely require more than three years of control effort. For more information about how costs were estimated, see the [Methods] appendix.

Possible funding structure

Organizations at the regional or local level could fund control activities or control could be funded through the programs described in [Costs and funding sources]. The BWSR CWMA Grant Program could help provide additional regional capacity.

Training and capacity needs

Core competencies for invasive *Phragmites* response partners include the ability to identify the plant, report and evaluate activities, decontaminate equipment, and follow permitting and herbicide use requirements. Those participating in surveillance will need to be capable of differentiating and reporting native and invasive *Phragmites*, or know how to submit specimens for identification. Coordinators of control activities must be aware of and follow herbicide use and permitting requirements when applicable. Contracted herbicide treatments can only be conducted by an MDA-licensed Commercial Pesticide Applicator. Determination of control approaches should be site-specific and only equipment that can be decontaminated following use should be employed. Reporting and evaluation of control actions is needed to support effective response.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Northeast region

Counties

- Lake
- Cook

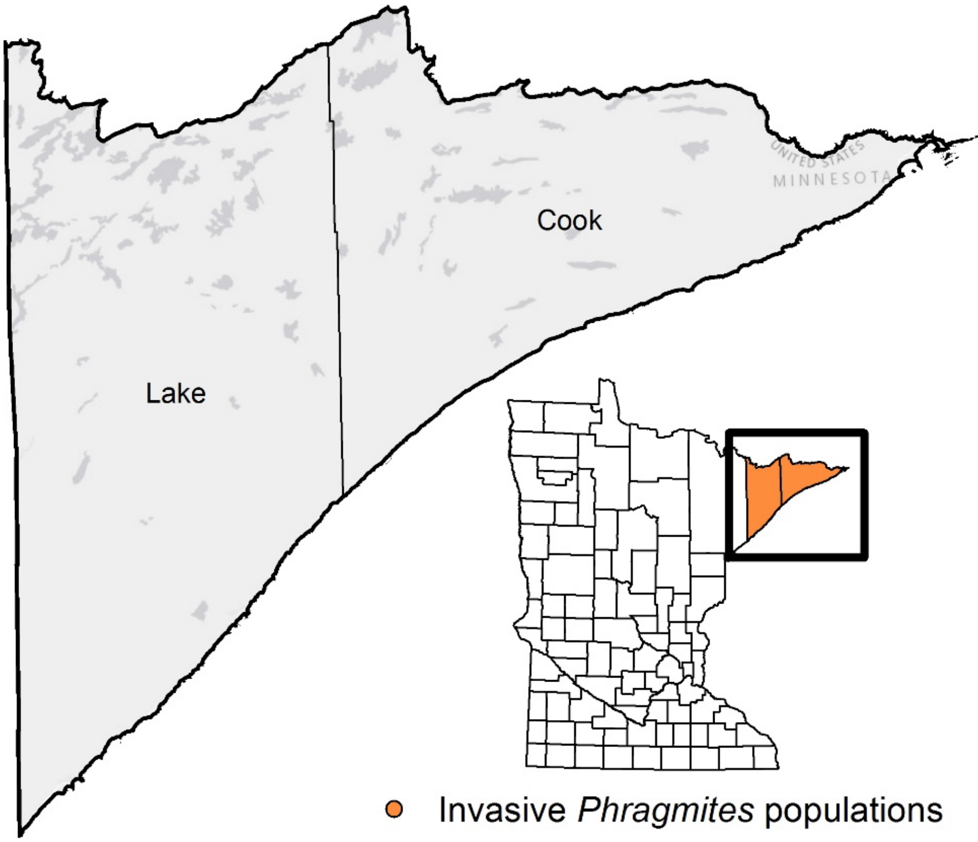
Invasive *Phragmites* status

No invasive *Phragmites* populations have been documented in the Northeast Region to date.

Invasive species response capacity

Both Lake and Cook Counties have CWMAs, which specialize in building partnerships and managing invasive species. This region includes MNDNR's Northeast Region aquatic invasive species specialists and wildlife managers. State and federal highway maintenance is coordinated through MNDOT's District 1. The Grand Portage Band of Lake Superior Chippewa is also in this region. Each county has an SWCD, working on natural resource issues, and a County Agricultural Inspector who oversees noxious weed law.

The vast remote acreages of wetland in the Northeast would be difficult to manage should invasive Phragmites establish in the Northeast Region.



Number of verified invasive *Phragmites* populations of different sizes, habitats, and property ownerships | Total: 0

Coverage area	Number of populations		Habitat types invaded	Number of populations		Property ownership	Number of populations
≤500 sq. ft.			Roadside			Private	
>500 sq. ft. – .25 acre			Lakeshore			Municipal	
>.25 – 1 acre			Wetland			County	
>1 – 2 acres			Mixed			Lake	
>2 acres			Stormwater pond			State	
Unknown			Industrial			MNDOT	
			Riverine			Federal	
			Other			Mixed	

Invasive *Phragmites* response options

While the Northeast Region is fortunate to have no documented invasive *Phragmites* populations, enhanced, coordinated surveillance would support early detection of and response to new reports. Communications with partners in the Saint Louis Region could assist in planning surveillance efforts and preparing response plans for potential populations.

Estimated control cost for region:
None at this time

Cost estimation notes

Some financial support may be needed in the development and implementation of surveillance programs in the Northeast Region. However, we did not estimate surveillance costs in this assessment.

Possible funding structure

While funding is not needed for invasive *Phragmites* control at this time, some of the programs described in the Costs and funding sources section may support surveillance and outreach efforts.

Training and capacity needs

Coordinated surveillance by partners capable of distinguishing and reporting native and invasive *Phragmites* (or ability to submit samples to an expert for verification) will be needed to prevent establishment. Partner organizations should also be aware of invasive *Phragmites* impacts and control approaches and requirements.

Reference sections

- Part II: Potential approaches for invasive *Phragmites* response
- Part III: Planning and networking
- Part IV: Resources for regional response teams

Part 2: Potential approaches for invasive *Phragmites* response

Control approaches for invasive *Phragmites* populations

We conducted a literature review of invasive *Phragmites* management guides and peer-reviewed research. Overall, this synthesis suggests that end-of-summer herbicide treatment (i.e., late August through September) is the most effective and practical approach for controlling invasive *Phragmites* (Kettenring et al. 2015, Peschel 2018). Herbicide treatment will be most effective at this time because invasive *Phragmites* is directing its energy to its roots rather than vegetative growth (MI DEQ 2014). The most effective herbicides are the broad-spectrum herbicides glyphosate or imazapyr, which are also used in combination (Kettenring et al. 2015).

While mowing alone is not effective for controlling invasive *Phragmites*, a winter or summer mow to reduce standing dead stems can facilitate uptake of herbicide. Studies have shown a combination of herbicide treatment with mowing can reduce invasive *Phragmites* cover by 60 to >90% (Back and Holomuzki 2008, Hallinger and Shisler 2009, Moore et al. 2012). These combination control activities (mowing or other site preparation approach plus herbicide treatment) have been shown to be significantly more reliable for controlling invasive *Phragmites* (Figure 4; Peschel 2018).

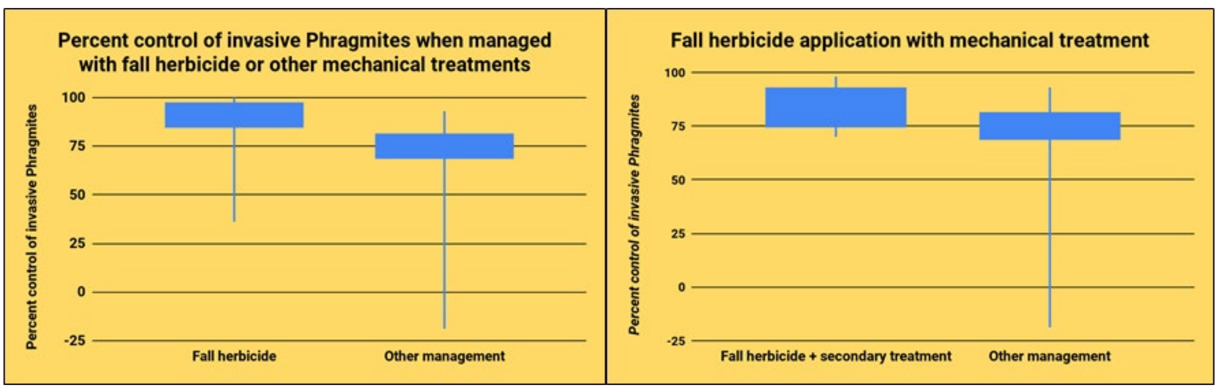


Figure 4. Results of a 2018 MNPhrag literature review conducted by Anna Peschel examining the efficacy of various invasive *Phragmites* control approaches, including fall herbicide treatment (26 studies, median = 94), fall herbicide treatment in combination with site preparation (8 studies, median = 81.5), and management approaches other than herbicide treatment (5 studies, median = 77.6).

It is likely that this control schedule will need to be repeated for three years to eliminate invasive *Phragmites* from most sites, though some sites may require longer-term effort (Farnsworth and Meyerson 1999). Continued monitoring is needed to enable rapid control of regrowth. We recommend five years of post-elimination monitoring at controlled sites, with routine monitoring protocols becoming sufficient after five years. The MNPhrag website further describes how this control approach can be used: www.mnphrag.org. Additional helpful resources include the Kettenring et al. (2015) report to the Utah DNR, the invasive *Phragmites* control guide developed by state agencies in Michigan (MI DEQ 2014), and publications available on the Great Lakes *Phragmites* Collaborative website.

Depending on the characteristics of the targeted site, herbicide treatment and mowing will require various types of equipment. The accessibility and hydrology of the target site, as well as the size and shape of the population, influence the type of equipment needed. For example, a linear roadside population can be readily treated using a hose connected to a tank transported on a truck, tractor, or UTV. A lakeshore population may require treatment from a boat or from shore, depending on the size and accessibility of the population. Large wetland populations may require a wetland-adapted vehicle, or aerial spraying via helicopter in extreme cases. Similarly, for mowing, a small population on a drier site might warrant a brush saw while a large population in a wetland may require employing an amphibious Marsh Master® or other tracked vehicle. Site and population characteristics, and associated equipment needs, determine the effort and costs associated with control. All equipment should be cleaned of plant propagules (including seeds, stems, rhizomes, stolons, and roots) between sites to avoid spreading invasive *Phragmites*. If a particular piece of equipment cannot be sufficiently cleaned, an alternative approach should instead be employed.

Burning, cutting, and water-level management alone have not proven to be effective control methods and can backfire by fueling root growth (van Der Toorn and Mook 1982, Thompson and Shay 1985). However, prescribed burns can be used in combination with herbicide treatment in place of mowing (Moore et al. 2012). Prescribed burning is likely to be more appropriate for populations in rural or undeveloped settings and should only be performed by a trained crew. There are some advantages of burning, including efficient removal of biomass and the potential to stimulate growth of native plants (Ailstock et al. 2001). Mowing or burning should be conducted in the winter or summer, avoiding the period from early March to mid-July when negative impacts to wildlife are more likely (Figure 5). Though flooding is unlikely to effectively control invasive *Phragmites*, it may help prevent reestablishment following reductions through previous years' herbicide treatments (MI DEQ 2014).

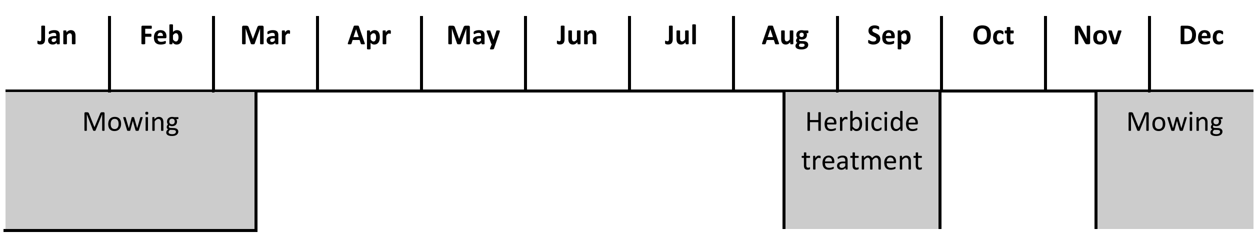


Figure 5. Visual timeline of control and site preparation schedule.

For any invasive plant control activities, key requirements and practices need to be followed to ensure they are effective, responsible, and legal. The "Training" section of this assessment provides further information regarding the best management practices described above. Prior to conducting any control, targeted populations should be verified by an expert as invasive *Phragmites*. For target populations in aquatic environments, a permit is typically needed from MNDNR and any herbicide used must be approved for aquatic use. Contracted herbicide applicators must have the appropriate commercial pesticide applicator license from MDA. Some herbicide formulations, including Habitat® which is a commonly used formulation containing imazapyr, must also be applied by a licensed applicator (either non-commercial or commercial). Organizations opting to conduct their own herbicide treatments should also be trained in appropriate, legal pesticide use. Monitoring and reporting of outcomes of control efforts are needed to verify effectiveness and support adaptive management. Care should be taken to clean seeds and plant fragments from equipment and dispose of plant material so that control activities do not contribute to invasive *Phragmites* spread. Finally, once invasive *Phragmites* appears to have been eliminated from a target site, revegetation or other post-treatment management may be needed to reduce risk of reinvasion.

Invasive *Phragmites* at wastewater treatment facilities

There are 16 wastewater treatment facilities in Minnesota that use or have used invasive *Phragmites* in their operations. Invasive *Phragmites* is used for dewatering biosolids, which are residual organic materials that remain following sewage treatment. The biosolids and invasive *Phragmites* are contained in a "reed bed," where invasive *Phragmites* removes water through evapotranspiration, consolidating the solids and reducing volume. This process is cost-effective for the facilities because it reduces the frequency with which biosolids need to be removed. Volume can be reduced more rapidly in a reed bed than a drying bed lacking water removal via plant transpiration. When early reed beds were constructed, designers assumed that invasive *Phragmites* was incapable of spreading by seed. As invasive *Phragmites* is now understood to produce viable seeds in general (Kettenring and Whigham 2009), including in Minnesota (Bohnen et al., unpublished data), these reed beds are recognized as sources for invasive *Phragmites* spread in Minnesota, and many wild populations are in close proximity to the facilities.

Once a bed is fully consolidated and no further material can be added, the biosolids and plant material must be removed so that operations can continue. MDA issues transport permits so that the solids can be moved to a landfill or applied to agricultural fields. Since the biosolids are nutrient-rich and can aid crop growth, the latter is seen as a beneficial use and is generally less expensive than landfill disposal. However, the biosolids are likely to contain potential propagules when transported. Field applications can only be made at dry sites where agricultural crops will be planted, and there are further restrictions based on proximity to surface waters and groundwater. While conditions at these sites are not optimal habitat for invasive

Phragmites, field-applied sites have not been formally surveyed to ensure that this practice is not contributing to invasive *Phragmites* spread.

Invasive *Phragmites* was recently replaced with the native subspecies at three wastewater treatment facilities in northern Wisconsin. The Treaty Natural Resources Division of the Red Cliff Band of Lake Superior Chippewa conducted a genetic study, which confirmed that nearby wild invasive *Phragmites* populations were related to those in the facilities' reed beds. They then hired a consultant to assess alternative biosolids dewatering strategies. The analysis suggested that removal of invasive *Phragmites* and replacement with the native subspecies would be the most cost-effective and environmentally sound alternative (Table 1). The contracted cost of replacing the beds with native *Phragmites* at all three facilities was ultimately close to $2.8 million, with the bulk of that cost deriving from disposal of the biosolids and plant material (which unexpectedly had to be moved about 80 miles to the nearest operable landfill due to flooding in northern Wisconsin; VanBergen 2019).

Reed beds are used by some smaller municipalities to remove water from sewage sludge, thereby reducing the volume of the biosolids.

Table 1. Summary of findings from Strand Associates, Inc.'s analysis of alternative dewatering strategies for three wastewater treatment facilities in northern Wisconsin. Costs presented are aggregate for all three facilities and were compiled in June 2016. 20-year total present worth includes the transition cost, operations and maintenance costs, replacement, and landfill costs over a 20-year period. The analysis also evaluated and estimated costs associated with transporting the biosolids to another facility for processing; those estimates are not included here as they were highly site-specific. Two of the facilities had four beds with dimensions of 40' x 100' each and the third facility had four beds of 50' x 100' each, for a total of 52,000 sq. ft. of reed beds.

Biosolids Dewatering Alternative	Advantages	Disadvantages	Estimated Transition Cost ($)	Estimated 20-Year Total Present Worth Costs ($)
Native Reed Beds: Sludge loaded to native *Phragmites* beds at slightly reduced rates for dewatering, then landfilled	Closely matches existing technology. Staff comfortable with operations. Similar operational costs.	Limited information on effectiveness. Does not eliminate risk of reinvasion.	1,772,000	3,076,000
Sand Drying Beds: Sludge mixed with polymer as needed, loaded into sand drying bed for dewatering, then land applied	Eliminates risk of reinvasion. Requires little mechanical equipment.	Labor intensive. Operations may be undesirable during, or restricted by, winter or wet weather, reducing available drying time.	3,423,000	4,943,000
Biosolids Thickening: Transfer of sludge to a mixed storage tank with mixer for dewatering, then land applied	Eliminates risk of reinvasion. Requires little mechanical equipment.	Increased waste generation. Increased carbon footprint and costs associated with hauling liquid sludge.	2,243,000	3,940,000
Biosolids Dewatering: Sludge mixed with polymer and a phosphorus-binding chemical, pumped into geotextile tubes for dewatering and eventually moved to a landfill	Eliminates risk of reinvasion. Requires little mechanical equipment.	Requires chemical use. Constraints on winter operations unless design allows operations during freezing conditions.	2,393,000	3,758,000

Transitioning to the use of different plant species would likely be the most cost-effective alternative for facilities in Minnesota as well. Another option for biosolids dewatering is storage in drying beds, which lack plants for enhanced water removal. While drying beds are designed and operated differently than reed beds, reed beds may be able to be operated as drying beds. The specific needs of each facility would determine if this is a feasible option. This approach may require facilities to remove biosolids more often, posing unanticipated costs. Other engineering methods for managing biosolids would entail high construction costs. While the estimated costs in Table 1 above are site-specific, they may provide a sense of the relative costs of different biosolids management strategies.

MNPhrag researchers are currently reviewing scientific literature related to the use and efficacy of various plant species for dewatering biosolids at wastewater treatment facilities, as well as physiological characteristics that could influence their effectiveness. Further research should evaluate the potential species' *in situ* effectiveness and identify short-term strategies to reduce the potential for invasive *Phragmites* spread from the facilities. Alternatives for biosolids dewatering must achieve similar performance to support sound wastewater treatment. Pilot projects testing the efficacy of alternative plant species in reed beds are needed. There may also be variability in practices such that optimal solutions may differ among facilities. An understanding of these practices would allow development of best management practices that could help contain *Phragmites* to the reed beds in the short-term.

Transitioning to an effective alternative for biosolids dewatering at wastewater treatment facilities is an integral part of coordinated, statewide response to invasive *Phragmites*. Reed beds will otherwise serve as sources of further spread and hinder response efforts targeting wild populations. At minimum, thorough, sustained surveillance around the facilities will be needed. Given the relatively limited distribution of invasive *Phragmites* in Minnesota, facilities would ideally shift to an alternative as soon as possible. However, it is critical that wastewater treatment processes are not hampered in the process. Current uncertainties regarding the use of alternatives must be addressed and funding for implementing those alternatives must be identified.

A more practical approach may be to identify funding to support required transition to alternative strategies as existing infrastructure reaches the end of its useful life. While the Minnesota Pollution Control Agency (MPCA) has not stopped construction of new reed beds that would use invasive *Phragmites*, they communicated to facilities' operators in 2013 that invasive *Phragmites* cannot be transported to facilities to be planted according to regulations under the jurisdiction of MDA. Reed bed structures have an expected lifespan of at least 20 years and biosolids and plant material are removed roughly every 4-10 years. Transition to an alternative approach could be required concurrent with updates to the facilities' infrastructure or solids removal (whichever occurs first), pending the identification of reliable alternatives. Possible funding sources to support transitions include the Minnesota Public Facilities Authority's Clean Water Revolving Fund Program (though eligible projects must meet certain criteria and minimum costs), some of the programs described in the Costs and funding sources section of this assessment, or other programs for maintaining and improving infrastructure in the state. Containment, necessary research, and surveillance and control of escapes, should continue in the meantime.

The transportation step of the biosolids management process may require a policy change to prevent invasive *Phragmites* spread as a result of movement and application to land-application sites. If invasive *Phragmites* appears to be spreading from land-applied sites, an MDA policy shift to only allow transport to landfill would be critical to response efforts. However, landfilling material may be more expensive than land application, so additional financial support to facilities may be needed to support this shift. If surveillance near land-applied sites does not suggest this practice contributes to invasive *Phragmites* spread, it may still be considered a viable method for reuse of material.

Solutions supporting coordinated response to invasive *Phragmites* and sound wastewater treatment operations are needed. Efforts to survey land-applied sites, identify effective alternatives, develop interim best practices prior to future transitions, fund transitions, and make appropriate policy changes should be initiated and communicated as soon as possible. For a comprehensive invasive *Phragmites* response, populations of both wild and reed bed invasive *Phragmites* must be addressed.

Part 3: Planning and networking

Coordination and networking strategies

A landscape-scale response to invasive *Phragmites* in Minnesota will require support from individuals and organizations at the local, regional, and statewide levels. Each of these levels is positioned to provide key contributions to response efforts. All levels can engage in education, outreach, and surveillance. For coordinating control and monitoring activities, we describe two possible strategies: 1) a statewide coordination and distribution of funding to regional and local organizations, or 2) organizations and individuals at the regional or local level seeking their own funding from various sources, with support at the statewide level to ensure a comprehensive response.

Under the first strategy, a state agency could administer a grant program to which regional and local entities could apply for funds. Potential sources for the underlying funds for controlling all known invasive *Phragmites* populations in Minnesota could include the Legislative-Citizen Commission on Minnesota Resources (LCCMR), Conservation Partners Legacy Grant Program, the Great Lakes Restoration Initiative (GLRI), or others listed in the Costs and funding sources section of this document or elsewhere. Pending receipt of sufficient funds, the agency could put out its own bids for control. For example, the Wisconsin Department of Natural Resources (DNR) has been coordinating invasive *Phragmites* control using GLRI funds, working with contractors directly. Alternatively, the agency could encourage regional and local entities to apply for the state-administered funding and coordinate control efforts. As identified in the region-specific sections of this report, Minnesota has substantial regional and local organizational capacity which could greatly benefit invasive *Phragmites* response efforts. Partnering with these entities could help ensure effective control and support continued surveillance, which will be critical to reversing invasive *Phragmites* spread. Another consideration is that the entity contracting for control will need to be responsible for the quality of the work completed. That is, the state agency or regional organization coordinating control must be able to supervise projects and monitor and evaluate their results to ensure successful efforts.

The second strategy would rely on regional and local entities providing or applying for funding from various sources to implement control. Locally and regionally, Minnesota is rich with organizations and resources that could lead or serve as partners in invasive *Phragmites* response. These include CWMAs, SWCDs, county programs and staff (such as county agricultural inspectors, natural resource managers, and highway and public works departments), lake associations, watershed districts, municipalities and their natural resource and parks departments, tribal governments, non-governmental and nonprofit organizations, private contractors and businesses, and regional aquatic invasive species (AIS) and wildlife specialists at MNDNR and USFWS. The Costs and funding sources section of this document provides an overview of possible programs that could support invasive species response efforts. Leaders at the regional and local level could develop partnerships to assist with outreach, education, and surveillance; contribute organizational funding or apply for grants; and coordinate, monitor, and evaluate control activities. There are already several organizations at the regional and local levels moving forward with these activities for invasive *Phragmites* response, and there are existing partnerships and networks developed for other natural resources issues that could be leveraged. At the county level, engaged individuals and organizations can work with their government representatives toward noxious weed or invasive species ordinances that could raise awareness and aid in control activities. In this strategy, statewide support would be needed for invasive *Phragmites* response efforts to be comprehensive. Assessment of control activities statewide, through communications with regional and local entities, will be needed to prevent geographic gaps in response efforts.

Regardless of the chosen strategy, multi-level partnerships will be critical in supporting efficiency and progress toward reversing invasive *Phragmites* spread. There are pros and cons for each strategy. For example, it could take longer to start a statewide grant program specific to invasive *Phragmites* response than to launch regional efforts. A combination of these two strategies is another possibility. This is already happening in Wisconsin, as some regional entities have applied for their own funding from GLRI to control invasive *Phragmites* populations in addition to those targeted by the Wisconsin DNR. Combination approaches may create unnecessary competition for grant funding and make it more difficult to achieve high standards of quality assurance. However, it is imperative that response efforts are rallied now, so the optimal strategy must consider how potential partners can best collaborate. If initiation of statewide efforts is delayed due to capacity or organizational issues, support should be provided to regional and local entities for more immediate planning and implementation.

A central, coordinating entity would greatly increase effectiveness of a statewide response, whether state-level or regional and local entities are administering funds and organizing control efforts. This coordination role may best be served through a staff position operating at a statewide level, fostering communication among partners and filling geographic gaps to support comprehensive control across the landscape. Needs for control evaluation and adaptive management could also be served by this role.

The following paragraphs describe key components of a comprehensive response to invasive *Phragmites* in Minnesota, regardless of the overall strategy employed. Cooperation with private landowners, efficient bidding for control activities, and government agency support are essential.

A significant number (around 25%) of known invasive *Phragmites* populations are located on private, individually or commercially owned properties. Successful coordination of invasive *Phragmites* response efforts will require engaging with private and commercial landowners about the detrimental effects of invasive *Phragmites* and the need to prevent its spread, and requesting property access to enable control activities. Ideally, such engagement can build on previous connections to landowners; in the absence of such connections, new relationships will need to be formed. Special contracting or permitting arrangements may need to be developed to foster agreement and collaboration between organizations. Private entities can also assist in invasive *Phragmites* response efforts by providing funds or other resources, educating neighbors, monitoring known populations and reporting suspected new populations, and, in some cases, attending trainings and conducting control activities.

Grouping target populations for permitting and contracting purposes based on proximity and equipment needs can help to increase invasive species response efficiency and reduce costs. MNDNR could issue bulk permits for multiple sites. This would make the permitting process simpler and less cumbersome for those coordinating control. Coordinators should also group populations when requesting bids from contractors, as grouping sites based on location, site characteristics, and equipment needs can make implementation more efficient, thereby reducing costs. Different contractors have different types of equipment available to them, which will also influence project costs. Some large equipment, such as a Marsh Master, may need to be rented out for a period of time, suggesting shared specialized equipment needs as another reason for grouping sites.

Several state agencies and organizations address issues related to noxious weeds and invasive species. Some of these already support noxious weed management by providing funding or hosting training workshops. The following paragraphs describe the roles of state agencies related to invasive *Phragmites* response efforts.

- **MNDNR** regulates invasive aquatic plant management activities and will be integral to response efforts. Depending on capacity, resources, and workload, they could coordinate invasive *Phragmites* control at the statewide level and/or apply for grant funding to be directly or regionally allocated (for example, the Wisconsin DNR has utilized funding from the Great Lakes Restoration Initiative to coordinate invasive *Phragmites* control projects for the past five years). At minimum, MNDNR would need to be involved in processing permits and providing technical assistance for invasive *Phragmites* control projects. They could provide bulk permits that would allow control efforts at multiple sites.

- Many documented invasive *Phragmites* populations in Minnesota are on state and federal highway rights-of-way. **MNDOT** coordinates roadside maintenance activities and could assist with invasive *Phragmites* response efforts by supporting control of verified populations by their staff or contractors. Alternatively, if regional or local entities are coordinating invasive *Phragmites* control projects, MNDOT could assist by providing access to rights-of-way.

- **MDA** is responsible for the states' Noxious Weed Law (Minnesota Statutes, sections 18.75-18.91) and coordinates with County Agricultural Inspectors who oversee local implementation. The MDA commissioner consults with and appoints members to the Noxious Weed Advisory Committee, which develops risk assessments to inform regulation. The categorization of a species on the Noxious Weed List defines how that species is regulated. Invasive *Phragmites* is currently regulated as a "restricted" noxious weed, which means the importation, sale, and transportation of propagating parts is prohibited. Species regulated as "prohibited control" means that effort must be made to prevent the spread, maturation and dispersal of propagating parts. "Prohibited eradicate" classification means that all above and below ground plant parts must be destroyed. Both prohibited control and prohibited eradicate do not allow the importation, sale, and transportation except as allowed by Minnesota Statutes, Section 18.82. The Noxious Weed Advisory Committee could potentially recommend regulating invasive *Phragmites* as prohibited eradicate or prohibited control based on new findings regarding its distribution and reproductive potential in the state. If the commissioner agrees to make this regulatory change, the stricter regulation could aid invasive *Phragmites* response efforts. This listing would increase the authority of County Agricultural Inspectors. Under the current restricted listing, Inspectors cannot require the destruction of existing populations; they can only enforce the prohibition of sale or movement. Under the prohibited listings, Inspectors could require landowners to destroy existing populations, or could have the eradication work done and charged to the landowner if necessary. Communications between MDA and County Agricultural Inspectors could facilitate response efforts. MDA could also possibly host species identification training for Inspectors.

- **MPCA** regulates the operations of wastewater treatment facilities in the state. For the wastewater treatment facilities that use invasive *Phragmites* in their operations, MPCA staff recommendations to facility operators on practices that ensure compliance with Noxious Weed Law and prevent invasive *Phragmites* spread from biosolids dewatering beds are likely to reduce some risks. MPCA staff could assist in identifying and connecting wastewater facilities with potential sources of funding such as Public Facilities Authority funding (a low interest loan program) to transition to another alternative. The MPCA could work with MNDNR and MDA to communicate why it is important these facilities receive funding.

- There are several other statewide organizations that may need to be involved in a landscape-scale invasive *Phragmites* response effort. **BWSR** administers grants to support the development of CWMAs and administers grants and contracts for wetland restoration and reconstruction projects. Several statewide associations that represent the interests of regional and local entities could support response efforts and facilitate communications between the state and regional-local levels, such as the **Association of Minnesota Counties, League of Minnesota Cities, Minnesota Association of Townships, Minnesota Association of Watershed Districts**, and others. State and federal non-governmental and non-profit natural resources organizations could also assist in coordinating and conducting invasive *Phragmites* control projects and providing public outreach.

Training

Knowledgeable participants are needed for successful invasive *Phragmites* response efforts. Managers must be capable of distinguishing between native and invasive *Phragmites*, conducting surveillance for new populations and monitoring known populations, and implementing best management practices for effective control and revegetation. This section describes key competencies for invasive *Phragmites* response related to these areas.

Continuous surveillance for new and undocumented invasive *Phragmites* populations is essential for reducing its spread in Minnesota. Early detection of new populations will make control more effective and less expensive because it can be applied to populations when they cover a smaller area, have a less established seed bank, and contain lower density of belowground structures that can lead to regrowth. Response partners can conduct targeted surveillance based on proximity to known populations. Public outreach can also help expand the network of individuals performing surveillance. There are opportunities to integrate with existing programs for outreach purposes, such as the BWSR Academy, UMN-Extension/MAISRC's AIS Detectors program (several Detectors participants have already been involved in reporting), and others.

It is critical that individuals submitting reports, and especially those planning control activities, be able to differentiate between native and invasive *Phragmites*. There are several publications that support identification, including the MNPhrag Identification Guide. Preliminary data show that observers using this guide achieved 95% accuracy in subspecies identification (relative to genetic testing). Suspected new invasive *Phragmites* populations can be reported online using the Early Detection and Distribution Mapping System (EDDMapS). To prevent destruction of native *Phragmites* populations, it is critical that the identities of all *Phragmites* populations targeted by control activities are verified by an expert as being invasive prior to control implementation. MNPhrag has accepted samples for verification for the past two years.

Determining the appropriate control approach for a given site requires significant expertise. Characteristics of the target population, the type of habitat invaded, the property on which it occurs, and social and cultural concerns all influence decisions related to control. Careful consideration should be dedicated to selecting the most effective control approach within each invasion context. Any removal of emergent vegetation (e.g., invasive *Phragmites* rooted below the ordinary high water line [OHL] in a lake, wetland, or river) using any control approach requires a permit from MNDNR (IAPM or APM) though there are some exemptions for agency staff on their lands). To ensure there are no rare plants or animals at the site that could be harmed by management activities, a data request can be submitted through MNDNR's Natural Heritage Information System. Some of the grant programs described in the section on Costs and funding sources require a Natural Heritage review as part of their application processes.

Practitioners must follow herbicide use regulations designed to ensure treatments are implemented responsibly and minimize non-target impacts. Treatment of populations near water must use herbicide formulations and surfactants that are approved for aquatic use, as some formulations are very harmful to aquatic organisms (Folmar et al. 1979, Relyea 2005, Bringolf et al. 2007). Anyone conducting herbicide applications should be trained in appropriate, legal pesticide use. Any individual hired to conduct herbicide treatments must hold a commercial pesticide applicator license in the appropriate category from MDA. Some herbicide formulations must also be applied by a licensed applicator (either non-commercial or commercial). This includes Habitat®, which is an herbicide formulation containing imazapyr that is commonly used to control invasive *Phragmites*. MDA's website describes the licensing process and different types of licenses and categories. University of Minnesota Extension has a pesticide applicator program that provides comprehensive training and education for applicators.

Reporting and evaluation of control activities will inform future invasive *Phragmites* control projects and facilitate adaptive management. Complete removal of invasive *Phragmites* from a site is expected to take 3-4 years of sustained effort (Farnsworth and Meyerson 1999). Tracking and assessing control activities will help determine if elimination of the target populations can be achieved by the approaches implemented, or if alternative approaches should be considered. Documentation of control activities should include the control (e.g., herbicide treatment) and site preparation (e.g., none, mowing) approaches implemented, equipment used, herbicide formulation and rates used (if applicable), environmental conditions during implementation, dates of implementation, area managed, and difficulties encountered. Documentation of the resulting effects on targeted invasive *Phragmites* will require assessments of population size and density both before and after control activities are conducted. Partners involved in coordinating invasive *Phragmites* response efforts may be best suited to track control activities and their effects. While it is still in development, the Invasive Species Management Tracking System (ISMTrack) is a web-based software being used by University of Minnesota Extension and state agencies in Minnesota and Wisconsin for tracking invasive species control and monitoring activities. Because it is integrated with EDDMapS, invasive populations reported in EDDMapS will appear in ISMTrack and changes in the status of invasive populations will be reflected in both databases, making it a promising tool for planning and evaluating invasive *Phragmites* response efforts. The Phragmites Adaptive Management Framework (PAMF) is another web-based initiative. PAMF uses statistical modeling to assist managers with site-specific control.

Once invasive *Phragmites* has been eliminated from a location, revegetation and restoration activities should begin where needed. Restoration at sites of high ecological value can assist in the recovery of native plants and wildlife habitat. Planting of desirable species in place of invasive *Phragmites* can also help prevent its reinvasion, or colonization by other undesired plants, and stabilize soil. Revegetation efforts are likely to be unsuccessful if invasive *Phragmites* is still prominent at the site. In such cases, revegetation should be delayed until follow-up control activities have eliminated invasive *Phragmites*. Dead invasive *Phragmites* biomass (standing dead stems and litter) will still be present at sites following control activities, possibly mixed in with remaining live stems. This dead biomass can prevent colonization by other undesired plants until all living invasive *Phragmites* has been eliminated; however, it can also hinder regrowth of beneficial native plants from the seedbank (Kettenring et al. 2015). If invasive *Phragmites* is nearly eliminated and the site is bare, inexpensive plantings may help prevent colonization by undesired plants (though there is risk that invasive *Phragmites* will be able to reinvade if the plantings do not take hold). Sites that have been revegetated or restored should continue to be monitored so that reemerging invasive *Phragmites* can be rapidly controlled.

To prevent invasive *Phragmites* spread, clothing and equipment must be properly decontaminated following control and other activities in invasive *Phragmites*-invaded sites. Vehicles, equipment, boots, and clothing should be cleaned prior to moving to another site. Because invasive *Phragmites*' reproductive potential increases with genetic diversity, there is risk that crews moving among sites could increase invasive *Phragmites*' invasibility by acting as unintentional vectors of genetic diversification. If equipment used in herbicide application or mowing cannot be adequately cleaned, it is recommended to employ an alternative approach rather than risk facilitating further spread. The Great Lakes *Phragmites* collaborative website suggests following the [decontamination guidelines] provided by the PlayCleanGo initiative and the Ontario Invasive Species Centre's Clean Equipment Protocol for Industry. MNDNR also has a policy outlining decontamination procedures that must be used by their staff ([MNDNR Operational Order #113]), which could serve as a decontamination guideline for others implementing control activities as well.

Image at right, top: Stem color and the tightness of the leaf sheath are good diagnostic features to distinguish native from invasive Phragmites.

Image at right, bottom: The height of the ligule is another strong diagnostic feature that helps to distinguish native from invasive Phragmites. The ligule in native Phragmites is 1 mm in height. In invasive Phragmites, the ligule is less than 1 mm in height.

Cost and funding sources

A comprehensive approach to invasive *Phragmites* response on a statewide scale will not be attainable without dedicated financial support. Through this assessment, we estimated cost for three years of control of Minnesota's verified invasive *Phragmites* populations to be $818,500-2,019,000 (Table 2). This does not include control and conversion costs associated with the wastewater treatment facilities in Minnesota that currently utilize invasive *Phragmites* in their operations.

Costs of monitoring, restoration and revegetation, equipment, and project administration by coordinators or contractees are additional real costs that we did not attempt to estimate (see Control cost estimations appendix for more information).

Table 2. Summary of verified invasive *Phragmites* populations, acres invaded, and estimated control costs across the 12 regions of Minnesota identified in this assessment.

Region	Number of documented populations	Acres of invasive *Phragmites*	Three year estimated control cost (Low end, $)	Three year estimated control cost (High end, $)
Metro	108	8.4	175,000	301,500
Central East	92	3.7	45,000	145,500
Saint Louis	67	23.0	309,500	842,000
Central South	64	11.1	171,000	454,000
Southeast	23	0.8	21,000	42,500
South Central	18	2.2	31,000	78,000
Southwest	4	0.7	13,500	28,000
North Central	4	0.1	2,000	3,000
Northwest	4	2.3	33,000	84,000
Central West	3	0.4	6,500	16,500
Central North	2	1.0	11,000	24,000
Northeast	0	0	0	0
Total	389	53.7	818,500	2,019,000

While these costs are substantial, it is instructive to compare them to the costs of invasive *Phragmites* control efforts in other states. Over approximately the past seven years, the Wisconsin DNR has spent roughly $700,000 on herbicide treatments to contain invasive *Phragmites* from expanding into western Wisconsin, and an additional $1.6 million for treatments along the Lake Michigan coastline. These figures do not include substantial control grants supporting work by regional partners in eastern Wisconsin, control conducted by GLIFWC in the Lake Superior basin, or treatments supported by the Wisconsin Department of Transportation along state and federal rights-of-way. In Nebraska, the Platte Valley and West Central Weed Management Areas have implemented highly effective invasive *Phragmites* control efforts around the Platte River, with approximately

$5.4 million spent on herbicide application and mechanical control from 2008-2018 (Platte Valley WMA 2019). While these efforts covered a sizeable portion of the state (approximately 43,000 acres around 336 miles along the Platte River), it does not represent all of the invasive *Phragmites* control conducted during that time period. Substantial control efforts have also been conducted along the lower segment of the Platte River, the Republican River, and the upper Missouri River, though cost information was not readily available for those projects (Jeff Runge, *personal communication*). The Maryland DNR has been actively managing invasive *Phragmites* for 25 years. In recent years, typical annual spending on aerial herbicide treatments in critical wetlands has ranged from $75,000-150,000. This is in addition to supplying approximately $20,000 worth of herbicides for licensed state applicators to conduct invasive *Phragmites* control on private lands (Donald Webster and Ned Gerber, *personal communication*).

As with our estimates, these costs from other states do not include staff time and project administration. Due to the extent of invasive *Phragmites* in these states, such efforts will likely need to be continued in some form in perpetuity, depending on management goals and policies. In Minnesota where invasive *Phragmites* is not yet dominant on the landscape, sufficient investment in control now would result in only small expenditures for responding to newly detected populations in the future.

We did not attempt to characterize costs associated with choosing not to respond to invasive *Phragmites* in the state. The costs of invasion are likely to be far beyond current control costs. Estimating the monetary cost of invasion is highly complex, requiring full consideration of the ecosystem services affected (Pimentel et al. 2005, 2006). Such investigation would require a multi-year project. Waiting to implement response until such an investigation were completed would allow invasive *Phragmites* to expand its distribution far beyond the controllable level currently documented and would likely greatly increase overall costs.

Here, several sources of funding are listed that could support invasive *Phragmites* response efforts in Minnesota (Table 3).

The Conservation Partners Legacy Grant Program

The Conservation Partners Legacy (CPL) Grant Program supports restoration projects (up to $575,000 per project in FY2019). Approximately $80 million for the program has been approved annually by the Minnesota legislature since 2009. Eligible applicants include local, regional, state, and national non-profit organizations, including government entities. Most projects are expected to be completed in a 3-4 year period and funded work may only be conducted on public lands or private lands where there is a permanent conservation easement. CPL grants could provide a significant source of funds for control of a few large invasive *Phragmites* populations or many small, distinct populations on public or conservation easement lands within a particular region. Funding for this program comes from the Outdoor Heritage Fund (made up of sales tax revenue which will be available until June 30, 2034 according to the Clean Water, Land, and Legacy amendment). More information about the CPL grant program can be found here.

Minnesota Department of Agriculture Noxious Weed and Invasive Plant Grant Program

MDA has a grant program for control of noxious weeds and invasive plants for which counties, municipalities, and other local government units are eligible. In FY2019, $300,000 was appropriated by the state legislature for this program. Whether or not the program will continue to be funded is currently being negotiated by the legislature. Should the program continue to operate similarly to previous years, applications would be accepted for all listed noxious weeds and Specially Regulated Plants, though Palmer amaranth or other species on the Prohibited-Eradicate Noxious Weed List assume priority. There is a maximum award of $20,000 per applicant. Depending on funding availability and the nature of competing projects, MDA Noxious Weed and Invasive Plant grants could assist with county-level invasive *Phragmites* control efforts on both private and public properties. More information can be found here.

Minnesota Board of Soil and Water Resources CWMA Grant Program

BWSR administers a grant program to support formation of and increase the capacity of CWMAs that can develop partnerships and coordinate control of invasive species. Since FY2014, $200,000 has been appropriated for this program biennially. Previously, SWCDs were the only eligible applicants for this funding. However, the program is now considering watershed districts, counties, and cities, and may consider others in the future (Dan Shaw, *personal communication*). This program may be particularly beneficial for supporting invasive *Phragmites* response efforts where organizational capacity is currently lacking. More information can be found on BWSR's website here.

Minnesota Aquatic Invasive Species Prevention Aid

Since 2014, $10 million has been allocated annually to Minnesota counties to assist in preventing the spread of AIS through the Aquatic Invasive Species Prevention Aid (AISPA) program. The amount allotted to each county is calculated as a function of the number of watercraft trailer launches and watercraft trailer parking spaces. A county-board designee is charged with developing and implementing county-level AIS prevention programs. The county and designee are able to determine how their funding from AISPA is directed, within broad guidelines dictated by Minnesota Statute 477A.19. Outreach, early detection and response, and managing existing AIS populations are all eligible activities that could benefit landscape-scale invasive *Phragmites* response efforts. One limitation is the variability in the amount of funding counties receive from AISPA. Because of the way allocations are calculated, the amount of funding counties receive varies greatly. Some counties are able to support dedicated AIS staff who could be valuable assets in invasive *Phragmites* response efforts, others receive funds sufficient to implement some control projects or raise awareness of invasive *Phragmites*, and other counties receive no AISPA funding. MNDNR's website on AISPA provides more information.

Greater Minnesota Parks and Trails Commission

The Greater Minnesota Regional Parks and Trails Commission distributes funding to support parks and trails through the Parks and Trails Fund (made up of sales tax revenue that will be available until June 30, 2034, per the Clean Water, Land, and Legacy amendment). The Parks and Trails Legacy Plan prioritizes preventing the spread of invasive species and restoring natural communities that have been degraded by invasive species. A number of documented invasive *Phragmites* populations are found in state and regional parks; the Parks and Trails Fund could be used to assist in controlling those populations. Additional information is available here.

Lessard-Sams Outdoor Heritage Council Funding

Funding for restoration projects with costs exceeding $400,000 can be applied for directly from the Lessard-Sams Outdoor Heritage Council (LSOHC). Approximately $100 million was available in this pool for FY2020 from the Outdoor Heritage Fund. LSOHC funds could possibly support invasive *Phragmites* control and large-scale restoration efforts at high-priority sites. More information can be found on the LSOHC website.

Legislative-Citizen Commission on Minnesota Resources

LCCMR is a 17-member group that makes recommendations to the Minnesota legislature for funding special environmental and natural resource projects. These funds come from the Environment and Natural Resources Trust Fund (ENRTF; which will be supported by income from the Minnesota State Lottery and investment income at least through 2024). LCCMR expects $53 million to be available for FY2020 for projects of all sizes that aim to protect, conserve, and enhance Minnesota's natural resources. While requests for LCCMR funding can be highly competitive, these funds could potentially assist with some of the most challenging invasive *Phragmites* control and restoration projects, or be used to support a coordinated response effort to control and monitor invasive *Phragmites* at a regional or statewide scale. More information can be found here.

National Fish and Wildlife Foundation

The National Fish and Wildlife Foundation (NFWF) has many grant programs, some of which support invasive species response efforts. In particular, the NFWF Pulling Together Initiative, which is a partnership with the Bureau of Land Management, USFWS, and U.S. Forest Service, exists to fund invasive plant management efforts by local communities. Approximately $420,000 was available for projects under this program in 2018. The purpose of the program is to help develop partnerships among landowners and plant management experts within a defined weed management area (such as a watershed, landscape, or county) to implement plant control plans and conduct public outreach and education. This program could assist in conducting landscape-scale invasive *Phragmites* response efforts. Another program which may be applicable is the National Wildlife Refuge Friends grant program, which provides funding to "Friends" organizations for projects supporting National Wildlife Refuges. This website has a full list of NFWF programs.

Great Lakes Restoration Initiative

The GLRI funds projects that protect and restore the Great Lakes, which include invasive species control and prevention efforts. GLRI has been allocated approximately $300 million annually for the past five years. The Wisconsin DNR and several regional and local organizations in Wisconsin have and continue to utilize GLRI funding to conduct invasive *Phragmites* control efforts in the Great Lakes basin. GLRI is another funding source that could potentially support regional invasive *Phragmites* response efforts in Minnesota. More information can be found here.

Great Lakes Fish and Wildlife Restoration Act

The Great Lakes Fish and Wildlife Restoration Act (GLFWRA) seeks to encourage cooperative conservation, restoration, and management activities in the Great Lakes Basin. This includes protecting, maintaining, and restoring fish and wildlife habitat, including wetlands. Partially supported by the GLRI, $1.1 million in GLFWRA funding is expected for FY2019. For more information, visit this website.

Minnesota State Department Budget Initiative

Most of the avenues for funding previously listed involve the issuance of grant funds to support relatively short-term projects. However, the challenges associated with invasive species response efforts are expected to be ongoing. A state budget allocation towards noxious weed management could support continuous coordination of statewide response efforts.

Table 3. Summary of funding sources which could support invasive *Phragmites* response efforts in Minnesota. Note: This information originated from the funding organizations' websites and notices of funding opportunities and may be subject to change.

Funding Source	Eligible Applicants	Purpose of Funding	Property Type Restrictions	Minimum or Maximum Award	Annual Appropriation
BWSR CWMA Grant Program	SWCDs, and possibly other local and regional entities	Support formation and increase capacity of CWMAs	N/A	None	$200,000
MDA Noxious Weed and Invasive Plant Grant Program	Counties, municipalities, and other local government units	Control of noxious weeds and invasive plants	None	≤$20,000	$300,000, pending negotiations by the state legislature
NFWF Pulling Together Initiative	Federal, state, local, and municipal government entities, Indian tribes, non-profit organizations, educational institutions	Develop partnerships, implement plant control plans and outreach programs	None	None	$420,000
NFWF National Wildlife Refuge Friends Grant Program	National Wildlife Refuge Friends Organizations	Support projects in National Wildlife Refuges	National Wildlife Refuges	None	$50,000
Great Lakes Fish and Wildlife Restoration Act	Federal, state, and local government entities, Indian tribes, non-governmental and conservation organizations, universities	Encourage cooperative conservation, restoration, and management in the Great Lakes Basin	None	None	$1.1 million

Funding Source	Eligible Applicants	Purpose of Funding	Property Type Restrictions	Minimum or Maximum Award	Annual Appropriation
MN AIS Prevention Aid	Counties	Prevent the spread of aquatic invasive species	None	Dependent on number of watercraft trailer launches and parking spaces per county	$10 million
LCCMR	All with demonstrated fiscal capacity	Fund environmental and natural resource projects	None	None	$53 million
CPL Grant Program	National, state, regional, and local non-profit organizations, including government entities	Support restoration projects	Public lands or private lands where there is a permanent conservation easement	≤$575,000	$80 million
LSOHC	Not specified	Support restoration projects	None	>$400,000	$100 million
GLRI	State, local, and Indian tribal governments, non-profit, for profit, and foreign organizations, foreign public entities, educational institutions	Protect and restore the Great Lakes	Lands within the Great Lake Basin, with some exceptions related to invasive species spread	None	$300 million
Greater MN Regional Parks and Trails Commission	Generally county and municipal governments with some additional groups depending on grant category	Support parks and trails	Some grant categories are only for areas outside the Twin Cities Metro	Dependent on grant category	Unknown

Potential challenges

Responding to invasive *Phragmites* at the statewide scale is an ambitious undertaking that will present many challenges. Some challenges are inherent to landscape-scale invasive species response, such as the long-term nature of the endeavor and momentum and organization needed to spur action (Simberloff et al. 2005, Epanchin-Niell et al. 2010). There are additional challenges driven by the availability of funding and how effort is coordinated and regulated. This section identifies likely challenges associated with responding to invasive *Phragmites* throughout Minnesota so they can be anticipated and overcome.

As described previously, there are many potential partners and funding sources that could support this effort and participation from all levels will provide the best chance for success. There are several state agencies with the ability to assist greatly in responding to invasive *Phragmites*, while the absence of their support would hinder efforts. This is also true for key regional and local organizations. Private landowners are potential partners who, if unwilling to allow access to properties occupied by invasive *Phragmites*, could house continuous sources of reinvasion. Capacity could also be reduced if decision-makers do not consider invasive *Phragmites* response efforts to be eligible for various funding sources. Lack of support in any of these forms would necessitate development of alternative strategies that would likely be more difficult to implement.

The short window of opportunity presented at this stage of invasion, as well as gaps in capacity, will require intensive and organized mobilization efforts up-front. The longer we wait to respond, the more difficult and expensive—and less likely to succeed—control efforts will become. State agencies often have to communicate and evaluate recommended actions broadly prior to implementation and may not be able to immediately participate as a result. In the meantime, regional and local momentum will need to be harnessed and nurtured. In some regions, interested partners will need to be identified and outreach and training programs implemented. Many scenarios will warrant applying for grant funding to support efforts. Equipment needs should also be assessed and addressed. Optimally, organizations with access to specialized equipment would share their equipment with partners under specific operating agreements, particularly if the equipment is not consistently used by the owner organization.

Additional scenarios and activities to consider include the presence of rare species at targeted sites and industry and infrastructure practices. Coordinators of control efforts will need to work with experts if there are sites where invasive *Phragmites* coincides with endangered, threatened, or otherwise rare species. Alternative approaches may need to be generated if traditional management is not permitted. The activities of some industries, such as plant nurseries, gravel suppliers, construction, and others, may unintentionally contribute to the spread of invasive *Phragmites* and other invasive species. Because of this, education, outreach, and enforcement to block these potential invasion pathways must accompany on-the-ground control efforts. This includes development and implementation of alternatives for wastewater treatment facilities in Minnesota currently using invasive *Phragmites* for biosolids dewatering.

Perhaps the greatest challenges associated with statewide invasive *Phragmites* response will be ensuring that control efforts are of sufficient quality and sustaining surveillance efforts. Reversing invasive *Phragmites*' spread will hinge upon those conducting the control work being highly competent and detail-oriented. Individuals conducting invasive *Phragmites* control must employ appropriate and thorough approaches, and understand the severity and opportunity of the issue such that adequate follow-up is provided. Part of employing thorough control includes equipment decontamination and making sure that control efforts do not contribute to spread. Partners coordinating invasive *Phragmites* response efforts can support sound management by holding contractors accountable for their results. Additionally, continued surveillance and early response must be persistent. A strong network of surveyors could best support this. Ongoing monitoring for new populations, and of sites where invasive *Phragmites* has been treated, will help ensure beneficial management outcomes. At the statewide and regional levels, identification and reassessment of 1-, 5-, and 10-year goals could reinforce the need to evaluate progress and maintain long-term surveillance.

Part 4: Resources for regional response teams

About invasive *Phragmites*

Invasive *Phragmites* is a perennial grass that can grow up to 20 feet tall and become dominant in wetlands, lakeshores, roadside ditches, and other wet habitats. In the United States, invasive *Phragmites* and its impacts are widespread throughout New England, the Great Lakes region, the mid-Atlantic, and in western states such as Nebraska and Utah. In Minnesota, fewer than 400 populations have been documented by the MNPhrag project. Most populations have been found in the Twin Cities metropolitan area and around the Lake Superior harbor in Duluth.

The ecological and economic impacts of invasive *Phragmites* are well-documented. It can outcompete and displace beneficial native plant species (Minchinton et al. 2006). It has also been shown to reduce diversity and abundance of fish, waterbirds, and invertebrates (Able and Hagan 2000, Meyer et al. 2010). Because of invasive *Phragmites*' proficiency in taking up water, it can dramatically alter hydrology and transform wetlands into environments resembling drier meadows (Windham and Lathrop 1999). It has also been shown to alter food webs, nitrogen cycling, primary productivity, and greenhouse gas fluxes (Windham and Meyerson 2003, Gratton and Denno 2006, Mozdzer and Megonigal 2013). Economic effects of invasive *Phragmites* involve recreation, commerce, transportation, and agriculture. Invasive *Phragmites* can grow densely along lakeshores, preventing access to lakes and other waterways and reducing property values (as has been shown with other invasive aquatic plants; Horsch and Lewis 2009). It can also obstruct sight lines along transportation corridors (MTO 2015) and compete for wild rice habitat. Invasive *Phragmites* monocultures also burn extremely quickly, presenting a potential public safety concern.

Effective approaches for controlling invasive *Phragmites* must take its basic biology into account. Invasive *Phragmites* can reproduce both sexually (by seed) and asexually (from rhizome, stolon, and stem fragments). While it was previously undocumented, the MNPhrag project has found that invasive *Phragmites* is capable of reproducing sexually in Minnesota's climate, particularly in the southern third of the state. Invasive *Phragmites* is self-incompatible, meaning that sufficient genetic diversity within populations is needed for sexual reproduction; introduction of invasive *Phragmites* from different locations and genetic strains will increase its ability to spread (Kettenring et al. 2010, Kirk et al. 2011). Invasive *Phragmites* flowers in late August and early September. Seeds are developed from September to October. While it will proceed into dormancy following the first frost, seeds can be spread throughout the winter by wind, water, and mechanical means.

Invasive *Phragmites* (*P. australis* subsp. *australis*, as has been referred to throughout this section) should not be confused with the native subspecies (*P. australis* subsp. *americanus*). Distinguishing characteristics include ligule thickness, stem texture and color, density of the flowering head, and others. Consideration of multiple characteristics is needed to reliably distinguish between subspecies. A guide to identifying invasive *Phragmites* can be found on the MNPhrag website. While hybridization between the native and invasive subspecies has been documented in the scientific literature, it is rare and has not been documented in Minnesota.

Invasive *Phragmites* is one of the most studied invasive species in the world (Meyerson et al. 2016). For further information, visit MNPhrag.org.

Appropriate herbicide use

This section provides scientific background for the imperative that anyone applying herbicides is well-trained in appropriate use. We have emphasized throughout this assessment the importance of using aquatic-approved herbicide formulations, as well as the legal requirements for contracting commercially licensed pesticide applicators. These are essential to ensuring that invasive *Phragmites* management activities do not cause unintentional environmental harm.

Terrestrial forms of glyphosate (e.g., Roundup®) contain a surfactant known as polyethoxylated tallowamine (POEA), which is lethal to many forms of aquatic life if applied directly to or near aquatic environments. Surfactants are used to improve herbicide performance. However, low concentrations of POEA have been shown to result in high mortality rates in fish, frogs, and freshwater mussels (Folmar et al. 1979, Relyea 2005, Bringolf et al. 2007). There are aquatic forms of glyphosate available that do not include POEA (e.g., Rodeo®), which are not effective unless mixed with a surfactant that is safe to use in aquatic environments (Annett et al. 2014). There are also special regulatory requirements for some herbicide formulations, including the imazapyr formulation Habitat®, which must be applied by a licensed applicator.

We recommend that anyone applying herbicides for invasive *Phragmites* control possess either a commercial or non-commercial pesticide applicator's license with aquatic certification. Pesticide applicators' licensing is designed to ensure that practitioners are knowledgeable about safe usage practices. Without training, it can be difficult to know which formulations of herbicides to use or the proper amount to apply and, more generally, how to conduct treatments safely and effectively. By law, anyone contracted to conduct herbicide treatments must hold a commercial pesticide applicator license. Comprehensive training and education programs are provided by the University of Minnesota-Extension pesticide applicator program.

Disposal and decontamination

Properly decontaminating equipment and disposing of plant material will be another crucial component of invasive *Phragmites* response efforts. Decontamination and disposal can be time and labor intensive but are needed to prevent management activities from contributing to further spread. After all, regeneration and establishment of new populations is possible from nearly all parts of invasive *Phragmites* (Packer et al. 2017). As described in the Training section of this assessment, there are several resources that provide instruction on how to decontaminate clothing and equipment. The most important thing is to remove all propagules between sites. Disposal of material, if needed, can be more difficult. While large amounts of biomass from well-established populations may need to be managed in some way to facilitate revegetation, material effectively treated with herbicide should no longer be viable and typically should not need to be removed. Some situations that would require disposal are the transitioning of invasive *Phragmites*-using wastewater treatment facilities to alternative dewatering strategies (VanBergen 2019), or where standing invasive *Phragmites* hampers other industrial activities. In some cases, managers or coordinators of control may choose to remove seed heads to prevent dispersal while waiting for the right time of year to conduct treatments. MDA provides recommendations for disposal of noxious weeds. They recommend leaving invasive plant material on site to prevent unintended spread. Burning the material may be the simplest approach for removing biomass, though this is not always feasible depending on the location of the site, its proximity to developed and natural areas, and regulations and permitting requirements. Alternatively, with a permit, it may be possible to carefully contain and transfer material to one of the approved disposal locations listed on MDA's website.

Further resources

General

- MNPhrag Annotated Bibliography on invasive *Phragmites* invasion biology, impacts, and control
- Great Lakes *Phragmites* Collaborative

Surveillance and reporting

- MNPhrag *Phragmites* Identification Guide
- Early Detection and Distribution Mapping System (EDDMapS)

Control recommendations and response planning

- USFWS and California Invasive Plant Council's "Land Manager's Guide to Developing an Invasive Plant Management Plan"
- MNPhrag Management Recommendations
- Invasive Species Management Tracking System (ISMTrack)
- UMN Pesticide Safety Training
- MDA Pesticide Applicator Licensing

Restoration

- How to Restore *Phragmites*-invaded wetlands (Utah State University, Utah Wildlife Resources and Forestry, Fire & State Lands Divisions):
- Restoring the Marsh: *Phragmites* removal and monitoring (Michigan Sea Grant)

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Photo credits

- **Figure 1b.** Secretive marshbirds Photo by ethan.gosnell2 / CC BY-SA 2.0; Mummichogs Photo by Northeast Coastal & Barrier Network / CC BY-SA 2.0
- **Figure 1d.** Photo by Heidi Springborn, provided by Brock Woods, Wisconsin Department of Natural Resources
- **Herbicide treatment photos:** Brandon Van Tassel
- **All other photos:** Julia Bohnen, University of Minnesota

Links

Part 1: Regional assessments of invasive *Phragmites* response needs

General

- Minnesota DOT districts: http://www.dot.state.mn.us/information/districts.html
- Minnesota DNR aquatic invasive species specialists: https://www.dnr.state.mn.us/invasives/ais/contacts.html
- Minnesota DNR wildlife managers: https://www.dnr.state.mn.us/areas/wildlife/index.html

Central East region

- Chisago-Lindstrom Lakes Association: https://clla-lakes.com/

Part 2: Potential approaches for invasive *Phragmites* response

Control approaches for invasive *Phragmites* populations

- Great Lakes *Phragmites* Collaborative website: https://www.greatlakesphragmites.net/resources/factsheets-guidelines/

Part 3: Planning and networking

Training

- MNPhrag Identification Guide: https://www.maisrc.umn.edu/identifying-phragmites
- EddmapS: https://www.eddmaps.org/
- MNDNR Invasive Aquatic Plant Management permits: https://www.dnr.state.mn.us/invasives/iapm.html
- MNDNR Aquatic Plant Management permits: https://www.dnr.state.mn.us/apm/index.html
- MDA website on licensing process: https://www.mda.state.mn.us/pesticide-fertilizer/pesticide-applicator-licensing
- University of Minnesota Extension's pesticide applicator program: https://extension.umn.edu/pesticide-safety-and-certification/private-pesticide-applicators
- Invasive Species Management Tracking System: http://www.ismtrack.org/index.cfmPhragmites Adaptive Management Framework: https://www.greatlakesphragmites.net/pamf/

- Great Lakes Collaborative decontamination guidelines: http://www.greatlakesphragmites.net/resources/factsheets-guidelines/
- MNDNR Operational Order #113: https://www.dnr.state.mn.us/invasives/dnrlands.html

Cost and funding sources

- The Conservation Partners Legacy Grant Program: https://www.dnr.state.mn.us/grants/habitat/cpl/index.html
- Minnesota Department of Agriculture Noxious Weed and Invasive Plant Grant Program: https://www.mda.state.mn.us/plants-insects/noxious-weed-and-invasive-plant-grant
- Minnesota Board of Soil and Water Resources CWMA Grant Program: http://www.bwsr.state.mn.us/grants/cwma/CWMA.html
- Minnesota Aquatic Invasive Species Prevention Aid: https://www.dnr.state.mn.us/invasives/ais/prevention/index.html
- Greater Minnesota Parks and Trails Commission: https://www.gmrptcommission.org/
- Lessard-Sams Outdoor Heritage Council Funding: https://www.lsohc.leg.mn/index.html
- Legislative-Citizen Commission on Minnesota Resources: https://www.lccmr.leg.mn/
- National Fish and Wildlife Foundation Pulling Together Initiative: https://www.nfwf.org/pti/Pages/home.aspx
- National Wildlife Refuges: https://www.nfwf.org/refugefriends/Pages/home.aspx
- Full list of National Fish and Wildlife Foundation programs: https://www.nfwf.org/whatwedo/programs/Pages/home.aspx
- Great Lakes Restoration Initiative: https://www.glri.us/index.php
- Great Lakes Fish and Wildlife Restoration Act: https://www.fws.gov/midwest/fisheries/glfwra-grants.html

Part 4: Resources for regional response teams

About invasive *Phragmites*

- Guide to identifying invasive *Phragmites*: https://www.maisrc.umn.edu/identifying-phragmites
- University of Minnesota-Extension pesticide applicator program: https://extension.umn.edu/pesticide-safety-and-certification/private-pesticide-applicators
- MDA recommendations for disposal of noxious weeds: https://www.mda.state.mn.us/plants/pestmanagement/weedcontrol/disposalnoxweed

Further resources

- MNPhrag Annotated Bibliography on invasive *Phragmites* invasion biology, impacts, and control: https://www.maisrc.umn.edu/phraginvasion-biology
- Great Lakes *Phragmites* Collaborative: https://www.greatlakesphragmites.net/
- MNPhrag *Phragmites* Identification Guide: https://www.maisrc.umn.edu/identifying-phragmites
- Early Detection and Distribution Mapping System (EDDMapS): https://www.eddmaps.org/
- USFWS and California Invasive Plant Council's "Land Manager's Guide to Developing an Invasive Plant Management Plan":

https://bugwoodcloud.org/mura/mipn/assets/File/USFS/2019%20Invasive%20Plant%20Mgmt%20Planning_BMP_USFWS.pdf

- MNPhrag Management Recommendations: https://www.maisrc.umn.edu/phrag-management
- Invasive Species Management Tracking System (ISMTrack): http://www.ismtrack.org/index.cfm
- UMN Pesticide Safety Training: https://extension.umn.edu/safety/pesticide-safety-and-certification
- MDA Pesticide Applicator Licensing: https://www.mda.state.mn.us/pesticide-fertilizer/pesticide-applicator-licensing
- How to Restore *Phragmites*-invaded wetlands (Utah State University, Utah Wildlife Resources and Forestry, Fire & State Lands Divisions): https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1001&context=uaes_pubs
- Restoring the Marsh: *Phragmites* removal and monitoring (Michigan Sea Grant): http://www.miseagrant.umich.edu/files/2012/11/12-720-phragmites-fact-sheet.pdf

Appendices

Methods

MNPhrag surveillance efforts

Due to the ease with which invasive *Phragmites* spreads along road corridors, surveillance of roadsides was determined to be an efficient means to assess distribution of invasive *Phragmites* in Minnesota. MNPhrag staff made nine separate trips covering many major roads throughout the state in an effort to detect invasive *Phragmites* along likely corridors, including state and county highways, secondary roads along railroad corridors, and in the vicinity of each of the 16 wastewater treatment facilities using invasive *Phragmites* in dewatering basins. In addition, routes to and from a subset of sites distributed across the state where invasive *Phragmites* leaf tissue and seed heads were collected (samples were collected three times during the project) were varied to add additional roadsides to the search effort. MNPhrag staff conducted some level of roadside surveillance in 80 of 87 Minnesota counties, driving more than 11,000 miles from September 2017 to May 2019.

MNPhrag staff also engaged 173 citizen volunteers or agency staff as observers to assist in the search for and documentation of populations of invasive *Phragmites* throughout Minnesota. All observers were sent a kit with a MNPhrag identification guide and instructions for submitting samples for expert identification. Plant samples and/or reports were submitted by 55 individuals. MNPhrag staff gave many presentations on invasive *Phragmites* to citizens, contractors, and county, state, and federal natural resource professionals at conferences, workshops, and pesticide recertification trainings. More than 500 individuals were reached through these presentations.

MNPhrag staff and other observers have provided some level of surveillance in 94% of Minnesota Counties over the project period from July 2017 to May 2019. Only 5 counties had no documented surveillance effort by MNPhrag staff or observers during the project period (Figure 6).

The size of each population, as reported in the region-specific sections of this assessment as well as the table in Locations of and basic information about documented invasive *Phragmites* populations, was estimated based on visual assessment upon visiting the site, reports in EDDMapS, or calculation of area from aerial imagery (Table 4). A description of the habitat invaded was also reported as part of our surveillance efforts (Table 5).

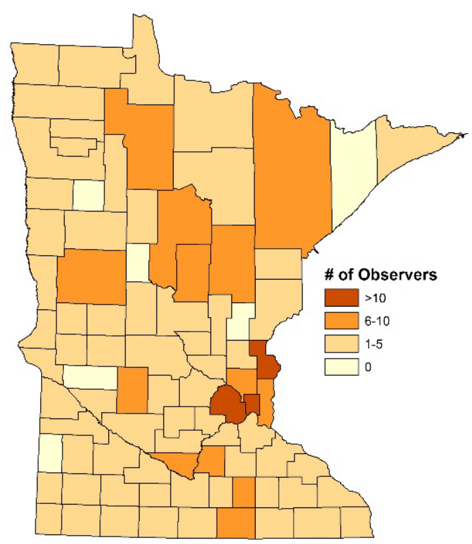

Figure 6. The number of observers contributing to MNPhrag surveillance efforts across Minnesota’s 87 counties, including MNPhrag staff surveillance and citizen and agency staff observers.

Table 4. Summary of sizes of verified invasive *Phragmites* populations in Minnesota.

Area Invaded	Number of invasive *Phragmites* populations
≤500 sq. ft.	156
>500 sq. ft. – ¼ acre	189
>¼ – 1 acre	28
>1 – 2 acres	4
>2 acres	2
Unknown	10

Table 5. Summary of habitats invaded by verified invasive *Phragmites* populations in Minnesota.

Habitat Invaded	Number of invasive *Phragmites* populations
Lakeshore	129
Roadside	98
Wetland	66
Mixed	52
Stormwater pond	26
Industrial	7
Riverine	5
Other	6

This assessment includes all invasive *Phragmites* populations documented and verified as of May 5, 2019. While there are undoubtedly invasive *Phragmites* populations in the state that have not yet been verified, surveillance efforts thus far provide an understanding of the plants' distribution in the state sufficient to support an effective landscape-scale response. Capacity for surveillance has increased statewide as a result of MNPhrag's outreach and will continue to improve with a concerted response effort from partner organizations.

Property ownership determination

Ownership of invasive *Phragmites*-occupied sites was determined using county-managed parcel data (either in ArcMap or web-based GIS interfaces or through conversations with county staff) acquired in late 2018, and used to categorize parcels as private, municipal, county, lake, state, MNDOT, federal, or mixed (Table 6). Our *Phragmites* data include coordinates, rather than polygons or areas, so there may be cases where a single population spans multiple ownership categories. We tried to categorize these populations as "mixed," though there may be other populations that span multiple ownerships. There were also some populations where parcel information was not available, primarily along roadsides or in and around lakes. The ownership category for these populations was assumed based on the type of roadway (state, county, or municipally managed) or the ownership category of the nearest adjacent parcel. Participants in invasive *Phragmites* response efforts should be certain of property ownership and acquire all necessary access permissions prior to implementing control.

Table 6. Summary of property ownerships where invasive *Phragmites* populations in Minnesota have been verified.

Property ownership category	Number of invasive *Phragmites* populations
Mixed	105
Private	96
MNDOT	75
Municipal	49
County	25
Lake	22
State	16
Federal	1

Identification of potential partners

The lists of potential partners in the regional "Invasive Species Response Capacity" sections were identified based on web-based investigation and personal communications. We tried to include all Tribes, CWMAs, SWCDs, watershed districts, County Agricultural Inspectors, and MNDOT and MNDNR operating units in each region. Lake organizations, non-individual private entities, federal agencies, and county highway maintenance departments were listed if invasive *Phragmites* has been documented in and around their properties. Other types of organizations that are already coordinating or conducting invasive *Phragmites* response efforts were also recognized if we were aware of them. Given this approach to identifying potential partners, we are likely to have missed other entities with capacity and interest in participating. Regional and local entities may be able to identify these additional partners, expanding capacity and networks beyond the groups described in this assessment; we apologize for any omissions, which were unintended.

Development of regional response options

We developed a list of control and site preparation approaches that could be used to manage invasive *Phragmites* in Minnesota and associated all documented populations with the approaches we anticipated would be most appropriate. Table 7 lists the control and site preparation approaches identified. The regional response options sections summarize the predominant control and site preparation approaches assigned to populations in each region. Managers may, and should when appropriate, choose to depart from the approaches described based on a more thorough knowledge of site conditions. The ability to decontaminate equipment to avoid facilitating invasive *Phragmites* spread should also be considered when determining a control approach.

Table 7. The control and site preparation approaches identified which may be used in controlling invasive *Phragmites* in Minnesota.

Control Approach Number	Habitat Type and Site Information	Control Approach Description
1	Lakeshores, lake, or riverine	Apply herbicide from boat with tank and hose
2	Lakeshores, lake, or riverine	Apply herbicide from land with backpack
3	Lakeshores, lake, or riverine	Apply herbicide from land with ATV and tank
4	Roadside, reachable with hose, wet	Apply herbicide with hose from tank on truck
5	Roadside, reachable with hose dry	Apply herbicide with hose from tank on truck
6	Roadside or vehicle accessible; square/non-linear shape; wet; too far for hose	Apply herbicide using truck, tractor, or UTV with mounted tank with hose reel; leave roadside to treat stems
7	Roadside or vehicle accessible; square/non-linear shape; dry; too far for hose	Apply herbicide using truck, tractor, or UTV with mounted tank with hose reel; leave roadside to treat stems
8	Wetland	Apply herbicide from tank on dry ground, dragging hose into wetland
9	Wetland	Apply herbicide with backpack sprayer
10	Wetland	Apply herbicide using a wetland-adapted vehicle with a large tank into the wetland
11*	Wetland; large non-linear population	Apply herbicide via helicopter
12*	Not too wet, chemicals undesirable	Physical removal or scrape
13	Dry; small or sparse stand	Apply herbicide with backpack sprayer or hand wick
Site Prep Approach Number	**Site Prep Approach Description**	
1	Winter knock down	
2	Brush saw	
2a*	Underwater brush cutter	
3	DR mower	
4	Forestry mow/brush hog	
5	Tractor with flail or sickle mower	
6	Marsh Master with amphibious cutter	
7	Mowing/knockdown not necessary (e.g., sparse or young populations)	

*These approaches were initially identified as being potentially useful for invasive *Phragmites* control in the state, though they were ultimately not assigned to any populations. There may still be situations where these approaches would be applicable or preferable, based on social and environmental considerations unknown to us.

Control cost estimations

The cost estimates in this assessment were developed based on cost information solicited from contractors and past contracts and available information about invasive *Phragmites* populations documented to date.

For each approach described in Table 7, cost information was solicited from eight entities, including both companies that perform vegetation management (contractors) and organizations that have contracted related work (clients). To be respectful of respondents' time, we accepted cost information in the form that was easiest for them to provide. Some individuals provided information from past projects they had been involved in, from which cost per acre was calculated. Others provided general per-acre cost estimates for the various control and site preparation approaches. From others, we requested costs for controlling multiple invasive *Phragmites* populations at specific locations likely to require similar management approaches. These multi-site costs were requested to account for contractors' administration and mobilization. The cost information received can be found in Figure 7.

We then used the cost information we received to assign control costs to the populations. Generally, populations were grouped together and given an overall cost estimate when there were multiple populations that could be controlled with the same approaches in a region, with similar ownership of sites or likely coordinators of control. Grouping populations in this way assumes some level of coordination as described in Coordination and networking strategies, with further assumptions described below. In some cases, there were populations that were not grouped because of a unique combination of location, equipment needed, and property ownerships. We predominantly used the multi-site cost information to assign cost estimates, assuming that these data better represented the costs associated with implementation. Costs were scaled to the total area of the target populations in each group. For very small sites, mobilization constituted the bulk of the cost. All estimates included a minimum and a maximum to account for the range in cost information provided by different contractors. All minimum and maximum values were above $400-600 for control and $300-400 for site preparation. To develop regional-level costs, the sum of the control and site preparation costs for all regional populations was then multiplied by three (and rounded to the nearest $500), assuming that the average population would need to be managed over the course of a three-year period. That is, regional control cost estimates include the costs of implementing herbicide treatment and site preparation once annually for three years for all documented populations.

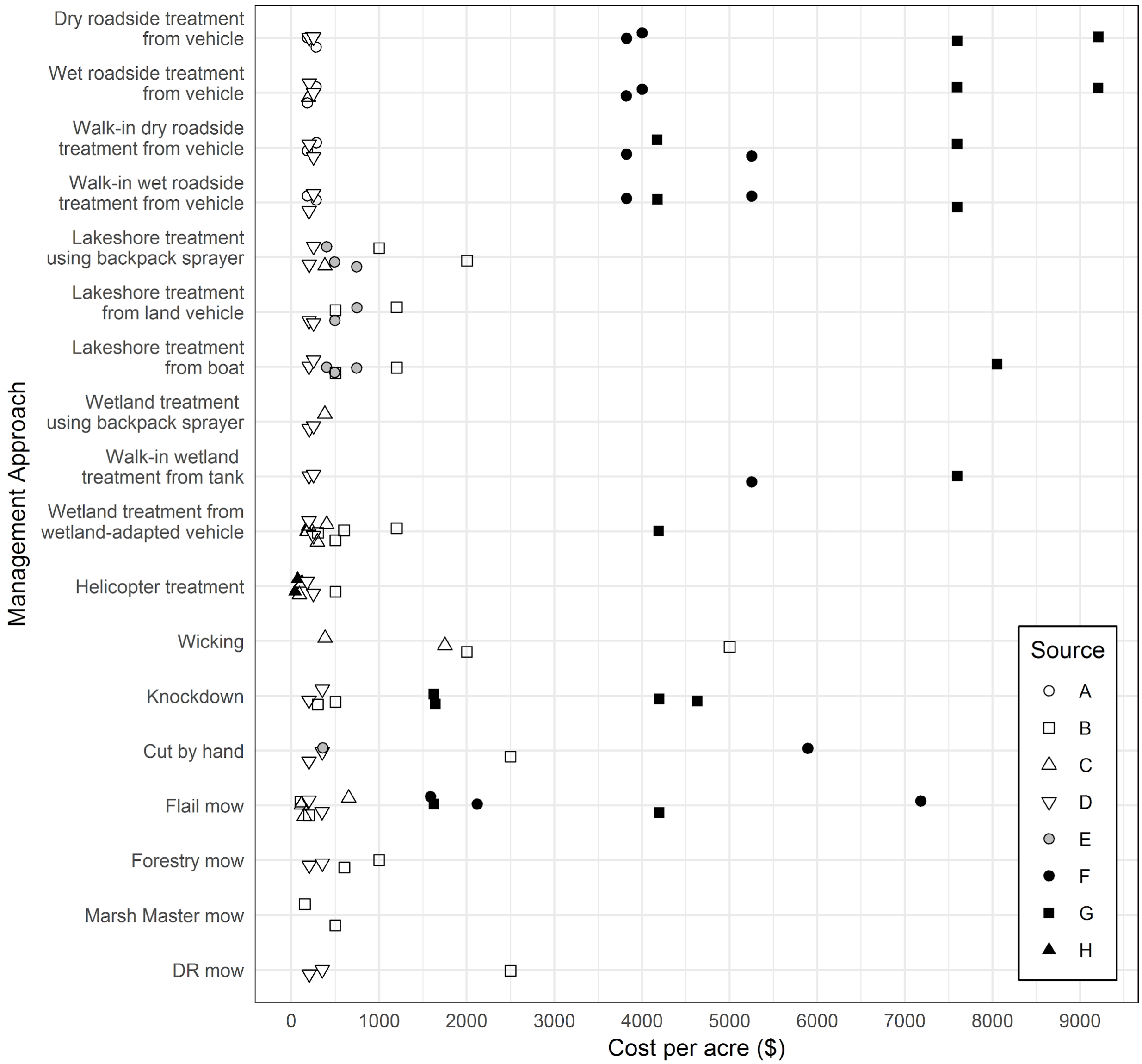

Figure 7. Control cost information provided by contractors and contractees for each control and site preparation approach identified for invasive *Phragmites* management in this assessment. Each source is a different contractor or contractee. White symbols indicate cost data that considered work at only a single site, black symbols indicate cost data that considered multiple sites, and the gray circles include both single and multi-site cost information from a single contractor.

Other assumptions and considerations regarding cost estimates are as follows.

- Cost estimates include the costs of herbicide treatment and site preparation only.

- The costs of restoration, surveillance, project administration by contractees and coordinators, equipment decontamination and purchasing, and other potential expenses are additional real costs that must be considered in planning invasive *Phragmites* response efforts. These costs will depend largely on which organizations participate in invasive *Phragmites* response and their partnerships. Because these details have yet to be determined, we could not estimate costs beyond those of herbicide treatment and site preparation.

- The costs of implementing alternative dewatering strategies at wastewater treatment facilities that currently use invasive *Phragmites* in their operations were also not included in regional estimates.

- It was assumed that control of all populations was contracted. This does not account for the possibility of some governmental, private, or other entities choosing to conduct control using internal staff or including invasive *Phragmites* control under existing plant management efforts, which could reduce costs.

- We assumed what we consider to be a minimal level of coordination among organizations. Generally, populations across county boundaries were not grouped for cost estimation. However, we assumed individual private landowners would not contract for control activities themselves, and would instead allow access to their property to contractors hired by a local, regional, or state entity. State agencies were assumed to contract for control of populations on their properties. The assumption of minimal coordination is not to suggest that that is the level of coordination needed, but is meant to provide a conservative estimate of control costs. Coordination beyond what was assumed in our cost estimation process could further reduce herbicide treatment and site preparation costs (e.g., by grouping populations in close proximity that require similar management approaches). However, additional time spent coordinating efforts could also increase costs in other areas.

- If management is effective, costs should decrease somewhat each year as populations are eliminated or reduced in size, though we did not account for this type of reduction over the three-year period for which costs were estimated.

- In some cases, it is likely that initial control efforts will not achieve elimination of targeted populations, necessitating more than three years of treatment. Several studies have examined efficacy of various control approaches depending on the size of the target population (Quirion et al. 2018, Rohal et al. 2019). In the regional sections of this assessment, we have indicated populations ≥ 0.5 acres as possibly requiring more than three years of control effort. The management approach employed, quality of management and follow-up, and site conditions are additional factors that could lead to the need for less than or greater than three years of control effort.

There are many factors that contribute to variability in control costs and we stress the importance of engaging contractors for quotes early in the planning process. Contractors and clients described many factors influencing costs, including the type of equipment used, water depth at the site, the density and area of target stands, the distance to and between sites, the number of sites, the quality of surrounding vegetation, and the type of herbicide used (costs are only affected to a small degree by this last point). While the cost estimates in this assessment provide reasonable approximations for regional herbicide treatment and site preparation costs to assist with planning response actions, the estimates also carry assumptions that may not reflect how responses are ultimately implemented. To ensure sufficient funds, we strongly recommend acquiring quotes from contractors in the early planning stages and budgeting for additional expenditures specific to how response efforts are ultimately implemented (e.g., project administration by contractees and coordinators, restoration, surveillance, equipment decontamination and purchasing).

Restoration site identification criteria

Each invasive *Phragmites* population documented as a part of the MNPhrag project was assigned one of three levels of post-control management: restoration of native species, revegetation, or no revegetation (Table 8). Generally, sites requiring some form of revegetation or restoration have large invasive *Phragmites* populations, steep slopes, or are vulnerable to reinvasion. Sites categorized for restoration had high quality plant communities and ecological value prior to invasion; these are the sites described in Part I of this assessment, in the sections specific to the Saint Louis, Southeast, Southwest, and Central South Regions. Sites categorized for revegetation include those having poor ecological quality or strictly functional plant communities (e.g., preventing erosion), and those with potential for erosion or reinvasion by invasive *Phragmites* or other invasive species. The goals of revegetation in these cases are to stabilize soils and provide affordable, robust non-invasive vegetative cover. Sites with small invasive *Phragmites* populations located in areas where the surrounding plant community will fill in openings resulting from control activities may not require revegetation (Rohal et al. 2019). The revegetation categorization assignments provided in Table 8 suggest potential candidate sites where restoration and revegetation could be beneficial. Managers should further assess the need for revegetation following elimination of invasive *Phragmites*, taking into account the risk of not revegetating and the potential benefits of revegetation.

Locations and basic information about verified invasive *Phragmites* populations

The following table includes the locations of all 389 verified invasive *Phragmites* populations as well as their estimated size, property ownership and restoration categorization, and EDDMapS identification numbers when possible. This list includes all populations verified as of May 5, 2019. A periodically updated digital version can be found at MNPhrag.org.

Table 8. Locations of and basic information about all documented invasive *Phragmites* populations in Minnesota as of May 5, 2019.

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
5168439	Central East	Chisago	Wyoming Park and Ride	45.3356	-93.0059	300	Mixed	None
5180875	Central East	Chisago	Chisago Lake, Chisago Blvd	45.3423	-92.8651	1000	Mixed	None
5180871	Central East	Chisago	Cty Rd 23 (Cty Rd 83)	45.3477	-92.8390	5000	Private	Revegetation
7812048	Central East	Chisago	Chisago Lake #7	45.3520	-92.8649	150	Mixed	None
7812049	Central East	Chisago	Chisago Lake #6	45.3533	-92.8668	450	Mixed	None
7812054	Central East	Chisago	Chisago Lake #5	45.3536	-92.8672	30000	Mixed	None
7812053	Central East	Chisago	Chisago Lake #1 Schlimmer's Slough	45.3579	-92.8593	500	Mixed	None
7812052	Central East	Chisago	Chisago Lake #2	45.3586	-92.8655	225	Mixed	None
7812051	Central East	Chisago	Chisago Lake #3	45.3588	-92.8654	10	Mixed	None
7812050	Central East	Chisago	Chisago Lake #4	45.3590	-92.8656	75	Mixed	None
7815888	Central East	Chisago	Chisago Lake #8	45.3598	-92.8649	450	Mixed	None
7815887	Central East	Chisago	Chisago Lake #9	45.3618	-92.8652	12	Mixed	None
7815890	Central East	Chisago	Chisago Lake #10	45.3649	-92.8667	1500	Mixed	None
7815892	Central East	Chisago	Chisago Lake #12	45.3701	-92.8700	500	Mixed	None
7815891	Central East	Chisago	Chisago Lake #13	45.3715	-92.8717	150	Mixed	None
7801883	Central East	Chisago	South Center Lake	45.3716	-92.8119	2500	Mixed	None
7801884	Central East	Chisago	South Center Lake	45.3736	-92.8076	200	Mixed	None
7801880	Central East	Chisago	South Center Lake	45.3741	-92.8378	900	Mixed	None
7801878	Central East	Chisago	South Center Lake	45.3745	-92.8310	300	Mixed	None
7801879	Central East	Chisago	South Center Lake	45.3745	-92.8375	400	Mixed	None
7815893	Central East	Chisago	Chisago Lake #14	45.3749	-92.8690	5	Mixed	None
7801877	Central East	Chisago	South Center Lake	45.3753	-92.8305	9500	Mixed	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801882	Central East	Chisago	South Center Lake	45.3767	-92.8147	400	Mixed	None
7815886	Central East	Chisago	South Lindstrom Lake #3	45.3771	-92.8577	5000	Mixed	None
7801885	Central East	Chisago	South Center Lake	45.3772	-92.8128	100	Mixed	None
7815883	Central East	Chisago	South Lindstrom Lake #2	45.3773	-92.8621	144	Mixed	None
7815884	Central East	Chisago	South Lindstrom Lake #1	45.3777	-92.8631	500	Mixed	None
7815885	Central East	Chisago	South Lindstrom Lake #4	45.3780	-92.8554	3000	Mixed	None
7801886	Central East	Chisago	South Center Lake	45.3796	-92.8134	100	Mixed	None
7801887	Central East	Chisago	South Center Lake	45.3800	-92.8130	300	Mixed	None
7801881	Central East	Chisago	South Center Lake	45.3807	-92.8196	25	Mixed	None
7801889	Central East	Chisago	South Center Lake	45.3808	-92.8077	150	Mixed	None
7801888	Central East	Chisago	South Center Lake	45.3809	-92.8123	400	Mixed	None
7826751	Central East	Chisago	Hwy 8, Shafer	45.3828	-92.7493	100	MNDOT	None
7826750	Central East	Chisago	Hwy 8, Shafer	45.3828	-92.7451	100	MNDOT	None
7801844	Central East	Chisago	Hwy 8 SB, Chisago City	45.3833	-92.8698	400	MNDOT	None
7801876	Central East	Chisago	South Center Lake	45.3843	-92.8261	400	Mixed	None
7801875	Central East	Chisago	South Center Lake	45.3849	-92.8254	4000	Mixed	None
7801890	Central East	Chisago	South Center Lake	45.3856	-92.8100	800	Mixed	None
7801891	Central East	Chisago	South Center Lake	45.3872	-92.8128	100	Mixed	None
7801874	Central East	Chisago	South Center Lake	45.3889	-92.8244	1500	MNDOT	None
7801893	Central East	Chisago	South Center Lake	45.3889	-92.8169	200	Mixed	None
7801873	Central East	Chisago	South Center Lake	45.3893	-92.8199	300	Mixed	Revegetation
5160566	Central East	Chisago	South Center Lake	45.3896	-92.8149	400	Mixed	None
7801843	Central East	Chisago	North Center Lake Boat Launch	45.3899	-92.8252	3000	State	Revegetation
7801892	Central East	Chisago	South Center Lake	45.3899	-92.8156	400	Mixed	None
4425578/5160569	Central East	Chisago	North Center Lake	45.3911	-92.8183	21780	Mixed	Restore
7801894	Central East	Chisago	North Center Lake	45.3923	-92.8258	2400	Mixed	None
7801872	Central East	Chisago	North Center Lake	45.3935	-92.8173	100	Mixed	None
7801846	Central East	Chisago	North Center Lake	45.3937	-92.8296	300	Mixed	None
7801847	Central East	Chisago	North Center Lake	45.3955	-92.8262	200	Mixed	None
7827783	Central East	Chisago	Cty Rd 19, Chisago City	45.3961	-92.8749	1000	Private	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801851	Central East	Chisago	North Center Lake	45.3964	-92.8314	100	Mixed	None
7801848	Central East	Chisago	North Center Lake	45.3966	-92.8281	300	Mixed	None
5159797	Central East	Chisago	The Ridges - Cty Rd 20 & Magnolia	45.3971	-92.8453	1000	Municipal	Restore
7854381	Central East	Chisago	Cty 37 (310th St)	45.3972	-92.7205	150	County	None
5178331	Central East	Chisago	North Lindstrom Lake	45.3973	-92.8472	2500	Mixed	None
7801852	Central East	Chisago	North Center Lake	45.3975	-92.8328	2400	Mixed	None
7801849	Central East	Chisago	North Center Lake	45.3976	-92.8276	250	Mixed	None
7801850	Central East	Chisago	North Center Lake	45.3984	-92.8293	200	Mixed	None
7801871	Central East	Chisago	North Center Lake	45.3985	-92.8233	400	County	None
7801870	Central East	Chisago	North Center Lake	45.3989	-92.8234	200	County	None
7801853	Central East	Chisago	North Center Lake	45.4001	-92.8333	200	Municipal	None
7801869	Central East	Chisago	North Center Lake	45.4004	-92.8229	6000	Mixed	None
7802967	Central East	Chisago	Cty Rd 19, Chisago City	45.4011	-92.8967	1200	Private	Revegetation
7801854	Central East	Chisago	North Center Lake	45.4012	-92.8321	800	Municipal	None
7801855	Central East	Chisago	North Center Lake	45.4027	-92.8339	300	Municipal	None
5160567	Central East	Chisago	Lincoln Rd (Cty 14) at 316th St	45.4027	-92.8635	600	County	None
7801858	Central East	Chisago	North Center Lake	45.4093	-92.8326	3600	Mixed	None
7801856	Central East	Chisago	North Center Lake	45.4102	-92.8334	200	Mixed	None
7801857	Central East	Chisago	North Center Lake	45.4103	-92.8316	100	Mixed	None
7801868	Central East	Chisago	North Center Lake	45.4117	-92.8259	300	Mixed	None
7801867	Central East	Chisago	North Center Lake	45.4124	-92.8244	1400	Mixed	None
7801866	Central East	Chisago	North Center Lake	45.4134	-92.8248	1000	Mixed	None
7801860	Central East	Chisago	North Center Lake	45.4139	-92.8352	2000	Mixed	None
7801865	Central East	Chisago	North Center Lake	45.4140	-92.8249	3600	Mixed	None
7801859	Central East	Chisago	North Center Lake	45.4143	-92.8357	1600	Mixed	None
7801864	Central East	Chisago	North Center Lake	45.4144	-92.8251	200	Mixed	None
7801862	Central East	Chisago	North Center Lake	45.4144	-92.8275	1000	County	None
7801863	Central East	Chisago	North Center Lake	45.4146	-92.8254	500	Mixed	None
7801861	Central East	Chisago	North Center Lake	45.4203	-92.8303	100	Mixed	None
5160568	Central East	Chisago	Lincoln Rd (Cty 14) at Lindo Trail (340th St)	45.4394	-92.8842	1100	County	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
5161673 & 5185238	Central East	Chisago	Cty Rd 18/Lent Rd; Peterson Slough W	45.4421	-92.9179	6000	Private	Restore
5185240	Central East	Chisago	Peterson Slough E shore	45.4470	-92.9102	4000	State	Restore
5164598	Central East	Chisago	Peterson Slough E shore	45.4476	-92.9102	10890	Private	Restore
5161042	Central East	Chisago	Falcon Ave N & Athens Trl (Cty 17)	45.4506	-93.0002	440	County	None
4900160/5161044/7801845	Central East	Chisago	I-35 SB at Athens Trl (Cty 17)	45.4541	-92.9914	2600	MNDOT	Revegetation
5161043	Central East	Chisago	Lincoln Trl at 360th St	45.4699	-92.9190	440	Mixed	None
5180764	Central East	Chisago	Janet Johnson Memorial WMA	45.4769	-92.9508	50	State	None
5184801	Central East	Chisago	410th St EB	45.5427	-92.9588	440	Private	None
7825928	Central East	Isanti	Cty Rd 9 EB	45.4563	-93.1369	500	County	None
7808901	Central East	Isanti	Cambridge Middle School	45.5370	-93.2076	40	Municipal	Revegetation
7801941	Central North	Aitkin	Aitkin, Co Rd 1/410th Ave	46.5523	-93.7077	43560	Mixed	Revegetation
None	Central North	Aitkin	Aitkin, Co Rd 1/410th Ave NB	46.5757	-93.7081	600	County	Revegetation
7801919	Central South	Kandiyohi	Kandiyohi, off Hwy 12	45.1326	-94.9768	3000	Mixed	Revegetation
7979158	Central South	Kandiyohi	Willmar, lakeshore	45.1351	-95.0431	Unknown	Private	Revegetation
5166545	Central South	Kandiyohi	Willmar, wetland	45.1363	-95.0422	10000	Private	Revegetation
5166890	Central South	Kandiyohi	Cty Rd 29, E of Swenson Lk	45.2623	-95.1338	43560	Private	Restore
7801918	Central South	Kandiyohi	Lake Andrew Twp	45.2673	-95.1293	Unknown	Private	Revegetation
None	Central South	Kandiyohi	160th St NE	45.2911	-94.8252	400	Private	None
5167881	Central South	Kandiyohi	Brown Property, 176th Ave NE	45.2952	-94.8394	174240	Private	Restore
4426272/4888810/5166893	Central South	Kandiyohi	Hwy 23, Hawick	45.3530	-94.8180	8000	State	Revegetation
5184208	Central South	McLeod	Hwy 7, Clouster Lake WMA	44.9065	-94.1241	600	State	Restore
5167903	Central South	Meeker	Calhoun Estates, Irving Twnshp	45.1705	-94.5030	65340	Private	Revegetation
7817792	Central South	Sherburne	Sherburne NWR	45.4797	-93.6871	2400	Federal	Restore
7817793	Central South	Sherburne	Princeton WWTP Wetland	45.5484	-93.5740	21780	Municipal	None
None	Central South	Sibley	441st Ave	44.6192	-94.1526	Unknown	County	None
7801917	Central South	Sibley	Hwy 6 - Scenic Byway Rd	44.6378	-93.7981	1000	Private	None
None	Central South	Stearns	Richmond Cement Plant	45.4477	-94.5103	1200	Private	None
7801842	Central South	Stearns	Richmond Cement Plant	45.4483	-94.5139	1000	Private	None
7801965	Central South	Wright	Delano Cty Rd 16 SE	45.0242	-93.7975	400	County	None
7801963	Central South	Wright	Delano Cemstone	45.0340	-93.7724	200	Private	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801962	Central South	Wright	Delano Cemstone	45.0343	-93.7730	3500	Private	Revegetation
7801964	Central South	Wright	Delano Cemstone	45.0347	-93.7721	1200	Private	Revegetation
7801970	Central South	Wright	Delano Cemstone	45.0348	-93.7734	2500	Private	Revegetation
7801969	Central South	Wright	Delano Cemstone	45.0351	-93.7731	900	Private	Revegetation
4706703	Central South	Wright	Delano-Hwy 12	45.0354	-93.7767	1000	MNDOT	Revegetation
4706696	Central South	Wright	Delano Cemstone	45.0354	-93.7731	401	MNDOT	Revegetation
7813797	Central South	Wright	Delano Stormwater Retention Pond	45.0443	-93.7812	1200	Private	Revegetation
7813784	Central South	Wright	Delano Stormwater Retention Pond	45.0452	-93.7814	1600	Private	None
7813785	Central South	Wright	Delano Wetland	45.0456	-93.7816	21780	Private	Revegetation
7801968	Central South	Wright	Delano Maple Ave & 4th St N	45.0458	-93.7849	600	Mixed	None
None	Central South	Wright	Delano, Wetland complex	45.0464	-93.7825	4000	Private	Revegetation
7813787	Central South	Wright	Delano Stormwater Retention Pond	45.0475	-93.7830	100	Municipal	None
7813786	Central South	Wright	Delano Wetland	45.0486	-93.7822	21780	Municipal	Revegetation
7801967	Central South	Wright	Delano Cty Rd 30 SE/70th St SE	45.0502	-93.7775	700	County	None
7801961	Central South	Wright	Delano WWTP	45.0504	-93.7842	1800	Municipal	Revegetation
7801966	Central South	Wright	Delano WWTP	45.0509	-93.7851	1800	Municipal	Revegetation
7813792	Central South	Wright	Hwy 12	45.0647	-93.8667	21780	MNDOT	Revegetation
7813794	Central South	Wright	Hwy 12 W of Delano	45.0648	-93.8872	1600	MNDOT	Revegetation
7813791	Central South	Wright	Hwy 12 W of Delano	45.0653	-93.8804	1600	MNDOT	None
7813788	Central South	Wright	Hwy 55 W of Rockford	45.0934	-93.7503	5000	MNDOT	Revegetation
7813789	Central South	Wright	Hwy 55 SE of Buffalo	45.1159	-93.8083	20	MNDOT	None
7813793	Central South	Wright	Cty Rd 12 S	45.1347	-93.9002	200	Private	None
None	Central South	Wright	Hwy 55 Buffalo	45.1534	-93.8468	1500	MNDOT	Revegetation
7813790	Central South	Wright	Buffalo, Settlers Pkwy & Wilder Way	45.1634	-93.8624	1200	MNDOT	Revegetation
7801950	Central South	Wright	St Michael Wastewater Trtment Plant	45.1995	-93.6488	100	Municipal	None
7801949	Central South	Wright	St Michael Wastewater Trtment Plant	45.1997	-93.6483	100	Municipal	None
7801948	Central South	Wright	St Michael Wastewater Trtment Plant	45.2000	-93.6481	100	Municipal	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801947	Central South	Wright	St Michael Wastewater Trtment Plant	45.2001	-93.6482	100	Municipal	None
7801946	Central South	Wright	St Michael Wastewater Trtment Plant	45.2007	-93.6487	400	Municipal	None
7801953	Central South	Wright	St Michael Wastewater Trtment Plant	45.2014	-93.6501	750	Municipal	Revegetation
7801952	Central South	Wright	St Michael CtyRd 119/45th St	45.2113	-93.6742	100	Municipal	None
7801960	Central South	Wright	St Michael Cty Rd 119/45th St	45.2117	-93.6743	600	Municipal	None
7801959	Central South	Wright	St Michael CtyRd 119/45th St	45.2121	-93.6756	1000	State	None
7801957	Central South	Wright	St Michael 3rd St NW	45.2123	-93.6697	900	Municipal	None
7801951	Central South	Wright	St Michael Cty Rd 119/Birch Ave	45.2123	-93.6744	100	County	None
7801958	Central South	Wright	St Michael 3rd St NW	45.2124	-93.6698	600	Municipal	None
7801954	Central South	Wright	St Michael Maciver Ave NE	45.2153	-93.6441	900	Mixed	None
7813796	Central South	Wright	Buffalo, Hwy 25	45.2176	-93.8498	1800	MNDOT	Revegetation
7801956	Central South	Wright	St Michael/Albertville	45.2218	-93.6648	700	County	None
7801955	Central South	Wright	St Michael/Albertville	45.2227	-93.6647	600	Mixed	None
7801978	Central South	Wright	Albertville, Kyler Ave	45.2278	-93.6662	3200	Municipal	Revegetation
7801977	Central South	Wright	Albertville I-94	45.2370	-93.6465	150	MNDOT	None
7801971	Central South	Wright	Albertville Memorial Park	45.2400	-93.6502	50	Municipal	None
7854374	Central South	Wright	Albertville, 63rd St NE & Marlowe Ave NE	45.2417	-93.6398	2500	Private	Revegetation
7854375	Central South	Wright	Albertville, Mackenzie Ave NE	45.2472	-93.6408	3000	Mixed	Revegetation
7854378	Central South	Wright	Albertville, 80th St NE	45.2664	-93.6462	200	Private	None
7801930	Central West	Grant	Wetland	46.0712	-96.1757	Unknown	State	Restore
7801939	Central West	Otter Tail	Central Lakes Trail	46.2104	-95.9734	800	MNDOT	Restore
3956003	Central West	Otter Tail	I-94	46.3593	-96.1574	6000	MNDOT	Revegetation
5184238	Metro	Anoka	I-35E	45.1381	-93.0392	440	MNDOT	None
7824018	Metro	Anoka	Coon Rapids Blvd ramp to Hwy 610	45.1412	-93.2810	17424	Mixed	Revegetation
5251712	Metro	Anoka	Coon Creek and Hwy 10	45.1698	-93.2948	5000	Mixed	Revegetation
7814494	Metro	Anoka	Blaine, Sunrise Lake Channel	45.1927	-93.1961	7500	Private	Revegetation
5160578	Metro	Anoka	W Freeway Drive	45.2474	-93.0268	18537	MNDOT	Revegetation
5184240	Metro	Anoka	I-35W just N of Lake Dr NE	45.2518	-93.0245	6000	MNDOT	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7628228	Metro	Anoka	Ham Lake Baptist Camp	45.2558	-93.2176	150	Private	Restore
5183924/5185257/7801920	Metro	Anoka	I-35W, Columbus	45.2568	-93.0205	2500	MNDOT	Revegetation
7826165	Metro	Carver	Jonathan Carver Pkwy	44.7879	-93.6424	100	Mixed	None
5178722	Metro	Carver	Clover Ridge Dr/RR ROW	44.8211	-93.6411	2000	Private	None
5162229	Metro	Carver	Big Woods Lake Chaska	44.8488	-93.6052	200	Municipal	Revegetation
7801915	Metro	Carver	Hwy 5	44.8669	-93.6331	100	MNDOT	None
7801916	Metro	Carver	Hwy 5	44.8669	-93.6447	100	Municipal	None
None	Metro	Carver	Hwy 5	44.8671	-93.6242	100	MNDOT	None
7801914	Metro	Carver	Hwy 5	44.8674	-93.6236	100	MNDOT	None
7801913	Metro	Carver	Carver Park Reserve Mitigation Pond	44.8754	-93.6849	6800	County	Restore
7801945	Metro	Dakota	Lebanon Hills Reg Park Visitor Ctr Entr Rd	44.7853	-93.1245	50	County	None
7801987	Metro	Hennepin	I-169/I-94 Interchange Bloomington/Eden Prairie	44.8589	-93.3959	600	MNDOT	None
7801986	Metro	Hennepin	Winter Park Bloomingon	44.8618	-93.4016	43560	Private	Revegetation
7801993	Metro	Hennepin	I-494 Roadside	44.8955	-93.4449	450	Private	None
7801988	Metro	Hennepin	Excelsior Covenant Church	44.9089	-93.5317	4000	Private	Revegetation
7801991	Metro	Hennepin	I-169 S of 7th St/2nd Ave S	44.9112	-93.4026	250	State	None
None	Metro	Hennepin	Little Long Lake	44.9399	-93.7051	400	Private	Revegetation
5184341/7637430/7801995	Metro	Hennepin	Lake of the Isles	44.9519	-93.3097	1000	Municipal	Restore
4425694/4998527	Metro	Hennepin	Cedar Lake Trail, St Louis Park	44.9597	-93.3560	36419	Private	Revegetation
5185251	Metro	Hennepin	Franklin Ave & Cedar Ave, S Mpls	44.9649	-93.2479	3300	County	Revegetation
7801994	Metro	Hennepin	I-494 overpass of Oakland Rd	44.9678	-93.4610	100	MNDOT	None
7801981	Metro	Hennepin	Hwy 12 Orono	44.9851	-93.5711	200	MNDOT	None
7801982	Metro	Hennepin	Hwy 12 Orono	44.9855	-93.5765	200	MNDOT	None
7801989	Metro	Hennepin	Hwy 12 Maple Plain	45.0010	-93.6382	1400	MNDOT	None
7801990	Metro	Hennepin	Hwy 12 Independence	45.0095	-93.6848	200	MNDOT	None
None	Metro	Hennepin	Hwy 12 Maple Plain	45.0105	-93.6783	100	MNDOT	None
7813795	Metro	Hennepin	Crystal Lake, Robbinsdale	45.0231	-93.3255	1400	Municipal	None
7818000	Metro	Hennepin	Hollingsworth Park	45.0302	-93.3280	18	Mixed	Revegetation
7817999	Metro	Hennepin	Hollingsworth Park	45.0303	-93.3274	36	Private	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801983	Metro	Hennepin	3905 Nature View Circle at 46th 1/2 Ave N	45.0398	-93.3301	400	Private	Revegetation
7813798	Metro	Hennepin	Delano, County Line Rd SE/Hwy 139	45.0485	-93.7667	200	MNDOT	None
7801992	Metro	Hennepin	Wetland S of Usher Smith	45.0744	-93.4443	5000	Private	None
7814501	Metro	Hennepin	Timber Crest Drive	45.0833	-93.4571	4356	County	Revegetation
7820767	Metro	Hennepin	3Rivers Reg Trl S of Weaver Lake Rd	45.1062	-93.4828	5700	County	Revegetation
7801984	Metro	Hennepin	I-94, Maple Grove	45.1266	-93.4846	500	MNDOT	None
5183925	Metro	Hennepin	Hwy 81 SB	45.1610	-93.5037	800	MNDOT	None
5183926	Metro	Hennepin	Hwy 81 SB	45.1620	-93.5054	200	MNDOT	None
7801985	Metro	Hennepin	I-94 at Brockton Ln N (Cty 101)	45.1636	-93.5210	900	MNDOT	None
5229628	Metro	Hennepin	Tucker Rd adj to Henry Lake	45.1676	-93.6010	200	County	None
5183922	Metro	Hennepin	I-94 at Cty Rd 81	45.1731	-93.5266	1000	MNDOT	None
7801980	Metro	Hennepin	Champlin Mill Pond	45.1842	-93.3992	10	Private	Revegetation
7817791	Metro	Hennepin	Hwy 81 SB	45.1895	-93.5497	800	MNDOT	None
4712842/5183927	Metro	Hennepin	I-94 at 101, Rogers	45.1917	-93.5459	15000	MNDOT	Revegetation
7801911	Metro	Ramsey	Victoria Park	44.9156	-93.1377	900	Municipal	None
7801912	Metro	Ramsey	Victoria Park	44.9157	-93.1379	400	Municipal	None
7979211	Metro	Ramsey	Victoria Park	44.9158	-93.1405	200	Municipal	None
7801910	Metro	Ramsey	Victoria Park	44.9160	-93.1380	100	Private	None
4707458	Metro	Ramsey	Victoria Park	44.9164	-93.1371	2500	Municipal	None
5182174	Metro	Ramsey	Pig's Eye Regional Park	44.9280	-93.0356	7875	Municipal	Revegetation
5178489	Metro	Ramsey	Swede Hollow Park-St Paul	44.9602	-93.0744	325	Municipal	Revegetation
5159642/5178491	Metro	Ramsey	Swede Hollow Park-St Paul	44.9603	-93.0742	325	Municipal	Revegetation
4202699	Metro	Ramsey	Maplewood, Adj to Priory Neighborhood Preserve	44.9877	-92.9891	2800	State	Revegetation
4202700	Metro	Ramsey	Maplewood, Adj to Priory Neighborhood Preserve	44.9878	-92.9888	130	State	None
4202698	Metro	Ramsey	Maplewood, Adj to Priory Neighborhood Preserve	44.9895	-92.9888	30492	Municipal	Revegetation
5788216	Metro	Ramsey	McCarrons Pond Apartment Raingarden	45.0008	-93.1076	4000	Private	Revegetation
7638249	Metro	Ramsey	I-35E SB to Hwy 36 E	45.0103	-93.0906	5600	MNDOT	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
5184343	Metro	Ramsey	Hwy 36 at McKnight	45.0129	-93.0062	1600	Mixed	Revegetation
5168437	Metro	Ramsey	I-35E/I-694E ramp	45.0452	-93.0614	4356	MNDOT	Revegetation
5285313	Metro	Ramsey	Tony Schmidt Reg Pk	45.0507	-93.1735	1000	County	Revegetation
5252262	Metro	Ramsey	I-35W NB	45.0641	-93.1860	Unknown	MNDOT	Revegetation
7814380	Metro	Ramsey	White Bear Lake	45.0707	-92.9890	21780	Lake	None
7814378	Metro	Ramsey	White Bear Lake	45.0708	-93.0053	400	Lake	None
7814386	Metro	Ramsey	White Bear Lake	45.0809	-92.9941	10	Lake	None
7814388	Metro	Ramsey	White Bear Lake	45.0810	-92.9947	400	Lake	None
7814382	Metro	Ramsey	White Bear Lake	45.0814	-92.9971	4356	Lake	None
4792397	Metro	Ramsey	White Bear Lake	45.0830	-93.0009	16770	Mixed	None
4792398	Metro	Ramsey	White Bear Lake	45.0842	-92.9992	400	Mixed	None
7814391	Metro	Ramsey	White Bear Lake	45.0895	-92.9988	400	Lake	None
7817790	Metro	Ramsey	Hammond Rd, White Bear Lake	45.0935	-93.0405	3600	Private	None
7814392	Metro	Ramsey	White Bear Lake	45.0965	-92.9847	21780	Lake	None
7814394	Metro	Ramsey	White Bear Lake	45.0972	-92.9896	900	Lake	None
3108803	Metro	Ramsey	Otter Lake, Tamarack NC	45.1217	-93.0455	220	Mixed	None
7801836	Metro	Scott	Hwy 5 - Hickory Blvd	44.5988	-93.7461	200	MNDOT	None
4494058	Metro	Scott	I-35 Median	44.6073	-93.2961	1000	MNDOT	Revegetation
7801838	Metro	Scott	Hwy 6 Belle Plaine	44.6224	-93.8132	200	MNDOT	None
7801837	Metro	Scott	I-169 Belle Plaine	44.6264	-93.7420	300	MNDOT	None
7801839	Metro	Scott	Hwy 25	44.6324	-93.7636	1500	MNDOT	None
7801929	Metro	Washington	I-494	44.8865	-93.0034	900	Private	Revegetation
7801925	Metro	Washington	I-494 at Exit 60 Lake Rd	44.9139	-92.9812	700	MNDOT	None
7801926	Metro	Washington	I-694 & Cty Rd 14 (34th St N)	44.9983	-92.9585	400	MNDOT	None
None	Metro	Washington	I-694 & Hwy 36 Interchange	45.0294	-92.9606	400	MNDOT	None
7814376	Metro	Washington	White Bear Lake	45.0560	-92.9659	100	Lake	None
7814390	Metro	Washington	White Bear Lake	45.0774	-92.9779	200	Lake	None
None	Metro	Washington	White Bear Lake	45.0786	-92.9650	400	Lake	None
7814393	Metro	Washington	White Bear Lake	45.0795	-92.9652	10890	Lake	None
7814385	Metro	Washington	White Bear Lake	45.0805	-92.9769	250	Lake	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7814395	Metro	Washington	White Bear Lake	45.0806	-92.9653	400	Lake	None
7814389	Metro	Washington	White Bear Lake	45.0815	-92.9651	20	Lake	None
None	Metro	Washington	White Bear Lake	45.0822	-92.9762	400	Lake	None
7814387	Metro	Washington	White Bear Lake	45.0824	-92.9754	400	Lake	None
7814381	Metro	Washington	White Bear Lake	45.0829	-92.9730	400	Lake	None
7814383	Metro	Washington	White Bear Lake	45.0834	-92.9719	10	Lake	None
7814377	Metro	Washington	White Bear Lake	45.0846	-92.9712	100	Lake	None
7814379	Metro	Washington	White Bear Lake	45.0851	-92.9716	20	Lake	None
7814396	Metro	Washington	White Bear Lake	45.0938	-92.9836	250	Lake	None
7801928	Metro	Washington	Geneva Ave, Hugo	45.1626	-92.9841	3500	Municipal	Revegetation
3215821	Metro	Washington	Scandia Trl & Hoekstra Ave N	45.2623	-92.9462	50	Private	None
5183923	Metro	Washington	I-35W NB, Forest Lake	45.2660	-93.0099	1000	MNDOT	Revegetation
5177908/5183929	Metro	Washington	1-35W NB, Forest Lake	45.2671	-93.0091	800	MNDOT	Revegetation
5177909/5184237	Metro	Washington	I-35 SB, Forest Lake	45.2683	-93.0095	600	MNDOT	Revegetation
5168438/5183917	Metro	Washington	I-35W Exit 131 to W Broadway Ave	45.2796	-93.0037	400	MNDOT	None
7801927	Metro	Washington	Meadowbrook Ave, Forest Lake	45.2883	-92.8508	900	Mixed	None
7826749	North Central	Lake of the Woods	Hwy 11 WB	48.7107	-94.7053	1200	Private	Revegetation
7826753	North Central	Lake of the Woods	Hwy 11 WB	48.7129	-94.6603	800	Private	Revegetation
7826748	North Central	Lake of the Woods	Hwy 11 WB	48.7734	-94.9804	500	Private	Revegetation
7819637	North Central	Lake of the Woods	Hwy 11 WB	48.7842	-95.0268	20	Private	Revegetation
5168434	Northwest	Becker	Hwy 10/RR ROW	46.8418	-95.9288	87120	MNDOT	Revegetation
None	Northwest	Becker	Hwy 10	46.8778	-96.0492	600	Private	None
None	Northwest	Clay	Hwy 10	TBD	TBD	2400	MNDOT	None
7801934	Northwest	Polk	Glacial Ridge NWR Cty Rd 45	47.7023	-96.3278	200	Mixed	None
None	Saint Louis	Carlton	Hwy 33 ROW	46.7633	-92.4533	10890	MNDOT	Revegetation
None	Saint Louis	St. Louis	S of Kilchlis Meadow	46.6820	-92.1804	10890	Mixed	Revegetation
None	Saint Louis	St. Louis	S of Mouth of US Steel Creek	46.6871	-92.2011	10890	Mixed	Restore
None	Saint Louis	St. Louis	Mouth of US Steel Creek	46.6880	-92.2030	10890	Mixed	Restore

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
None	Saint Louis	St. Louis	Island	46.6941	-92.1959	10890	Mixed	Restore
7823447	Saint Louis	St. Louis	No description	46.6951	-92.2048	10890	Private	None
None	Saint Louis	St. Louis	S of Munger Landing	46.6987	-92.2082	10890	Private	None
7823445	Saint Louis	St. Louis	S of Munger Landing	46.6997	-92.2081	10890	Private	None
None	Saint Louis	St. Louis	S of Munger Landing	46.7006	-92.2073	10890	Mixed	None
None	Saint Louis	St. Louis	N of Munger Landing	46.7015	-92.2072	10890	Mixed	None
7823454	Saint Louis	St. Louis	N of Munger Landing	46.7017	-92.2073	10890	Mixed	None
None	Saint Louis	St. Louis	N of Munger Landing	46.7020	-92.2075	10890	Mixed	None
None	Saint Louis	St. Louis	N of Munger Landing	46.7024	-92.2076	10890	Private	None
None	Saint Louis	St. Louis	Swenson Ave	46.7028	-92.2136	10890	Mixed	Revegetation
7823439	Saint Louis	St. Louis	N of Munger Landing	46.7030	-92.2073	10890	Private	None
7823440	Saint Louis	St. Louis	N of Munger Landing	46.7037	-92.2073	10890	Private	None
7823438	Saint Louis	St. Louis	N of Munger Landing	46.7042	-92.2071	10890	Private	None
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7051	-92.2048	10890	Mixed	None
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7053	-92.2046	10890	Mixed	None
None	Saint Louis	St. Louis	No description	46.7056	-92.2067	10890	Private	Revegetation
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7059	-92.2042	10890	Private	None
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7066	-92.2046	Unknown	Private	Revegetation
None	Saint Louis	St. Louis	Spring Street	46.7070	-92.2055	10890	Private	Revegetation
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7071	-92.2044	50	Private	None
None	Saint Louis	St. Louis	Spirit Lake Marina	46.7081	-92.2017	43560	Private	Revegetation
None	Saint Louis	St. Louis	Celeste's Island	46.7185	-92.1847	10890	Mixed	None
None	Saint Louis	St. Louis	No description	46.7198	-92.1649	10890	Private	Revegetation
None	Saint Louis	St. Louis	No description	46.7216	-92.1629	10890	Private	None
None	Saint Louis	St. Louis	No description	46.7232	-92.1627	10890	Private	None
None	Saint Louis	St. Louis	No description	46.7241	-92.1629	7500	Private	None
4202302	Saint Louis	St. Louis	Grassy Point, Duluth	46.7245	-92.1535	63772	State	Restore
None	Saint Louis	St. Louis	No description	46.7252	-92.1622	43560	Private	None
None	Saint Louis	St. Louis	No description	46.7263	-92.1604	10890	Private	None
None	Saint Louis	St. Louis	No description	46.7266	-92.1604	21780	Private	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
5159381	Saint Louis	St. Louis	Grassy Point	46.7272	-92.1604	43560	Private	Revegetation
None	Saint Louis	St. Louis	Grassy Point	46.7274	-92.1590	43560	Municipal	Revegetation
None	Saint Louis	St. Louis	Waseca Industrial Rd Overpass	46.7278	-92.1626	10890	Private	Revegetation
None	Saint Louis	St. Louis	No description	46.7283	-92.1650	10890	Private	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7403	-92.1420	10890	Municipal	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7404	-92.1421	10890	Municipal	Revegetation
7823449	Saint Louis	St. Louis	Oneota	46.7406	-92.1415	10890	Municipal	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7406	-92.1417	10890	Municipal	Revegetation
7823444	Saint Louis	St. Louis	Oneota	46.7408	-92.1484	10890	Mixed	Revegetation
7823457	Saint Louis	St. Louis	Oneota	46.7411	-92.1404	10890	Municipal	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7415	-92.1399	10890	Municipal	Revegetation
7823444	Saint Louis	St. Louis	Oneota	46.7416	-92.1495	10890	Mixed	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7417	-92.1493	Unknown	Private	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7418	-92.1498	10890	Mixed	Revegetation
7823458	Saint Louis	St. Louis	Oneota	46.7419	-92.1503	10890	Mixed	Revegetation
None	Saint Louis	St. Louis	Oneota	46.7422	-92.1492	Unknown	Municipal	Revegetation
5159381	Saint Louis	St. Louis	Duluth Hallett Dock Area	46.7479	-92.1377	107593	Private	None
None	Saint Louis	St. Louis	Rice's Point	46.7529	-92.0999	10890	Private	None
None	Saint Louis	St. Louis	Rice's Point	46.7532	-92.0985	100	Mixed	None
None	Saint Louis	St. Louis	Courtland St	46.7561	-92.1288	Unknown	Mixed	Revegetation
7801932	Saint Louis	St. Louis	Rice's Point - Duluth Seaway Port Authority	46.7570	-92.1060	21780	Private	Revegetation
None	Saint Louis	St. Louis	Rice's Point	46.7585	-92.1045	750	Private	None
None	Saint Louis	St. Louis	Rice's Point	46.7589	-92.1056	Unknown	Private	None
None	Saint Louis	St. Louis	Rice's Point	46.7590	-92.1051	100	Private	None
None	Saint Louis	St. Louis	Hearding Island	46.7594	-92.0854	10890	State	Revegetation
None	Saint Louis	St. Louis	Harbor Point Circle	46.7644	-92.0875	21780	Private	Revegetation
7823453	Saint Louis	St. Louis	Rice's Point	46.7661	-92.1039	10890	Mixed	None
7823453	Saint Louis	St. Louis	No description	46.7662	-92.1036	10890	Mixed	None
7801933 & 8067311	Saint Louis	St. Louis	Duluth Haines Road	46.8161	-92.1746	800	County	Restore
None	Saint Louis	St. Louis	Hwy 53	46.9644	-92.4638	43560	MNDOT	Revegetation

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
5162173	Saint Louis	St. Louis	Hwy 53, Cotton	47.1523	-92.4726	10890	MNDOT	None
None	Saint Louis	St. Louis	Hwy 7	47.2966	-92.6032	2100	Mixed	Revegetation
7801931	Saint Louis	St. Louis	Hwy 53/RR ROW	48.1818	-92.8839	500	State	Revegetation
4792145	South Central	Blue Earth	Fernwood Rd N of RR tracks	44.1743	-94.1242	150	Private	None
4494028	South Central	Freeborn	I-35 N of Exit 42	43.5361	-93.3547	3000	MNDOT	Revegetation
4498339	South Central	Freeborn	Cty Rd 14 to 700th Ave	43.6914	-93.4685	120	Private	None
5181870 & 4498342	South Central	Freeborn	Hwy 13/RR ROW, S of Manchester	43.7086	-93.4396	2200	Private	Revegetation
4498342 & 7801979	South Central	Freeborn	Hwy 13/RR ROW, S of Manchester	43.7112	-93.4410	17424	Private	Revegetation
7801921	South Central	Le Sueur	Ludwig Island, Lake Emily	44.3067	-93.9190	2000	County	Restore
5178885 & 5182768	South Central	Le Sueur	110/107 (Lake Emily Rd) & 21 (Golf Course Rd)	44.3101	-93.9319	43560	Private	Revegetation
5181867	South Central	Le Sueur	Le Center, Cty Rd 5	44.4156	-93.6871	2200	Private	Revegetation
5182572	South Central	Nicollet	Hwy 14 and I-169 Ramp	44.1913	-94.0180	400	MNDOT	None
5183181	South Central	Nicollet	Swan Lake WMA	44.2710	-94.2447	600	MNDOT	Revegetation
5181869	South Central	Steele	Owatonna, Bridge St	44.0842	-93.2500	870	MNDOT	None
4795628	South Central	Steele	Owatonna - off intersection Partridge Ave SE & Rose St, S of Rose St	44.0878	-93.1953	3000	Mixed	Revegetation
7847066	South Central	Steele	Rice Lake State Park	44.0942	-93.0641	300	State	None
4711241	South Central	Steele	I-35 NB, Owatonna	44.0989	-93.2450	10000	MNDOT	Revegetation
5159796	South Central	Steele	I35W N-bound, Under Exit 43 sign, ramp to NW 26th St	44.1067	-93.2456	130	MNDOT	None
None	South Central	Steele	380th Ave Janesville	44.1089	-93.7153	400	Private	None
5184803	South Central	Steele	Owatonna, I-35 at Exit 45	44.1424	-93.2534	3000	Private	Revegetation
5159674	South Central	Steele	I-35 NB Medford	44.1648	-93.2585	4356	MNDOT	Revegetation
7801896	Southeast	Dodge	Hwy 14 E of Kasson	44.0254	-92.6994	2000	MNDOT	Revegetation
7801900	Southeast	Fillmore	Mabel Hwy 44	43.5236	-91.7659	400	MNDOT	None
7801902	Southeast	Fillmore	Mabel WWTP	43.5242	-91.7603	900	Municipal	None
7801903	Southeast	Fillmore	Mabel WWTP	43.5244	-91.7603	100	Municipal	None
7801899	Southeast	Fillmore	Mabel WWTP	43.5247	-91.7627	400	Private	None
7801904	Southeast	Fillmore	Mabel WWTP	43.5247	-91.7590	6400	Municipal	None
7801907	Southeast	Fillmore	Mabel WWTP	43.5249	-91.7631	100	Private	None

EDDMapS Number	Response Region	County	Description	Latitude	Longitude	Area Invaded (sq. ft.)	Property Ownership	Restoration Category
7801901	Southeast	Fillmore	Mabel WWTP	43.5252	-91.7624	400	Private	None
7801906	Southeast	Fillmore	Mabel WWTP	43.5253	-91.7607	1600	Municipal	None
7801905	Southeast	Fillmore	Mabel WWTP	43.5254	-91.7596	2400	Municipal	None
7801898	Southeast	Fillmore	Mabel WWTP	43.5258	-91.7606	200	Municipal	Revegetation
7801908	Southeast	Fillmore	Chatfield	43.8368	-92.1800	2500	Private	None
7801895	Southeast	Goodhue	Frontenac State Park	44.5106	-92.3304	50	State	None
5209042	Southeast	Olmsted	SW corner of Cty 117 & US Hwy 63	43.9621	-92.4659	200	Mixed	None
7801923	Southeast	Olmsted	Hwy 14	44.0289	-92.6058	150	MNDOT	Revegetation
7801922	Southeast	Olmsted	Hwy 52	44.0923	-92.5118	2100	MNDOT	Revegetation
5160840	Southeast	Wabasha	McCarthy WMA Hwy 61	44.2401	-91.9569	108	Private	None
7801937	Southeast	Wabasha	N of Cty Rd 24	44.3306	-91.9793	3000	County	Restore
7801938	Southeast	Wabasha	N Cty Rd 24	44.3433	-91.9779	4000	Private	Restore
7801935	Southeast	Wabasha	N Cty Rd 24	44.3437	-91.9788	4000	Private	Restore
7801936	Southeast	Wabasha	N Cty Rd 24	44.3449	-91.9758	5000	County	Restore
None	Southeast	Winona	Hwy 61 Frontage Rd	43.9702	-91.4228	300	MNDOT	Revegetation
7801944	Southeast	Winona	Hwy 61, Minneiska	44.1907	-91.8649	200	MNDOT	Revegetation
None	Southwest	Lac Qui Parle	Lac Qui Parle WMA	45.2167	-96.2364	21780	State	Restore
7826752	Southwest	Lyon	Hwy 14	44.2396	-95.9467	1600	MNDOT	None
5157823	Southwest	Lyon	Hwy 23	44.3100	-95.9648	4000	MNDOT	Revegetation
7801940	Southwest	Redwood	Hwy 14 Lamberton WMA	44.2396	-95.2174	3000	Mixed	Restore

MINNESOTA AQUATIC INVASIVE SPECIES
RESEARCH CENTER *in partnership with*
UNIVERSITY OF MINNESOTA | EXTENSION

MNPhrag

Minnesota Non-native *Phragmites* Early Detection Project

Guide to Identifying Native and Non-native Phragmites australis

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Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND

Introduction

Distinguishing native from non-native *Phragmites australis* can be challenging. Here we provide guidance to assist you in making this distinction. The morphological characters presented here are in order of stronger characters to weaker characters. Characters most readily identifiable in the field are leaf sheath adherence to the stem and stem glossiness. These characters are best used after mid-summer and in winter. Ligule height can be a strong character, but is not as readily identifiable in the field, although note that the thickness of the band of color along the ligule can be used in the field. Stand density, stem height, leaf color, and inflorescences are variable characters that are not reliable on their own for identification. A solid ID depends on using as many as 6 different characters. Information is provided here on each of these characters to provide additional context for distinguishing native from non-native *Phragmites*.

Report populations of suspected non-native Phragmites in the EDDMapS app. Along with your report, submit several photos including photos of the whole stand and images that show details of the inflorescences, leaf sheaths, and stem color/texture.

The EDDMapS app can be downloaded for free from Bugwood and the GreatLakes Early Detection Network (GLEDN)

Thank you for your contribution to efforts in the early detection of invasive Phragmites in Minnesota.

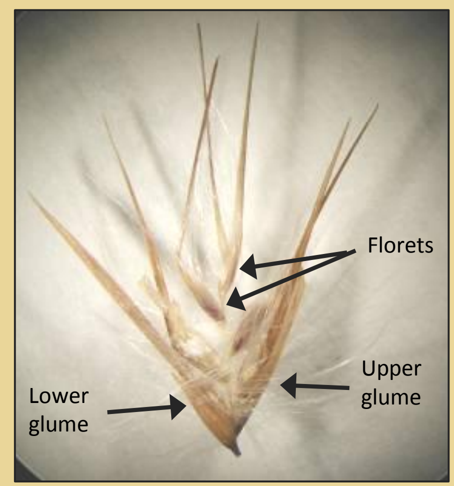
Photo Credits

- Bernd Blossey - Cornell University, Ecology and Management of Invasive Plants; Ithaca, NY. Pages 1 and 8.
- Julia Bohnen – University of Minnesota; Department of Fisheries, Wildlife and Conservation Biology; St Paul, MN. Pages 1-8.
- Robert Meadows – North Delaware Wetland Rehabilitation Program; Delaware Mosquito Control Section; Newark, DE. Page 9.
- Kristin Saltonstall – Smithsonian Tropical Research Institute; Panama City, Panama. Pages 2 and 9.

Get acquainted with terms used in this guide

Grass vegetative structures

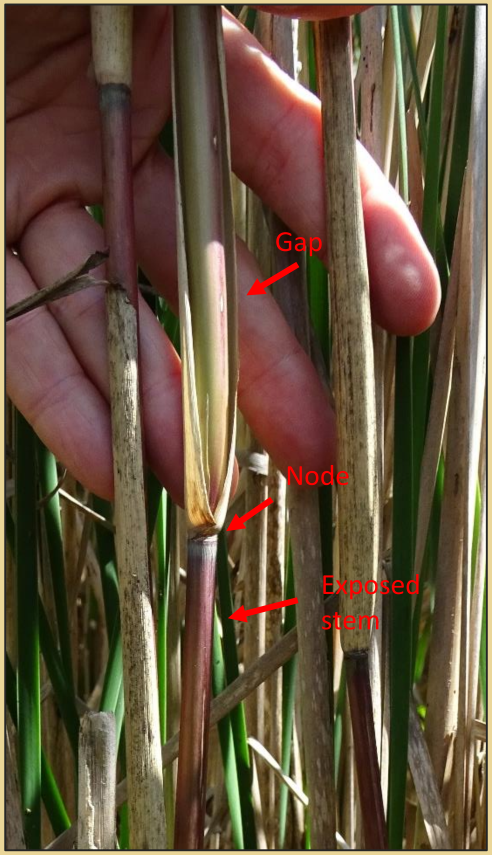
Grass floral structures



Inflorescence photo courtesy R. Blossey. Spikelet photo courtesy K. Saltonstall.

Leaf Sheath Adherence to Stem

Leaf Sheaths on Current Year's Stems



Native
Sheaths loosely attached and gap away from the stem; some may be open down to their attachment at the node.

Non-native
Sheaths closely attached to the stem with no gaps.

These photos taken in August

ID Tips:
In early to mid summer, the leaf sheaths on the upper stems of **native *Phragmites*** are also tightly adhering. Lower sheaths may be somewhat loose, but may not gap yet. Note that the sheaths of **native *Phragmites***, particularly on the lower stems, do not consistently overlap each other and the stem is exposed in the gap between the two adjacent sheaths. In early summer, the stems will already be red where they are not covered by the sheath and they will be smooth and shiny.

The sheaths of **non-native *Phragmites*** more consistently overlap each other, so the stem appears to be more consistently green. Sometimes on the lower stem, the sheaths do not overlap, and where the stem is exposed, it may have a reddish blush This seems to be more typical of young stems and stems growing in standing water. Where the stem is exposed, it will be dull and rough, as described on page 5.

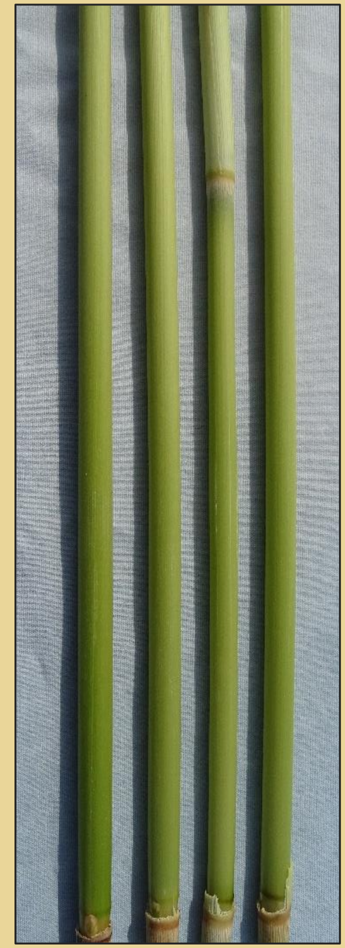
Stem Texture and Color

Native
Stem glossy and feels smooth to the touch; typically chestnut-red in the lower part of the plant.

Non-native
Stem feels rough due to ridges in the stem; typically green, but may be red on the lower stem.

Note: For color and texture, be sure to assess the stem and not the sheath which covers the stem.

Stem color with sheaths removed



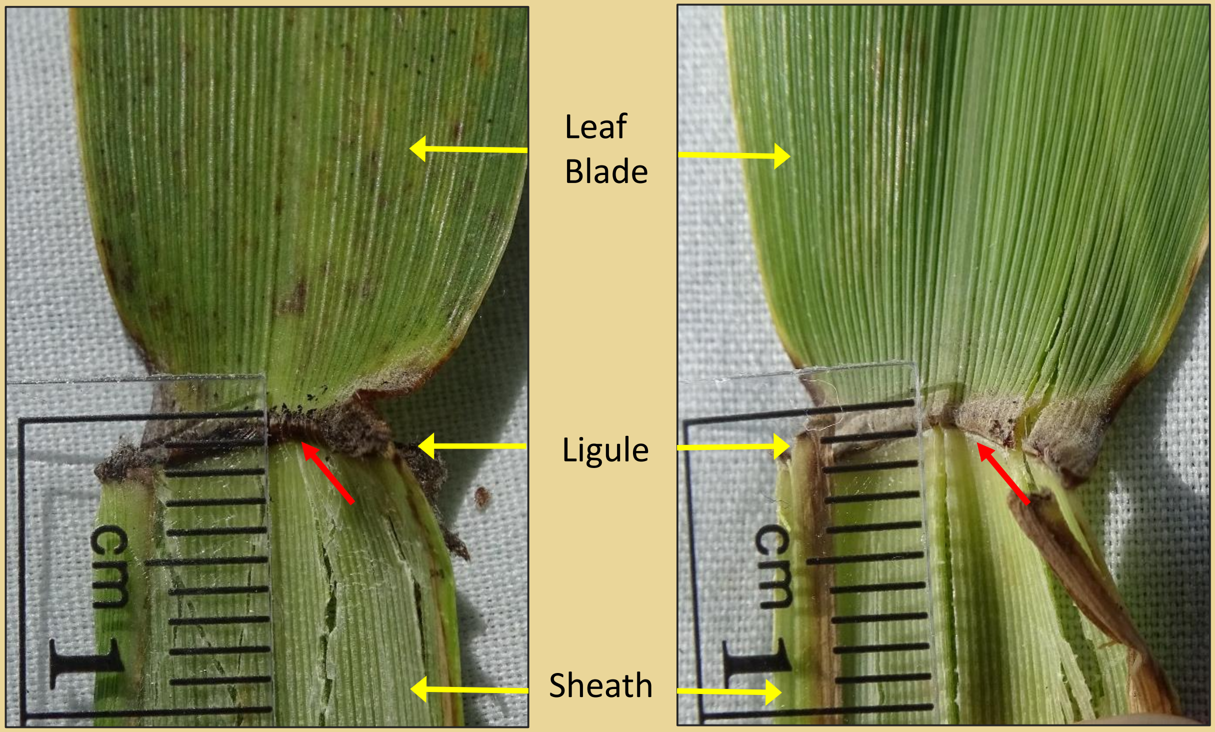
Native
Stems glossy and rosy to chestnut-red in the lower half of the plant, especially where exposed to light; stems green where sheath was removed.

Non-native
Stems dull and typically green throughout, but may be red on the lower stem.

Ligule Height (Thickness)

Ligule height (thickness) is one of the stronger characters for identifying non-native *Phragmites*. Although it may not be easy to measure in the field, it can be visually determined with a little practice using the cues described here.

Measure ligule height on leaves from approximately the middle third of the plant. Ligules on upper, newly emerging leaves are not as well-developed. On lower leaves, ligules may be degraded.



Native
Thick smudgy line (red arrow)
>1 mm (1.0-1.7 mm)

Non-native
Thin discrete brown line (red arrow)
<1 mm (0.4-0.9 mm)

To find the ligule (see the red arrows), hold a leaf blade in one hand and the culm in the other, pull the leaf blade away from the culm to expose the ligule. Measure the height of the ligule from the point of attachment as indicated by the red arrows. Include the membranous tissue and the short, stiff fringe of hairs in the measurement. Do not include any longer thread-like hairs. A hand lens is helpful to determine the area to measure.

ID Tips: In early to mid summer, the ligule of the native type is brown and does not look smudged. In late summer and fall, the ligule of the native type is described as a thick smudged line as if drawn with a lead pencil. In summer and fall, the ligule of the non-native type can be described as a discrete thin, brown to black line as if drawn by a fine point marker.

Stem Density, Persistence, and Height

Native
Stem density is often low (upper inset), allowing mixed species communities, though high density monocultures also occur. Dead stems persist through winter, but may not be as abundant the following season as in non-native stands. Plant height is up to 12 feet tall. The stand will be dark green early in the season, but will begin turning yellowish-green as early as mid-August, as it senesces earlier than the non-native (lower inset).

Non-native
Stem density is typically high with live and dead stems forming a dense monoculture; newly established populations may be less dense (inset). Standing dead stems persist into the following season. Plant height is as much as 15-18 feet tall. The stand may appear bluish-green and by late summer is usually darker than most populations of the native form. Stays green after early frosts.

Leaf Blade Color

Native - Leaf blade color is deep green in early summer as the plants emerge. Plants begin to senesce and yellow as early as August and can readily be picked out by their yellow tone by early September (inset).

Non-native - Leaf blade color is typically darker bluish-green. Dark green lasts until after the first hard frost.

Inflorescence

The large fluffy inflorescences along with the height of the plants may be the first thing that draw your attention to *Phragmites*. Don't rely on these characteristics alone to make an ID. Confirm the ID using characteristics of the sheath, stem texture, stem color, and ligule.

Native
Emerging inflorescences are green to purplish-green; may be more sparse compared to the invasive form; persist through winter at a lower density.

Non-native
Emerging inflorescences are green to purplish-green; may be more dense compared to the native form; persist through winter at a higher density.

Inset winter inflorescence photos courtesy B. Blossey.

Late Winter and Early Summer ID Tips

Inflorescences on Previous Year's Stems

Native
Inflorescence thin and few branched

Non-native
Inflorescence full and much branched

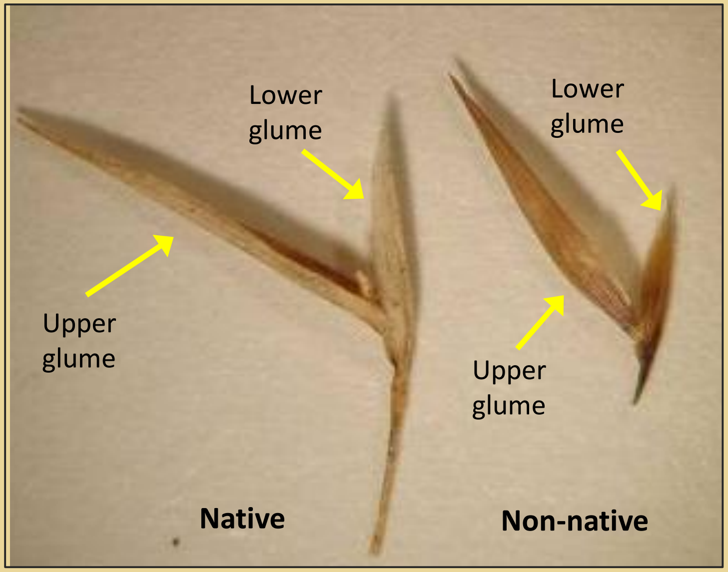
Leaf Sheaths on Previous Year's Stems

Native
Sheaths loosely attached; most readily fall off stem when leaf blades die, leaving smooth glossy bare stems the following season. "Naked = Native"

Non-native
Sheaths closely attached; more likely to persist on stems the following season.

More Difficult/Less Reliable Characteristics

Glumes



Native
Lower glume
3.0-6.5 mm,
most >4 mm

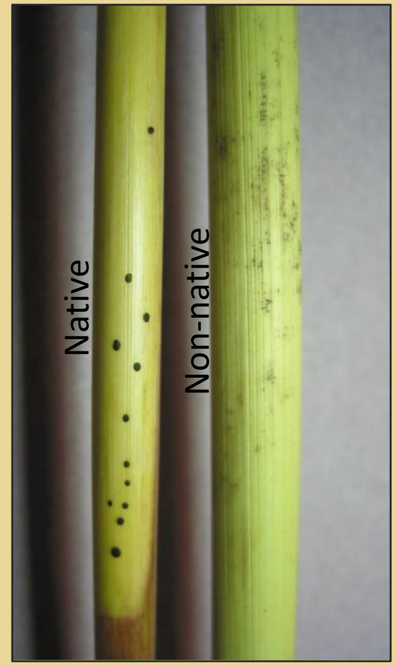
Upper glume
5.5-11.0 mm,
most >6.0 mm

Non-native
Lower glume
2.5-5.0 mm,
most <4 mm

Upper glume
4.5-7.5 mm,
most <6.0 mm

Glume characters are not easy to use in the field. Measurable glumes are not present in every season and measurement requires a microscope.

Spots on Stems



Native
Fungal spots may occur on the stem after mid-summer. Many stands will not have spots.

Non-native
This image shows mildew on the stem. Some non-native stands have now been found with fungal spots as well.

Fungal spots alone should not be relied upon as an identifying characteristic.

Glume photo courtesy K. Saltonstall. Spots on culm photo courtesy R. Meadows.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota
SUBPROJECT MANAGER: Raymond M Newman
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $221,375
AMOUNT SPENT: $220,412
AMOUNT REMAINING: $963

Sound bite of Subproject Outcomes and Results

We determined the distribution of hybrid, Eurasian, and northern watermilfoil in Minnesota and assessed factors related to this distribution. We also assessed genetic variation (diversity) and distribution of specific genotypes and began an assessment of the response of watermilfoil and genotypes to management with herbicides.

Overall Subproject Outcome and Results

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most problematic invasive aquatic plants in Minnesota. It can hybridize with the native northern watermilfoil (*M. sibiricum*) and reproduce sexually. Previous studies show that some genotypes of hybrid are resistant to specific herbicides and some may be more invasive. We determined the distribution of hybrid, Eurasian, and northern watermilfoil in Minnesota and assessed factors related to this distribution. We also assessed genetic variation (diversity) and distribution of specific genotypes and began an assessment of the response of watermilfoil and genotypes to management with herbicides. We sampled 64 lakes across the state stratified by county, size, and duration of infestation and collected milfoil from random points. The DNA from the milfoil samples was analyzed to determine taxon (Eurasian, northern or hybrid) and specific genotypes.

We found Eurasian in 43 lakes, hybrid in 28 lakes, and northern in 23 lakes. Hybrid was much more common in the metro, whereas Eurasian was broadly distributed. Northern watermilfoil was the most diverse with 84 genotypes, none shared across lakes. In contrast, we found one widespread genotype of Eurasian and six others found in indivdual lakes. Hybrid was intermediate in diversity with 53 genotypes; most lakes had only 1 unique genotype but 40% had multiple hybrid genotypes. Several genotypes were found in multiple lakes indicating clonal spread. The high diversity of hybrid watermilfoil indicates there is much potential for selection of problematic genotypes that are resistant to herbicides or that are competitively superior. There are numerous hybrid genotypes that could become problematic, but few have been widely distributed. We have not yet identified any clearly problematic genotypes in Minnesota but lakes with unexplained treatment failures, and populations with high diversity should be assessed. We will implement a strategy to identify and test problematic genotypes in Phase II of this project – MAISRC Subproject 18.2: Genetics to improve hybrid and Eurasian watermilfoil management.

Subproject Results Use and Dissemination

We disseminated our results with presentations at the MAISRC Research & Management Showcase, several regional meetings and the national Aquatic Plant Management Society. We met with DNR Specialists, lake managers, consultants and other stakeholders twice to present results and to seek input on further work. In conjunction with MAISRC staff, we developed a Google Map indicating the locations we sampled and found Eurasian, hybrid and northern watermilfoil (https://www.maisrc.umn.edu/hybrid-distribution). This map will be updated as we get new information. We also generated a preliminary report in March 2019 and a final report detailing the background, methods, results and conclusions for distribution to managers and stakeholders and posting on the MAISRC website. The DNR and managers are starting to take this information into account when planning control activities.

Eurasian and hybrid watermilfoil genotype distribution in Minnesota

Final Report to the Minnesota Aquatic Invasive Species Research Center

August 2019

Raymond M. Newman, Project Manager, University of Minnesota

Ryan A Thum, Co-PI Montana State University

With assistance and input from Graduate Student Jasmine Eltawely

Department of Fisheries, Wildlife and Conservation Biology

University of Minnesota

St. Paul, MN 55108

Email Address: RNewman@umn.edu

Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR)

Abstract:

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most problematic invasive aquatic plants in Minnesota. It can hybridize with the native northern watermilfoil (*M. sibiricum*) and reproduce sexually. Previous studies show that some genotypes of hybrid are resistant to specific herbicides and some may be more invasive. We determined the distribution of hybrid, Eurasian, and northern watermilfoil in Minnesota and assessed factors related to this distribution. We also assessed genetic variation (diversity) and distribution of specific genotypes and began an assessment of the response of watermilfoil and genotypes to management with herbicides. We sampled 64 lakes across the state stratified by county, size, and duration of infestation and collected milfoil from random points. The DNA from the milfoil samples was analyzed to determine taxon (Eurasian, northern or hybrid) and specific genotypes.

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Background

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most troublesome aquatic weeds in North America. It occurs in over 350 waterbodies in Minnesota (https://www.dnr.state.mn.us/invasives/ais/infested.html) in 35 counties. In addition to suppressing native plant communities, inhibiting recreation and use and suppressing property values, hundreds of millions are spent annually on its control, with over $2 million per year in Minnesota. Recently concern has arisen for hybrid watermilfoil, which may respond differently to management or be more invasive than pure Eurasian (LaRue et al. 2013b, Taylor et al. 2017, Thum and McNair 2018). This study aims to determine the distribution and extent of the hybrid milfoil problem in Minnesota to define the scope of the problem and develop specific hypotheses that can be tested with future studies to improve management.

Eurasian watermilfoil hybridizes with the native northern watermilfoil (*M. sibiricum*) (Moody and Les 2002, 2007; Zuellig and Thum 2012, LaRue et al. 2013b). Hybrids are difficult to distinguish from Eurasian watermilfoil (Moody and Les 2007), and as a result, populations identified as “Eurasian watermilfoil” may be composed of “pure” Eurasian watermilfoil, hybrids, or both. Although managers and aquatic botanists increasingly recognize Eurasian and hybrid watermilfoil as distinct taxa, they are not frequently distinguished when it comes to operational management strategies, control tactics, or evaluations of management actions. Recent molecular genetic studies demonstrate that genetic diversity is much higher in watermilfoils than previously recognized (Zuellig and Thum 2012). Several studies have identified clear tolerance by some hybrid genotypes to some herbicides, including fluridone (Berger et al. 2012, 2015; Thum et al. 2012) and the auxin mimics 2,4-D (LaRue et al. 2013a; Taylor et al. 2017) and triclopyr, whereas studies on other genotypes have not found any evidence for tolerance (e.g., Poovey et al. 2007, Slade et al. 2007, Glomski and Netherland 2010, Berger et al. 2012). Netherland and Willey (2017) found that some genotypes that were relatively tolerant to one herbicide were relatively susceptible to others, and vice versa. Although hybrid watermilfoil has been documented in Minnesota since the early 2000s (Moody and Les 2002, 2007) and additional occurrences have since been reported, a comprehensive assessment of the distribution and genetic diversity of hybrid watermilfoil in Minnesota has not been conducted.

To address this gap, we assessed the distribution and occurrence of hybrid watermilfoil in Minnesota and examined relations to factors that may affect its ecology and management. Specifically, our project had the following objectives:

Objective 1: Describe the frequency of occurrence and the geographic distribution of hybrid watermilfoil in Minnesota in order to determine the extent of this AIS problem and evaluate factors that are relevant to its biology and management. Specifically, test whether it is a) geographically widespread versus restricted to the Metro Region, b) more likely to occur in lakes with native northern watermilfoil, or c) more likely to occur in lakes with a longer invasion history.

Objective 2: Delineate and quantify genetic variation in hybrids in order to determine the role different genotypes and genetic diversity might play in its distribution and management. Specifically, A) assess whether specific genotypes are associated with a) geography and distribution extent, b) invasion history, or c) management history. B) Determine whether genetic diversity or the occurrence of specific genotypes is related to a) local environment and aquatic plant communities or b) management history or actions.

Methods

To determine the occurrence and distribution of hybrid watermilfoil in Minnesota we sampled 62 lakes with varying size and duration of infestation in 24 counties across the state. We determined the number of lakes to sample per county based on the relative numbers of lakes with documented Eurasian watermilfoil infestations (includes hybrid) as of 2017 from the Minnesota Department of Natural Resources' (MNDNR) infested waters list: https://www.dnr.state.mn.us/invasives/ais/infested.html). Lakes sampled ranged from 12.5 to 51,891 hectares in size, 2.5 to 135 m in maximum depth, and the durations of infestation ranged from 1 to 31 years (Appendix A). Because the MNDNR does not differentiate between Eurasian and hybrid when indicating invasive milfoil infestations, the year first infested may be based on sighting of either Eurasian or hybrid watermilfoil. We sampled and recorded presence of northern watermilfoil at each location, but our data does not fully reflect the distribution of northern watermilfoil in Minnesota because we sampled from only lakes listed as Eurasian/hybrid infested and northern occurs in many non-infested lakes.

Field sampling and data collection

At each lake we navigated to ~100 pre-selected random points within a predefined littoral zone (depth ≤ 4.6m). At each point, at least one individual stem (top 10-15 cm of plant) was collected for each unique watermilfoil taxon found at that location and placed in a labeled sealable bag on ice in a cooler. Taxa were identified visually based on morphological features and leaflet counts. The following leaflet counts were used to identify each taxon: Eurasian 14-21 leaflet pairs, northern 5-9 pairs, and hybrid 10-13 pairs (Moody and Les 2007). At each surveyed point the depth and number of plant stems per taxa collected were recorded. Plants were returned to the laboratory, rinsed of any debris, and meristem tips (top 1-2 cm) were flash frozen and stored at -80 ˚C until analysis.

Genetic identifications

Total genomic DNA was extracted from cleaned plant samples using DNeasy Plant Mini Kits (Qiagen). To distinguish Eurasian, hybrid, and northern watermilfoil, plants were identified to taxon using a genetic assay based on internal transcribed spacer DNA sequence (ITS; Moody and Les 2007, Grafé et al. 2015). The same DNA samples were then used to determine genetic composition. Genetic variation was quantified and specific clones were delineated using eight microsatellite markers developed by Wu et al. (2013) (Myrsp 1, Myrsp 5, Myrsp 9, Myrsp 12, Myrsp 13, Myrsp 14, Myrsp 15, and Myrsp 16). Each microsatellite locus was amplified using the protocols detailed in Wu et al. (2013). Fluorescently labeled microsatellite PCR products were sent to University of Illinois – Urbana-Champaign's Core Sequencing Facility for fragment analysis on an ABI 3730xl sequencer. Microsatellites were scored using GeneMapper, version 5.0 (Applied Biosystems). Because EWM, NWM, and hybrids are hexaploid, exact genotypes cannot be determined because the numbers of allele copies are ambiguous. Therefore, we treated microsatellites as dominant, binary data (i.e., presence or absence of each possible allele at each locus) using the R-package POLYSAT (Clark and Jasieniuk 2011).

We delineated distinct genotypes using Lynch distances and a threshold of 0 in POLYSAT (Clark and Jasieniuk 2011). We genetically analyzed 20 randomly selected samples from each lake when available; if genetic variation was present or for lakes assessed more intensively or on several occasions we analyzed additional plants. Over 1600 plants were genotyped.

Distribution Data Analysis

Based on the genetically determined taxon identifications, all surveyed lakes were mapped with ArcGIS 10.5 to indicate presence/absence of each milfoil taxon. The geographic distribution of hybrid watermilfoil was determined, as well as relative distances between infestations. Hybrid watermilfoil infestations were assessed to determine if they were more commonly found in the Twin Cities metro versus greater Minnesota. To determine the influence of lake and environmental attributes associated with the presence of hybrid watermilfoil in Minnesota and to make comparisons between lakes, we assessed the following factors for each lake (or bay of Lake Minnetonka): age of infestation, number of vehicle/trailer parking spaces at public water accesses, lake area, maximum depth and littoral area (water depth ≤ 4.6m) as obtained from the MNDNR's LakeFinder database <https://www.dnr.state.mn.us/lakefind/index.html>.

Water quality variables including mean Secchi depth and trophic state index were obtained from the Minnesota Pollution Control Agency (MPCA) lake and stream water quality assessment database <https://cf.pca.state.mn.us/water/watershedweb/wdip/index.cfm>. Data for both variables were based on the ten-year average from state index data collected between June and September 2008 to 2017. Lakes were given milfoil management ratings on a scale of 0-3 to describe the extent of milfoil management, which include both chemical and mechanical control, based on DNR permit approval data from 2012 to 2017. A zero indicates no management during this 6-year period, one indicates 1-2 treatments, two indicates 3-4 treatments, and three indicates 5-6 treatments. A total of four lakes were excluded from these lake attribute analyses because sampling methods were inconsistent; however they were included in the taxa distribution map and assessment to indicate presence/absence.

To assess relationships for each attribute described above, lakes were separated into groups based on milfoil taxon presence (EWM, HWM, NWM lake), making it possible for the same lake to be in more than one group if it contained multiple milfoil taxa. To determine if significant differences existed between the means of each group, a one-way analysis of variance (ANOVA) was used to compare means for the various attributes (lake area, maximum depth, littoral area, Secchi depth, distance from nearest infestation, parking spaces at water access, milfoil management rating, and age of infestation) with a p-value of 0.05 used to determine significance.

Genetic Diversity and Response to Management

We used the microsatellite genotype IDs to first look at the distribution of genetic diversity within and among taxa, and across the state and by lake attributes. We then looked at the distribution of specific genotypes among lakes and identified lakes that share genotypes.

To assess genetic variation in more detail and the potential response of hybrid watermilfoil to management with herbicides, ten lakes were selected to be intensively sampled based on recommendations by the DNR, consultants, and applicators. The five treatment lakes were Bald Eagle (62-0002), Ham (02-0053), Schmidt (27-0102), and North Arm (27-013313), and Grays' Bay (27-013301) of Lake Minnetonka. Schmidt Lake and North Arm Bay of

Minnetonka were treated with a lake-wide fluridone application, Ham Lake and Grays Bay received partial lake treatments with ProcellaCOR, and Bald Eagle had a partial lake treatment with 2-4,d. The control lakes were Christmas (27-0137), Smith's Bay of lake Minnetonka, Upper Prior (70-0072), and Otter (02-0003).

Control and treated lakes were surveyed in 2018 to characterize milfoil abundance and the plant community with Point Intercept Surveys (e.g., Madsen 1999, Nault et al. 2018; > 100 littoral points per lake) and samples of watermilfoil were collected at each site present and frozen for genetic analyses. Treated lakes were resurveyed in August to characterize the response to herbicide treatment and characterize the native plant community. Milfoil and native plant frequency of occurrence and density were compared before and after treatment lake-wide and within the areas of treatment. Changes in frequency and distribution of genotypes was also assessed.

Results

Occurrence and geographic distribution in Minnesota

A total of 62 Eurasian watermilfoil infested lakes were sampled (2 non-infested lakes containing northern watermilfoil were also sampled). We did not find any milfoil in two lakes (Gervais 62-0007 and Locke 86-0168), 43 contained Eurasian, 28 contained hybrid, and 23 contained northern (Table 1). We found various taxa combinations in surveyed lakes where milfoil was found (Table 2). Of the 28 lakes that we found containing hybrids, 13 had only hybrid watermilfoil and no other milfoil taxa, and the remaining 15 had some combination with either Eurasian, northern, or both (Table 2). In assessing all hybrid infested lakes containing one or the other parental taxon, it was found that hybrid was more likely to be present in a lake with Eurasian (13 lakes) versus northern (3 lakes). There were also significant geographic relationships. Hybrid-only infestations were mostly present in the metro (91%); only one hybrid exclusive infestation was found in greater Minnesota (Figure 1). The hybrids found in lakes outside of the metro were largely from populations that also had Eurasian and/or northern. We found four lakes that contained all three taxa, half of which were in the metro and half in greater Minnesota (Table 2).

Eurasian was evenly distributed across the state (Figure 1) and it was most commonly found in lakes that contained another taxon rather than existing alone (Table 1). In lakes where another taxon was present with Eurasian, it was more commonly found with northern (60%) over hybrid (40%). We found that 83% of lakes where both Eurasian and northern were present were outside of the metro, indicative of northern being most commonly found there as well.

Northern watermilfoil was more common outside the Twin Cities metro: 30% of lakes with northern were in the metro and 70% were outside (Figure 1). This may be due to the longer invasion history in the metro (Eurasian displacing northern) or better water clarity and more diverse plant communities outstate. Hybrid watermilfoil tended to be clustered in the metro and specifically the central and eastern metro (Figure 1). Very few lakes (5) outside the 7-county metro had hybrid (Table 2) and somewhat surprising, no lakes in Carver county (western metro) had hybrid (Table 1) despite the long occurrence of Eurasian watermilfoil in Carver county (since 1989) and large number of infestations (27).

Table 1. Summary of genetic analyses of lakes surveyed in 2017-2018. The number of each taxon identified from samples collected in each lake is presented and the number of distinct genotypes is indicated for each taxon in each lake.

		Counts per taxon			Number of genotypes		
Lake	**County**	**EWM**	**HWM**	**NWM**	**EWM**	**HWM**	**NWM**
Coon	Anoka	11	29		1	2	
Crooked	Anoka		20			3	
Ham	Anoka		97	6		1	1
Otter	Anoka		64			2	
Ballantyne	Blue Earth	20			1		
Chub	Carlton	1		19	1		1
Auburn	Carver	24			1		
Piersons	Carver	19			1		
Riley	Carver	21			1		
Steiger	Carver	20			1		
Swede	Carver	13			1		
East Rush	Chisago		18	2		1	1
South Lindstrom	Chisago		9	19		1	4
Bay	Crow Wing	14		6	1		3
Emily	Crow Wing	2		6	1		6
Alimagnet	Dakota		20			1	
Cobblestone	Dakota		2			1	
Fish	Dakota		20			1	
Lac Lavon	Dakota		20			5	
Orchard	Dakota			5			4
Thomas	Dakota		5			2	
Oscar	Douglas	5		15	1		5
Cedar	Hennepin	5			1		
Christmas	Hennepin	48		33	1		5
Harriet	Hennepin	20			1		
Independence	Hennepin	43	44		1	1	
Minnetonka-Grays	Hennepin		54			5	
Minnetonka-North Arm	Hennepin		20			7	
Minnetonka-Smiths	Hennepin	14	37	6	2	10	4
Mitchell	Hennepin	24		16	1		3
Rebecca	Hennepin	21	8		1	1	
Schmidt	Hennepin		62			2	
Staring	Hennepin	8			1		
Spectacle	Isanti	3		22	1		4
Green	Kandiyohi	2			1		
German	Le Seuer	1	9	1	1	5	1
Minnie-Belle	Meeker	1		25	1		5
Mille Lacs	Mille Lacs	2		10	1		2
Pokegama	Pine	5			1		

Gilchrist	Pope	20			1		
Bald Eagle	Ramsey	35	43	50	1	1	3
Gervais	Ramsey						
Josephine	Ramsey		19			1	
McCarron	Ramsey	21	11		1	1	
Phalen	Ramsey	4			1		
Turtle	Ramsey	6	6		1	1	
Fox	Rice	20			2		
McMahon	Scott	4			1		
Upper Prior	Scott	14	10		2	2	
Mitchell	Sherburne	5		34	1		3
Gilbert Pit	St. Louis	9			1		
Little Birch	Todd	4		15	1		6
Big Carnelian	Washington			5			3
Big Marine	Washington	12		13	1		8
Bone	Washington		19			1	
Elmo	Washington	16	23		1	1	
White Bear	Washington	24	12		1	1	
Cedar	Wright			20			6
Constance	Wright	17			1		
Howard	Wright	9	10	1	1	6	1
Indian	Wright		1			1	
Locke	Wright						
Somers	Wright	2			1		
Sugar	Wright	1		19	1		5

Table 2. Occurrence of taxa in lakes in the seven county metro, greater Minnesota, and statewide for combinations present in all surveyed lakes.

	EWM only	**HWM only**	**NWM only**	**EWM & HWM**	**NWM & HWM**	**EWM & NWM**	**All three taxa**	**Total**
Greater Minnesota	8	1	1	0	2	10	2	24
Metro	10	12	0	8	1	3	2	36
Total	18	13	1	8	3	13	4	60

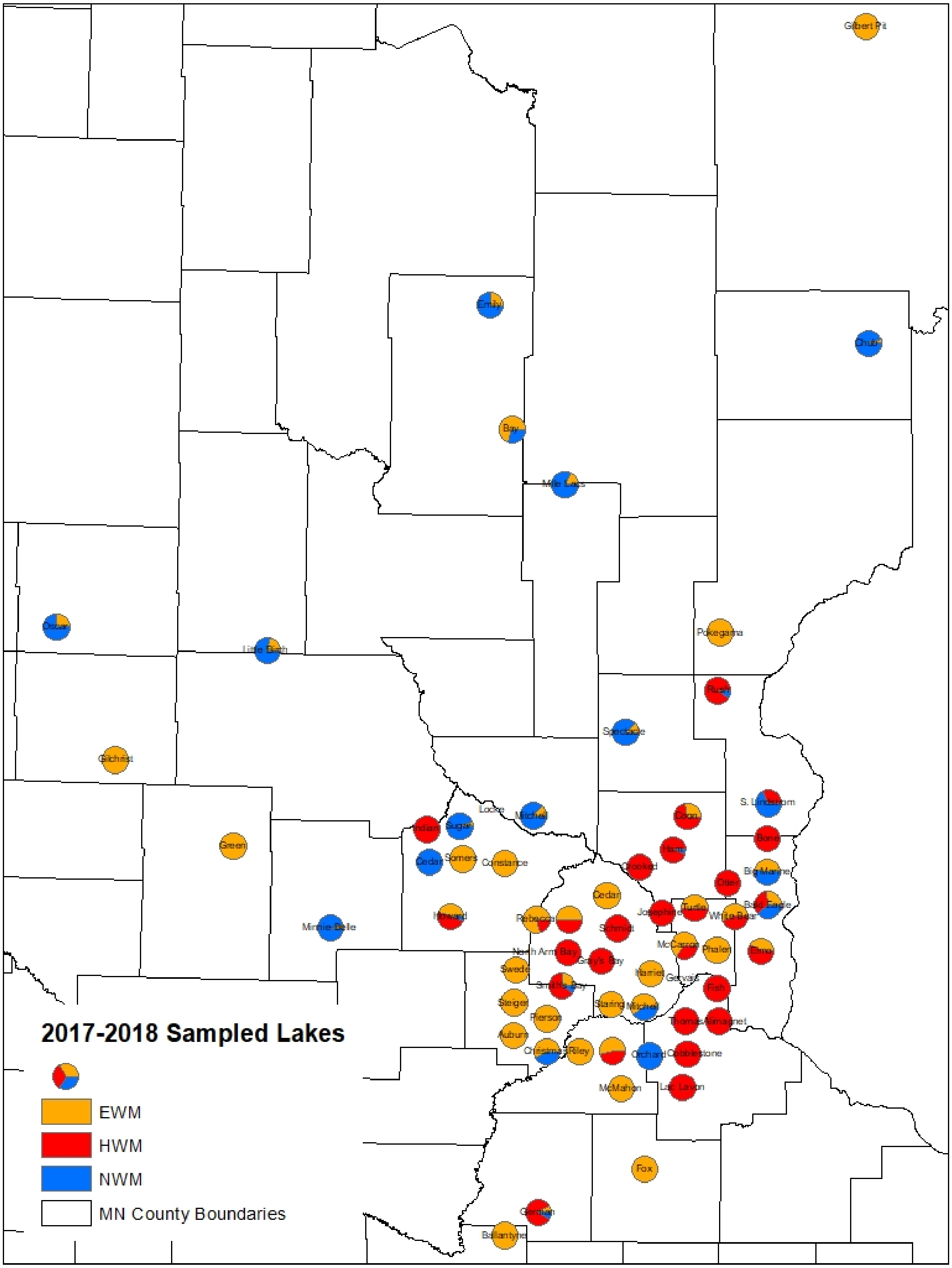

Figure 1. Statewide occurrence and proportions of Eurasian (EWM orange), hybrid (HWM red), and northern (NWM blue) watermilfoil based on genetic analyses for lakes sampled 2017-2018.

Four lakes of our total 62 lakes were left out of the environmental attribute analysis due to no milfoil being found in two lakes and limited sampling in two other lakes. Compared to lakes containing Eurasian or northern, those containing hybrid were on average smallest in size, maximum depth, and littoral area (Table 3). Average Secchi depth values for lakes with Eurasian and hybrid were similar, but lakes with northern on average had deeper Secchi depths. Across all three taxa most lakes (94%) had a trophic state index (TSI) within the range of meso- to eutrophic. Hybrid infestations were on average closer to one another in comparison to Eurasian and northern lakes across the state (Table 3).

Table 3. Mean values and standard errors for environmental characteristics of 58 sampled Minnesota lakes classified as containing either Eurasian (EWM), hybrid (HWM), or northern (NWM) watermilfoil.

Lake type[a]	**Lake area (ha)**	**Max depth (m)**	**Secchi Depth – water clarity of a lake (m)**	**Littoral Area (ha)**	**Trophic state index**	**Average distance from nearest infestation (km)**
EWM Lakes	299 ± 62	17.5 ± 3.2	2.5 ± 0.3	159 ± 28	Meso-Eutrophic	20.8 ± 3.5
HWM Lakes	202 ± 45	12.3 ± 1.5	2.4 ± 0.2	122 ± 29	Meso-Eutrophic	11.5 ± 2.2
NWM Lakes	314 ± 52	14.3 ± 1.7	2.8 ± 0.3	177 ± 31	Meso-Eutrophic	29.4 ± 5.3

[a] Lake types include all lakes with the taxon present and therefore a lake may be represented in more than one category.

We further analyzed factors associated with conditions in the metro, greater Minnesota and statewide for the same group of 58 lakes (Table 4). For all three categorized lakes (EWM, HWM, NWM), on average we found that Eurasian watermilfoil infestations were oldest in the metro in comparison to greater Minnesota, and had higher numbers of parking spaces at the water access (Table 4), however, these differences were not significant. Milfoil taxa were collected from deeper average depths from lakes in greater Minnesota versus the metro; this relationship was found across all three taxa but hybrid had the shallowest statewide average depth. Overall, sampled lakes were not heavily managed; we found that the median scores for hybrid lakes in the metro and greater Minnesota were both one. Northern lakes in the metro were less managed with a median score of 0.5 compared to greater Minnesota, which had a score of one. Eurasian lakes in the metro had a median score of zero and in greater Minnesota had a score of 0.5. The two attributes we found to be significant ($p < 0.05$) when comparing the three taxa were distance from nearest infestation ($p = 0.01$) and presence in the metro versus outstate ($p = 0.0007$).

Table 4. Average values of explanatory variables for Minnesota lakes classified as containing either Eurasian (EWM), hybrid (HWM), or northern (NWM).

		Number of lakes	Average age of infestation (years)	Average number of parking spaces at water access	Median milfoil management score	Average number of unique genotypes per lake	Average depth of collected taxa (m)
EWM	Statewide	41	16.6	22	0	1.0	1.9
	Metro	21	19.7	32	0	1.0	1.7
	Greater MN	20	13.2	11.5	0.5	1.0	2.0
HWM	Statewide	26	19.2	27.7	1	2.5	1.5
	Metro	21	20.2	29.5	1	2.5	1.5
	Greater MN	5	15	21.6	1	2.8	1.7
NWM	Statewide	21	17.8	23	1	3.6	1.8
	Metro	6	21.2	35.8	0.5	4.0	1.7
	Greater MN	15	16.4	17.8	1	3.5	1.8

Genetic diversity

We identified unique genotypes of each taxon based on microsatellites. Amongst the three taxa, EWM was the least diverse. Overall, we identified 7 Eurasian genotypes, 84 northern genotypes, and 53 hybrid genotypes in Minnesota (Table 5). For Eurasian watermilfoil, most lakes sampled in 2017-2018 (40 lakes) contained the same genotype that was the dominant genotype. There was very little within-lake diversity for Eurasian (2 lakes with > 1 genotype), and overall we have found six Eurasian genotypes that were different from the common widespread genotype. A unique Eurasian genotype was found in Chub, German, Smith's Bay, Upper Prior and two in Fox.

Hybrid watermilfoil showed intermediate genetic diversity in comparison to EWM and NWM (Table 1, Table 5). Twelve lakes had multiple hybrid genotypes, with there being particularly high diversity (≥ 5 genotypes) in three lakes (Lac Lavon, German and Howard) and three bays of Lake Minnetonka (Gray's, Smith's, and North Arm). The greatest number of hybrid genotypes in a single lake or bay was 10 found in Smiths' Bay of Lake Minnetonka of Hennepin County; Grays Bay had 5 genotypes and North Arm had 7 genotypes. Overall, Minnetonka had 17 different genotypes of hybrid watermilfoil. We found the same genotype in two sets of two lakes in Dakota county (Alimagnet and Lac Lavon share a genotype and Cobblestone and Lac Lavon shared a genotype). We also found a different, but common genotype in the following seven lakes: Bald Eagle, Bone, Fish, Josephine, Otter, South Lindstrom and White Bear, which spanned five counties (Ramsey, Washington, Dakota, Chisago, and Anoka). The bays in Lake Minnetonka also shared genotypes of HWM, but each also had unique genotypes. These common hybrid genotypes are indicative of clonal spread of hybrids in Minnesota. There are numerous hybrid genotypes that could become problematic, but there are relatively few hybrid genotypes that have been more widely distributed.

Northern watermilfoil was the most diverse, with most lakes having multiple different genotypes within (Table 1) and no genotypes shared between lakes (Table 5). The genetic diversity present in hybrids is linked to this diversity in its northern parent. They further suggest that northern watermilfoil is reproducing sexually within lakes and we have no evidence of spread of northern watermilfoil between lakes.

Comparing genetic diversity by taxa and across the state, we found that northern had an average of 3.6 genotypes per lake: 3.5 different genotypes per lake in greater Minnesota and 4 in the metro (Table 4). Eurasian had an average of 1 genotype per lake statewide and in both the metro and greater Minnesota. Hybrids had an average of 2.5 genotypes per lakes in the metro and 2.5 in greater Minnesota. In comparing the ages of infestation of hybrid lakes containing a single hybrid genotype and lakes with greater than 2 hybrid genotypes, we found that the age of infestation was significantly older ($p = 0.03$) for hybrid lakes containing 3 or more genotypes (23.7) versus those with one genotype (15.9) (Figure 2).

Table 5. Occurrence of clones (genotypes) of Eurasian (EWM), hybrid (HWM), or northern (NWM) in Minnesota. The clone number is followed by the number of plants of that clone identified in the lake (Clone:N).

			Clone: N	
Lake	**County**	**EWM**	**HWM**	**NWM**
Coon	Anoka	1:11	40:1, 55:28	
Crooked	Anoka		67:11; 68:8; 69:1	
Ham	Anoka		14:97	15:6
Otter	Anoka		3:63, 144:1	
Ballantyne	Blue Earth	1:20		
Chub	Carlton	87:1		86:19
Auburn	Carver	1:24		
Piersons	Carver	1:19		
Riley	Carver	1:21		
Steiger	Carver	1:20		
Swede	Carver	1:13		
East Rush	Chisago		88:18	89:2
South Lindstrom	Chisago		3:9	19:6; 20:10; 21:2; 22:1
Bay	Crow Wing	1:14		117:4; 118:1; 119:1
Emily	Crow Wing	1:2		75:1; 76:1; 77:1; 78:1; 79:1; 80:1
Alimagnet	Dakota		81:20	
Cobblestone	Dakota		84:2	
Fish	Dakota		3:20	
Lac Lavon	Dakota		81:5; 82:3; 83:8; 84:3; 85:1	
Orchard	Dakota			50:2; 51:1; 52:1; 64:1
Thomas	Dakota		45:4; 46:1	
Oscar	Douglas	1:5		70:6; 71:3; 72:3; 73:2; 74:1
Cedar	Hennepin	1:5		
Christmas	Hennepin	1:48		105:1; 133:6; 134:4; 135:21; 136:1
Harriet	Hennepin	1:20		
Independence	Hennepin	1:43	99:44	

Minnetonka-Grays	Hennepin		7:10; 12:32; 137:2; 138:6; 139:4	
Minnetonka-North Arm	Hennepin		6:4; 7:11; 8:1; 9:1; 10:1; 11:1; 12:1	
Minnetonka-Smiths	Hennepin	1:13; 141:1	7:19; 9:2; 12:2; 106:1; 107:4; 108:3; 109:3; 114:1; 140:1; 143:1	110:1; 111:2; 112:2; 113:1
Mitchell	Hennepin	1:24		16:12; 17:3; 18:1
Rebecca	Hennepin	1:21	56:8	
Schmidt	Hennepin		53:61, 142:1	
Staring	Hennepin	1:8		
Spectacle	Isanti	1:3		41:19, 42:1, 43:1, 44:1
Green	Kandiyohi	1:2		
German	Le Seuer	63:1	57:1; 58:2; 59:1; 60:4; 61:1	62:1
Minnie-Belle	Meeker	1:1		34:3, 35:5, 36:12, 115:1, 116:4
Mille Lacs	Mille Lacs	1:2		65:9; 66:1
Pokegama	Pine	1:5		
Gilchrist	Pope	1:20		
Bald Eagle	Ramsey	1:35	3:43	2:33, 4:16, 5:1
Josephine	Ramsey		3:19	
McCarron	Ramsey	1:21	13:11	
Phalen	Ramsey	1:4		
Turtle	Ramsey	1:6	54:6	
Fox	Rice	90:19; 91:1		
McMahon	Scott	1:4		
Upper Prior	Scott	1:13; 32:1	31:1; 33:9	
Mitchell	Sherburne	1:5		37:23; 38:7; 39:4
Gilbert Pit	St. Louis	1:9		
Little Birch	Todd	1:4		127:8; 128:3; 129:1; 130:1; 131:1; 132:1
Big Carnelian	Washington			47:1; 48:3; 49:1
Big Marine	Washington	1:12		23:2; 24:1; 25:2; 26:1; 27:1; 28:4; 29:1; 30:1
Bone	Washington		3:19	
Elmo	Washington	1:16	55:23	
White Bear	Washington	1:24	3:12	
Cedar	Wright			121:7; 122:8; 123:2; 124:1; 125:1; 126:1
Constance	Wright	1:17		
Howard	Wright	1:9	92:4; 93:1; 95:1; 96:1; 97:2; 98:1	94:1
Indian	Wright		120:1	
Somers	Wright	1:2		
Sugar	Wright	1:1		100:10; 101:3; 102:1; 103:3; 104:2

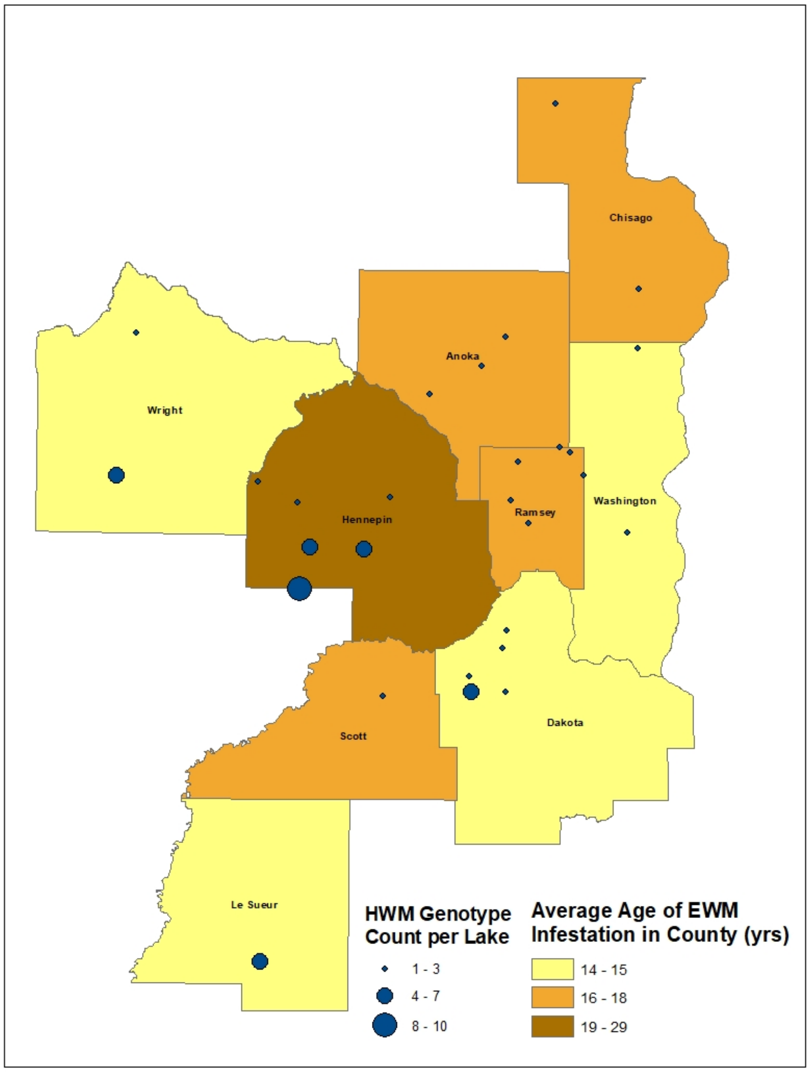

Figure 2. Hybrid watermilfoil (HWM) genotype counts per lake (blue) by average age of invasive milfoil (EWM) infestation in county (yrs).

Within lake variation and response to management

We assessed 5 reference lakes and 5 treated lakes to look at spatial and temporal changes in milfoil and hybrid genotype occurrence as well as the response of these taxa and native plants to management. All lakes had at least 1 genotype of hybrid present, except Christmas, which was previously determined to have hybrid present, but no definitive hybrids were found during our sampling in 2017-2018. Most lakes were sampled in 2017 for presence of hybrids with a random survey and then again in 2018 with point intercept surveys (higher point density) that characterized the entire plant community. The point intercept surveys will be repeated in 2019 and 2020 as part of a continuation project to assess response to management.

The lakes had a range of milfoil occurrences and densities (Table 6). In the control lakes milfoil frequency of occurrence in the littoral ranged from 4% in Upper Prior to 65% in Christmas Lake. Water clarity limited the plant community in Upper Prior (Figure 3), which also had low occurrence of native plants (31%). Both milfoil and native plant occurrence remained relatively similar between early and late summer in the two lakes that were sampled twice (Independence and Christmas) (Figure 4). These lakes have better water clarity and support a more abundant plant community than Upper Prior. Otter Lake and Smith's Bay also have good clarity and supported the most abundant native plant communities (Table 6). Milfoil was widely distributed in these lakes and northern watermilfoil was common in shallower portions of Smiths Bay (Figure 5). Milfoil was found at half the sites in Smiths Bay (Table 6).

In Otter Lake we found one hybrid genotype lake-wide in 2017 (20 samples), but with more intensive sampling in 2018 found 1 plant of a second genotype (43 plants were the same genotype found in 2017); no EWM was found. It should be noted that hybrid has been found in Otter since 1999 (Moody and Les 2001) and repeated genetic analyses since (e.g., Roley and Newman 2006, Moody and Les 2007). In Independence, one genotype of Eurasian and one of hybrid was found and no change in frequency was noted between early and late summer. In Christmas, there were no significant changes in composition of Eurasian (one genotype) and northern watermilfoil (several genotypes combined) between "early" (July) and "late" (August) samples in 2018 (χ^2=3.40, p=0.19), or between 2016 and 2018 (χ^2=1.27, p=0.26). The lake-wide frequency of occurrence in 2018 decreased from 65% to 45% between early and late samples (Table 6) and there was an increase in northern watermilfoil (Figure 4). Both taxa are distributed around the lake.

At Smiths Bay northern was present but, restricted to shallower sites and Eurasian/hybrid was more widespread (Figure 5). There was a significant change in the composition of Eurasian, hybrid, and northern watermilfoil between 2016 and 2018 (χ^2=21.59, p=0.00002); specifically, there was an increase in hybrid and a decrease in Eurasian over this time. This is consistent with hybrid expansion. There was no significant change in the composition of hybrid genotypes (χ^2=1.63, p=0.82).

Independence had a lower occurrence of milfoil (28-33%; Table 6). About half the milfoil was EWM and half was HWM (Table 5). There was no change in proportion of the two taxa between early and late summer and only one genotype of each was found. We did find some of the hybrid with 5 leaflet whirls, but there was no difference in genetic identity between the 4- and 5-leaved whirled hybrids.

For the managed lakes, Schmidt Lake and North Arm Bay of Minnetonka were treated with a lake-wide fluridone application and both had significant decreases in milfoil abundance following treatment, with almost complete elimination of milfoil (<2% frequency remaining) (Table 6, Figure 6). Only one genotype of hybrid was found in Schmidt, but future sampling can determine if other genotypes emerge. North Arm, by contrast, had much greater diversity with 7 genotypes. Previous results (Thum et al. 2017a) found a significant change in hybrid genotype composition between pre- and post-treatment with the auxin mimic triclopyr in 2015 (χ^2=9.97, p=0.02). Specifically, the "North Arm" genotype (clone 7) increased in relative frequency post-treatment. And, concomitantly, several genotypes that were found before treatment were not found after treatment (overall diversity went down). There was not a significant change in composition between pre-treatment 2015 and 2017, although it was close (χ^2=7.29, p=0.06). This is interesting, because although clone 7 increased after treatment in 2015, its relative abundance decreased back to a similar level over time. The marginal significance can be attributed to an increase in relative frequency of clone 6 and some "new" clones found in 2017. This is a diverse bay, and it looks like there is some level of introduction of new genotypes (either recruitment from seed or introductions from other bays/lakes). There was also no significant change in composition between post-treatment 2015 and 2017 (χ^2=5.23, p=0.16). The potentially tolerant clone 7, increased in 2015 after treatment, but then went back down a bit in 2017 but still stayed at a higher proportion than it was before treatment in 2015. Tolerance to herbicide and competitive or growth abilities are not necessarily correlated and further assessment of this genotype is warranted. The fluridone treatment in 2018 may have further reduced or eliminated this genotype.

The lakes treated with 2,4-D and ProcellaCOR had more focused treatments, less herbicidal coverage (8-15% of lake area treated) and less overall control (Table 6). About half of the lakewide milfoil was controlled in Bald Eagle with 2,4-D; however milfoil occurrence decreased from 53% to 5% within the treatment areas (Table 6, Figure 7). Lakewide native plant frequency increased after treatment and some northern watermilfoil expanded in the untreated areas (Figure 7). Between 2017 and pre-treatment in 2018 there was significant increase of Eurasian and hybrid relative to northern. However, there was a significant decrease in hybrid and Eurasian in 2018 from pre to post treatment and northern increased after treatment. This appears mainly due to treatments focusing on areas with abundant Eurasian and hybrid and leaving untreated areas with northern to expand (Figure 7).

There was less control observed with the use of ProcellaCOR, and lakewide milfoil abundance increased following treatment in both lakes (Table 6). On Gray's Bay the treatment-area milfoil abundance decreased from 53% to 7% following the ProcellaCOR treatment, but on Ham Lake the treatment-area milfoil abundance increased from 47% to 82% (Table 6). The lakewide increase in occurrence at Gray's was due mainly to increases in areas outside the treatment plots, although some milfoil remained in treated areas (Figure 8). At Ham, milfoil increased within and outside the treatment plots (Figure 8) after treatment. Ham also had a significant decrease in native plant coverage (82% to 70%) following treatment including a virtual loss of northern watermilfoil (Figure 8). There were, however, no significant changes in composition of one hybrid genotype and one northern watermilfoil genotype between pre- and post-treatment in 2018 (χ^2=2.01, p=0.16), or between 2017 and 2018 (χ^2=0.02, p=0.86). At Gray's Bay, there was no significant change in the composition of the five hybrid genotypes that were present across sampling times in our study and in Thum et al. (2017). For these genotypes, there were no significant differences in composition between pre- and post-treatment sampling in 2018 (χ^2=2.05, p=0.73), or between 2015 and 2018 (χ^2=2.58, p=0.46). The hybrid clone 7 genotype that increased in North Arm in 2015 and increased in Smiths between 2016 and 2018 was present in Grays Bay in 2018; it deserves further monitoring.

Table 6. Summary of intensive lakes results including milfoil and native plant frequency of occurrence (FOC) pre- and post-treatment based on 2018 surveys within the lake wide littoral zone (shallower than 4.6m) and within treated areas.

Lake	County	Treat	Lake wide Milfoil FOC (pre-treat)	Lake wide milfoil FOC (post treat)	Native plant FOC (pre-treat	Native plant FOC (post treat)	Within treated Milfoil FOC (pre-treat)	Within treated Milfoil FOC (post treat)
Ham	Anoka	Procella COR – 14 acres	23%	34%	82%	70%	47%	82%
Gray's Minnetonka	Hennepin	Procella COR – 28 acres	22%	27%	94%	98%	53%	7%
North Arm Minnetonka	Hennepin	Fluridone – lakewide	61%	0.6%	92%	97%	Lake wide	Lake wide
Schmidt	Hennepin	Fluridone – lakewide	79%	2%	100%	96%	Lake wide	Lake wide
Bald Eagle	Ramsey	2,4d – 42 acres	60%	32%	73%	92%	53%	5%
Otter	Anoka	Control	49%		96%			
Smith's Minnetonka	Hennepin	Control	53%		97%			
Independence	Hennepin	Control	28%	33%	55%	66%		
Christmas	Hennepin	Control	65%	45%	89%	91%		
Upper Prior	Scott	Control	4%		31%			

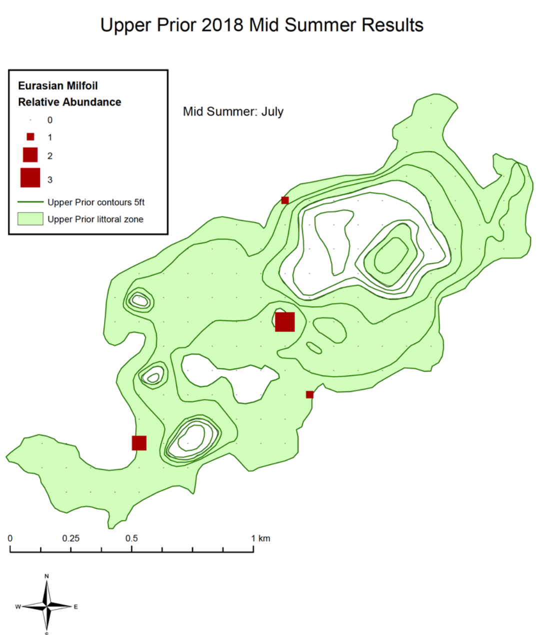

Figure 3. Occurrence and relative abundance of milfoil in Upper Prior Lake, July 2018.

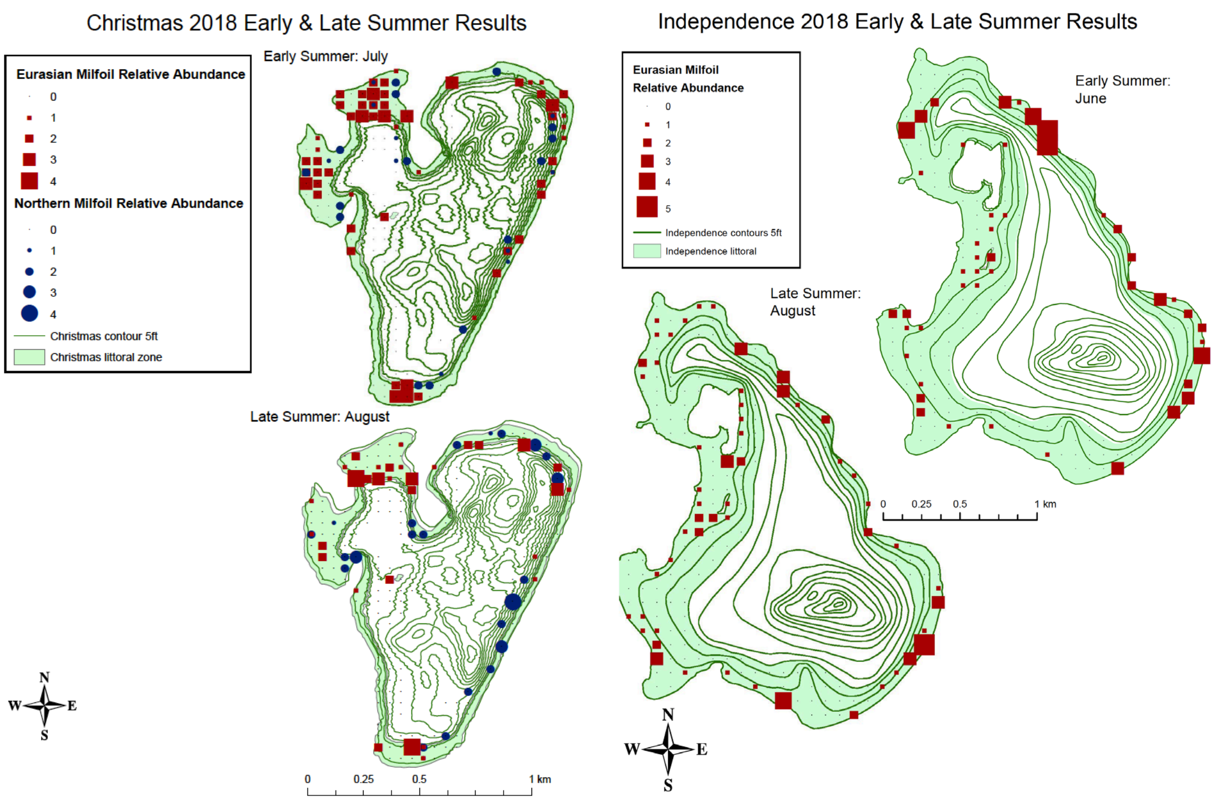

Figure 4. Occurrence and relative abundance of Eurasian (includes hybrid) and northern watermilfoil in reference lakes Christmas and Independence in early and late (August) summer.

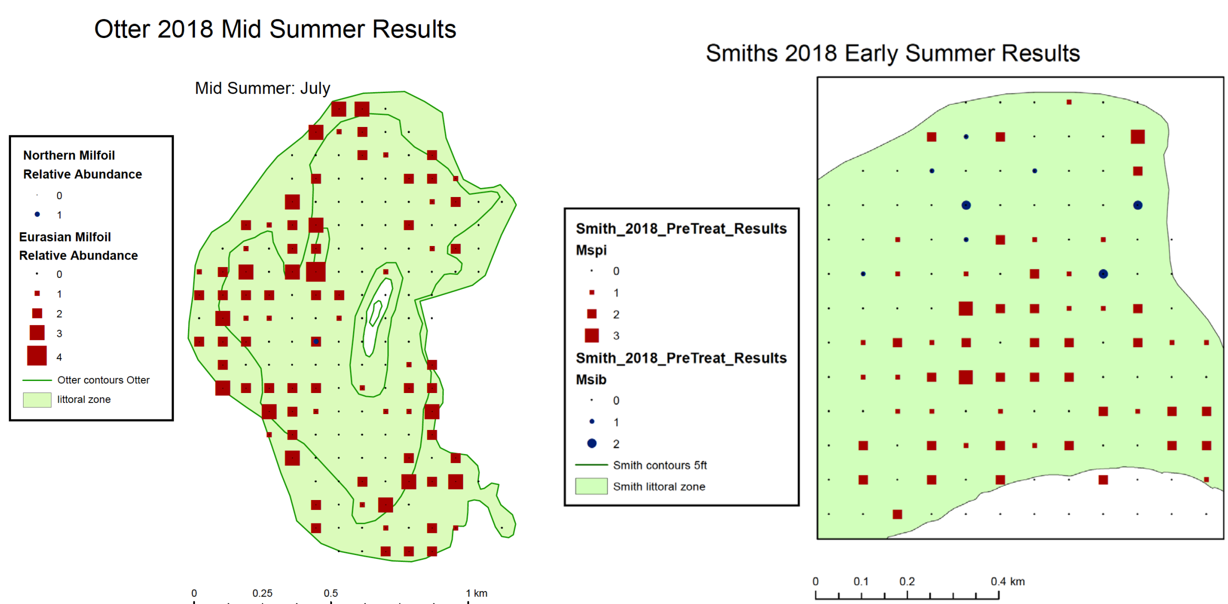

Figure 5. Occurrence and relative abundance of Eurasian (includes hybrid) and northern watermilfoil in reference lakes Otter and Smith's Bay.

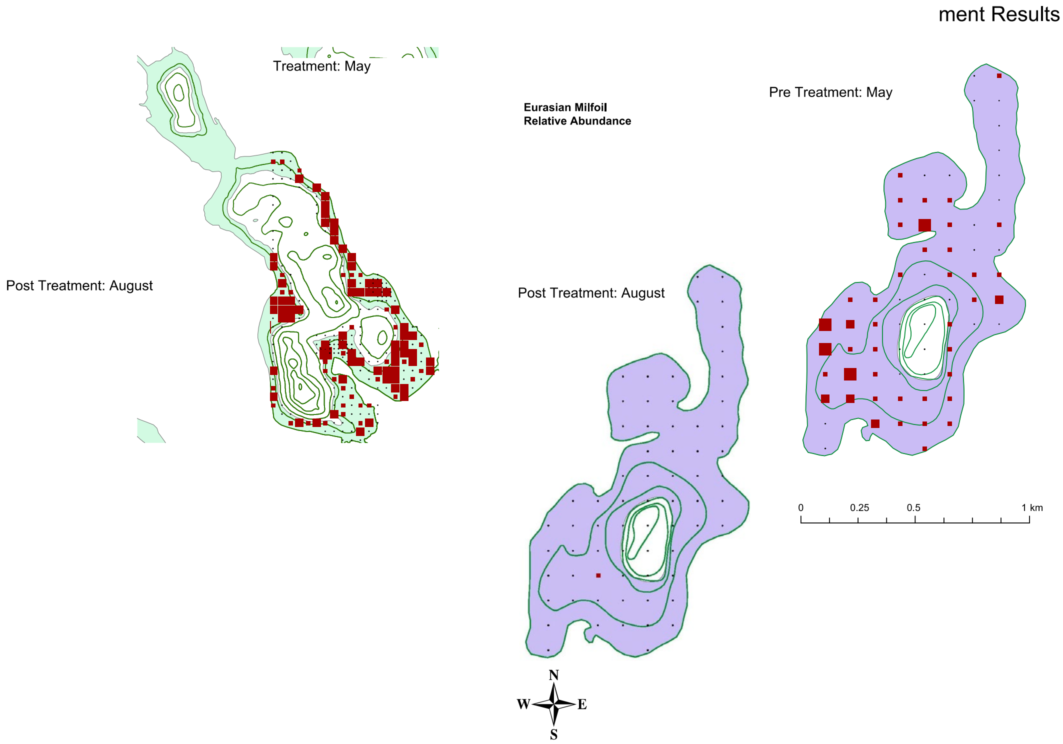

Figure 6. Pre and post-treatment occurrence and relative abundance of milfoil in North Arm Lake Minnetonka and Schmidt Lake. Both lakes were treated with fluridone in May.

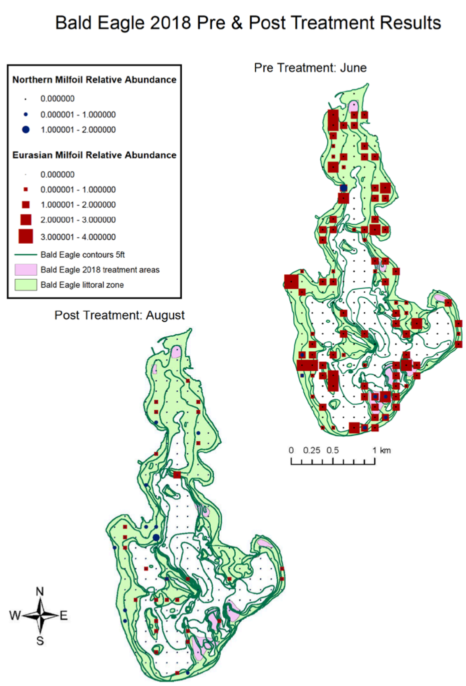

Figure 7. Pre and post-treatment occurrence and relative abundance of milfoil in Bald Eagle Lake. The lake was treated with 2,4-d in localized treatment areas in July.

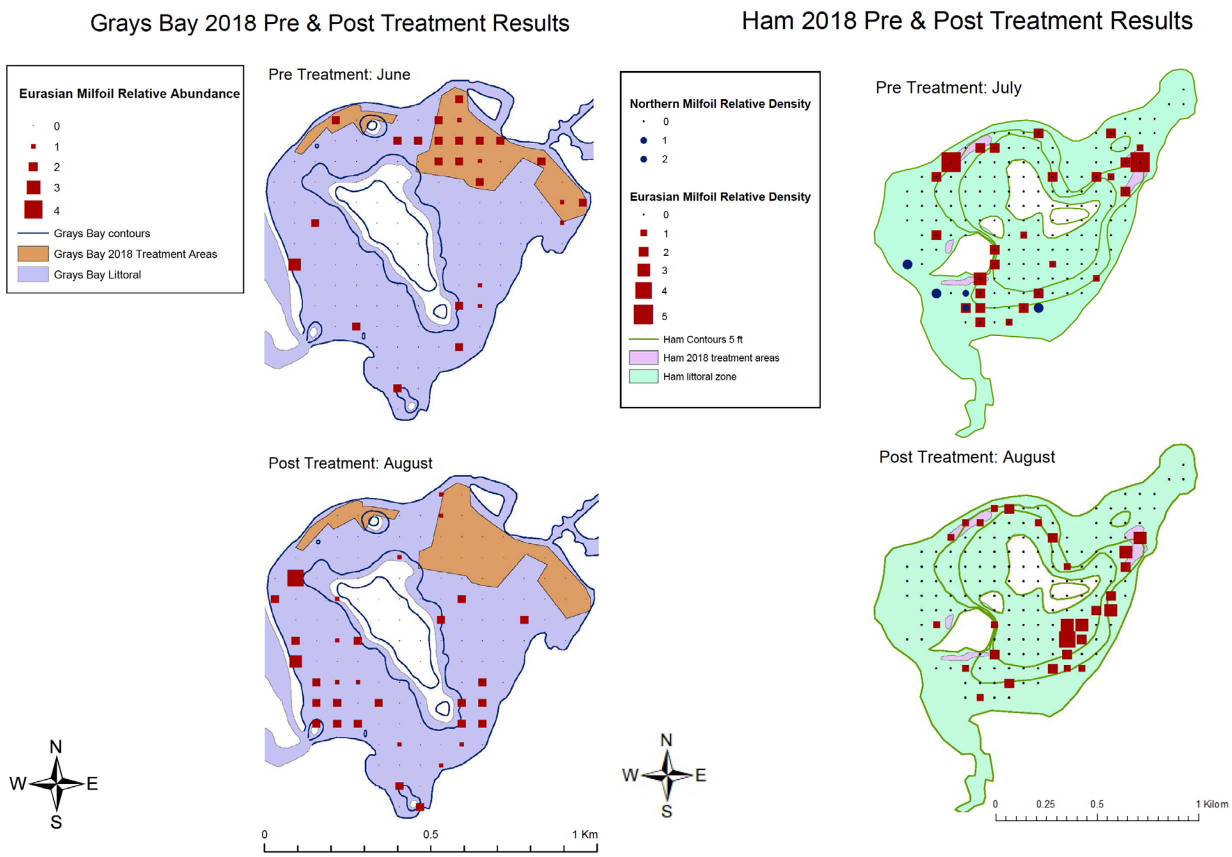

Figure 8. Pre and post-treatment occurrence and relative abundance of milfoil in Grays Bay Lake Minnetonka and Ham Lake. Both lakes were treated with ProcellaCor in mid-summer.

Discussion

Hybrid watermilfoil is common in Minnesota occurring in almost half the lakes assessed, but it is most common in the Twin Cities metro where it occurred in more than 60% of infested lakes. Eurasian watermilfoil is more broadly distributed and northern watermilfoil is more common in greater Minnesota, beyond the metro. Northern watermilfoil is the most genetically diverse with each lake having unique genotypes and many lakes have multiple genotypes of northern. In contrast, there is one widespread and dominant Eurasian genotype and 6 other genotypes that are found only in one lake each. Hybrid watermilfoil is of intermediate diversity with 53 genotypes; it is likely that hybrid watermilfoil is reproducing sexually (LaRue et al. 2013b) and Eurasian and northern are reproducing to produce more hybrids (Zuelig and Thum 2012). Although most lakes only have one genotype of Eurasian or hybrid, there are lakes with multiple genotypes of hybrid. This genetic diversity has the potential to produce plants that a tolerant to herbicides or are more invasive.

These data indicated that the only significant differences in lakes containing hybrids, in comparison to Eurasian and northern, is that hybrid lakes on average were more common in the Twin Cities metro, and were closer to one another in distance. The analysis of all other lake attributes (lake area, maximum depth, age of infestation, littoral area, Secchi depth, parking spaces at water access, and milfoil management score) indicated that the differences in these averages between taxa were insignificant. These data inform us that the types of lakes that hybrid watermilfoil inhabits are very similar to those of Eurasian and northern in regards to these lake attributes. Wu et al. (2015) found that hybrid was more common in areas where northern and Eurasian occupied the same habitat. In Minnesota, northern was likely present in all lakes infested with Eurasian but may have subsequently disappeared from competition with Eurasian (Nichols 1994) or as non-target impacts of Eurasian herbicidal control.

We found hybrids in six of the seven counties of the Twin Cities metro. We found no hybrids in Carver County, although we did find Eurasian in numerous Carver County lakes. It is likely that hybrids will be found in Carver County, with hybrid's location dependent upon lake distance from current hybrid infestations, but the lack of hybrids in the county is puzzling. On average, the metro lakes we surveyed overall had higher parking spot counts at lake accesses in comparison to greater Minnesota, indicating that metro lakes have increased opportunities to introduce hybrids or Eurasian. In order to predict where hybrids will infest next, it is important to look at where it is currently present. Although hybrid milfoil was most common in the metro it was found in 5 lakes outside the metro, however, none were further than 80 km from Lake Minnetonka.

In lakes where hybrids were present with a parental taxon, hybrids were more often present with Eurasian rather than northern. This may be due to northern being outcompeted by the invasive milfoil species over time (Nichols 1994). It is important to note we were sampling based on documented Eurasian/hybrid infestations, so it makes sense that northern would be found in fewer lakes because our data do not truly describe its distribution. We had 13 lakes where we found hybrid watermilfoil only, which indicates that hybrids do not necessarily require their parental taxa be present in a lake. This suggests that hybrids are capable of infesting a lake through either asexual propagation, or sexual reproduction or that once present, they outcompete their parents. We had initially predicted that hybrids would most likely be present in lakes with older ages of infestation, but our analysis did not find this difference to be significant. Although Eurasian infested lakes on average had older ages of infestation, and hybrids were more commonly found in the metro, this did not directly translate to hybrid infestations being older.

LaRue et al. (2013a) found that hybrids were more common in lakes that had been treated whereas parentals were more common in lakes without treatment history. Similarly, Parks et al. (2016) found the relative frequency of Eurasian went way down following treatment whereas the relative frequency of hybrids went way up. This suggests that perhaps hybrids had a greater competitive advantage in treated lakes and can displace the pure parental genotypes. In these cases the competitive advantage may in part be due to tolerance to the herbicide.

In assessing our genetic data, we found a significant difference ($p < 0.01$) in average genotypes found per taxon. Hybrids were found to be intermediately diverse compared to Eurasian and northern. Hybrid had a statewide average of 2.5 genotypes present in a lake, whereas Eurasian had one and northern had 3.6. This suggests that Eurasian hybridizes more with northern than it reproduces with itself, or that hybrids undergo more sexual reproduction than Eurasian allowing it to create genetically diverse lake infestations. In terms of managing

Eurasian infestations, this is quite promising because it means that Eurasian watermilfoil is not sexually reproducing very often and therefore won't likely develop new genotypes that may later be tolerant to commonly used herbicides (although somatic mutations could confer resistance, e.g., Michel et al. 2004). The diversity in hybrid means there are more opportunities for genotypes that are tolerant of or resistant to an herbicide. This also indicates that hybrids have most likely inherited their genetic diversity from northern watermilfoil rather than Eurasian. Hybrid lakes containing a single hybrid genotype were significantly younger than hybrid lakes with more than 2 genotypes. All of the lakes with 3 or more genotypes of hybrid have been listed infested since 2003. This observation indicates that older invasive milfoil infestations are prone to developing numerous hybrid genotypes and may be locations of interest for management if herbicide tolerance becomes apparent with specific hybrid genotypes.

Although diversity of hybrid milfoil may be associated with age of infestation, many of the east metro lakes that shared hybrid genotypes were relatively new infestations, consistent with clonal spread after development in a source lake (such as White Bear, Bald Eagle or Lac Lavon). In contrast to Eurasian watermilfoil, where one genotype is dominant and widespread, we have not been able to identify any wide-spread genotype of hybrid that might be particularly problematic, but that is the aim of our ongoing work. There does not yet appear to be a few genotypes that are being widely spread. In Michigan, Thum's lab has found one hybrid genotype in six lakes across Michigan that is the same genotype as a known fluridone-resistant genotype isolated from Townline Lake, Michigan (Berger et al. 2012, 2015; Thum et al. 2012) and that also appears to exhibit diquat resistance (Netherland and Willey 2017).

There were varied responses to management and continued assessment during the next two years will provide more complete interpretation. In general, abundance and genetic structure remained fairly consistent over time in the reference lakes. As with our larger data set, hybrid diversity within lakes is not prevalent and only Smith's Bay had a number of genotypes (but the treated bays North Arm and Grays also had numerous genotypes). There was an increase in hybrid relative to Eurasian between 2016 and 2018, but no change in hybrid genotypes in this untreated bay. The fluridone treatments were quite effective at controlling milfoil and ongoing sampling will be needed to determine if there are any shifts in genetic composition. Due to the limited treatment areas, there was a more variable response to the auxin mimics 2,4-d and ProcellaCor. In Bald Eagle, Eurasian and hybrid increased across years but decreased after treatment and northern, which was largely untreated, responded conversely. Because only one genotype of Eurasian and one of hybrid has been found in Bald Eagle, shifts in genotypic composition have not been seen.

Lakewide results with ProcellaCor were more mixed. It is not known if the lesser control on Ham Lake was due to ineffective treatment or to a tolerant hybrid genotype or both. The poor control in Ham Lake was likely due to under dosing, but the Ham Lake genotype has been identified as potentially tolerant (Beets and Netherland 2018). A follow up treatment in late Fall 2018 appears to have been more effective and genetic analyses of milfoil found in early summer 2019 has not been completed. It will be important to find out whether the increase in milfoil abundance in 2018 had to do with the targeting or scale of these treatments or response of tolerant genotypes. The decrease in native plants after treatment at Ham raises questions regarding the effect ProcellaCOR has on native plant communities, or whether this has to do with specific lake dynamics on Ham. Although there was considerable genetic diversity in Grays Bay, there were no significant shifts in genetic composition despite bay-wide increases in hybrid watermilfoil. With ProcellaCOR being a new herbicide, it will be interesting to continue to monitor these two lakes to assess the milfoil population in the future.

Continued monitoring of these various herbicide treatments will be needed to determine if problematic genotypes are present in Minnesota and we will expand our statewide assessments to better identify potentially problematic genotypes in Minnesota. The response to fluridone in North Arm and Schmidt Lake suggest that fluridone tolerant genotypes were not present in these lakes but there has been limited prior use of fluridone in Minnesota and none in these lakes. It likely will be several years before we can determine what genotypes return in these lakes.

Hybrid watermilfoil is widespread in Minnesota and has much more genetic diversity than its parent Eurasian watermilfoil. The greater genetic diversity increases the likelihood that problematic genotypes will emerge. Although we have yet to identify particularly problematic genotypes this study has provided the background data and direction to better assess for problematic genotypes in Minnesota.

Acknowledgements

This work was funded by a grant administered by the Minnesota Aquatic Invasive Species Research Center (MAISRC) based on funding from Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). Additional support was provided by the Minnesota Agricultural Experiment Station USDA National Institute of Food and Agriculture, Hatch grant MIN-41-081. A large number of people provided input and assistance; we probably forgot a few. We greatly appreciate the support and cooperation from the Minnesota Department of Natural Resources, particularly AIS Specialists Kylie Cattoor, Allison Gamble, Christine Jurek, Eric Katzenmeyer, April Londo, Keegan Lund, Tim Plude, Mark Ranweiler, and Rich Rezanka and Donna Perleberg and Chip Welling. Matt Kocian, Rachel Crabb, Mike Sorensen, Tony Brough, Caleb Ashling, Jessie Koehle, Eric MacBeth, Tim Ohmann, and Justin Townsend helped with access and logistics. In addition to advice and input the following consultants, watershed and park professionals shared data and assisted with sample collection: James Johnson and Steve McComas, Brian Vlatch and Justin Valenty, Eric Fieldseth, and Patrick Selter. T.J Ostendorf, grad student Jacob Olson and undergrads Alex Franzen, Kyle Blazek, Matt Gilkay, and Matt Manthey assisted with field collection and data entry. Emma Rice, Leah Simantel, Jeff Pashnick, Anna French, and Greg Chorak contributed to molecular work in the Thum lab. Thanks to all for making this work possible.

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M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 19: Decision-making tool for optimal management of AIS
SUBPROJECT MANAGER: Dr. Nicholas Phelps
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $172,465
AMOUNT SPENT: $80,469
AMOUNT REMAINING: $91,996

Sound bite of Subproject Outcomes and Results
We optimized network models for water connectivity and boater movement in Minnesota to predict zebra mussel and Eurasian watermilfoil invasion patterns. We then developed county-based recommendations to prioritize the optimal location of watercraft inspectors. The approach was piloted with Crow Wing, Ramsey, and Stearns Counties, and the results broadly disseminated.

Overall Subproject Outcome and Results
Understanding the patterns of historic AIS invasion can provide the framework for forecasting future invasions. To that end, we used a big data approach to combine hydrologic connectivity and boat movement to create a multiplex metacommunity model for both zebra mussel and Eurasian watermilfoil. We found that the hydrological corridors are important pathways of spread, even more so that previous research has suggested. While overland dispersal of AIS via boater movement is still a significant factor, additional management strategies should be developed to include intervention of hydrological pathways.

Using connectivity networks of boater movement, we developed county-based AIS management optimization models that prioritize inspection locations that will intercept the highest number of 'risky boats' (e.g. moving from infested to uninfested lakes). We piloted the models in Crow Wing, Ramsey, and Stearns Counties and had a very productive collaboration with county managers and citizen advisory boards during the development and evaluation for each. Ultimately, the application of this approach was well received and helped inform allocation of their inspection hours at the county level (for example: https://www.crowwing.us/1004/Aquatic-Invasive-Species-AIS).

Dissemination and usability of the models was a priority of this project. We created online tools to 1) visualize the spread risk for zebra mussels and Eurasian watermilfoil based on model predictions made in Activity 1, and 2) visualize and modify the decision optimization model at the county level based on management thresholds or funding availability. These tools and more detailed descriptions of the project has been disseminated through in-person stakeholder meetings and presentations to diverse audiences, including managers, researchers and the public.

Subproject Results Use and Dissemination
Efforts were made throughout the project to engage end-users, share findings and make deliverables broadly available. We used a combination of formal and informal dissemination strategies for this project given the

direct application to AIS managers and broad interest among other stakeholders. We held in-person meetings with County representatives and citizen advisor boards from Crow Wing, Ramsey and Stearns Counties to present results and update our models according to their input. These meetings were highly valuable to the project team and the outcomes of the project. In addition, we provided scientific and/or outreach presentations at the International Conference on Aquatic Invasive Species, the Aquatic Invaders Summit, the Cass County Watercraft Inspectors annual training, the annual AIS Roundtable, and MAISRC's Research and Management Showcase. Several publications are currently in late-stage drafts and will be submitted for peer-review in the coming months.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 21: Early detection of zebra mussels using multibeam sonar
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $96,549
AMOUNT SPENT: $96,175
AMOUNT REMAINING: $374

Sound bite of Subproject Outcomes and Results
This project tested the utility of a swath mapping system (multibeam sonar) to detect the presence/abundance of zebra mussels. Acoustic backscatter data was collected and machine-learning was used to identify what is present in the substrate. Researchers were able to differentiate by mussel type (native vs. invasive) and density.

Overall Subproject Outcome and Results
Zebra mussels pose a serious threat to Minnesota lake and river ecosystems. However, monitoring zebra mussel populations is challenging because current methods for detecting and counting zebra mussel colonies rely on time consuming and expensive diving surveys, video imaging, or sampling of veligers (larvae), which limits the areas surveyed. Remote sensing techniques have been shown to quickly and efficiently gather spatially extensive information. Using this technology to detect zebra mussels would likely be much more efficient and more effective than traditional methods and could be used for early detection and warning in rivers, lakes and reservoirs and to track changes in zebra mussel density.

This project was the first phase of research designed to test the utility of a swath mapping system, multibeam sonar, for detecting the presence and abundance of invasive mussels. Laboratory experiments were conducted to test the feasibility of using multibeam sonar to distinguish zebra mussel containing substrates. Acoustic backscatter data were collected in a two meter deep tank over sand, gravel, and mixed substrate containing high and low densities of zebra mussels and with native mussels using combinations of different sonar settings (frequencies and pulse lengths). Machine-learning was used to differentiate the acoustic backscattering signatures in a data-driven substrate classifier approach. Using these methods, we were able to classify substrate by size and mussel density. Classification errors decreased with more sonar settings. For minimum errors of less than 20%, 8 sonar settings are required, and for minimum errors of 10% or less for all substrates, 12 sonar settings. Each sonar setting corresponds to a separate boat survey of an area with a multibeam sonar in the field. Therefore, the next phase of this research is to further develop and test multibeam sonar monitoring approaches in the field (MAISRC Subproject 21.2: Field validation of mulitbeam sonar zebra mussel detection).

Subproject Results Use and Dissemination
Research results from Phase I will be disseminated through a peer-reviewed publication (in preparation) and will inform Phase II field testing starting July 2019 (MAISRC Subproject 21.2: Field validation of mulitbeam sonar zebra mussel detection). During this one-year project, we participated in MAISRC Fellows meetings and presented our project to the public at the annual MAISRC Research & Management Showcase. The Minnesota

Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 26: Updating an invasive and native fish passage model for locks and dams

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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $90,827
AMOUNT SPENT: $88,296
AMOUNT REMAINING: $2,531

Sound bite of Subproject Outcomes and Results

This project updated the Computational Fluid Dynamics Agent-Based fish passage model using the field and experimental data through Lock and Dam 2. This new model will better stop invasive Asian carp moving up the Mississippi River in case of blocking or help native fish to swim upstream through navigation dam.

Overall Subproject Outcome and Results

The main purpose of the project was to develop an updated version of the Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model (Zielinski, et al., 2018) using the field/experimental data of fish passage through Lock and Dam #2. This updated CFD-AB model can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams.

The subproject has been fulfilled for all the goals that were declared:

1. The computational code CFD-AB directed to enhance the simulation of swimming fish trying to pass through the navigation dams was updated/developed. The analysis of different fish passage index (FPI) showed that the values of FPI for the modified algorithm for a model channel (Gilmanov, et al., 2019, Water, under review) were greater than the FPI of the original algorithm at about 16%. At this moment, no essential differences in fish passage index FPI for the original and modified model at LD2 and LD8 have been found. This effect can be explained by the special gate adjustments, which generate a rather high fluid flow prevented fish to pass through the dams. In other words, in case of blocking invasive species, the modified algorithm does not change the final results of FPI at LD2 and LD8. But the modified algorithm could play a positive role to help native fish to pass through the navigation dams in the case of changing gate adjustments leading to decrease flow velocity.
2. The modified algorithms now account for more realistic fish behavior, including placement of “attraction points”, such as resting zones characterized by low recirculating fluid flow. These parameters have been informed by the literature and unpublished field data collected on other projects.
3. Based on investigations of (Larson, et al., 2017, Kokotovich et al, 2017) it was reported that the “Invasive Front" is currently positioned in southern Iowa between Pool 14 and Pool 16. Therefore, the strategy of blocking bigheaded carp at Lock and Dams of Minnesota should be reconsidered. It is well documented that the navigational dams have significantly altered the movement, spawning, feeding and other

activities of native fish (Wilcox et al. 2004). Hence, managers should consider alternative strategies whereby navigation dams are adjusted to *help* native fish pass, instead of *blocking* invasive fish. This strategy could help with ecosystem restoration efforts and potentially improve natural resistance to invasion by bigheaded carps. To evaluate this strategy, simulations of walleye passing through LD2 have been executed. It has been shown that by changing gate adjustments, FPI=4% is for the original algorithm and FPI=12% for the modified algorithm. We have to note, that for current gate adjustments from USACE the FPI=0% for original and modified CFD-AB models. By utilizing active monitoring data of bigheaded carp managers could *instantly* change gate adjustments at LD2-LD8 by using our CFD-AB approach if the invasion front threatens Minnesota.

Subproject Results Use and Dissemination

The results of the “MAISRC Subproject 26: Updating an invasive and native fish passage model for locks and dams” were/will be presented at the following events:

- MAISRC Research & Management Showcase (2018) with a poster presentation "A computational model provides a way to stop invasive carp at two key Minnesota Lock and Dams." Discussions and conversation with different groups of people were very informative and helpful.
- 2018 Upper Midwest Invasive Species Conference that was held with a joint conference of North American Invasive Species Management Association on October 15-18, 2018 - Mayo Civic Center - Rochester, MN and made an oral presentation "Computational model of fish swimming through Mississippi River locks and dams demonstrates ways to stop carp."
- The paper (Gilmanov, et al., 2019, under review) with the description of development/modification of CFD-AB model was submitted to the “Water” (an Open Access Journal from MDPI).
- MAISRC Research & Management Showcase (2019) with a poster “Mississippi River Dams: blocking invasive fish, helping natives”.
- Additional paper "Spillway gate settings in Mississippi River navigation lock and dams can be used to help native fish upstream passage" is in process and will be submitted for review in October-November 2019.
- The computer code of fish swimming through the navigation dam LD2 will be prepared and put in the publicly accessible Data Repository and the University of Minnesota (DRUM) system.

M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2022

SUBPROJECT TITLE: MAISRC Subproject 15: Determining Highest Risk Vectors of Spiny Water Flea Spread
SUBPROJECT MANAGER: Dr. Valerie Brady
ORGANIZATION: University of Minnesota Duluth,
COLLEGE/DEPARTMENT/DIVISION: Natural Resources Research Institute
MAILING ADDRESS: 5013 Miller Trunk Hwy
CITY/STATE/ZIP: Duluth, MN 55811
PHONE: 218-788-2753
E-MAIL: vbrady@d.umn.edu
WEBSITE: https://maisrc.umn.edu/swf-spread
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $119,513
AMOUNT SPENT: $119,337
AMOUNT REMAINING: $176

<u>**Sound Bite of Project Outcomes and Results**</u>

Spiny water fleas are an invasive zooplankton that threaten Minnesota lakes. In tests of recreational fishing gear, fishing lines entangled the most spiny water fleas and should be the focus of cleaning efforts. In addition, all water should be removed from bait buckets and livewells to prevent spreading this invader.

<u>**Overall Subproject Outcome and Results**</u>

Spiny water fleas are a predatory non-native zooplankton that threatens the ecology and recreational value of Minnesota lakes. Estimates are that >40% of northern Minnesota lakes are vulnerable to invasion. These invaders are primarily spread by human recreational activity, but we do not know exactly how this is happening. Our project goals were to 1) determine which types of recreational fishing gear would entangle (and thus spread) spiny water fleas, and 2) widely disseminate our results and gear-cleaning tips. We conducted 7 sampling events on Lake Mille Lacs, collecting 718 samples including zooplankton tows and spiny water flea counts on fishing gear and anchor ropes. We found that fishing lines accumulated the most spiny water fleas and thus should be the focus of angler cleaning efforts. In addition, it is critically important that all water be removed from bait buckets and livewells to prevent spread. To help recreational anglers clean their fishing gear, we printed and/or coordinated the distribution of over 20,000 cellulose dish cloths that were printed with cleaning instructions. 8,000 cloths were printed and distributed to 18 community partners (lake associations, AIS prevention staff, agency partners) as a part of this project and an additional 12,000 were printed and distributed through coordination with partner organizations and additional funders. Cloths were distributed to recreational anglers, focusing on those who move between spiny water flea infested lakes and uninfested lakes. In addition, we launched the stopspiny.org website to disseminate research findings and share prevention resources and created three PSA videos that demonstrated how to use the cloth to clean fishing lines. The videos played on YouTube, Facebook, Twitter, and TV in the Lake Superior, Lake of the Woods, Mille Lacs, Twin Cities markets. Facebook advertising was used to extend the stop spiny PSAs, reaching over 208,000 individual people and resulting in 442,000 impressions. PSA ads were also placed in local, online and print publications with an estimated reach of 103,000 readers. The research team also wrote one scientific manuscript and presented their results 19 times to about 1,500 people.

Subproject Results Use and Dissemination

All outreach was done with strong collaboration and support from MAISRC staff.

Stop Spiny Cloths: To help recreational anglers clean their fishing gear, we printed a simple image of a spiny water flea and what they look like when ensnared on fishing lines, along with cleaning instructions and funder logos, on 8,000 cellulose dish cloths. These cloths look like a steam-rolled sponge. Use of these cloths (or any cloth) to wipe fishing line prior to leaving an infested lake will help prevent the spread of spiny water flea from lake to lake. In testing, we found that these cloths are easy to use to clean fishing lines (and a more useful product than our original idea of a sticker). These cloths were distributed this spring to about 18 partners (lake associations, AIS spread prevention staff, agency partners, etc.). In addition, we facilitated the Minnesota Lakes and Rivers Advocates to help about 25 other groups (mostly lake associations and conservation districts) order over 9,000 more spiny wipe cloths for distribution. In total, we have or are in the process of facilitating distribution of over 20,000 cloths (3,000 of these were part of our companion project funded by St. Louis County) to wipe spiny water fleas from angler fishing lines.

To support distribution of the cloths and assist those distributing them, MAISRC staff worked with us to create an outreach campaign that we called the "Stop Spiny" campaign.

Website: The Stop Spiny campaign was chiefly hosted on the MAISRC website at stopspiny.org, which redirects to www.maisrc.umn.edu/stopspiny. The web page was created in Fall 2020 by MAISRC staff. Since its creation, the Stop Spiny campaign page has been viewed over 4,721 times. The average time a visitor spends on the page is nearly two minutes and thirty seconds. The Stop Spiny campaign webpage, as of Jan. 2022, is the seventh most popular page on the entire MAISRC website over the last year and a half.

The Stop Spiny campaign page gives an overview of spiny water flea invasion history and impacts and explains how water recreationists can help prevent the spread of spiny water fleas. A video about the project results is linked on this page. Additional information includes an interactive map showing current spiny water flea invaded lakes in Minnesota and links to additional spiny water flea research and species pages.

MAISRC staff also created a Stop Spiny campaign resources web page. This page hosts a variety of Stop Spiny factsheets, images, videos, fliers, and more for the free use and distribution of educators, resource managers, lakeshore associations, and/or any others hoping to help prevent the spread of spiny water fleas. The average time spent on this page by users was six minutes, which is very long by web page viewing standards and indicates that visitors are taking the time to read and download the information on this webpage.

Videos: To help share the Stop Spiny message in a visually interesting format, we worked with MAISRC and UMD to produce multiple high-quality videos. Three different video lengths were created—15 seconds, 30 seconds, and a full length (~2:30 min). The videos were shared on multiple social channels, including MAISRC's Facebook and Twitter accounts. The videos were also used in different combinations for Facebook advertisements and a television advertisement. On YouTube alone, the videos have accumulated over 850 views.

Advertisements: The Stop Spiny campaign included a combination of digital and print advertising. Print advertising included placements in the Lake Country Journal (based near the spiny water flea-infested Lake Mille Lacs), the Ely Summer Times (distributed along the Minnesota Iron Range, in the heart of spiny water flea-infested lakes), and Northern Wilds Magazine (another Northern Minnesota distributor). The estimated reach, per outlet, as provided by their respective company websites are as follows; Lake Country Journal—40,000; Ely Summer Times—28,000; Northern Wilds Magazine—18,000.

Northern Wilds Magazine, which also has an online edition and active online community, was contracted for Stop Spiny banner ads. The ads were placed on the Northern Wilds Magazine website at the top column of their side bar. The company estimates that their web pages see roughly 17,000 page views per month. Stop Spiny advertisements were placed on the top side bar for three consecutive months, from June to August 2021.

In addition, extensive Facebook advertising was used to enhance the Stop Spiny campaign. Multiple rounds of advertisements were planned to coincide with time of year and spiny water flea population increases. Since the launch of the campaign in spring 2021, Stop Spiny advertisements on Facebook reached over 208,000 individual people and resulted in 442,000 impressions. Included in all the advertisements were hyperlinks to the Stop Spiny campaign website for additional information and resources. In total, over 1,500 people clicked from the advertisement to the Stop Spiny campaign page.

On average the amount of time an individual person will watch a video on Facebook is six seconds. Engaging users to watch more than six seconds is a huge engagement success. By the end of the Stop Spiny campaign, over 29,000 users watched the Stop Spiny video they were served to completion (15-30 seconds) and over 60,000 users watched over 50% of the video they were served (7-15 seconds).

Finally, we have had numerous radio and print articles about our project and how to stop the spread of this invasive species, including an outreach article by MAISRC personnel in a Minnesota angling magazine (Activity 2, Outcome 4). Additional outreach has included working with Lake Minnetonka local government staff to use their lighted electronic boards to promote Stop Spiny messages, creating Stop Spiny factsheets and handouts, and sidebar online advertisements on the Northern Wilds website. Our Stop Spiny website hosts all these videos, factsheets, an interactive map, the radio scripts, and presentations for watercraft inspectors. The PIs published one scientific manuscript, and gave 19 presentations to over 1,500 people in total.

Peer-Reviewed Publications

- Donn K. Branstrator, Joshua D. Dumke, Valerie J. Brady & Holly A. Wellard Kelly (2021): Lines snag spines! A field test of recreational angling gear ensnarement of Bythotrephes, *Lake and Reservoir Management*, DOI: 10.1080/10402381.2021.1941447

Presentation Recordings/Videos

- 2021 MAISRC Research & Management Showcase Presentation https://z.umn.edu/2021ShowcaseSpiny
- 2020 MAISRC Research & Management Showcase Presentation https://z.umn.edu/2020ShowcaseSpiny
- AIS Detectors Webinar: Lines Snag Spines! Preventing the Spread of Spiny Water Flea https://z.umn.edu/DetectorsWebinarLinesSnagSpines
- MAISRC Video: Preventing the Spread of Spiny Water Flea https://z.umn.edu/MAISRCPreventingSpinySpread

Select Media Coverage

- Minnesota Opinion: Avoid catches you don't want this fishing season – West Central Tribune https://www.wctrib.com/opinion/editorials/minnesota-opinion-avoid-catches-you-dont-want-this-fishing-season
- New ways to stop spiny water flea spread – Mesabi Tribune https://www.mesabitribune.com/opinion/columnists/new-ways-to-stop-spiny-water-flea-spread/article_daea21e8-bca9-11eb-ae17-0b26c8aa0317.html

Spine: 0.243928 inches

Trim: 8.5 × 11 inches

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Cover Photos: The invasive spiny water flea *Bythotrephes* ensnared on an angling line on Rainy Lake, northern Minnesota (each black dot is an eyespot of a *Bythotrephes*). Branstrator et al. revealed that ensnarement rates of this aquatic invasive species were higher on angling lines than on any of the other gear tested, including downrigger cables, livewells, bait buckets, and anchor ropes. Photo by Jeff Gunderson, retired Director of Minnesota Sea Grant.

The *Lake and Reservoir Management* Instructions for Authors are available on the NALMS and Taylor & Francis websites.

LAKE AND RESERVOIR MANAGEMENT
2021, VOL. 37, NO. 4, 391–405
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Lines snag spines! A field test of recreational angling gear ensnarement of *Bythotrephes*

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ABSTRACT

Branstrator DK, Dumke JD, Brady VJ, Wellard Kelly HA. 2021. Lines snag spines! A field test of recreational angling gear ensnarement of *Bythotrephes*. Lake Reserv Manage. 37:391–405.

Recreational angling gear is a high-risk pathway of dispersal for the invasive spiny water flea (*Bythotrephes cederströmii*). We measured the number of *Bythotrephes* individuals ensnared on trolled shallow angling lines (3 line materials), a trolled downrigger angling line, a trolled downrigger steel cable, a trolled simulated livewell, a trolled bait bucket, and stationary anchor ropes (3 rope materials) in 2 Minnesota (United States) lakes. The shallow angling lines and the downrigger angling line had the greatest mean ensnarement rates (number of *Bythotrephes* individuals ensnared/transect transit), followed by the downrigger cable and the livewell, followed by the bait bucket and the anchor ropes. Added together, the shallow angling lines (as a mean of the 3 line materials) and the downrigger angling line accounted for 87–88% of the mean total ensnarement rate. Among the shallow angling lines, monofilament and fluorocarbon lines had greater mean ensnarement rates than braided line but the distinction was only statistically significant in one of the 2 lakes. The ensnarement rate of all angling gear combined was positively related to the density of *Bythotrephes* in the water column at the time of study (ambient density). On the downrigger angling line (monofilament), instar-3 *Bythotrephes* were ensnared at a relative frequency disproportionately greater than ambient density would predict, while instar-1 *Bythotrephes* were ensnared at a relative frequency disproportionately less than ambient density would predict. Our results suggest that education and outreach messaging should include instructions on removing *Bythotrephes* from angling lines in addition to the reminder to drain all water.

KEYWORDS
Angling gear; aquatic invasive species; *Bythotrephes*; dispersal; ensnarement

Prevention of propagule dispersal is the most important management strategy in the global effort to minimize environmental and economic impacts associated with nonindigenous invasive species (Leung et al. 2002, Vander Zanden et al. 2010, Sinclair et al. 2020). For many invasive species, human recreation is considered to be the leading pathway of propagule dispersal. In the United States, there is a nationally recognized "Stop Aquatic Hitchhikers!" campaign that directs people to clean, drain, and dry their equipment before moving it between waterbodies. This message is research based and should be effective if followed stringently. Nonetheless, it is broad and fails to emphasize the pathways that pose the highest risk for specific invasive species, and thus where decontamination could be focused or where usage could be minimized or avoided.

The spiny water flea (*Bythotrephes cederströmii*, formerly known as *Bythotrephes longimanus* [Korovchinsky and Arnott 2019], and hereafter *Bythotrephes*) is a nonindigenous aquatic invasive species of considerable concern in North America. Its impacts on food webs pose serious threats to the ecology and recreational value of lakes (Azan et al. 2015), including reductions in the biomass and production of native zooplankton (Pangle et al. 2007, Kerfoot et al. 2016), reductions in the growth rates of sport fishes (Staples et al. 2017, Hansen et al. 2020), and potential changes in water clarity (Walsh et al. 2016). Although *Bythotrephes* has spread to hundreds of lakes in the Midwestern region of North America (Kerfoot et al. 2011, Azan et al. 2015), it still occupies only a fraction of its potential range (Branstrator

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et al. 2006, Walsh et al. 2020), underscoring the value of improving best management practices to prevent its dispersal (Vander Zanden and Olden 2008, Sinclair et al. 2020).

Because *Bythotrephes* is small-bodied (maximum length of about 1 cm), open-water dwelling, widely dispersed in the water column, and often reaches high population densities (10–100 individuals/m^3), it is not amenable to mitigation or eradication once a lake becomes infested. Efforts to control its range focus on expedient and cost-effective ways of eliminating its dispersal. The primary pathway by which *Bythotrephes* is believed to disperse between invaded and uninvaded lakes is on recreational equipment. Evidence that recreational equipment is the primary pathway of dispersal comes from (1) scientific studies that show that rates of human visitation to lakes and associated transport of recreational equipment significantly predict invasion likelihood (MacIsaac et al. 2004, Weisz and Yan 2010) and (2) anecdotal information such as photographs and word-of-mouth testimonials of *Bythotrephes* fouling angling lines and downrigger cables. While aquatic birds are often discussed as couriers of dormant life stages of zooplankton (e.g., as resting eggs that can stick to feet or feathers, or be carried in the gut), there is little support for overland dispersal of *Bythotrephes* by birds (Charalambidou et al. 2003, MacIsaac et al. 2004, Branstrator et al. 2013).

Among the various types of recreational equipment of concern for *Bythotrephes* dispersal, recreational angling gear is considered a high-risk pathway (MacIsaac et al. 2004). Several factors may affect the dispersal risk of angling gear. Thin-gauge gear such as angling line is particularly vulnerable to ensnaring the barbed portion of a *Bythotrephes* tailspine; thus, such gear types may pose the greatest dispersal risk. As *Bythotrephes* grow, they lengthen their tailspine, and this could increase the dispersal risk of angling gear for larger *Bythotrephes* instars. *Bythotrephes* vertically migrate up in the water column on a nightly basis (Young and Yan 2008, Brown et al. 2012), which could increase their contact rates with angling gear during twilight compared to daytime hours. Also, *Bythotrephes* densities vary considerably within and between lakes (Brown et al. 2012, Kelly et al. 2013); thus, dispersal risk of angling gear is likely to be time and location specific.

Previous research has begun to address ensnarement rates of nonindigenous crustacean zooplankton invaders on recreational angling gear. MacIsaac et al. (2004) used surveys of anglers to help rank the risk of angling gear to disperse *Bythotrephes*. They reported that fishing line/nets and anchor ropes were perceived as higher risk whereas livewells and bait buckets were perceived as lower risk, suggesting that differences in ensnarement rate should be expected among different gear types. Jacobs and MacIsaac (2007) measured in situ rates of ensnarement of the fishhook water flea, *Cercopagis pengoi* (hereafter *Cercopagis*), on various brands of commercially available angling line in Lake Ontario. They reported that lines trolled deeper and farther through the water ensnared more *Cercopagis*, and that different brands of angling line ensnared different numbers of *Cercopagis*. We suspect that similar patterns of ensnarement on recreational angling gear exist for *Bythotrephes*; however, a comprehensive study has not yet been conducted. To address this gap, we tested 5 hypotheses in the field: (1) Thin gauge angling lines are more susceptible than other types of angling gear to ensnarement of *Bythotrephes*, (2) ensnarement of *Bythotrephes* on angling gear is positively related to the developmental instar (number of tailspine barb pairs), (3) ensnarement of *Bythotrephes* on angling gear is positively related to the density of *Bythotrephes* in the water column at the time of study (ambient density), (4) ensnarement of *Bythotrephes* on angling gear is greater during twilight compared to daytime hours, and (5) ensnarement of *Bythotrephes* on angling gear differs between lakes.

Methods

We deployed common types of angling gear along transects established in 2 Minnesota (United States) lakes for a prescribed distance or time, and then removed and counted the ensnared *Bythotrephes*. The lakes were Island Lake Reservoir (surface area = 32 km^2, maximum depth = 29 m) in 2017 and Lake Mille Lacs (surface area =

519 km^2, maximum depth = 13 m) in 2018 (MNDNR 2020). We chose these 2 lakes because of their invaded status, accessibility, and popularity among anglers. *Bythotrephes* was first recorded in the water column of Island Lake Reservoir in 1990 (Gravelle 1990) and in the water column of Lake Mille Lacs in 2009 (MNDNR 2009). Island Lake Reservoir (Secchi depth = 1–2 m) is less transparent than Lake Mille Lacs (Secchi depth = 3–4 m; MNDNR 2009). The reservoir's low transparency is due in part to its tannin staining (total organic carbon = 11.7 mg/L, apparent color = 58 Platinum-Cobalt Scale units; Sorensen et al. 2005).

At the beginning of each field season, we recorded Global Positioning System (GPS) coordinate locations for the start and end positions of 3 transects (each 1 linear km long) and used the same 3 transects every time we visited the lake. In Island Lake Reservoir, the transects were located in the east basin where bottom depth was 7–19 m. In Lake Mille Lacs, the transects were located in the northwest region, near the town of Garrison, where bottom depth was 7–8 m.

We visited each lake on 6 different dates in August and September, the time of year when high but also variable densities of *Bythotrephes* can be expected. On every date we conducted a set of 3 daytime and 3 twilight transect transits, for a total of 36 transect transits (18 daytime and 18 twilight) per lake, or 72 total. Sampling was only conducted when there was very little or no precipitation, wind, and wave action, which ensured that rain and boat motion did not interfere with accurate sample collection. Daytime sampling occurred during 09:00–14:00 h, while twilight sampling occurred during 17:00–22:00 h, with some adjustments, especially to twilight sampling times made in late September when daylight hours diminished. We elected to contrast these 2 time periods because *Bythotrephes* are known to engage in diel vertical migration, rising up in the water column on a nightly basis (Young and Yan 2008, Brown et al. 2012), and people commonly target twilight for angling, suggesting the potential for an interaction with time of day relevant to ensnarement rate. Generally, a set of 3 transect transits required 3–4 h to complete.

We collected vertical profiles of water temperature and dissolved oxygen concentration with a YSI 85 hand-held meter at increments of 1 m at either the start or end position of every transect transit.

On each transect transit we deployed 10 gear types (Table 1). We deployed 7 gear types at the start position and trolled them from a boat moving at 3 km/h to the end position, where we stopped the boat and retrieved the gear. Exposure time was 18–20 min. We deployed 3 anchor ropes at the start position, which were left unattended. The anchor ropes were exposed for 1.5–2.5 h with a few exceptions.

Shallow angling lines

We spooled angling lines on 3 matching rods that were 2.3 m (7 ft 6 in) long with 3 matching bait casting reels (Shakespeare Ugly Stick). Angling lines were 4.5 kg (10 lb) test Berkeley Trilene XL Smooth Casting (hereafter monofilament), 4.5 kg (10 lb) test Berkley Trilene 100% Fluorocarbon XL (hereafter fluorocarbon), and 13.6 kg (30 lb) test Sufix Performance Braid Digital Y6 Braiding (hereafter braided). Each angling line was 0.25 mm (0.01 inch) diameter. We outfitted the terminal end of each angling line with an 85 gm (3 oz) weight tied to a swivel. At the start of a transect transit, 10.7 m (35 ft) of angling line was paid out on each reel. A preliminary field test determined that with 10.7 m of angling line paid out, the terminal weight rode at 3 m (10 ft) depth in the water column when the boat traveled at 3 km/h. Three rod holders

Table 1. Summary of the recreational angling gear deployed. One of each gear type was deployed per transect transit.

Angling gear	Description
Shallow angling line (monofilament)	4.5 kg (10 lb) test, 0.25 mm (0.01 in) diameter
Shallow angling line (fluorocarbon)	4.5 kg (10 lb) test, 0.25 mm (0.01 in) diameter
Shallow angling line (braided)	13.6 kg (30 lb) test, 0.25 mm (0.01 in) diameter
Downrigger angling line (monofilament)	4.5 kg (10 lb) test, 0.25 mm (0.01 in) diameter
Downrigger steel cable	68 kg (150 lb) test
Livewell simulation	1.6 cm (5/8 in) diameter intake hose
Bait bucket	5.7 L (6 qt) capacity
Anchor rope (twisted nylon)	1 cm (3/8 in) diameter
Anchor rope (braided nylon)	1 cm (3/8 in) diameter
Anchor rope (twisted polypropylene)	1 cm (3/8 in) diameter

secured to the boat stern held the rod tips over the sides of the boat with the lines trailing behind the boat away from the outboard motor. Angling line angles at the point of contact with the water were about 30° from horizontal. We estimated that about 6 m (20 ft) of each angling line was submerged.

We retrieved angling lines at the end of each transect transit. Most ensnared *Bythotrephes* accumulated on the first rod eyelet. We rinsed them into a plastic bag using a stream of water. Using forceps, we manually removed *Bythotrephes* that passed the first eyelet. We preserved specimens in 75% ethanol.

Downrigger angling line and cable

On the boat stern we mounted a downrigger apparatus (Cannon Easi-Troll ST) outfitted with a stainless steel downrigger cable that was 68 kg (150 lb) test and weighted at the terminal end with a 3.6 kg (8 lb) keeled downrigger ball. We used the same brand of rod, reel, and monofilament line used for the shallow angling lines. We clipped the angling line to the cable at the terminal end near the downrigger ball using the same style line release clip that anglers would use when fishing. At the start of a transect transit, we lowered the weighted cable and angling line to 1 m above the lake bottom, as determined by an electronic depth finder that displayed the downrigger ball. We adjusted the depth of the downrigger ball, cable, and angling line periodically while transiting a transect to maintain 1 m distance from the lake bottom.

At the end of a transect transit, we unclipped the angling line from the cable by applying slight tension to the angling line so it would detach from the downrigger ball release clip, and then we retrieved both the angling line and the cable. We removed *Bythotrephes* from the angling line as already described for the shallow angling lines. We removed *Bythotrephes* from the cable by continually spraying it with a stream of water into a Nitex mesh bag immediately before it entered the terminal guide. Using forceps, we manually removed *Bythotrephes* that passed the terminal guide. We preserved specimens in 75% ethanol.

Livewell

We simulated how a boat livewell would be used during trolling by continuously pumping water into a plankton net during a transect transit. Water was pumped from about 0.5 m below the lake surface through a 1.6 cm (5/8 in) diameter intake hose, mounted on the port side of the boat's transom, at a rate of 14.2 L/min. This produced about 256–284 L during the 18–20 min transect transit. We preserved specimens in 75% ethanol.

Bait bucket

We trolled a bait bucket (Frabill Flow Troll, 5.7 L [6 qt] capacity) on the lake surface using a rope (about 1.5 m long) attached to the starboard side of the boat. Water flowed passively through the ventilation holes. At the end of a transect transit, we rinsed the contents of the bait bucket into a Nitex mesh bag. We preserved specimens in 75% ethanol.

Anchor ropes

At the start of each transect transit, we deployed 3 anchor ropes of 1 cm (3/8 in) diameter including 1 of twisted nylon, 1 of braided nylon, and 1 of twisted polypropylene material. Separate floats and weights vertically suspended each rope to cover the entire water column. Ropes were deployed within a 10 m diameter circle.

At the end of the exposure time, we retrieved the anchor ropes by gently coiling them into separate plastic bags, which we stored on ice in a cooler. We processed the anchor ropes within 24 h of retrieval by visually examining each rope with a desktop, lighted magnifying lens. Each rope required 15–30 min to search with a few exceptions. Using forceps, we manually removed *Bythotrephes* and other ensnared organisms (daphniids, isopods, leeches, and mollusks) and stored them in 75% ethanol. For quality control, a subset of the anchor ropes (27% in Island Lake Reservoir and 12% in Lake Mille Lacs) was reexamined immediately by a second researcher. We reduced the level of quality control in Lake Mille Lacs due to the absence of ensnared *Bythotrephes* on the anchor ropes in Island Lake Reservoir.

Ambient density

Simultaneous with the deployment of angling gear from the first boat, from a following second boat we collected *Bythotrephes* from the water column. At 3 locations along each transect transit (both end positions and the middle position), we vertically towed standard zooplankton nets (0.5 m diameter mouth opening, 500 µm aperture mesh) in triplicate from 1 m above the lake bottom to the surface. We collected tows within 15 min of the time that the trolled angling gear had passed the sampling location. We preserved specimens in 75% ethanol.

Laboratory and data analysis

Using dissecting microscopes, we sorted and counted *Bythotrephes* by its 3 developmental instars based on the number of tailspine barb pairs (Branstrator 2005). We included specimens with damaged or missing tailspines in the total counts but not in the instar-specific analysis.

To estimate the density (individuals/m^3) of *Bythotrephes* along each transect transit at the time of angling gear deployment (ambient density) we computed the mean of the triplicate zooplankton net tows collected at each of the 3 locations (both end positions and the middle position) and then computed the mean of those 3 values.

We used SYSTAT 13 for statistical analyses, and a cutoff of $P = 0.05$ for statistical significance.

Ambient density, time of day, lake, sampling date, transect. We used a multiple linear regression model and analysis of variance (ANOVA) and F ratio to evaluate the overall effect of 5 fixed-effect, independent variables on the dependent variable “total ensnarement rate.” “Total ensnarement rate” was defined as the number of *Bythotrephes* individuals ensnared on a transect transit (number/transect transit) on all angling gear combined. For the 3 shallow angling lines and the 3 anchor ropes, we first computed a mean ensnarement rate for each gear type. The 5 independent variables were “ambient density,” “time of day” (twilight vs. daytime), “lake” (Island Lake Reservoir vs. Lake Mille Lacs), “sampling date” (the 6 dates that we sampled, given as 1–6), and “transect” (given as 1–3). We included sampling date as a fixed-effect variable because we were concerned that our sampling efficiency (removal of *Bythotrephes* from angling gear) might have improved as a field season progressed. We included transect as a fixed-effect variable because we always transited the same transects in each lake in the sequence 1–3.

Having found no significant effect of time of day, lake, sampling date, or transect on total ensnarement rate, we thereafter treated the 72 transect transits as independent replicates. We plotted total ensnarement rate as a function of ambient density for the 72 transect transits, and used a simple linear regression model to estimate a best-fit relationship and test for slope = 0 (ANOVA, F ratio).

Angling gear. To test for differences in the ensnarement rate of *Bythotrephes* among angling gear types, we first computed a mean ensnarement rate (number/transect transit) for each gear type and then compared the mean ensnarement rates among the shallow angling lines, downrigger angling line, downrigger cable, and livewell within each lake using one-way ANOVA models and Tukey pairwise comparisons. For the shallow angling lines, we used the mean of the 3 line materials. We excluded the bait bucket and the anchor ropes due to their low ensnarement rates (4 total *Bythotrephes* were recovered from all bait buckets and anchor ropes, combined, across the entire study). We also used one-way ANOVA models and Tukey pairwise comparisons to compare the mean ensnarement rates among the 3 shallow angling line materials within each lake. We log10 transformed the data before analysis and evaluated the assumption of homogeneity of variances using Levene’s test. Untransformed zero values were included in the calculations of the means but not in the statistical models. In 3 of the 4 ANOVA models, the assumption of homogeneity of variances was met ($P > 0.05$). The exception was the ANOVA model that compared the 4 gear types in Lake Mille Lacs ($P = 0.03$). We considered that result to be marginally statistically significant and not in severe violation of the model assumption.

In order to further evaluate differences between gear types, we tabulated the number of transect transits in each lake for which the ensnarement rate was greater for the shallow angling lines (as a mean of the 3 line materials) than for the downrigger angling line, or vice versa. The classification for each transect transit was categorical as either a "yes" (a greater ensnarement rate for the shallow angling lines) or a "no" (a lesser ensnarement rate for the shallow angling lines). We used Fisher's exact test to determine whether the proportion of transect transits in the 2 classifications was different between lakes.

Developmental instar. To test for proportional differences in ensnarement frequency among the 3 developmental instars of *Bythotrephes*, we used Chesson's α (Chesson 1983) to determine whether the relative frequencies of the 3 instars of ensnared *Bythotrephes* on angling gear were proportionate to their relative frequencies in ambient density as

$$\alpha = \left(r_i/p_i\right)/\Sigma\left(r_i/p_i\right),\ \text{for i} = 1-3 \quad (1)$$

where r_i is the number of an instar ensnared on the gear at the end of a transect transit, and p_i is ambient density of an instar along a transect transit. Because *Bythotrephes* has 3 instars, $\alpha = 0.33$ indicates that the relative frequency of an instar ensnared on the gear is proportionate to its relative frequency in ambient density. Values of $\alpha > 0.33$ or $\alpha < 0.33$ indicate a disproportionately greater or lesser relative frequency on the gear compared to the relative frequency in ambient density, respectively. We used Kruskal–Wallis models to compare the α values among the 3 instars in each lake. This nonparametric test was more appropriate than ANOVA because the data failed to meet the assumption of homogeneity of variances (Levene's test) for the Lake Mille Lacs data. For this analysis we did not merge the data sets for the 2 lakes because the mean proportions of developmental instars between the 2 lakes were notably different.

We estimated α for the downrigger angling line only. In addition, we used box plots to show the relative frequency of *Bythotrephes* by developmental instar ensnared on the downrigger angling line compared to ambient density. The downrigger angling line was one of 3 gear types (with the other 2 being the downrigger cable and the anchor ropes) for which physical exposure spanned the entire water column that we sampled with the zooplankton vertical net tows. This was important because spanning the entire water column eliminated potential bias that could have been caused by vertical spatial variation by instar of *Bythotrephes* at the time of sampling (Brown et al. 2012). There were too few ensnared *Bythotrephes* (with many zeros) on the downrigger cable and anchor ropes to allow for a robust analysis of α for either of those gear types.

Results

The main portion of the water column where we conducted transect transits in Island Lake Reservoir (0–12 m) was well oxygenated (>2 mg/L dissolved oxygen) and 11.9–23.4 C. Deeper regions of the water column (12–19 m), which represented minor portions of the transect transits, were often cooler (but never <11.0 C) and often contained <2 mg/L dissolved oxygen. The water column where we conducted transect transits in Lake Mille Lacs (0–8 m) was consistently well oxygenated (>2 mg/L dissolved oxygen) and 15.6–23.7 C.

Ambient density, time of day, lake, sampling date, transect

A multiple linear regression model, with total ensnarement rate as the dependent variable, was significant overall (ANOVA, $F_{5,66} = 7.4$, $P < 0.01$). Of the 5 independent variables, only ambient density was statistically significant ($P < 0.05$). The P values for the other variables were as follows: time of day ($P = 0.32$), lake ($P = 0.86$), sampling date ($P = 0.69$), and transect ($P = 0.72$). These results do not support the hypothesis that ensnarement of *Bythotrephes* on angling gear is greater during twilight compared to daytime hours, or the hypothesis that ensnarement of *Bythotrephes* on angling gear differs between lakes. However, they do support the hypothesis that ensnarement of *Bythotrephes* on angling gear is positively related to ambient density.

Specifically, total ensnarement rate is predicted by ambient density (Figure 1) as:

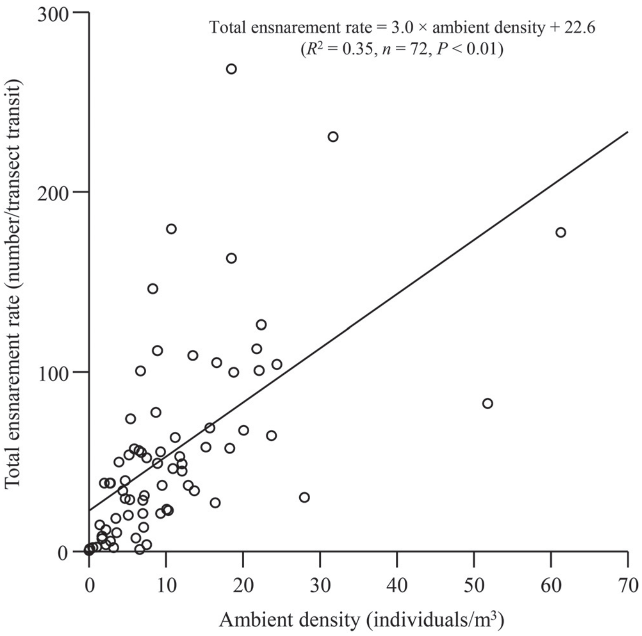

Figure 1. *Bythotrephes* total ensnarement rate as a function of ambient density as defined by a best-fit linear relationship for Island Lake Reservoir and Lake Mille Lacs.

$$\text{total ensnarement rate} = 3.0 \times \text{ambient density} + 22.6 \tag{2}$$

(R^2 = 0.35, $n = 72$ transect transits). The slope of the relationship is significantly different from zero ($F_{1,70}$ = 37.3, $P < 0.01$). Across both lakes, the mean ± 1 standard deviation for ambient density was 11.1 ± 10.6 individuals/m^3 ($n = 72$ transect transits, range = 0.1–61.4), and the mean ± 1 standard deviation for total ensnarement rate was 56.0 ± 54.0 individuals/transect transit ($n = 72$ transect transits, range = 0.0–268.0).

Angling gear

The mean ensnarement rate of *Bythotrephes* ranged widely among angling gear types (Table 2). The general trend in both lakes was that the mean ensnarement rates were greatest for the shallow angling lines (as a mean of the 3 line materials) and the downrigger angling line, intermediate for the downrigger cable and the livewell, and least for the bait bucket and the anchor ropes (as a mean of the 3 rope materials). ANOVA (which excluded the bait bucket and the anchor ropes due to so few individuals ensnared) revealed overall statistical significance among the shallow angling lines (as a mean of the 3 line materials), the downrigger angling line, the downrigger cable, and the livewell in both lakes

Table 2. Ensnarement rates of *Bythotrephes* as number/transect transit presented as the mean ± 1 standard error (n = number of transect transits) for the recreational angling gear tested. For the shallow angling lines and the anchor ropes, values are the means of the 3 materials. Shared letters within lake (column) indicate that the mean values are not statistically significantly different ($P > 0.05$) based on ANOVA and Tukey pairwise comparisons that included 4 variables (shallow angling lines, downrigger angling line, downrigger steel cable, and livewell simulation). We excluded the bait bucket and anchor ropes from the ANOVA models due to the predominance of zero values. The last row represents the sums of the means of the individual gear types.

	Ensnarement rate	
Angling gear	Island Lake Reservoir	Lake Mille Lacs
Shallow angling lines	12.5 ± 1.7 (36) a	37.5 ± 6.3 (36)
Downrigger angling line	37.0 ± 6.5 (33)	14.7 ± 5.8 (35) a
Downrigger steel cable	0.8 ± 0.3 (34) b	6.0 ± 1.8 (36) a,b
Livewell simulation	6.3 ± 1.9 (36) a,b	0.9 ± 0.3 (21) b
Bait bucket	0.0 ± 0.0 (36)	0.03 ± 0.03 (35)*
Anchor ropes	0.0 ± 0.0 (36)	0.03 ± 0.02 (36)*
Total (sum of the means)	56.6	59.2

*Note additional decimal places.

(Island Lake Reservoir, $F_{3,98}$ = 15.2, $P < 0.01$; Lake Mille Lacs, $F_{3,88}$ = 12.3, $P < 0.01$). Tukey pairwise comparisons for Island Lake Reservoir revealed that the mean ensnarement rate for the downrigger angling line was greater than for any of the other 3 gear types. By contrast, Tukey pairwise comparisons for Lake Mille Lacs revealed that the mean ensnarement rate for the shallow angling lines (as a mean of the 3 line materials) was greater than for any of the other 3 gear types. These results support the hypothesis that thin gauge angling lines are more susceptible than other types of angling gear to ensnarement of *Bythotrephes*.

There was a difference between lakes in the actual number of transect transits for which the ensnarement rate was greater for the shallow angling lines (as a mean of the 3 line materials) than for the downrigger angling line. Only 10 of 33 transect transits in Island Lake Reservoir had an ensnarement rate that was greater for the shallow angling lines. By contrast, 29 of 33 transect transits in Lake Mille Lacs had an ensnarement rate that was greater for the shallow angling lines. Fisher's exact test revealed strong statistical departure ($P < 0.01$) from random proportions.

We recovered few *Bythotrephes* from the bait bucket or the anchor ropes (Table 2). Specifically, we recovered no *Bythotrephes* from the bait bucket in Island Lake Reservoir and only one *Bythotrephes* from the bait bucket in Lake Mille

Lacs. We recovered no *Bythotrephes* from the anchor ropes in Island Lake Reservoir and only 3 *Bythotrephes* from the anchor ropes in Lake Mille Lacs, including one on twisted nylon and 2 on braided nylon ropes. However, we recovered a variety of other taxa on the anchor ropes in both lakes, including 10 daphniids (Island Lake Reservoir) and 87 isopods, 53 leeches, and 38 mollusks (Lake Mille Lacs).

Using our results in Table 2, we calculated a mean total ensnarement rate in each lake by summing the individual mean rates for each gear type. This produced values of 56.6 (Island Lake Reservoir) and 59.2 (Lake Mille Lacs) individuals/transect transit. This calculation of the mean total ensnarement rate differs slightly from that presented in the preceding for the entire dataset (equation 2 and Figure 1) where we first calculated a total ensnarement rate per transect transit, not by individual gear type. Using our second formulation (based on Table 2), the sum of the mean ensnarement rates for the shallow angling lines (as a mean of the 3 line materials) and the downrigger angling line accounted for 87% (Island Lake Reservoir) and 88% (Lake Mille Lacs) of the mean total ensnarement rate. Using our results in Table 2, we plotted the projected cumulative percentage of *Bythotrephes* that would be removed from angling gear as it is cleaned (Figure 2). Using greatest percentage ensnarement to prioritize cleaning, our results indicate that shallow and downrigger angling lines should receive the most attention.

There were notable differences in the mean ensnarement rates among the 3 shallow angling line materials (Table 3). For Island Lake Reservoir, the overall ANOVA model was statistically significant ($F_{2,93}$ = 8.2, $P<0.01$) and Tukey pairwise comparisons indicated that the mean ensnarement rate for monofilament and fluorocarbon was each greater than for braided. For Lake Mille Lacs, the overall ANOVA model was not statistically significant ($F_{2,90}$ = 0.6, $P=0.55$) despite trends in the same direction as the Island Lake Reservoir results.

Developmental instar

The relative frequency of *Bythotrephes* by developmental instar ensnared on the downrigger angling line departed notably from the relative frequency in ambient density (Figure 3). Most notably, instar 1 was strongly underrepresented on the gear and instar 3 was strongly overrepresented on the gear in comparison to ambient density.

In both lakes, the α values were generally least (below 0.33) for instar 1, intermediate (near 0.33) for instar 2, and greatest (above 0.33) for instar 3. Specifically, in Island Lake Reservoir the mean α values were 0.12, 0.33, and 0.55 for instars 1, 2, and 3, respectively ($n=30$ transect transits per value); in Lake Mille Lacs the mean α values were 0.03, 0.20, and 0.77 for instars 1, 2, and 3, respectively ($n=24$ transect transits per value). The distributions of the α values were statistically significantly different within each lake based on Kruskal–Wallis models (Island Lake Reservoir, $H=51.4$, $P<0.01$; Lake Mille Lacs, $H=50.9$, $P<0.01$). These results support the hypothesis that ensnarement of *Bythotrephes* on angling gear (specifically, downrigger angling line) is positively related to the developmental instar.

Discussion

Transportation of recreational equipment among waterbodies is believed to be the single most important pathway of dispersal for *Bythotrephes* among North American lakes (MacIsaac et al. 2004). To better understand the problem, we tested 5 hypotheses related to the ensnarement rate (number/transect transit) of *Bythotrephes* on recreational angling gear in Island Lake Reservoir and Lake Mille Lacs, Minnesota (United States).

Angling gear

Our results support the hypothesis that thin gauge angling lines are more susceptible than other types of angling gear to ensnarement of *Bythotrephes* (Table 2). Angling lines, whether they were trolled in a shallow fashion behind the boat or lowered with aid of a downrigger that spanned the entire water column, had the greatest mean ensnarement rates among all gear types tested. By contrast, the downrigger cable and the livewell had intermediate mean ensnarement

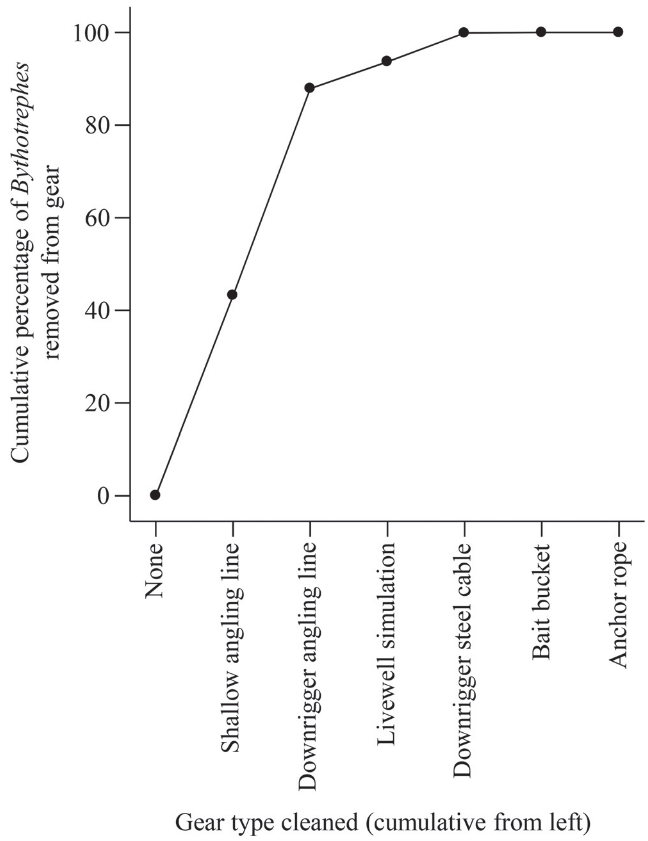

Figure 2. A projection of the cumulative percentage of ensnared *Bythotrephes* that would be removed from recreational angling gear if the gear (*x* axis) were cleaned in sequence from left to right. Numbers are based on the ensnarement rate results in Table 2.

Table 3. Ensnarement rates of *Bythotrephes* (number/transect transit) presented as the mean ± 1 standard error (*n* = number of transect transits) for the 3 shallow angling line materials tested. Shared letters within lake (column) indicate that the mean values are not statistically significantly different ($P > 0.05$) based on ANOVA and Tukey pairwise comparisons.

	Ensnarement rate	
Angling line material	Island Lake Reservoir	Lake Mille Lacs
Monofilament	18.6 ± 3.2 (36) a	38.9 ± 9.0 (36) a
Fluorocarbon	13.1 ± 2.2 (36) a	43.6 ± 8.6 (36) a
Braided	5.8 ± 1.2 (36)	29.9 ± 5.8 (36) a

rates, while the bait bucket and the anchor ropes had the lowest mean ensnarement rates. In a survey of >800 recreationalists in Ontario (Canada), MacIsaac et al. (2004) found that fishing line/nets and anchor ropes were perceived to be more likely than livewells and bait buckets to disperse *Bythotrephes*. A recent survey of Minnesota anglers (MNDNR 2019) found that more than 50% do not believe that angling gear can move aquatic invasive species among waterbodies. Our results indicate that people may not always correctly identify which gear types pose the greatest ensnarement risk.

It is possible that our anchor rope deployments did not sufficiently mimic true anchor rope use by boaters. We attempted to mimic a common usage of anchor ropes by deploying them in a stationary fashion in water with no obvious directional current. It is possible that anchor ropes being dragged behind a drifting boat or deployed in flowing water (e.g., in a river or near a lake inlet or outlet) could yield greater ensnarement rates than we found. Evidence for other groups of biota ensnared on the anchor ropes suggests that chemical toxicity of the rope materials, physical shape or texture of the rope materials, or inadequate methodology to detect ensnared biota on our part are unlikely explanations for the almost complete absence of ensnared *Bythotrephes* on the anchor ropes.

We observed an interesting contrast in our results between the livewell and the bait bucket. Both were deployed near or at the lake surface, but the mean ensnarement rates were much greater for the livewell. One difference was that

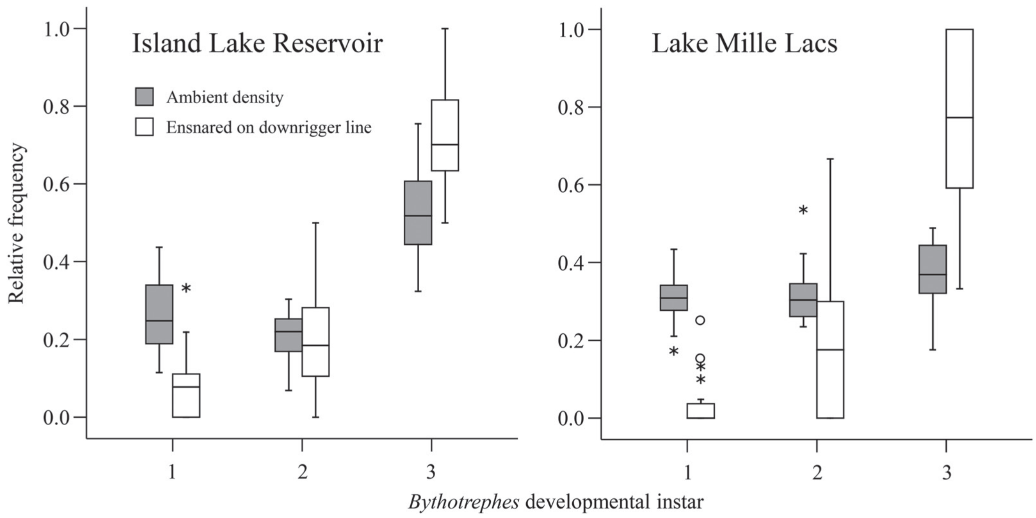

Figure 3. Box plots of the relative frequency (0–1) of *Bythotrephes* by developmental instar in ambient density (gray bar, left) and those ensnared on the downrigger angling line (open bar, right). Centerline = median, box edges = first and third quartiles, whisker ends = range, circles and asterisks = outliers ($n = 30$ transect transits per bar in Island Lake Reservoir, and $n = 24$ transect transits per bar in Lake Mille Lacs). Within each panel, mean relative frequencies (not shown) sum to 1 for each variable across the 3 instars.

the livewell was actively drawing lake water in through an unscreened intake, whereas the bait bucket was accepting lake water more passively through the bucket holes. It is probable that more water and plankton entered the livewell than the bait bucket because water was not pumped into the bait bucket, and because the bait bucket only has holes on the top surface, which reduces water exchange (i.e., water must flow in and out of the same holes, a design feature intended to protect baitfish from being injured from high-volume through flow). A second explanation is that any *Bythotrephes* ensnared in the livewell simulation would have been retained in a net that we installed specifically for capturing plankton. By comparison, *Bythotrephes* initially ensnared in the bait bucket could have been washed back out through the bait bucket holes before the end of a transect transit. A similar phenomenon may occur in real-life ensnarement of *Bythotrephes* in angler livewells, as boat manufacturers build into their livewells overflow drains that may allow some *Bythotrephes* that are pumped into a livewell to passively flush out. Our livewell simulation did not include an overflow drain. We acknowledge that our livewell simulation may represent greater *Bythotrephes* retention than may be experienced by anglers since we captured all *Bythotrephes* entering a livewell, rather than just the individuals retained there. However, we consider our livewell test to still be valuable because boat and livewell designs vary greatly among manufacturers, and there is no way to predict how *Bythotrephes* would interact with many livewell designs once contained within. Thus, our results indicate the ensnarement potential that any livewell could pose.

In addition to the contrasts in the mean ensnarement rates among the gear types, there were differences among the angling line materials (Table 3). Specifically, in Island Lake Reservoir, the mean ensnarement rate for the monofilament and fluorocarbon lines was each greater than for the braided line. The directional trend in Lake Mille Lacs was consistent with that of Island Lake Reservoir in that the mean ensnarement rate was least for the braided line, but there was no statistical difference among the 3 line materials. Monofilament and fluorocarbon lines are made of single-stranded, extruded polymers that have a smooth feel, whereas braided line is made of a multistranded, woven thread that has a rougher texture. Monofilament and fluorocarbon lines also stretch more than braided line before breaking, which would give the line materials different capacities to respond to agitation during trolling and retrieval. Both factors could have influenced the tendency of *Bythotrephes* to become ensnared and remain ensnared. We and others have anecdotally noted that *Bythotrephes* tend to slide on angling line and that this often leads to clumping, which may increase the tendency of *Bythotrephes* to remain attached. Perhaps *Bythotrephes* slid and clumped more frequently on the monofilament and fluorocarbon lines than on the braided line. Jacobs and MacIsaac (2007) measured ensnarement rates of *Cercopagis* on sport fishing lines trolled from a boat in Lake Ontario. Their evaluation of 4 different monofilament line brands showed that the ensnarement rate for Berkeley Trilene XL Smooth Casting, which was the same brand that we used here, was greater than for the other 3 monofilament brands, and that there was considerable variation among all 4 of the monofilament brands they tested. Their study and ours together suggest that the interaction of angling line and ensnarement rate of zooplankton is determined by factors beyond just line material.

Angling lines accounted for the vast majority of ensnared *Bythotrephes* in both lakes. Specifically, the sum of the mean ensnarement rates for the shallow angling lines (as a mean of the 3 line materials) and the downrigger angling line accounted for 87% (Island Lake Reservoir) and 88% (Lake Mille Lacs) of the mean total ensnarement rate. Thus, just cleaning angling lines would remove the majority of *Bythotrephes* ensnared on angling gear (Figure 2). This is an important message to convey to anglers, especially considering that results from a Minnesota angler survey (MNDNR 2019) indicate that more than 50% of anglers may not be thinking about cleaning their gear because they do not believe it can transfer invasive species.

It is prudent to keep in mind that we made specific choices in how we used the angling gear and that these undoubtedly influenced our results. As mentioned, we deployed the anchor ropes in a stationary fashion for 1.5–2.5 h while all other angling gear was trolled for a distance of 1 km from a moving boat for 18–20 min. A host of variables, including the length of time that we deployed the gear, our deployment of the anchor ropes in stationary water (as opposed to in a current), distance from the boat that we trolled the gear, our specific uses of the gear (e.g., trolling as opposed to casting and retrieving angling lines), the types of terminal tackle that we used on the angling lines (e.g., weights as opposed to lures), the lack of any flushing of the livewell (i.e., overflow drains) in our simulation, and the lack of baitfish in the bait bucket, might all have influenced the outcomes. Nonetheless, we believe that our results are a robust index of relative ensnarement rates in relation to how we deployed the gear, and are thus relevant to informing the dispersal risk of angling gear.

Developmental instar

Our results support the hypothesis that ensnarement of *Bythotrephes* on angling gear is positively related to the developmental instar. We tested this hypothesis for the downrigger angling line in both lakes and found that there was a strong and significant trend that indicated that the distribution of the α values was statistically greatest for instar 3 and least for instar 1. These results are consistent with the disproportionate relative frequency of developmental instars ensnared on the downrigger angling line compared to ambient density (Figure 3). During morphological development from instar 1–3, *Bythotrephes* grow progressively in core body size, spine length, and number of tailspine barb pairs (Branstrator 2005). Larger, longer, and more adorned (number of barb pairs) animals present a larger and more complex profile in the water that could increase the frequency of ensnarement on a moving angling line. These same 3 morphological characters could also feasibly increase an individual's retention rate after ensnarement. It seems unlikely that variation in swimming speed could have played a role in the pattern. Swimming speeds of *Bythotrephes* are reported as 16–20 mm/sec (Muirhead and Sprules 2003) with instar 3 being the fastest, but this pace is minimal compared to the velocity that our angling line was moving (2–3 km/h or 555–833 mm/sec).

From the standpoint of dispersal and establishment risk, this result is particularly relevant because instar-3 individuals are the sexually mature portion of a population. When *Bythotrephes* are gravid, instar-3 individuals commonly carry 2–7 offspring (parthenogenetic embryos or resting eggs) per clutch (Branstrator 2005). A strong relationship between propagule pressure and establishment risk has been experimentally demonstrated for *Bythotrephes* in mesocosms (Gertzen et al. 2011, Branstrator et al. 2019). Thus, factors that increase propagule pressure during a dispersal event, including the transport of individuals as embryos or resting eggs, will likely simultaneously enhance establishment risk. This dynamic magnifies the overall threat of dispersal associated with certain types of angling gear such as angling line that selectively ensnares instar-3 *Bythotrephes*.

Ambient density

Our results support the hypothesis that ensnarement of *Bythotrephes* on angling gear is positively related to ambient density. Our results can thus be used to predict ensnarement rate within and among lakes over a range of natural densities of *Bythotrephes* (Figure 1). Because natural densities of *Bythotrephes* commonly fluctuate in lakes by an order of magnitude over short (weekly) time frames (Brown et al. 2012, Kelly et al. 2013), anglers may observe widely different ensnarement rates in the same lake on the same angling gear from week to week. This underscores the need for anglers to avoid vigilance fatigue and to clean gear every time an infested lake is visited. Jacobs and MacIsaac (2007) reported a positive relationship between distance trolled and number of *Cercopagis* ensnared on fishing lines in Lake Ontario. We did not test for an effect of trolled distance on ensnarement rate, and we caution that our results should not be projected to longer or shorter distances because the relationship might be nonlinear and could lead to false predictions. Likewise, if angling gear is periodically retrieved to a boat we suspect that the movement and agitation of the gear could help remove ensnared *Bythotrephes* and reduce total accumulation.

Time of day

Our results do not support the hypothesis that ensnarement of *Bythotrephes* on angling gear is greater during twilight compared to daytime hours. Further investigation of the data did not reveal any reversals in trends in the rank order of the mean ensnarement rates among the gear types in Table 2 when twilight vs. daytime was considered. Sampling even later after dark than we did could reveal different results.

Lake

Our results do not support the hypothesis that ensnarement of *Bythotrephes* on angling gear differs between lakes. There was, however, a notable interaction in ensnarement rate for the shallow and the downrigger angling lines between the 2 lakes. Among other differences, Island Lake Reservoir and Lake Mille Lacs have contrasting presence of cisco (*Coregonus artedi*), a planktivorous predator of *Bythotrephes*. Young and Yan (2008) reported that cisco influence the vertical position of *Bythotrephes,* causing them to occupy shallower portions of the water column during both day and night than they otherwise would in lakes that lack cisco. Our results are directionally consistent with the possibility that *Bythotrephes* were shallower in the water column in Lake Mille Lacs (which supports cisco) than in Island Lake Reservoir (which lacks cisco) in a way that increased their relative exposure in Lake Mille Lacs to the shallow angling lines compared to the downrigger angling line and vice versa in Island Lake Reservoir.

Alternatively, it is possible that the length of line paid out played a role, particularly in Island Lake Reservoir, where more line was typically paid out on the downrigger angling line than the shallow angling lines. In Lake Mille Lacs, however, the length of line paid out (and submerged in the lake during transect transits) was remarkably similar between the 2 gear types, yet the mean ensnarement rate was far greater for the shallow angling lines. We caution that the 2 lakes differ in many ways and that more research is needed to understand how other factors such as bathymetry and water clarity might influence ensnarement rate of *Bythotrephes* on angling gear.

Education and outreach

This study is the first to empirically characterize ensnarement rates of *Bythotrephes* on common types of angling gear. Our results reveal a variety of trends that we believe are relevant to education and outreach messaging around human-assisted dispersal of *Bythotrephes*. In particular, our results demonstrate that angling lines (monofilament, fluorocarbon, and braided) pose major risks for ensnaring large numbers of *Bythotrephes* when trolled in an infested lake.

We caution that turning our results into education and outreach recommendations should be done with consideration for what we measured (ensnarement rate) and what still remains unknown (risk of transfer of living individuals to another lake). Our study did not determine how likely *Bythotrephes* are to survive should they become ensnared and transported on gear. Survival during transport is a critical stage in the range expansion of any invasive species (Sinclair et al. 2020). To this end, we cannot lose sight of the fact that livewells and bait buckets, despite their lesser ensnarement rates measured here, could enhance survival of ensnared *Bythotrephes* if they remain wet for longer periods of time than angling lines.

Bythotrephes are likely to experience a wide range in survival rate depending on whether they remain wet. Research has shown that *Bythotrephes* resting eggs are vulnerable to drying and cannot survive exposure to dry conditions for 6 h or longer (Branstrator et al. 2013). While it is believed that the planktonic stages of zooplankton are far less tolerant of drying than their corresponding resting (dormant) egg stages, this has not yet been tested for *Bythotrephes*. Nonetheless, the absence of a protective carapace around the body of *Bythotrephes* would suggest that tolerance of the planktonic stage to drying is far less than for its resting egg, and thus likely less than 6 h. However, if ensnared individuals remain wet, the window of survival could be prolonged. For example, large masses of *Bythotrephes* clumped on angling line would likely provide a degree of protection against drying that could prolong survival during transfer out of water, particularly during humid or rainy conditions. Likewise, livewells and bait buckets that contain internal crevices that remain damp even after draining could provide safe microhabitats for *Bythotrephes* between lakes. This reinforces the importance of continuing to communicate the imperative to drain all water when leaving a lake. We suggest that wiping down internal crevices after draining would further help remove water and *Bythotrephes*.

In conclusion, our results demonstrate that trolled angling lines are highly susceptible to ensnarement of *Bythotrephes* and thus represent a high-risk pathway of dispersal for this invader. Nonetheless, decontamination of other equipment should not be overlooked. In addition to physical removal or draining water, approaches such as drying equipment surfaces for 6 h or longer (Branstrator et al. 2013) or exposure to lethal levels of heat or chemical disinfectant (e.g., Virkon) for prescribed periods (Branstrator et al. 2013, De Stasio et al. 2019) offer managers and citizens a range of options to decontaminate equipment.

Acknowledgments

We acknowledge the contributions of Kari Hansen, Robert Hell, Matthew Santo, John Utecht, Alexandra Quinn, Nick Pierce, Sean Loftus, Nichole DeWeese, Maxwell Brubaker, and Sam Zrust in the field and laboratory.

Authors' contributions

DKB, JDD, and VJB conceived the project. All authors developed the methodology and collected the data. DKB led the data analysis and writing. All authors contributed critically to the drafts and gave final approval for publication.

Funding

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Final Report – Map
MAISRC Subproject 15: Determining Highest Risk Vectors of Spiny Water Flea Spread
Dr. Valerie Brady

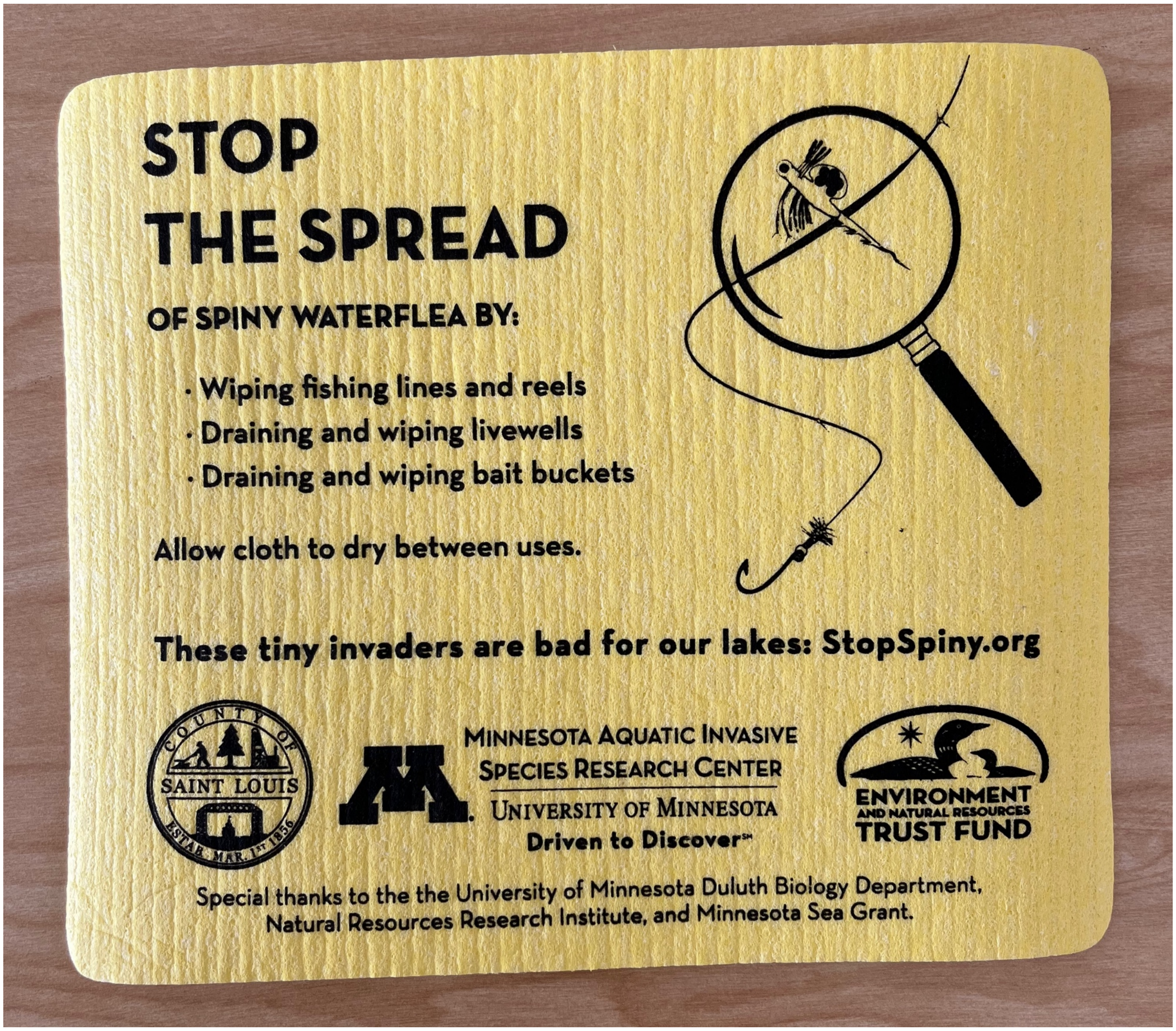
Field work site on Lake Mille Lacs.

Final Report – Visual Component

MAISRC Subproject 15: Determining Highest Risk Vectors of Spiny Water Flea Spread

Dr. Valerie Brady

Visual of spiny water flea cellulose dish clothes that were produced and distributed to help recreational anglers clean their fishing gear. Additional resources and materials are available on the stopspiny.org website.



Minnesota Aquatic Invasive Species Research Center
University of Minnesota
Driven to Discover℠

Environment and Natural Resources Trust Fund

M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2020

SUBPROJECT TITLE: MAISRC Subproject 20: A Novel Technology for eDNA Collection and Concentration
SUBPROJECT MANAGER: Dr. Abdennour Abbas
ORGANIZATION: University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Food Agriculture and Natural Resource Sciences/College of Science and Engineering; Department of Bioproducts and Biosystems Engineering
MAILING ADDRESS: 2004 Folwell Ave
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PHONE: 612-624-4292
E-MAIL: aabbas@umn.edu
WEBSITE: **(1)** http://abbaslab.com/ **(2)** https://www.maisrc.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $190,863
AMOUNT SPENT: $186,527
AMOUNT REMAINING: $4,336

Sound bite of Project Outcomes and Results

The development of a novel filter capable of efficiently extracting Environmental DNA (eDNA) from water, and enabling rapid filtration of large volumes of samples at a reasonable cost, is expected to help convert the eDNA technology from a research curiosity into a routine tool for ecosystem protection and monitoring, and evidence-based management of invasive species.

Overall Subproject Outcome and Results

Background/Context: Environmental DNA (eDNA) is the genetic material (genomic DNA) obtained directly from environmental samples such water. Collection and analysis of eDNA has the potential to provide actionable information on the presence and distribution of aquatic invasive species.

Challenge: The major challenge is that the results obtained from eDNA techniques currently do not always correlate with traditional netting data due to the size and quality of sampling. Unlocking the potential of eDNA requires disruption in sampling methods and tools.

Objectives: This project aimed to develop a novel aquatic eDNA collection and concentration technology for more efficient, reliable and cost-effective screening for not only invasive aquatic organisms and pathogens but also native and endangered species. The technology would significantly enable and empower aquatic ecosystem survey and management programs in Minnesota. Specifically, we aimed to 1) develop an eDNA nanofilter that specifically and rapidly captures nucleic acids (DNA, RNA) from water and enable the processing of large volumes of samples within a short period of time, 2) Verify increased eDNA sampling efficiency of the new nanofilter in field settings (proof-of-concept)

Results and Accomplishments: We have successfully developed a new eDNA filter that captures 50-100% of eDNA within 10 seconds. Commercial kits are incapable of capturing free eDNA. The loading capacity of the new filter is up to 5 mg/g, meaning that 1 g of filter can capture up to 5 mg of DNA. This is a record-breaking capacity that enables the filtration of large volumes of water with one filter, knowing that surface water contains usually 10 ng/L of eDNA.

Following the COVID-19 pandemic, we have adapted the nonfilter to develop an RNA extraction kit for SARS-CoV-2. The new kit was evaluated by the University of Minnesota COVID-19 Diagnostic Laboratory on 80 patient samples, and it showed that our kit has a 100% specificity and 94% sensitivity, which is respectively 12.8% and 5.4% higher than the widely used Qiagen kits

Significance and Impact to Minnesota: Ecosystem conservation managers have been relatively reluctant to use eDNA as a routine tool for ecosystems monitoring. The results obtained here can have a significant impact on the widespread adoption of eDNA technology, which will help the State enhance the accuracy and quality of the data and improve decision making for the management of invasive species. This work has also led to starting a new company, which is expected to accelerate the transfer of the technology to the market, and enhance the industry capacity to respond to the State's need for AIS management.

Subproject Results Use and Dissemination

The results obtained in this project have been presented at three conferences and meetings and will be published through four scientific publications that are currently in process. The work has also been highlighted by the University of Minnesota news service and more media coverage is expected after manuscript publication. The work conducted in this project has also led to the foundation of a new technology company that is expected to take the eDNA filter technology to the market during 2021.

Presentations:

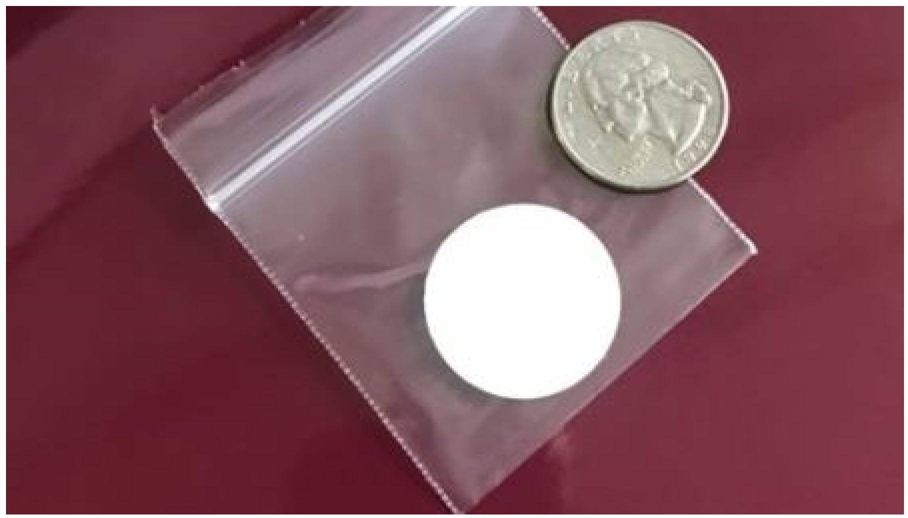
- Zarouri, A., A. Abbas. September 2019. Enhancing fish surveys: A novel technology for environmental DNA capture. MAISRC Research and Management Showcase. Saint Paul, MN.
- Quichen, D., A. Zarouri, A. Abbas. September 2019. A Novel Technology for Environmental DNA Collection and Concentration. American Fisheries Society and The Wildlife Society Conference. Reno, NV.
- Zarouri, A., Q. Dong, A. Abbas. October 2019. A Novel Technology for Environmental DNA Collection and Concentration. 2019 Department of Bioproducts and Biosystems Engineering Research Poster Session. Saint Paul, MN.

Media:

- Detection connections. CFANS News. 9 July 2020. https://cfans.umn.edu/news/abbas-lab-covid-19-update

Attachments:

- Photo of the eDNA nanofilter that was developed as a part of this project.



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2020

SUBPROJECT TITLE: MAISRC Subproject 22: Copper-based control: zebra mussel settlement and non-target impacts
SUBPROJECT MANAGER: James A. Luoma
ORGANIZATION: U.S. Geological Survey
COLLEGE/DEPARTMENT/DIVISION: Upper Midwest Environmental Sciences Center
MAILING ADDRESS: 2630 Fanta Reed Road
CITY/STATE/ZIP: La Crosse, WI 54603
PHONE: 608-781-6391
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WEBSITE: **(1)** https://www.usgs.gov/centers/umesc **(2)** https://www.maisrc.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a; M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $218,956
AMOUNT SPENT: $214,526
AMOUNT REMAINING: $4,430

Sound bite of Project Outcomes and Results

A 10-day low-dose copper treatment of an enclosed bay in Lake Minnetonka (Minnesota) was highly effective at reducing the abundance of zebra mussel veligers and preventing zebra mussel settlement success. The treatment did cause some nontarget effects including, but not limited to, reductions in native zooplankton and benthic invertebrate abundance.

Overall Subproject Outcome and Results

This study evaluated a low-dose copper treatment for zebra mussel (*Dreissena polymorpha* Pallas 1771) suppression by maintaining a mean copper concentration of 60 µg/L in waters above the thermocline for 10 consecutive days in St. Albans Bay (66.3-ha) of Lake Minnetonka, Minnesota. Robinson Bay (37.2-ha, Lake Minnetonka) was a control site. The volume of EarthTec QZ applied during five every-other-day applications was determined using copper concentrations measured in the field.

Treatment effects on zebra mussels lifestages were evaluated by analyzing changes in veliger abundance, juvenile settlement, benthic abundance, and adult survival. Treatment effects on nontargets were evaluated by analyzing changes in water chemistry properties, chlorophyll a, native fish (4 species) survival, native mussel (1 species) survival, native zooplankton abundance and richness, and native benthic invertebrate abundance and richness.

The copper concentration was maintained above 60 µg/L during the treatment period and returned to background levels between 60 and 90 days after treatment. The treatment adversely affected all life stages of zebra mussels throughout the study period. In the treated bay, veliger density was near zero 14 days after treatment, a strong reduction in juvenile settlement was observed, zebra mussel benthic density was sparse after treatment, and the odds of adult survival was substantially reduced. Detectable nontarget treatment-related effects included reductions in zooplankton abundance, chlorophyll a, and fathead minnow survival. Elevated copper residues in fish and mussel tissues were also observed. Decreases in benthic invertebrate abundance, secchi disk readings, and dissolved oxygen concentration were also observed after the treatment.

The data from this study can be used to assist in assessing if low-dose copper treatments are an appropriate zebra mussel management strategy for a waterbody. Any use of trade, firm, or product names in this report is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Subproject Results Use and Dissemination

Publications:

- Luoma J.A., Barbour M.T., and Severson T.J. (2020). Data Release: Copper-based control: zebra mussel settlement and non-target impacts. U.S. Geological Survey. Data Release. https://doi.org/10.5066/P9B9NUQM.

Presentations:

- Barbour M.T., Luoma J.A., Severson T.J., Wise J.K., and Dahlberg A. (2019). Low-dose copper-based control: zebra mussel settlement and non-target impacts. MAISRC Research and Management Showcase, University of Minnesota Continuing Education and Conference Center, Saint Paul, Minnesota.
- Dahlberg A., Phelps N., Waller D., Luoma J., and Barbour M. (2020). Low-dose copper-based control: zebra mussel settlement and non-target impacts (webinar). AIS Detectors Program, August 26, 2020, https://www.maisrc.umn.edu/ais-detectors/webinars.
- Dahlberg A., Phelps N., Waller D., Luoma J., and Barbour M. (2020). Low-dose copper-based control: zebra mussel settlement and non-target impacts (webinar). Invasive Mussel Collaborative, August 27, 2020.

Media:

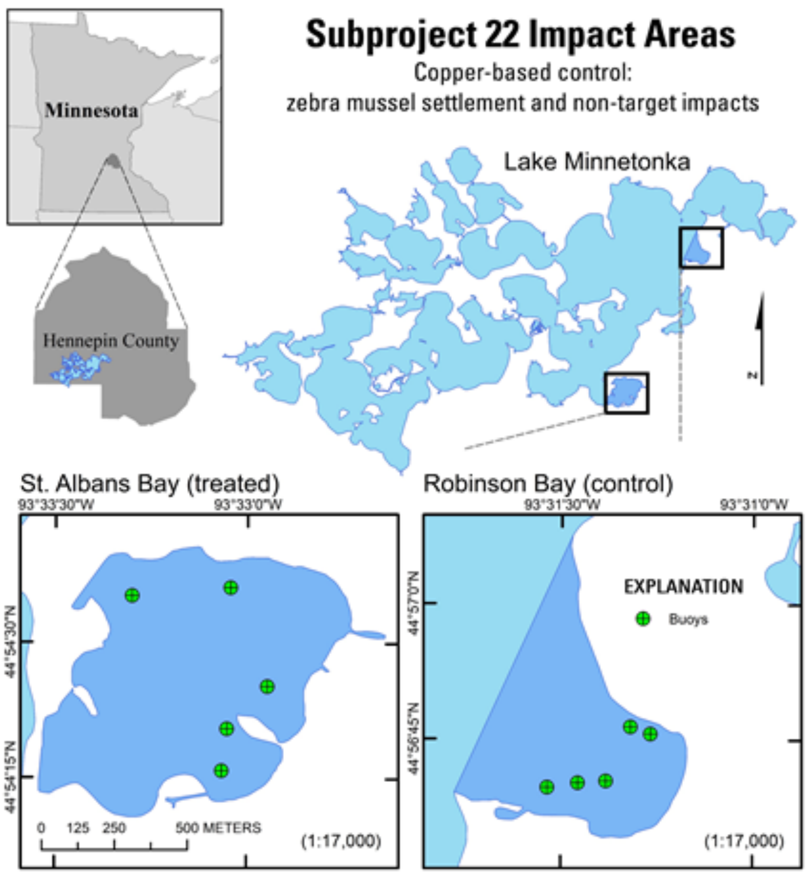
- UMN Driven to Discover video: Guardians of the Lake (2019). https://twin-cities.umn.edu/discover/guardians-lake
- Zebra mussels research project planned for Lake Minnetonka this summer. Melissa Turtinen, Southwest News Media. 23 April 2019. https://www.swnewsmedia.com/lakeshore_weekly/news/local/zebra-mussels-research-project-planned-forlake- minnetonka-this-summer/article_750497a4-a492-5020-868b-6d752887fa0b.html
- St. Alban's, Robinson's bays will be site of zebra mussel research project. Sabina Badola, Sun Sailor. 16 April 1029. https://www.hometownsource.com/sun_sailor/free/st-alban-s-robinson-s-bays-will-be-site-of-zebra-musselresearch-project/article_fe8a1ea4-607c-11e9-aafc-63c0878d1728.html

Attachments:

- Zebra Mussel Control with Low-Dose Copper (handout)
- Photos from field work
- Effects Map

MAISRC Subproject 22: Copper-based control - zebra mussel settlement and non-target impacts

Two bays of Lake Minnetonka (Minnesota) were test locations for this project. St. Albans Bay was treated with EarthTec QZ with a target copper concentration of 60 µg/L for 10 days with five, every-other-day applications (i.e. 5 applications over 10 days). Robinson Bay served as an untreated control reference site. Each bay had five sampling locations that were marked with a buoy. All samples for the study were collected in the vicinity of the buoys, except for the benthic quadrat surveys used to assess zebra mussel abundance. Zebra mussel settlement plate samplers and test animal holding cages were also deployed at each sampling buoy location.



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2022

SUBPROJECT TITLE: MAISRC Subproject 23: AIS Management: An Eco-economic Analysis of Ecosystem Services
SUBPROJECT MANAGER: Dr. Amit Pradhananga
ORGANIZATION: University of Minnesota Twin Cities
COLLEGE/DEPARTMENT/DIVISION: Department of Forest Resources, Center for Changing Landscapes
MAILING ADDRESS: 1530 Cleveland Avenue N
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PHONE: 612-624-6726
E-MAIL: prad0047@umn.edu
WEBSITE: https://maisrc.umn.edu/public-values
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $242,090
AMOUNT SPENT: $241,394
AMOUNT REMAINING: $696

Sound bite of Project Outcomes and Results

Minnesotans hold great value for Aquatic Invasive Species Management, both to lakes they visit and to waterbodies in the state as a whole and are willing to pay significantly for it. Minnesotans are concerned about AIS and are generally supportive of AIS management actions and policies.

Overall Subproject Outcome and Results

Minnesota hosts a number of aquatic invasive species (AIS), which have far-reaching impacts on Minnesota's waterbodies, and subsequently its population. However, little was known about how Minnesotans value AIS, as well as costs associated with AIS management. To address this, we collected data on aquatic invasive species management and costs, public perceptions, values, knowledge, and willingness to pay for aquatic invasive species management via several surveys of different types spanning 2019 to 2021. Surveys of watershed districts and soil and water conservation districts provided data from 92 lakes across 12 counties, showing that carp management is a priority in Minnesota. We also were able to collect data on costs and types of management employed. On the individual side, an onsite survey of approximately 1000 people visiting lakes in the summer showed us visitors are willing to pay for AIS management at the lakes they are visiting and hold significant value for Minnesota's water resources, though individual AIS species present are not impactful for these social values. We also collected data through a mail survey of about 300 people, which confirmed Minnesotans' intrinsic value for water resources. Many residents are willing to pay for AIS management statewide, meaning they do not have to directly visit or use a lake to find value in it. This project is important as it provides data to support the viewpoint that Minnesotans do in fact have great value for AIS management and are willing to pay to expand management across the state.

Subproject Results Use and Dissemination

This project's findings have been disseminated through nine oral and poster presentations to researchers, resource professionals (e.g., Minnesota Department of Natural Resources), lake associations, policy makers, and the general public (e.g., lakeshore residents) at professional conferences (e.g., Minnesota Water Resources Conference), Minnesota Aquatic Invasive Species Research Center (MAISRC) Research & Management

Showcase, and invited seminars (e.g., Minnesota DNR, AIS Detectors' Aquatic Invasive Species Webinar Series). We have published one open access article in a peer-reviewed journal (PLOS ONE). We have developed a fact sheet highlighting findings from the statewide survey conducted with Minnesota residents. In coordination with MAISRC, we developed a handout of findings from the survey conducted with recreationists at four Minnesota lakes. We plan to continue to disseminate study findings through presentations and peer-reviewed journal articles. We have submitted two abstracts to the International Association for Society and Natural Resources Conference and Universities Council on Water Resources Annual Conference to be held in June, 2022 and are currently preparing three additional manuscripts for submission to peer-reviewed journals.

Peer-Reviewed Publications

- Levers, L., & Pradhananga, A. (2021). Recreationist Willingness to Pay for Aquatic Invasive Species Management. *PLOS ONE*. https://doi.org/10.1371/journal.pone.0246860

Presentation Recordings/Videos

- 2021 MAISRC Research & Management Showcase Common Carp Panel https://z.umn.edu/2021ShowcaseCommonCarpPanel
- AIS Detectors Webinar: Recreationists' Willingness to Pay for Aquatic Invasive Species Management https://z.umn.edu/DetectorsWebinarWillingnessToPay
- MAISRC Video: Valuing Aquatic Invasive Species Management https://z.umn.edu/MAISRCValuingAISManagement

Minnesota Aquatic Invasive Species Research Center

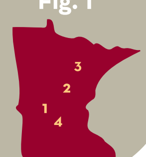
PAYING TO PROTECT MINNESOTA'S WATERS

Daily pay-to-play

Minnesota is renowned for its beautiful lakes, but do residents place a high value on the state's water quality, habitat, and recreational aspects? And if so, would recreational water users in Minnesota be willing to pay a daily access fee that would be applied toward aquatic invasive species management at the lake that they use? Researchers at the Minnesota Aquatic Invasive Species Research Center conducted in-person surveys at four Minnesota lakes in 2019 to find out.

Polling lakeside users

Fig. 1



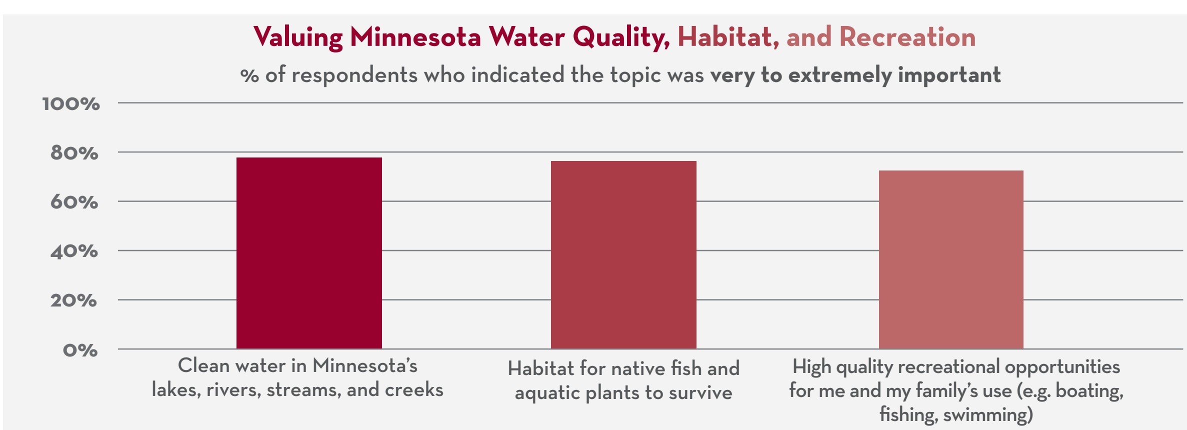
The surveys were conducted at the public water accesses of Minnewaska (1), Gull (2), Pokegama (3), and Koronis (4) Lakes in summer 2019 (**Figure 1**). The lakes were chosen by researchers for a number of reasons. Gull and Pokegama attract a number of out-of-town visitors, whereas Minnewaska and Koronis generally have a more local user base—researchers wanted to include both perspectives. Additionally, the lakes have varying levels of infestations—from Gull, which is heavily infested with zebra mussels, to Pokegama which did not have any aquatic invasive species infestations at the time of the survey. Surveys were conducted on both weekdays and weekends, and at a variety of times throughout the day.

What we learned

$9

The survey was completed by 994 people. Respondents affirmed that they highly value Minnesota's water purity, habitat, and recreational opportunities (**Figure 2**). Of the 994 people who completed the survey, roughly half were willing to pay a daily user fee that would be applied to aquatic invasive species management at the lake that they use. Of those willing to pay the daily user fee, the mean amount they were willing to pay was approximately $9 per day. There was no significant difference in daily willingness to pay between any of the surveyed lakes. In 2021, a paper version of the survey will be sent to 2,000 households in Minnesota. Researchers are aiming to build upon the data collected lakeside and employ the mail survey as a means of ensuring the broadest participation of recreationists possible, stretching across geographic and socioeconomic barriers—wider feedback is needed from Minnesotans before a statewide attitude on the fee can be assessed.

Fig. 2



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RESEARCH ARTICLE

Recreationist willingness to pay for aquatic invasive species management

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Abstract

We estimated willingness to pay for local aquatic invasive species lake management in the form of a daily lake access fee by conducting summer lake surveys in Minnesota, USA. Similar pairs of lakes with differing infestations of zebra mussels, *Dreissena polymorpha*, and starry stonewort, *Nitellopsis obtuse*, were used as study sites to infer how being at an infested lake vs. being at an uninfested lake and different local species would impact responses. We also examined recreationists' visit motivation, and aquatic invasive species perceived risk, knowledge, and awareness of problem. We estimated mean willingness to pay about nine to ten dollars per day, which did not differ significantly by lake. Additionally, perceived risk, awareness of problem, and visit motivation were significant in predicting willingness to pay, which could have important ramifications for aquatic invasive species management.

1 Introduction

Aquatic invasive species (AIS) are a growing problem in freshwater systems throughout the world, negatively affecting native biota and human populations. The aquatic environment poses some unique challenges to invasive species management, particularly as it relates to detection and control [1–3]. In areas which have high numbers of water bodies and high numbers of water craft (1 out of 6 Minnesotans owns a boat—in more northern areas, it is 1 out of 3 [4]), invasive species are easily moved from one site to the next and have gained substantial foothold. In Minnesota alone, the Department of Natural Resources (DNR) lists 37 different aquatic invasive species [5]. Some of which, like common carp, *Cyprinus carpio*, and curlyleaf pondweed, *Potamogeton crispus*, are considered naturalized and are no longer tracked by DNR. Some have been present for decades, like zebra mussels, *Dreissena polymorpha*. Others are relatively new, like starry stonewort, *Nitellopsis obtuse*. With such a variety of species, it is understandable that effects are as varied as the species themselves. When multiple species are infesting the same water body, impacts become even more difficult to tease out. Combined with climate change, and other anthropogenic influences on environmental quality and ecosystem health, the true impacts of a particular species can be extremely difficult to determine for scientists, let alone laypersons. Ideally, to understand how people value invasive species, we would need to fully understand 1) underlying causal relationships between invasive species and environmental impacts, and 2) how individuals in question value those impacts.

While much attention has been paid to understanding ecological and economic impacts of invasive species, linkages between human systems and ecosystem impacts of invasive species are not well understood. In recent years, there has been an increase in understanding the economic impacts of invasive species using contingent valuation methods (e.g., [6, 7]). Yet, there is little clarity of the underlying motivations for individuals' support and willingness to pay for invasive management. From a management perspective, understanding people's motivations for and constraints to actions that prevent or manage the spread of invasive species can help resource managers develop programs that are based on public needs and concerns.

Very few studies have focused on the social-psychological determinants of willingness to pay for invasive species management. In a study of residents, tourists, and conservationists in Spain [8], the authors found that attitudes about invasive species and invasive species management were related to willingness to pay. In a similar study [9], the authors reported that demographic variables such as higher levels of income and smaller household size were positively associated with willingness to pay for invasive species management. Further, reported interest in nature (e.g., membership in environmental organizations), knowledge about invasive species, concern about invasive species impacts on cultural identity, and sense of place (i.e., emotional connection people feel for a geographic area) were positive predictors of willingness to pay. Other researchers have also linked income, interest in nature [6, 10], and age [10] with willingness to pay. While not in the context of invasive species management, a subset of studies have argued that it is critical to examine the underlying social-psychological factors that influence willingness to pay [1, 2]. These studies have generally concluded that the inclusion of social-psychological theories and variables improves the explanatory power of models examining willingness to pay. For example, a study applying different theoretical models to willingness to pay [1] found that personal norm and awareness of responsibility, variables from the norm-activation theory [3], have higher explanatory power than variables derived from theories such as the theory of planned behavior and the theory of public goods. Further, environmental concern has been reported as a significant predictor of willingness to pay for public environmental goods (i.e., forest biodiversity). Another study [2] provides empirical support for the influence of variables from the theory of planned behavior [4] on willingness to pay for increase in biodiversity. Attitudes about biodiversity, subjective norms (i.e., social pressure to take action), and perceived behavioral control (i.e., ease or difficulty of performing a behavior) were significant predictors of willingness to pay [2]. Studies have also linked knowledge, past environmental activism, trust in governing agencies [5], value orientations, awareness [6], perceived effectiveness of policy, social capital [7] and perception of other actors' actions [5, 7] with willingness to pay for environmental goods in the context of energy consumption, emission reductions, and waste management. This study builds on this body of research by exploring the social-psychological factors that influence willingness to pay for invasive species management. This project employed on-site summer lake surveys to understand how recreationists using public lake access points perceived and valued aquatic invasive species management, and whether different species and magnitudes of infestations (proxied by different lakes) influenced their perceptions of AIS and their willingness to pay for aquatic invasive species management.

2 Conceptual framework

This study's conceptual framework integrated multiple lines of research that link environmental awareness, risk perception, motivations, and environmental behaviors. While environmental behaviors have been studied from a wide range of theoretical perspectives [8], research in the human dimensions of natural resources suggests that a cognitive structure of values, attitudes, and beliefs affect general pro-environmental behaviors, as well as behaviors targeted at invasive species [9, 10]. According to the cognitive hierarchy theory, human cognitions are organized hierarchically from values, which are centrally held and stable, to elements such as attitudes, beliefs, and ultimately behaviors, which are numerous and more easily changed [11, 12]. This framework has been applied in various natural resource contexts [11–14]. We build on the cognitive hierarchy theory framework by integrating it with risk perception theory.

Researchers have extensively studied risk perceptions and its influence on environmental behavior [15–17]. Risk perception is defined as the process of "discerning and interpreting signals from diverse sources regarding uncertain events and forming a subjective judgement of the probability and severity of current or future harm associated with these events" ([15], p. 1). The concept of risk perception has been applied extensively to behaviors related to climate change. For example, a nationwide study of US residents found a significant influence of risk perception on voluntary climate change action (e.g., choice of transportation) and voting intentions (e.g., support for climate change-related government programs) [18]. Similarly, recent studies have reported risk perceptions as determinants of energy conservation [19], general pro-environmental behaviors (e.g., recycling, buying organic food) [15], willingness to participate in organic farming programs [20], and travel behavior [21]. While literature linking risk perception to invasive species prevention and control behaviors is scant, Estévez et al. [9] provide an integrated risk perception and cognitive hierarchy theory framework to study the human and social dimensions of invasive species management.

We also included knowledge about AIS and awareness of AIS problem as determinants of willingness to pay in our framework. While some studies have found weak to no relationship between knowledge and environmental behavior [22], a few studies provide support for the relationship between knowledge and AIS control behaviors, e.g. [23], as well as willingness to pay for environmental protection in the context of invasive species [24], greenhouse gas emissions [5], and stormwater management [25, 26]. Thus, some knowledge and awareness about environmental issues may be necessary for environmental actions [27].

Past work, particularly in the area of leisure and recreation management, has investigated people's motivations for engaging in leisure and recreation [28, 29]. The question of 'why' people engage in recreation or the desired outcomes of engaging in recreation (i.e., visit motivation) has been linked with recreationist behaviors, preferences, and satisfaction [29–31]. Since the survey sample in our study consisted of recreationists intercepted at lakes, it is plausible to hypothesize that their motivations for visiting the lake would have an influence on the actions aimed at protecting the lake from AIS. Thus, we included visit motivation in our conceptual framework as a cognitive element that influences recreationists' willingness to pay for AIS management.

3 Study sites

Priority species for management in Minnesota include zebra mussels, *Dreissena polymorpha*, and starry stonewort, *Nitellopsis obtuse*. Zebra mussels attach themselves to a myriad of surfaces in lakes, smothering native species, increasing water clarity, and causing property damages [18]. Additionally, walleye, *Sander vitreus*, a recreationally and ecologically important fish species in North America, grow more slowly in their first year when zebra mussels are present [19]. Starry stonewort is a macro-algae that forms thick mats just below the lake surface, reducing navigability and impacting native species [20]. We chose two pairs of lakes, with each pair representing a spectrum of infestation for each priority species, yet being as similar as possible in other measures such as limnological factors, typical recreational use, size, proximity, public access similarity, and nearby human population types. Sampling locations at each lake were public lake access points managed by the Minnesota Department of Natural Resources. They all included a boat ramp, parking facilities, and restrooms. A special use permit covering all locations and dates was obtained from the Minnesota Department of Natural Resources Division of Parks and Trails prior to data collection.

Because we knew that the public may not be aware of the local AIS, we chose lakes that had very obvious water quality impacts of infestation. We also needed to ensure the lakes had similar public access quality, visitation rates were high enough for adequate sampling, and preferably that the lakes were close together. Though, because of the nature of AIS spread, lakes nearby to one another are often infested with the same species. With consultation and data of the Minnesota Department of Natural Resources, expert opinion of Minnesota Aquatic Invasive Species Center affiliates, and ground-truthing, we chose the following lake pairs (See Fig 1 for locations):

a. *Gull Lake and Pokegama Lake.*
Gull Lake is heavily infested with zebra mussels, with the first found in 1990. The water is very clear. Mussel shells litter the beaches. At the time of the survey, Pokegama Lake (referred to as Lake Pokegama locally) was connected to a water body that is infested with zebra mussels, but no zebra mussels had been found in its waters (however, it was listed as infested later in the year). No additional invasive species have been noted at either lake. Both lakes have similar recreational activities and are popular vacation locations.

b. *Lake Koronis and Lake Minnewaska.*
The first lake in Minnesota found to host starry stonewort (in 2013), Lake Koronis is heavily infested with starry stonewort, but has no other AIS. The starry stonewort fills the water column in large patches of Koronis. Lake Minnewaska has a small infestation of starry stonewort, isolated to a marina (the survey locations were not located in or by the marina). Minnewaska also hosts zebra mussels and Eurasian watermilfoil, *Myriophyllum spicatum L.*, a rapidly growing aquatic plant that forms dense mats at the water's surface. Both lakes have similar recreational activities and are considered more "local" lakes, which are not as popular for vacationing as Gull and Pokegama.

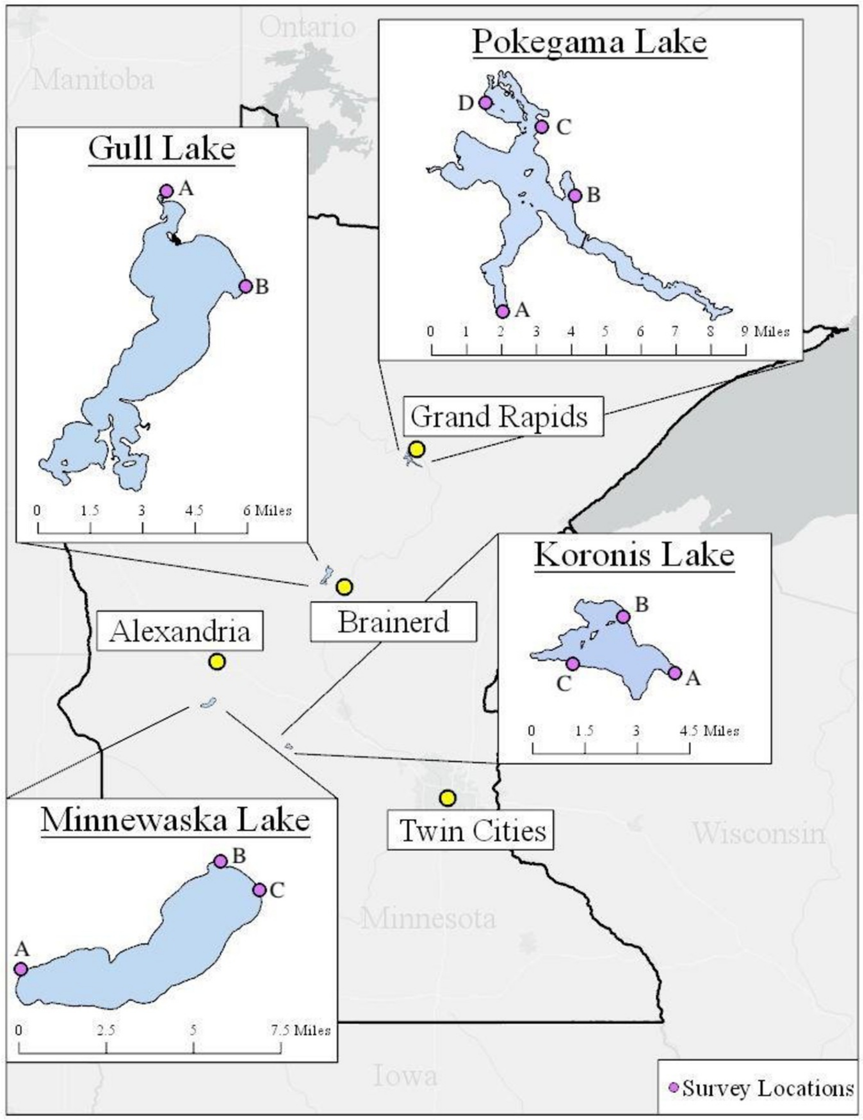
4 Survey design and statistical model

4.1 Survey design and administration

The survey protocol was developed using standard survey methodology [21, 22], and implemented via Qualtrics. Surveys were administered on tablets using Qualtrics at public lake access points during June, July, and August, 2019. As survey locations included boat ramps, many respondents were using boats, though this was not a requirement for survey participation. Each lake was surveyed for a total of 140 hours, spread out over four five-day periods, spanning weekends and weekdays. All lakes had multiple public lake access points (see Fig 1)—survey locations were rotated. Survey time blocks (morning, afternoon, evening) were also rotated.

Survey administrators (undergraduates) were trained and conducted mock surveys prior to collecting data. During surveying periods, administrators wore matching University of Minnesota hats, shirts, and nametags. They also put up University branded signs saying "Your opinion needed." Working in twos, they approached recreationists, many of whom were either launching or loading a boat. They identified themselves as University of Minnesota students

(a): Map of study sites



(b): Coordinates of study sites

	Site A	Site B	Site C	Site D
Koronis Lake	45.325501, -94.669602	45.348655, -94.701283	45.328333, -94.727969	--
Gull Lake	46.509939, -94.343929	46.471234, -94.295856	--	--
Minnewaska Lake	45.606259, -95.527086	45.653957, -95.411755	45.640769, -95.387382	--
Pokegama Lake	47.136746, -93.600299	47.185893, -93.556688	47.212234, -93.579791	47.223547, -93.610308

Fig 1. Location of study sites in the state of Minnesota.

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collecting data, and asked if the recreationists would participate in a voluntary survey about aquatic invasive species. Estimated time of completion, 7 to 10 minutes, was provided (actual median response time was measured as 7.8 minutes). Respondents were asked if they were over 18 before they were allowed to proceed. Respondents were then handed the tablets. Occasionally, administrators were asked to read the survey questions. If so, the administrators complied. Administrators were instructed not to answer any questions except for clarifying ones (e.g., if a respondent did not understand a word used in a question). The questionnaire and the administration protocol were reviewed by the University's Institutional Review Board.

4.2 Survey measures

The survey collected data on willingness to pay to access the lake (see Section 4.1 below), as well as data on multiple variables that could potentially impact willingness to pay, including respondents' visit (e.g., length of visit, activities), perceived AIS risk, knowledge about AIS, AIS awareness of problem, socio-demographics (e.g., gender, income, education), and travel patterns (e.g., whether they were coming from/returning to home [i.e. whether they were locals]).

Recreational activities. The questionnaire included two questions about respondents' current recreational activities during the visit. The first question asked participants to report the activities they participated in during the visit (e.g., fishing, boating, hiking, socializing, swimming). A follow up question asked respondents to identify the activity that was the primary reason for their visit.

Visit motivation. Recreationists' motivation to visit the lake was measured using eight items [23, 24]. People's motivation to participate in an activity (e.g., visiting a lake) provides an explanation for why people engage in that activity [23]. In the context of recreation and leisure science, visit motivation has been linked to intention to revisit tourist destination [25], and satisfaction with tourist or visit experience [26, 27]. Respondents were provided with a list of possible reasons why people visit lakes: "to be close to nature", "to be physically active", "to be on my own", "to socialize", "to view the scenery", "to get away from the usual demands of life", "to relax", and "to experience silence and quiet". They were asked to rate how important each of the reasons were to them on a five point scale from "not at all important (0)" to "extremely important (4)". Past work has identified several domains of visit motivation including autonomy (e.g., "to be on my own), nature enjoyment (e.g., to view the scenery, to be close to nature), health/physical rest (e.g., to relax), solitude (e.g, to experience silent and quiet), escape from personal/social pressures (e.g., to get away from usual demands of life), and social motivations (e.g., to socialize) [23, 24].

Perceived AIS risk. Perceived risk of AIS was measured using six items. Respondents were asked to rate the extent to which they believe AIS is a risk to various water-related ecosystem services: "habitat for native fish and aquatic plants", "quality of recreational opportunities (e.g., boating, fishing)", navigability of waterways", "economic viability of recreation and tourism businesses", "cost of water treatment", and "water quality in Minnesota's lakes, rivers, and streams". Response was on a five-point scale from "no risk at all (0)" to "extreme risk (4)".

Awareness of AIS problem. One item was used to measure awareness of AIS problem. Respondents were asked to rate the extent to which they believe AIS are a problem in Minnesota on a four-point scale from "not a problem at all (1)" to "severe problem (4)".

Knowledge about AIS. As the infestation type and magnitude of the particular lake was of importance, we asked the respondents general questions about AIS, including with what AIS they were familiar. We included photographs and general information about three invasive species of concern in Minnesota: Eurasian watermilfoil, zebra mussels, and starry stonewort

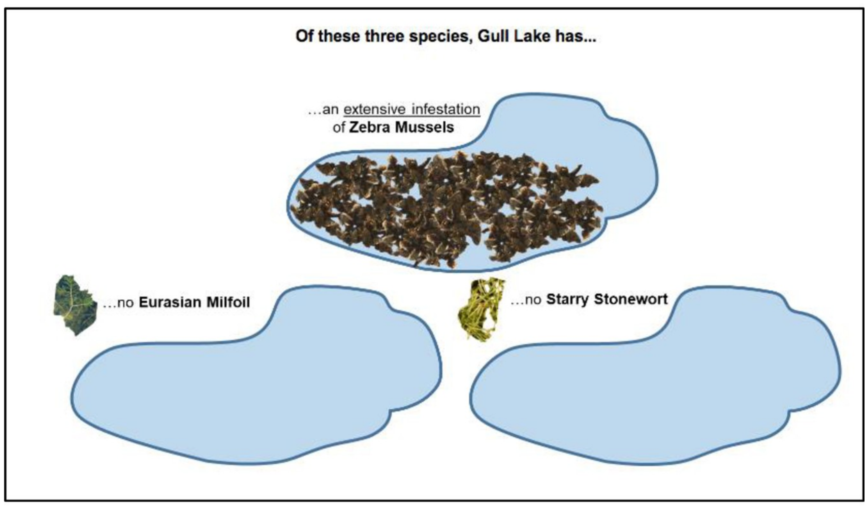
(S1 Appendix question). We also asked respondents to identify the invasive species that were present in the lake.

The invasive species in the response list, as well as the photos and descriptions, were the same as in the familiarity question listed above, and remained constant across the four lakes. A fourth option, “None of these species are in <lake name>” was also included. While the respondents were required to advance linearly through the survey, there is no guarantee they read the entire question. As such, we designed the survey with a degree of repetitiveness to give the respondents the best chance to retain information regarding the species.

4.3 Willingness to pay

Survey design. Willingness to pay questions were designed using recent stated preference guidance [28, 29]. First, respondents were provided with lake specific AIS information (and could no longer back track in the survey to avoid correcting themselves) in a graphical form which emphasized the infestation magnitude (Fig 2, Question 1a). Respondents were then provided information regarding management strategies and potential impact of management, while still emphasizing infestation magnitude (Fig 2, Question 1b), to ensure respondents had information on what is currently possible for AIS management.

Question 1a: Species infestation (Gull Lake question shown)



Question 1b: Species management (Gull Lake question shown)

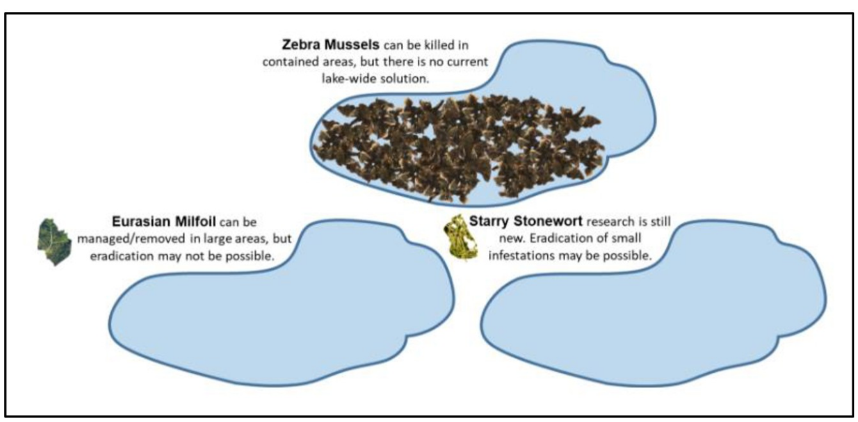
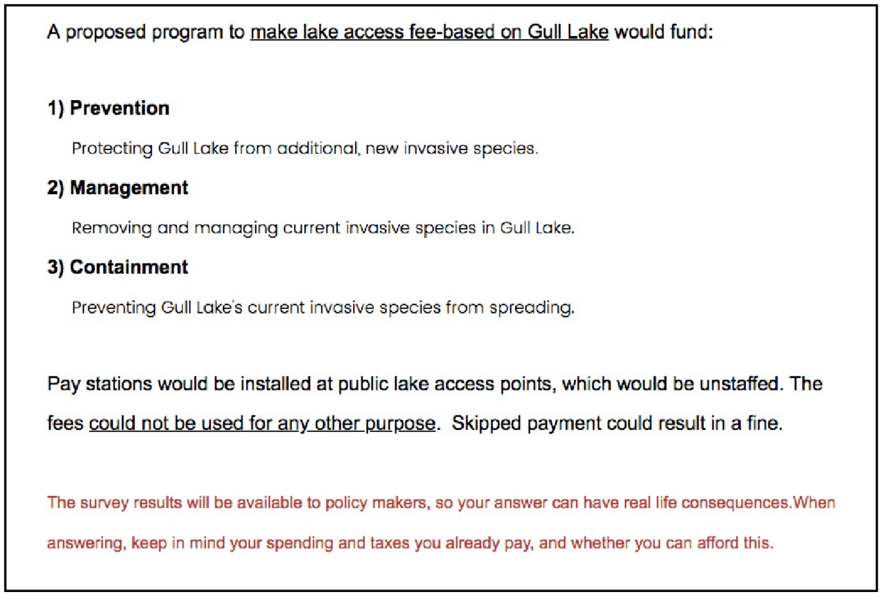


Fig 2. Question 1: Lake infestation.

https://doi.org/10.1371/journal.pone.0246860.g002



A proposed program to make lake access fee-based on Gull Lake would fund:

1) Prevention

Protecting Gull Lake from additional, new invasive species.

2) Management

Removing and managing current invasive species in Gull Lake.

3) Containment

Preventing Gull Lake's current invasive species from spreading.

Pay stations would be installed at public lake access points, which would be unstaffed. The fees could not be used for any other purpose. Skipped payment could result in a fine.

The survey results will be available to policy makers, so your answer can have real life consequences.When answering, keep in mind your spending and taxes you already pay, and whether you can afford this.

Fig 3. Question 2: Proposed program.

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Next, they were provided information on current statewide AIS spending including a comparison to other types of state spending (S2 Appendix question). They were also given information on a proposed program to make lake access fee-based (Fig 3, Question 2). This program stated that funds from making the lake access fee-based would go towards prevention, management, and containment at the individual lake via unstaffed pay stations. Failure to pay could result in a fine. Respondents were also warned that the results would be available to policy makers and that they should keep in mind their spending and taxes, and whether or not they could afford the fees. All this information is important for the collection of willingness to pay data, else respondents are more likely to answer unrealistically, potentially skewing results [32].

And, finally, respondents were asked if they would support a daily fee to access the lake in question (S3 Appendix question) following a double-bounded dichotomous choice format [30]. The dollar values were random—at first the values ranged from $8 to $14. If the respondent answered "No", they were asked again, with a lower value—between $1 and $7. If they answered, "Yes", they were asked a higher value—between $15 and $21. Values were chosen with consultation from the Department of Natural Resources.

Model. The individual *i*'s willingness to pay, WTP_i is:

$$WTP_i = \boldsymbol{x}_i\boldsymbol{\beta} + \boldsymbol{\varepsilon}_i \tag{1}$$

where $\boldsymbol{x_i}$ is a vector of explanatory variables, with their coinciding coefficients, $\boldsymbol{\beta}$. $\boldsymbol{\varepsilon}$ is the normally distributed error term. Explanatory variables include individual lake, socio-demographics, and measures described in Section 4.2.

The double-bounded question format results in four possible cases, with each case having a different probability of occurring. This is essentially a modified probit model (for full derivations, see [31]), which assumes the error is normally distributed. This is required by the model, as the probability estimates rely upon the cumulative distribution function. Φ. Maximum likelihood estimation (in this case the doubleb command in Stata) is used to estimate model parameters, $\widehat{\boldsymbol{\beta}}$, and $\widehat{\sigma}$, standard deviation.

Let v_i^1 be the first value seen by respondent *i*, and v_i^2 be the second. The probability that respondent *i* will answer "Yes" to both questions is:

$$\begin{aligned} Prob\ (Yes; Yes) &= Prob[WTP_i \geq v_i^2] \\ &= 1 - \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \end{aligned} \tag{2}$$

The probability that respondent *i* will answer "Yes" to the first question, and "No" to the second:

$$\begin{aligned} Prob\ (Yes; No) &= Prob[v_i^1 \leq WTP_i < v_i^2] \\ &= \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] - \Phi\left[\frac{v_i^1 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \end{aligned} \tag{3}$$

The probability that respondent *i* will answer "No" to the first question, and "Yes" to the second:

$$\begin{aligned} Prob\ (No; Yes) &= Prob[v_i^2 \leq WTP_i < v_i^1] \\ &= \Phi\left[\frac{v_i^1 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] - \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \end{aligned} \tag{4}$$

And finally, the probability that respondent *i* will answer "No" to both is:

$$\begin{aligned} Prob\ (No; No) &= Prob[WTP_i < v_i^2] \\ &= \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \end{aligned} \tag{5}$$

5 Results

5.1 Response summary

We had a total of 994 respondents who fully answered the survey, a response rate (RR) of 60% (Pokegama: n = 190, 64% RR; Gull: n= 273, 56% RR; Minnewaska; n= 350, 59% RR; Koronis: n= 181, 65% RR). The average respondent identified as a white male, held a college degree, and earned more than 70K per year. Responses are consistent with previous DNR surveys and demographics of Minnesota [33–36]. See Table 1 for demographic and qualitative responses.

There are a number of differences between the lakes; in addition to the information in Table 2, summary statistics for all included variables are provided in S2 Appendix table, grouped by lake. Awareness of problem is fairly consistent across all four lakes, and is not significantly different between any lakes. Knowledge of AIS is highest at Lake Koronis, significantly different from all other lakes. Koronis is very well known for having starry stonewort. Gull is relatively well known for its zebra mussel infestation, but it is frequented by non-locals who may not be as familiar (being a local was correlated with being correct about the AIS in the lake). Pokegama has no AIS; Minnewaska had three, and neither are well known for their AIS. Perceived Risk is highest at Koronis, lowest at Minnewaska (significant at 95% level). Again, the higher perceived risk at Koronis may be due to the well-known nature of the starry stonewort infestation. Koronis was also interesting because it had far more people who indicated that fishing was their primary reason for visiting—about double that of any other lake. It also had the highest score for "to be on my own" visit motivation, which is correlated with fishing being the primary purpose. There were a number of people kayaking and fishing at Koronis, which may have helped generate these higher numbers. Pokegama has the highest percent of locals, which could be influencing why its estimated willingness to pay scores are on the

Table 1. Demographics and qualitative responses (n=994). (a). Demographics. (b). Values.

		(a)					
		Full Sample		**Koro**	**Minn**	**Gull**	**Poke**
Characteristic	Range	n	%	%	%	%	%
Gender	Female	260	29	23	34	76	33
	Male	644	71	77	66	24	67
Race	White	822	83	85	83	82	82
	Non-white	172	17	15	17	18	18
Age	Median	45	-	44	42	48	44
	Minimum	18	-	18	18	19	18
	Maximum	90	-	90	85	87	82
Formal education	Did not finish high school	0	0	0	0	0	0
	Completed high school	131	15	20	15	12	15
	Some college but no degree	133	15	21	15	15	10
	Associate or vocational degree	176	20	22	22	16	21
	College bachelor's degree	257	30	24	28	35	30
	Some post-graduate work	55	6	3	8	5	9
	Completed post-graduate degree	117	13	10	11	18	16
Household income	Under $20,000	34	5	2	10	1	3
	$20,000-$39,999	69	10	11	12	6	9
	$40,000-$59,999	110	15	17	17	12	15
	$60,000-$79,999	98	14	14	15	10	15
	$80,000-$99,999	96	13	16	11	16	11
	$100,000-$149,999	148	21	22	19	19	23
	$150,000 or more	167	23	17	17	36	23
Visitation Frequency	Several times a week	225	26	29	29	17	31
	Once a week	88	10	10	9	9	14
	Several times a month	166	19	16	21	18	20
	Once a month	69	8	5	10	8	7
	Several times a summer	152	18	25	13	19	19
	Once during the summer	121	14	9	13	22	9
	Every few summers	43	5	5	6	8	0
Residence[1]	Local	454	60	67	63	42	72
	Non-Local	307	40	33	37	58	18
Primary Visitation Purpose	Fishing	419	43	75	32	41	35
	Boating	209	21	11	16	29	31
	Watersports	95	10	3	9	14	12
	Swimming	87	9	3	16	3	10
	Socializing	50	5	2	8	56	3
	Relaxing	44	5	2	8	2	4
	Hiking	16	2	2	3	1	0
	Picnicking	8	1	1	2	0	1
	Art	8	1	1	2	0	0
	Other	39	4	2	5	4	5
		(b)					
Characteristic	**Range**	**n**	%				
AIS Knowledge	Correct	279	31	43	26	34	25
	Incorrect	616	69	57	74	66	75

(*Continued*)

Table 1. (Continued)

Awareness of AIS Problem	Not a problem at all	32	3	2	3	5	3
	Slight Problem	107	11	14	12	8	10
	Moderate Problem	398	40	40	40	40	41
	Severe Problem	413	42	43	41	43	40
	Don't know/Not sure	39	4	2	4	4	5
Perceived AIS Risk[2]	Median	Moderate/ High Risk		Mod/ High Risk	Mod/ High Risk	Mod/ High Risk	Mod/ High Risk
Visit Motivation "To be on my own"[3]	Median	Moderately Important		Mod Import	Mod Import	Mod Import	Mod Import

[1] Locals were respondents who indicated they lived at the lake, were the lake for a day trip and were coming from their primary residence, or were staying at the lake for multiple nights but were staying at home.

[2]AIS Risk is represented by the sum across the risk categories provided in Section 3, where the options are represented as [2] Perceived AIS Risk is represented by the sum across the risk categories provided in Section 3, where the options are represented as scalar variables (0: No risk at all, 1: Slight risk, 2: Moderate risk, 3: High Risk, 4: Extreme Risk). The median response was 2.7, or between moderate and high risk.

[3] Eight visitation reasons were provided (see Section 3), one of which was "To be on my own", which represented as scalar variables (0: Not at all important, 1: Slightly important, 2: Moderately important, 3: Very important, 4: Extremely important). The median response was 2, or moderately important.

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lower end (even if not significantly). Gull has the lowest percent of locals, but high income and high education.

To check that the willing to pay responses (S3 Appendix question) followed a downward sloping demand curve—i.e. that fewer people are willing to pay higher amounts, we estimated the frequency of "Yes" responses for each value offered. The initial values are given (random values from \$8 to \$14). Those who answered "No" to the first question were provided with

Table 2. Willingness to pay model (n =538; Wald = 656)[1].

Coefficient	Variable Description	Mean	Std. Error	P Value
$\widehat{\beta}_1$	*Initial Value* (v_i^1)	0.2901**	0.1472	0.049
$\widehat{\beta}_2$	*Awareness of AIS problem*	1.9109*	0.6697	0.004
$\widehat{\beta}_3$	*North * Awareness of AIS problem*	-0.7187**	0.3389	0.034
$\widehat{\beta}_4$	*Perceived AIS risk*	0.2025**	0.0963	0.035
$\widehat{\beta}_5$	*Visit motivation*	-1.0045*	0.3195	0.002
$\widehat{\beta}_6$	*Local*	-2.2465*	0.8464	0.008
$\widehat{\beta}_4$	*Education*	0.3588	0.2505	0.152
$\widehat{\beta}_5$	*Gender*	1.6922	0.9021	0.061
$\widehat{\beta}_6$	*Aged 45 or Greater*	1.8544**	0.8545	0.030

*indicates significance at the 99% confidence level.

**indicated significance at the 95% confidence level

[1] *Gender* (1:Female; 0:Male), *Local* (1: respondents indicated they lived at the lake, were the lake for a day trip and were coming from their primary residence, or were staying at the lake for multiple nights but were staying at home; 0: respondents indicated otherwise), *Gender* (1: female; 0: male); no respondents choose non-binary, *Aged 45 or greater* (1: respondent is 45 years of age or older; 0: respondent is less than 45), and *North* (1: Gull or Pokegam; 0:Minnewaska or Koronis);, *Initial Value* is the first bid offered in the WTP question. Scalar variables are *Awareness of AIS Problem* (See S1 Appendix question) (1: Not a problem at all, 2: Slight Problem, 3: Moderate Problem, 4: Severe Problem), *Perceived AIS Risk* is the sum across the risk categories, where the options are (0: No risk at all, 1: Slight risk, 2: Moderate risk, 3: High Risk, 4: Extreme Risk), *Visit Motivation* measured how important it was for the respondent to "be on my own" (0: Not at all important, 1: Slightly important, 2: Moderately important, 3: Very important, 4: Extremely important), and *Education* (1: Did not complete high school, 2: Completed high school, 3: Some college but no degree, 4:Associate degree or vocational degree, 5: College bachelor's degree, 6:Some postgraduate work but no degree, 7: Completed graduate degree).

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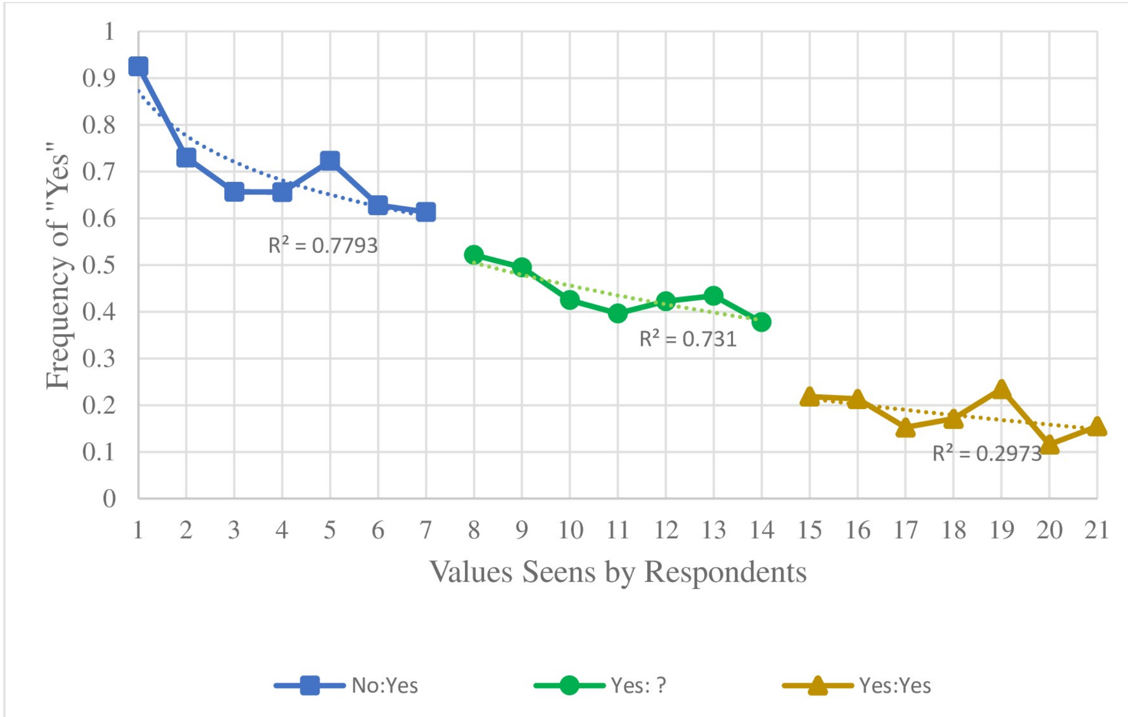

[1] [Yes: ?] values are the actual frequencies of "Yes" responses to the initial willingness to pay question (Appendix Question 3). [No: Yes] values are calculated with $\frac{\left(Y^1 + F_j^{1:No}(n-Y^1)\right)}{n}$ where $Y^1$is the number of respondents who chose "Yes" to the first question, $F_j^{1:No}$is the frequency of a "Yes" response to the second question with a value of j, $j \in \{1, \ldots, 7\}$, for those whom chose "No" to the first question. n is the total number of respondents to the first question. [Yes: Yes] values are calculated with $\frac{(Y^1 F_k^{1:Yes})}{n}$ where $Y^1$is the number of respondents who chose "Yes" to the first question, $F_k^{1:Yes}$is the frequency of a "Yes" response to the second question with a value of k, $k \in \{15, \ldots, 21\}$, for those whom chose "Yes" to the first question.

Fig 4. Calculated frequency of "Yes" responses to willingness to pay questions by value amount, v_i^1 and v_i^2 (overall $R^2 = 0.937$)[1].

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lower values in the second question (random values from $1 to $7), and those who answered "Yes" to the first question, and were provided with higher values in the second question (random values from $15 to $21). "Yes" response frequency to the initial willingness to pay question were lower for higher values, ranging from upper 30%s to lower 50%s, with a statistically significant negative slope (Fig 4). To estimate the frequencies of the responses to the second values, we assumed that people who answered "Yes" to the first set would also answer "Yes" to the lower values and people who answered "No" to the first set would answer "No" to the higher values.

The chart shows a clearly negative relationship between frequencies of "Yes" response and valued proffered. There are noticeable gaps between the sets of values, which are likely evidence of response biases. People who answered "No" initially may be experienced some guilt or pressure to answer "Yes" when faced with a lower value, which may increase the frequency —higher levels of affirmative responses can be a result of a multi-question format [32]. However, people who answered "Yes" initially may be experiencing some annoyance at being asked a higher value, which may decrease the frequencies. Still, we do not see extremely high response rates at the highest levels, which can be an issue in contingent valuation work [37].

Protest votes, which are willingness to pay values of zero given due to reasons beyond an actual value of zero (i.e. philosophical arguments), were excluded from the estimation of willingness to pay and were determined by a follow-up question to those choosing "No" to both valuation questions. Respondents were given the following options: "I already pay enough in taxes/fees for aquatic invasive species", "I would support more taxes/fees for aquatic invasive species in the whole state, but not for <lake name> alone", I will not support making <lake name> public lake access fee-based, regardless of the amount or what the fee is for", "I would support a fee, but this amount is too expensive/I cannot afford this much", "I do not believe we should fight aquatic invasive species", and "Other: please specify:". Determining which answers are protest votes and which are true zeros is not an exact science. However, if the answer indicates the respondent has no value for the public good (e.g. "I do not believe we should fight aquatic invasive species") or cannot afford the fee (e.g. "I would support a fee, but this amount is too expensive/I cannot afford this much"), the answers are considered true zeros [33, 34]. For the write-in answers, we used our best judgment as to whether the responses were protests or not—if they did not write anything in, we considered it a true zero. Protest votes were determined to make up about one-half of people who chose "No" to both questions, 23% of the total sample.

5.2 Willingness to pay estimation

We estimated mean willingness to pay for the total viable sample (n = 705) with a simple model that included no explanatory variables (Eq 1 with a constant and no vector of variables); the result was \$9.37. We also estimated the willingness to pay for each lake. The lowest was for Pokegama, \$8.15; the highest was for Koronis, \$9.80. Interestingly, all values were within each other's confidence intervals (95%), seemingly suggesting there are fewer differences in willingness to pay between the lakes than we had posited (Table 3). We next calculated that the mean willingness to pay as \$9.87 by estimating the parameters in Eq 1 (Table 2), which is 50 cents higher than in our simple model–for a discussion of non-response bias, see Section 5.3. Our preferred model included initial value offered (significant at the 95% level), perceived risk of AIS (significant at 95% level), magnitude of desire to "be on your own" (significant at 99% level), whether a respondent is a local (significant at 99% level), education (significant at 85% level), whether a respondent identifies as female (significant at 90% level), whether a respondent is aged 45 years or older (significant at the 95% level), AIS awareness of problem (significant at 99% level), and an interaction variable between AIS awareness of problem and whether the lake is one of the northern pair (Pokegama or Gull) (significant at the 95% level)

We compared our preferred model (Table 2) with alternative models that included the different lakes, alternate primary purposes of visit (fishing was predictive but the others were

Table 3. Comparison of WTP estimates from simple model and preferred model.

	Simple Model			Preferred Model		
	n	WTP	CI (95%)	n	WTP	CI (95%)
All	705	\$9.37	\$8.60 to \$10.13	538	\$9.87	\$9.07 to \$10.67
Koronis	124	\$9.80	\$7.94 to 11.66	102	\$10.40	\$9.28 to 11.51
Minnewaska	244	\$9.64	\$8.42 to \$10.85	185	\$10.34	\$9.27 to 11.40
Pokegama	142	\$8.15	\$6.09 to \$10.22	103	\$8.96	\$7.77 to 10.14
Gull	195	\$9.37	\$8.09 to \$10.89	148	\$9.56	\$8.38 to 10.74

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not), alternate motivation categories (the other options were not predictive as compared to "to be on my own"), alternate risk categories, and demographics. Alternative models did not result in discordant results or interpretation. Two alternative models are presented in S1 Appendix table, which provides similar willingness to pay results. These models were selected to show lake variables and three variables that were predictive in certain combinations: income, AIS Knowledge, and fishing as a primary visit purpose. Models were compared in terms of statistical fit (Wald's tests, Akaike's / Bayesian information criteria) and the estimated significance of impact on WTP. Our preferred model was chosen based on overall fit, significance of variables, correlation of variables (See S3 Appendix table) and sample size. We also tested our methodology by looking at classification error for a test set (80/20 split) and found good agreement (not shown).

Initial value presented impacted willingness to pay, by about 30 cents for each dollar increase in initial offer, evidence that anchoring may be occurring, which is common with this format of questioning [38]. Anchoring is a phenomenon where people base their willingness to pay on the apparent price of an item. As such, a higher initial value may increase respondents' final willingness to pay. Locals, people who said they were staying at/returning to home were willing to pay $2.25 less than people on trips. This may be because people who live at the lake feel they should not pay visitation fees or because they visit with more frequency–the fee proposed was a daily fee, not a yearly. Visitation frequency was significantly different between the two groups. Locals visited an average of several times a month, whereas as others visited once a month on average—however inclusion of both visitation variables did not increase model fit, nor did solely including visitation frequency (not shown).

Education was not very significant ($P = 0.16$), but showed the expected sign—where more educated people are willing to pay more. Education is correlated with income (S3 Appendix table), which we discuss more below, but feel is important. Gender was more significant ($P=0.06$), indicating that people who identify as women are willing to pay quite a bit more–$1.69. Being a woman was correlated somewhat with risk perception, which has been found before [39]. Adams et al [40] found that demographic variables changed willingness to pay by about |$1| for invasive plant control. Nunes and Van der Bergh [41] found very little impact of socio-demographics on willingness to pay for harmful algal bloom prevention; Chakir et al. [42] found the same for socio-demographics impact on willingness to pay to reduce negative impacts of an invasive beetle.

Perceived AIS risk, AIS awareness of problem, and desire to "be on your own", however, were significant. Risk was represented as a sum of equivalent risk responses across five categories. Extrapolating with our model results, the person who perceived AIS the most risky would be willing to pay about $4.86 more than the person who perceived the least risk. Problem awareness impact was large (and significant at 99%). Someone who viewed AIS as a severe problem would be willing to pay about $5.73 more than someone who viewed AIS as not a problem, if they were at Koronis or Minnewaska. If they were at Gull or Pokegama, this impact is lower–$3.58. This may have to do with the type of lake—both Pokegama and Gull are clearer lakes, viewed as recreation destinations. Minnewaska and Koronis are more known for having water quality impacts. Perhaps even if people at the northern lakes feel that AIS in Minnesota are a problem, their willingness to pay is lower at a lake they view as less impacted by AIS. Someone who ranked "to be on your own" as "extremely important" was willing to pay $4.02 less than someone who ranked "to be on your own" as "not at all important". AIS awareness of problem is less correlated with other variables than perceived AIS risk; the two are quite correlated with each other (S3 Appendix table). We chose to use still use both since they measure different social-psychological constructs.

It is interesting that we did not find more significance related to the individual lakes. What is apparent is that individual respondents' characteristics may be more important than which AIS is present or magnitude of infestation.

5.3 Problematic variables and non-response bias

We did not include income in our preferred model because a third of respondents refused to provide this information. A number of demographics are correlated with income, including education and whether a respondent is a local. Additionally, including income did not result in a better overall model. Though, we did include it in alternative models (S2 Appendix table), and it behaved as expected– Income increased willingness to pay—the difference between the smallest income bracket (up to $20,000) and the largest (over $200,000) equated to about $2.78. We also did not include AIS Knowledge in our preferred model. While AIS Knowledge was associated with higher willingness to pay in some model formulations (see S2 Appendix table), its inclusion reduced overall model fit. We felt it was problematic due to the questions being "harder" at some lakes (i.e. at Minnewaska you had to select three to be correct; at Pokegama, the correct answer was none, but zebra mussels were found there after the survey).

We took a look at the people who answered the income question vs. the people who did not, as we thought it may tell us something about non-response bias. Those who did not answer did not differ vary significantly from those who did in any of the variables tested, except for in education. We added a binary variable to our preferred model to account for answering the income question or not, and found that answering the question was associated with $2.85 more in willingness to pay (significant at 99% level [not shown]), which is about the magnitude of the income impact from the lowest to highest income category. It is quite possible that people who do not answer for income have lower income, which jives with the lower education level.

We also examined time to complete the survey, which averaged less than 8 minutes. While overall time was not significant in willingness to pay [not shown], those who were in the lower quartile (less than 6 min) were willing to pay about $1.70 less, with the significance at the 90% level (not shown). People who did not answer the income question were more likely to be in this lower quartile, but their mean time to completion was not significantly different that the remaining respondents. Still, this may point at a form of non-response bias. Additional evidence for potential non-response bias lies in the results of willingness to pay estimates on different subsamples. The simplest model uses the entire sample, and gives us slightly lower WTP score than the preferred sample (Table 3), while these may not be significantly lower than the preferred model, it made us curious about the one-quarter of the respondents in the usable sample who were not included in the preferred. These respondents had not responded to all included questions, so there is overlap with the folks who did not answer the income question. We used the simple model on this "non-responder" group and found that their willingness to pay was indeed lower (S4 Appendix table), $7.14. All of this implies that there could certainly be a concern over non-response.

6 Discussion and conclusion

We assessed recreationists' willingness to pay an access fee for AIS management, prevention, and containment in four Minnesota lakes: Gull, Pokegama, Koronis, and Minnewaska. The four chosen lakes were in two pairs with each pair chosen to be as similar as possible with the exception of their aquatic invasive species (AIS) infestation magnitude. The northern pair was Gull and Pokegama. Gull is host to an extensive zebra mussel, *Dreissena polymorpha*, population. Pokegama was considered free of infestation as of the time of the survey, however was listed as infested with zebra mussels later in the year. The southern pair was Koronis and Minnewaska, where Koronis was one of the first lakes in the state infested with starry stonewort, *Nitellopsis obtuse*, and has an extensive population. Minnewaska has a minor, contained starry stonewort population, but also has Eurasian watermilfoil, *Myriophyllum spicatum L*, and zebra mussels.

We found that respondents are willing to pay $9 to $10 daily to access these lakes, a not unsubstantial sum. We did not find that presence at infested lakes versus uninfested/less infested lakes affected willingness to pay—in other words, we could not detect willingness to pay differences within the lake pairs once we had accounted for other variables. We did find that certain demographic, social-psychological, and travel-related variables were predictive.

Locals were willing to pay less than visitors to the region. This could be related to frequency of visitation, which was higher for locals. A daily use fee could result in a significant amount over the course of a season with high visitation rates. While we did not include income in our preferred model due to sampling issues, it was predictive of higher willingness to pay values in alternate models. Education and gender were not highly significant, but each had the expected sign. Those over the age of 45 were willing to pay more, as well.

Demographic and descriptive variables provide information about *who* is willing to pay, but not about *why* they are willing to pay. We found that social-psychological variables: awareness of AIS problem, perceived risk, and visit motivation, were important determinants of willingness to pay for AIS management.

Our findings suggest that people who believe to a greater extent that AIS are a problem are more willing to pay for AIS management. The norm activation theory [3] suggests that awareness of a problem is an important first step in a cognitive process that leads to action. Thus, people who believe AIS is a problem are more likely to take action (i.e., willingness to pay). Interestingly, we also found that the influence of awareness of AIS Problem was stronger at the southern lake pair than the northern lake pair. The northern lakes are more clear and generally seen as "nicer" than the southern lakes. It is possible the even though the measurement of general awareness of AIS problem (the question was not directed specifically to the lake in question) did not differ between lakes, being at a lake that seemed "nicer" might reduce the willingness to pay.

We also found that recreationists who perceive greater risk of AIS to ecosystem services are willing to pay more for AIS management. Past work on risk perception, particularly around climate change beliefs and actions, indicate that perceptions of risk are an important predictor of public willingness to take actions to address climate change [16, 43]. Our findings provide support for the link between risk perception and environmental action in the context of invasive species management.

Finally, we found that recreationists with a privacy or autonomy motive [28] (i.e., desire to be on their own) are willing to pay less. Past work has found that the autonomy motive is related to affective attachment (i.e., emotional bond with a place or setting), as well as place identity (i.e., a place or setting becoming part of one's identity) [28]. This means that people who value autonomy and privacy generate strong sense of emotional connection with a physical space (e.g., city, lake). In our study, it is possible that people with an autonomy motive may have developed strong emotional connection to the lake, strong enough where they feel they should not have to pay to access the space they have an emotional connection with. However, since we did not measure attachment or place identity, the links among autonomy motive, attachment, and behavior is unclear and provides an interesting area for future research.

From a management perspective, our findings suggest that strategies that highlight the extent of the AIS problem, and draw links between AIS and their risks to ecosystem services may be successful in garnering more support for AIS management, regardless of local lake infestations. While these strategies can help garner public support for AIS management (through their willingness to contribute money to a hypothetical market), policy makers and resource managers should be cognizant of any resistance to the payment vehicle, in this case, a public access fee / parking fee. Public access to lakes in Minnesota is currently free. Protest votes from almost a quarter of our sample indicate that a substantial proportion of recreationists may be opposed to access-based fees. Additionally, like many willingness to pay studies, non-response bias may be a concern. Public acceptability of public access fees, and other policy options is a promising area for future research.

AIS are a growing and ecologically complicated problem worldwide, with substantial support for management from the public. Perhaps surprisingly, we highlight that AIS species and infestation levels may not always be predictive of willingness to pay for management, even when the proposed fees are lake specific. However, we show that recreationists' willingness to pay *is* influenced by their beliefs about whether AIS is a problem, their perceptions of risks associated with AIS, and motivations for visiting a lake, providing support for the inclusion of social-psychological variables in willingness to pay models, and in AIS management discussions.

Supporting information

S1 Appendix question. AIS awareness.
(TIF)

S2 Appendix question. Current spending.
(TIF)

S3 Appendix question. Willingness to accept (Gull Lake version).
(TIF)

S1 Appendix table. Preferred model vs. alternate models[1].
(DOCX)

S2 Appendix table. Comparison of explanatory variables by lake[1].
(DOCX)

S3 Appendix table. Variable correlation matrix (Spearman's).
(DOCX)

S4 Appendix table. Estimated willingness to pay for responders in preferred model and non-responders.
(DOCX)

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Public perceptions of aquatic invasive species management

Findings from a survey of Minnesota residents

2022

The purpose of this study was to assess Minnesota residents' beliefs about aquatic invasive species (AIS), AIS management, and their engagement in the prevention and control of AIS. We conducted a mail survey of 2000 Minnesota residents from April to June 2021. We received 298 completed surveys for an adjusted response rate of 19%. More than half of the respondents were male (52%). A vast majority report their race/ethnicity as white, not of Hispanic, Latino or Spanish origin (88%). Respondents' age ranged from 22 to 96. Most respondents have at least a college bachelor's degree (60%), and most reported annual household income of less than $100,000 (51%).

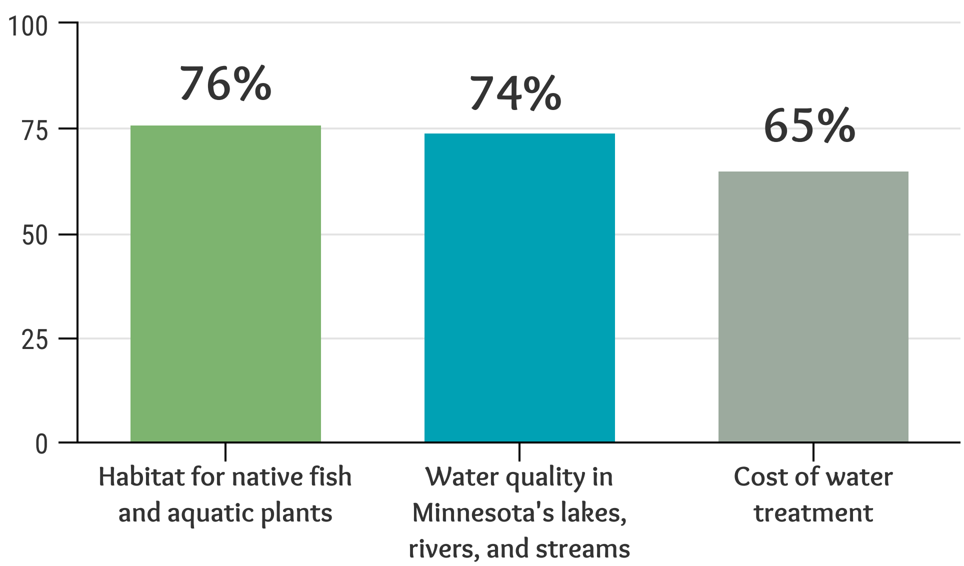
88% of respondents believed that AIS are a ***moderate to severe problem*** in Minnesota

72% of respondents believed that AIS are ***very to extremely common*** in Minnesota

66% of respondents reported that they have ***personally observed*** AIS in Minnesota's waters

To what extent did respondents believe AIS threaten ecosystem services?

*(Percentage of respondents that believed AIS pose **high to extreme risk** to the resource)*



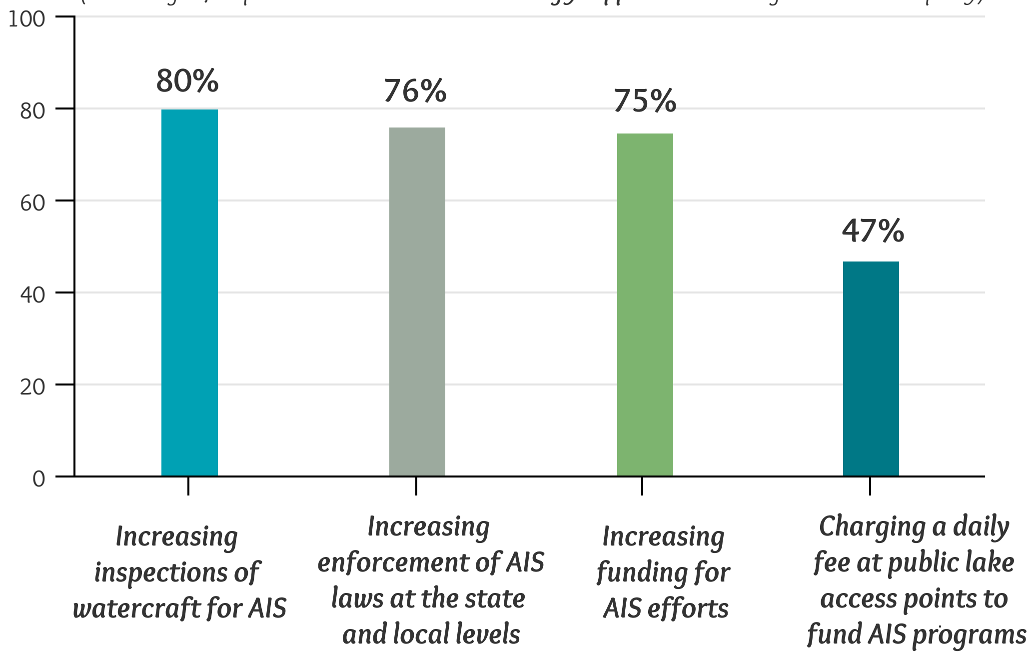
Respondents reported generally low engagement in AIS control and prevention

- 31% of respondents reported talking to others about AIS in the past 12 months.
- 11% reported donating money to an organization that works to control or prevent the spread of AIS
- Only 5% of respondents have an organization that works to control or prevent the spread of AIS
- Only 4% reported volunteering to improve habitat affected by AIS

The study was conducted by the Center for Changing Landscapes and Water Resources Center, University of Minnesota, in partnership with Minnesota Aquatic Invasive Species Research Center (MAISRC). Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by MAISRC and the Legislative-Citizen Commission on Minnesota Resources (LCCMR).For more information about this survey contact Amit Pradhananga at prad0047@umn.edu.

To what extent did respondents support AIS management actions and policies?

*(Percentage of respondents that **somewhat to strongly supported** the management action or policy)*



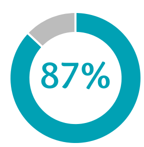
Only 25% of respondents were very to extremely confident that their personal actions can help reduce the spread of AIS in Minnesota.

57% of respondents somewhat to strongly agreed that they do <u>not</u> personally know enough about how to prevent the spread of AIS

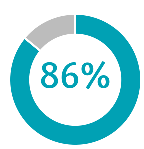
Only 19% of respondents reported that they were very to extremely confident that the residents in their community can solve the problem of AIS, and only 9% were very to extremely confident that everyone is working together when it comes to AIS control in their communities.

86% of respondents either disagreed or were unsure that their community has the financial resources it needs to manage AIS.

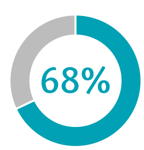
Where did respondents turn for information about AIS?



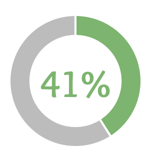
87% of respondents somewhat to strongly trust **MN Department of Natural Resources**



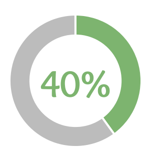
86% of respondents somewhat to strongly trust **university researchers/Extension**



68% of respondents somewhat to strongly trust **environmental organizations**



Only 41% of respondents trust **fishing, boating, or sporting organizations**



Only 40% of respondents trust **bait shops and sporting goods stores**

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Environment and Natural Resources Trust Fund

M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2021

SUBPROJECT TITLE: MAISRC Subproject 24: Genetic method for control of invasive fish species
SUBPROJECT MANAGER: Dr. Michael Smanski
ORGANIZATION: University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Biological Sciences; Department of Biochemistry, Molecular Biology, and Biophysics
MAILING ADDRESS: 1479 Gortner Ave, Room 140
CITY/STATE/ZIP: Saint Paul, MN 55108
PHONE: 612-624-9752
E-MAIL: smanski@umn.edu
WEBSITE: http://www.maisrc.umn.edu
http://www.bti.umn.edu/labs/smanski/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $250,116
AMOUNT SPENT: $249,004
AMOUNT REMAINING: $1,112

Sound bite of Project Outcomes and Results

MAISRC has laid the groundwork to develop innovative genetic biocontrol approaches to be used in the fight against invasive carp.

Overall Subproject Outcome and Results

Invasive fish species present an estimated $5.4 billion burden on our domestic economy, and much of that extends to the lakes and rivers of Minnesota. For example, the foraging habits of the invasive common carp, *Cyprinus carpio*, diminishes water quality, reduces vegetative cover and waterfowl numbers, and reduce the ability of lakes to absorb nutrients that enter water systems through agricultural runoff. Current control methods have not been able to stem the tide of invasive carp and other fish species, so improved strategies are needed. The overall goal of this project is to demonstrate a novel approach for controlling aquatic invasive species using invasive carp species as proof-of-concept. Success of this project would lead to its implementation in other aquatic invasive species (AIS), including Asian carp and zebra mussels.

Several major obstacles had to be overcome on this project to lay the foundation for genetic biocontrol of invasive carp. These included (i) Developing husbandry for year-round carp spawning in the MAISRC Containment Lab, (ii) Demonstrating transgenesis of *C. carpio*, (iii) Testing genetic reagents in a model laboratory fish that will be needed to engineer carp, and (iv) Performing a survey to gauge public perceptions of carp genetic biocontrol. We accomplished these project goals within a one-year no-cost extension to the project funding.

The impact of our results is that we are now primed to engineer carp genetic biocontrol agents in the lab during the next phase of this award, which will begin January 2022. There is still substantial work to be done before this will directly benefit Minnesotans. Specifically, we need to demonstrate a proof-of-concept carp biocontrol system in the laboratory; perform safety/efficacy testing; obtain permits for field trials; and eventually work with key stakeholders to use this new tool in the fight against invasive carp. The overall process is expected to take 10-15 years.

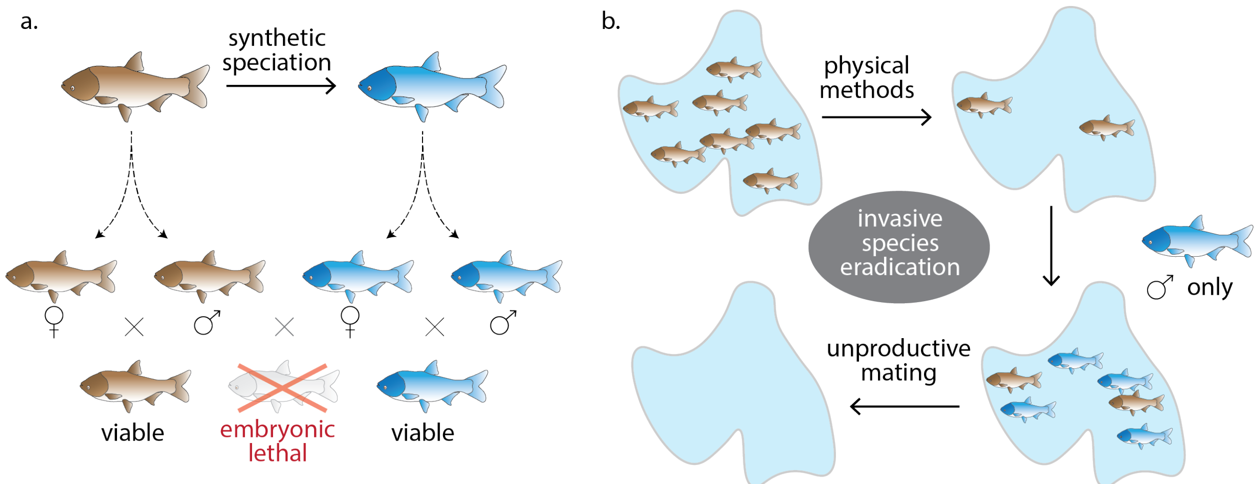
Subproject Results Use and Dissemination

Data generated from this subproject is expected to be included in three peer reviewed publications. These include results from the public survey (expected submission Summer 2021), results from the carp husbandry/transgenesis procedure (expected submission Winter 2021), and agent-based modeling results (waiting for accompanying wet-lab experimental confirmation).

In addition to these primary research reports, one book chapter that describes the techniques developed under this subproject has already been published:

Bajer P, Ghosal R, Maselko M, Smanski MJ, Lechelt JD, Hansen G, Kornis M (2019) Biological control of invasive fish and aquatic invertebrates: a brief review with case studies. *Management of Biological Invasions.* 10: 200-226.

Final Report – Visual Component
MAISRC Subproject 24: Genetic method for control of invasive fish species
Dr. Michael Smanski



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2020

SUBPROJECT TITLE: MAISRC Subproject 25: What's in your bucket? Quantifying AIS Introduction Risk
SUBPROJECT MANAGER: Nicholas Phelps
ORGANIZATION: Minnesota Aquatic Invasive Species Research Center, University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Food, Agriculture, and Natural Resource Sciences; Department of Fisheries, Wildlife, and Conservation Biology
MAILING ADDRESS: 135 Skok Hall, 2008 Upper Buford Circle
CITY/STATE/ZIP: Saint Paul, MN 55108
PHONE: 612-624-7450
E-MAIL: phelp083@umn.edu
WEBSITE: http://www.maisrc.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a
M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $199,784
AMOUNT SPENT: $185,634
AMOUNT REMAINING: $14,150

<u>Sound Bite of Project Outcomes and Results</u>

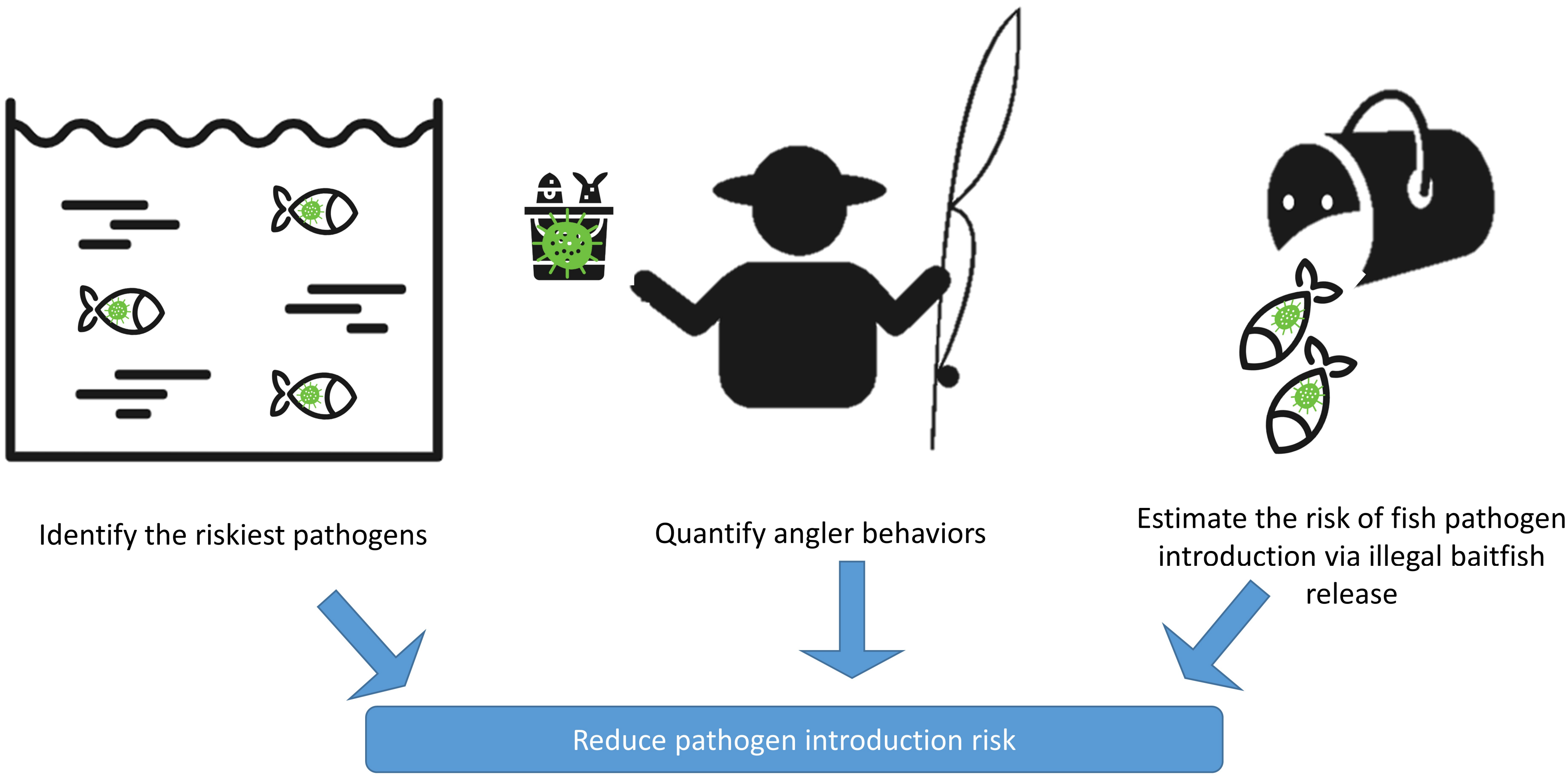
Live baitfish are popular among Minnesota anglers, but their illegal release is a known risk factor for spreading harmful diseases to wild fish populations. Our research identified high-risk pathogens in Minnesota, estimated the number of times anglers release an infected baitfish each year, and identified opportunities for strategic management intervention.

<u>Overall Subproject Outcome and Results</u>

In Minnesota, the illegal release of live baitfish by anglers has been identified as a weak point in our efforts to prevent the spread of aquatic invasive species and pathogenic microbes, however the magnitude of the risk and evidence-based opportunities for intervention had not been well studied. The purpose of this project was to assess the risk of fish pathogen introduction via illegal release of live baitfish by Minnesota anglers to inform strategic management strategies to reduce that risk. First, we created a semi-quantitative framework to evaluate the threat of baitfish pathogens in Minnesota and used it to rank pathogens so managers can prioritize resources. We then conducted a statewide survey of anglers to quantify risky behaviors and used those data to parameterize a risk assessment model for high-risk pathogens to estimate the number of risky trips that occur in a given year under a variety of scenarios. Our results were variable, indicating a wide range of outcomes depending on current management strategies and pathogen prevalence. For example, with strong surveillance and controls in place for the viral hemorrhagic septicemia virus, the number of risky trips is limited in most scenarios. However, for high-risk pathogens (Ovipleistophora ovariae, Asian fish tapeworm) for which no controls are in place, the large number of anglers, frequency of illegal release, and the popularity of susceptible baitfish species, can result in hundreds of thousands of risky trips each year, even in low-prevalence scenarios. Ensuring a safe, pathogen-free bait supply and decreasing the percentage of anglers who release their baitfish can reduce pathogen introduction risk while preserving the important cultural and economic benefits of recreational angling. Our project provides evidence-based tools for prioritizing scarce resources and identifying weak points in our management strategies so we can improve them to protect our valuable fish and fishing resources.

Subproject Results Use and Dissemination

Throughout this process we have communicated and collaborated with technical experts, managers, and members of the public alike. In addition to the three manuscripts either published or in prep for this project, we have presented this material in a variety of settings. Results from this project have been shared via presentations to local (UMN Ecosystem Health Group, MAISRC Research Showcase, MNDNR AIS Working Group meetings, Minnesota Lakes and Rivers Advocates), statewide (MN Chapter of the American Fisheries Society, UMN Extension Webinars), regional (Upper Midwest Invasive Species Conference), and national (North American Invasive Species Management Association, American Fisheries Society Fish Health Seminar) audiences and hundreds of individual participants. We have also maintained close contact with DNR Fisheries and AIS staff who have periodically served as unfunded collaborators and advisers on the project, and we worked with a number of AIS Detector volunteers in implementing the survey portion of the project.


Identify the riskiest pathogens
Quantify angler behaviors
Estimate the risk of fish pathogen introduction via illegal baitfish release
Reduce pathogen introduction risk