M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2021

SUBPROJECT TITLE: MAISRC Subproject 4.3: Social Learning and Carp Removal
SUBPROJECT MANAGER: Dr. Przemek Bajer
ORGANIZATION: University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Food, Agriculture, and Natural Resource Sciences; Department of Fisheries, Wildlife, and Conservation Biology
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E-MAIL: bajer003@umn.edu
WEBSITE: http://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: $189,475
AMOUNT SPENT: $189,475
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

A new method for removing common carp to improve water quality and habitat was developed. It uses bait to attract carp and remove them. It is very selective and easily scalable. While its efficacy is being improved, it is already being used by lake managers in Minnesota.

Overall Subproject Outcome and Results

Common carp is a widespread, invasive fish that negatively impacts habitat and water quality in lakes. Practical and selective removal strategies are needed for carp. Previous research showed that bait can be used to selectively attract large numbers of carp in lakes. In this experiment, we documented how the carp are attracted to the bait (Objective 1; social structure) and whether they could be removed using nets (Objective 2). In a lake with multiple baiting sites (8 sites in a 258-acre lake) and 300 carp tagged with electronic tags, 54% of carp (164 tags) were attracted to the bait over the whole summer, and ~ 20% of population were attracted daily (60 tags). Some carp ("Superfeeders") visited baited sites nearly every day, while others only every few days. The Superfeeders were significantly larger than other carp. The carp visited the bait mainly at night. Feeding aggregations were very dynamic – individual feeding bouts included 2-9 tagged carp, lasted <1 minute to over 30 min, and continuously formed and dissolved for several hours each night. We attempted three removal events at the baited sites, on 3 separate nights, collectively capturing 27% of the population (3,602 carp). Native fish bycatch was <1% (released).

Our results indicated that carp foraging is social, easily induced by species-specific bait, dominated by large-bodied individuals, and predictable (nightly). However, only a fraction of carp attracted to the bait were removed because individual feeding groups visited that bait at different times of the night. We suggest that next steps should address how to synchronize carp aggregations at the bait to increase removal efficiency (starts in January 2022 using acoustic conditioning). This line of research resulted in carp removal methods that are already being applied in Minnesota, often involving volunteers to bait the carp. Future optimizations will increase the efficacy of this new management method.

Subproject Results Use and Dissemination

To disseminate the results of this work we have presented two talks at scientific conferences focusing on invasive species and lake management:

Bajer P. G. et al. 2019. A new approach to manage common carp: Citizen-aided carp management. International Conference on Aquatic Invasive Species ICAIS, Montreal, Canada.

Hundt, P. J., Bajer P. G. 2020. Common Carp Feeding Aggregations: Responses of Invasive Carp and Native Fish to Corn Baiting, North American Lake Management Society, Minneapolis, MN.

We have published two peer-reviewed manuscripts:

Hundt, P. J., Amberg, J., Sauey, B., Vacura, K., & Bajer, P. G. (2020). Data from: Tests in a semi-natural environment suggest that bait and switch strategy could be used to control invasive Common Carp. Management of Biological Invasions.

Hundt PJ, While LA, Craft ME, Bajer PG. In review. Social associations in common carp: Insights from induced feeding aggregations for targeted management strategies. Ecology and Evolution.

We were featured in a Star Tribune article from January 30, 2021: Corn, Conveyor Belts and a Virus show promise in removing invasive carp from Minnesota Waters. We were also featured in a Minnesota Bound episode on common carp management in Lake Parley https://www.youtube.com/watch?v=3sS-Ej3VU4w.

We have also presented twice at the MAISRC Research & Management Showcase and conducted a webinar on common carp management that included 150 participants from several states. https://youtu.be/zNXcB1IfhqM.

Final Report – Visual Component
MAISRC Subproject 4.3: Developing carp removal schemes using social learning behaviors
Dr. Przemek Bajer

A new management strategy for common carp has been developed by exploiting carp's social feeding behaviors. Large numbers of carp can be attracted to sites baited with corn and then removed using specialized nets. This approach does not impact native fish because they are not attracted to corn. This new management strategy can be applied throughout Minnesota, often engaging citizens who conduct carp baiting. Photo below shows the process of carp removal from a trap baited with cracked corn.

Photo credit: Cameron Swanson

M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2021

SUBPROJECT TITLE: MAISRC Subproject 8.2: Impacts of invader removal on native vegetation recovery
SUBPROJECT MANAGER: Dr. Daniel Larkin
ORGANIZATION: University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Food, Agriculture, and Natural Resource Sciences; Department of Fisheries, Wildlife, and Conservation Biology
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E-MAIL: djlarkin@umn.edu
WEBSITE: http://www.maisrc.umn.edu
http://larkinlab.cfans.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: $119,034
AMOUNT SPENT: $119,034
AMOUNT REMAINING: $0

<u>**Sound bite of Project Outcomes and Results**</u>

This project adds to the growing understanding that invasive species are often only one of multiple stressors that drive declines in the health of our lakes. Controlling invasive plants is not a silver bullet for restoring turbid, degraded lakes—we have to think more holistically about what's dragging down our lakes' health.

<u>**Overall Subproject Outcome and Results**</u>

Controlling dominant invasive aquatic plants is a common goal of many stakeholders around the state. These invader-reduction efforts are often motivated as ways to promote the health or recovery of native plant communities—but the potential for these efforts to actually meet those goals is uncertain. We hypothesized that, in addition to potential competitive effects of invasive species, insufficient water clarity and native plant recolonization can also be "rate-limiting" components of restoring lake vegetation. If so, these limitations must be addressed and invader control alone will be inadequate for restoration. We addressed this issue in two ways: (1) By evaluating responses of native plants to actual, on-the-ground management efforts in invaded lakes in MN through synthesis and analysis of monitoring data. This can tell us how management is working across the state at scales relevant to lake managers. (2) We compared those conclusions to results of field experiments designed to untangle how invaders, light limitation, and reproduction can hinder native plant recovery. Overall, our work resulted in the aggregation of more than 4,000 surveys that will be used to evaluate responses of native plants to curlyleaf pondweed, Eurasian watermilfoil, and the management of each of these AIS. The funding supported the completion of all experimental fieldwork, bringing four years of work to a conclusion. In short, our experiments and data synthesis reveal that native plant recovery following invader control is a realistic outcome—but only under certain conditions, i.e., where water clarity and propagule availability are sufficient to foster native plant recovery. In addition, our results show that Eurasian watermilfoil exerts a stronger negative effect on native plants than curlyleaf pondweed. Thus, control of Eurasian watermilfoil is more likely to foster native recovery than is control of curlyleaf pondweed. If lake management is to restore native macrophytes, it must target the factors that are limiting native species recovery, and we show that invasive species are one of multiple limiting factors in Minnesota lakes.

Subproject Results Use and Dissemination

This project has produced materials of interest to a wide variety of stakeholders covering a wide breadth of the work the project entailed. Among these products are peer-reviewed publications, videos, presentations, posters, databases, and a data dashboard. Videos include a webinar on the statewide plant survey database, an instructional video describing point-intercept and delineation plant-survey methods for student and extension audiences, and two short presentations—one describing analysis of statewide data for management evaluation, and another describing ecological work using statewide data to define the niches of macrophytes. A poster and a presentation detail much of the work that went into developing aquatic plant revegetation methods. The statewide database is available as a database and through a beta-version dashboard. Multiple publications will detail the work as it pertains to contributions to the state of knowledge on the ecology and management of aquatic plants. All of these materials are available upon request.

Peer-reviewed publications:

Verhoeven, M. R., D. J. Larkin, and R. M. Newman. (2020). Constraining invader dominance: Effects of repeated herbicidal management and environmental factors on curlyleaf pondweed dynamics in 50 Minnesota lakes. Freshwater Biology, 65(5), 849–862. https://doi.org/10.1111/fwb.13468

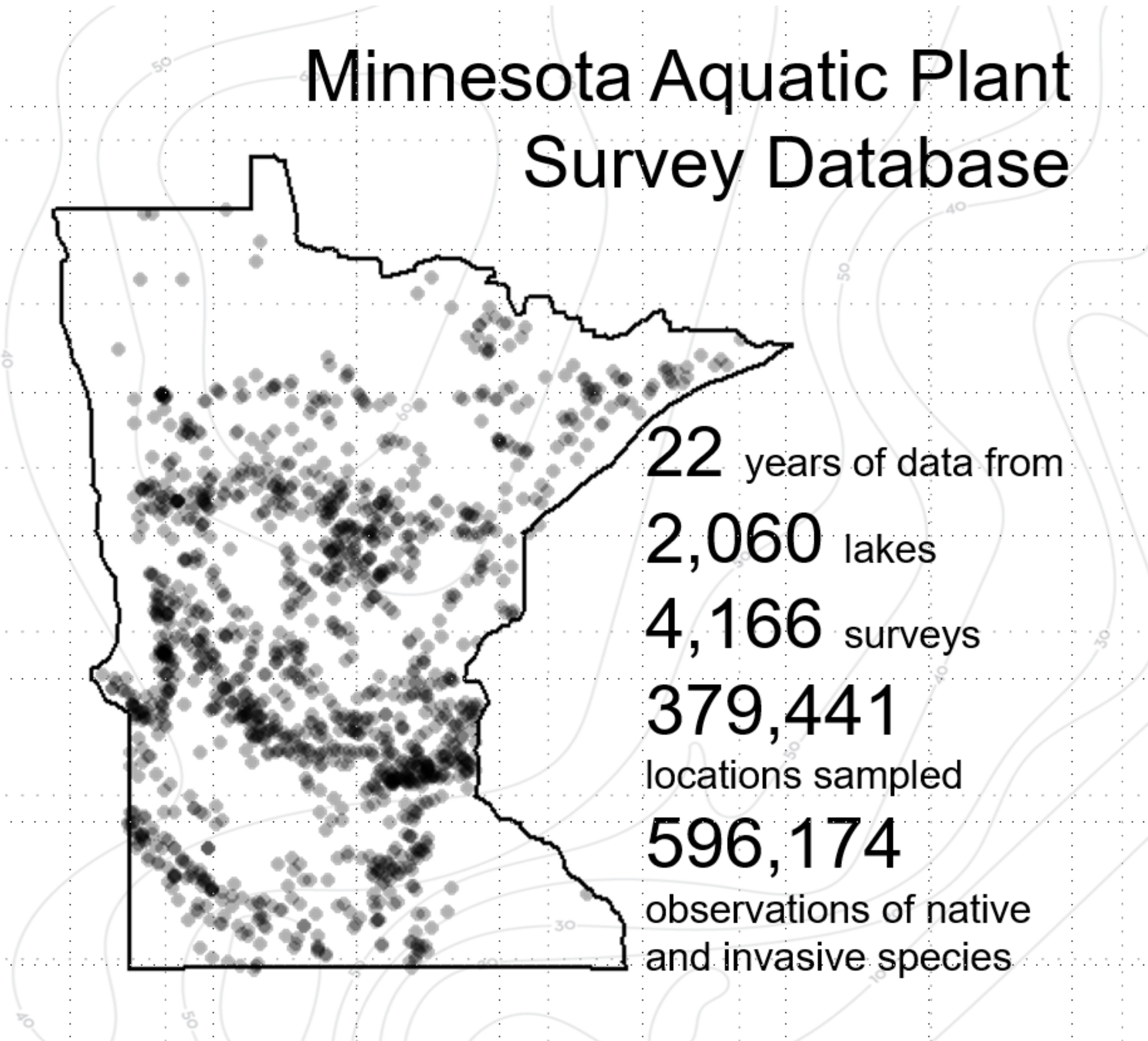
Verhoeven, M. R., W. J. Glisson, and D. J. Larkin. (2020). Niche models differentiate potential impacts of two aquatic invasive plant species on native macrophytes. Diversity, 12, 162. https://doi.org/10.3390/d12040162

Published datasets and R code:

Verhoeven, M. R., D. J. Larkin, and R. M. Newman. (2020). Complete data and analysis for: Constraining invader dominance: Effects of repeated herbicidal management and environmental factors on curlyleaf pondweed dynamics in 50 Minnesota lakes. Data Repository for the University of Minnesota. https://doi.org/10.13020/aw92-e606

Verhoeven, M. R., W. J. Glisson, and D. J. Larkin. (2021). Complete data and analysis for: Niche models differentiate potential impacts of two aquatic invasive plant species on native macrophytes. Data Repository for the University of Minnesota. https://doi.org/10.13020/cwqe-ge69

Final Report – Visual Component
MAISRC Subproject 8.2: Impacts of invader removal on native vegetation recovery
Dr. Daniel Larkin



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2021

SUBPROJECT TITLE: MAISRC Subproject 12.2: Historical analyses of spiny water flea invasion patterns
SUBPROJECT MANAGER: Dr. Donn Branstrator
ORGANIZATION: University of Minnesota Duluth
COLLEGE/DEPARTMENT/DIVISION: Swenson College of Science and Engineering; Department of Biology
MAILING ADDRESS: 1035 Kirby Drive
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WEBSITE: http://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: $53,795
AMOUNT SPENT: $53,795
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

After spiny water flea was first recorded in North America in the 1980s, sediment core analysis was heralded as a method to document the timing of lake invasions with implications for understanding and managing spread and threats. Our study casts concern on the method, revealing limitations to pinpoint early detection.

Overall Subproject Outcome and Results

Spiny water fleas threaten Minnesota's lakes, including walleye health, but we do not understand how many years it takes for the threats to manifest once they invade. This project was a continuation of Subproject 12 where we sought to use evidence in lake sediments to determine the timeline of first presence and growth of spiny water fleas in Lake Kabetogama and Lake Mille Lacs. The results of Subproject 12 demonstrated that spiny water fleas have been present in both lakes continuously since the early 1900s. This timeline conflicts with data on first sightings that do not place spiny water fleas in either lake until the early 2000s. This gap of about 100 years suggests that our sediment analysis methods are biased. With Subproject 12.2, our main objective was to conduct two additional lines of inquiry to determine the suitability of our methods by 1) measuring natural rates of mixing in surface sediments of Lake Kabetogama and Lake Mille Lacs, and 2) searching sediment cores that were collected before first sightings of spiny water fleas in Lake Kabetogama. The results demonstrate that 1) natural rates of sediment mixing are not sufficient to explain the early presence of spiny water flea body remains in Lake Kabetogama or Lake Mille Lacs sediments, and 2) there is no evidence in historical core material that places spiny water fleas in Lake Kabetogama before their reported year of first detection in the water. We combined our results with results from scientists at Queen's University (Canada) who have recently used similar methods to ask similar questions, into a forthcoming publication in the Journal of Paleolimnology. In that publication we review our findings and caution the use of our methods to pinpoint early detection of spiny water fleas in lakes until further study of the methods is conducted.

Subproject Results Use and Dissemination

We widely disseminated the results of Phase I of Subproject 12 (M.L. 2013) but we have not thus far disseminated any of the results in Phase II for two reasons. First, the COVID pandemic limited our opportunities. Second, the controversial nature of our results led to a hesitancy among us to share them until we had fully analyzed all evidence and lines of inquiry, both in our data set and the data set contributed by our collaborating scientists at Queen's University. Peer-reviewed publications are in process and presentations on results will be given as a part of the MAISRC Showcase.

Final Report – Visual Component
MAISRC Subproject 12.2: Historical analyses of spiny water flea invasion patterns
Dr. Donn Branstrator

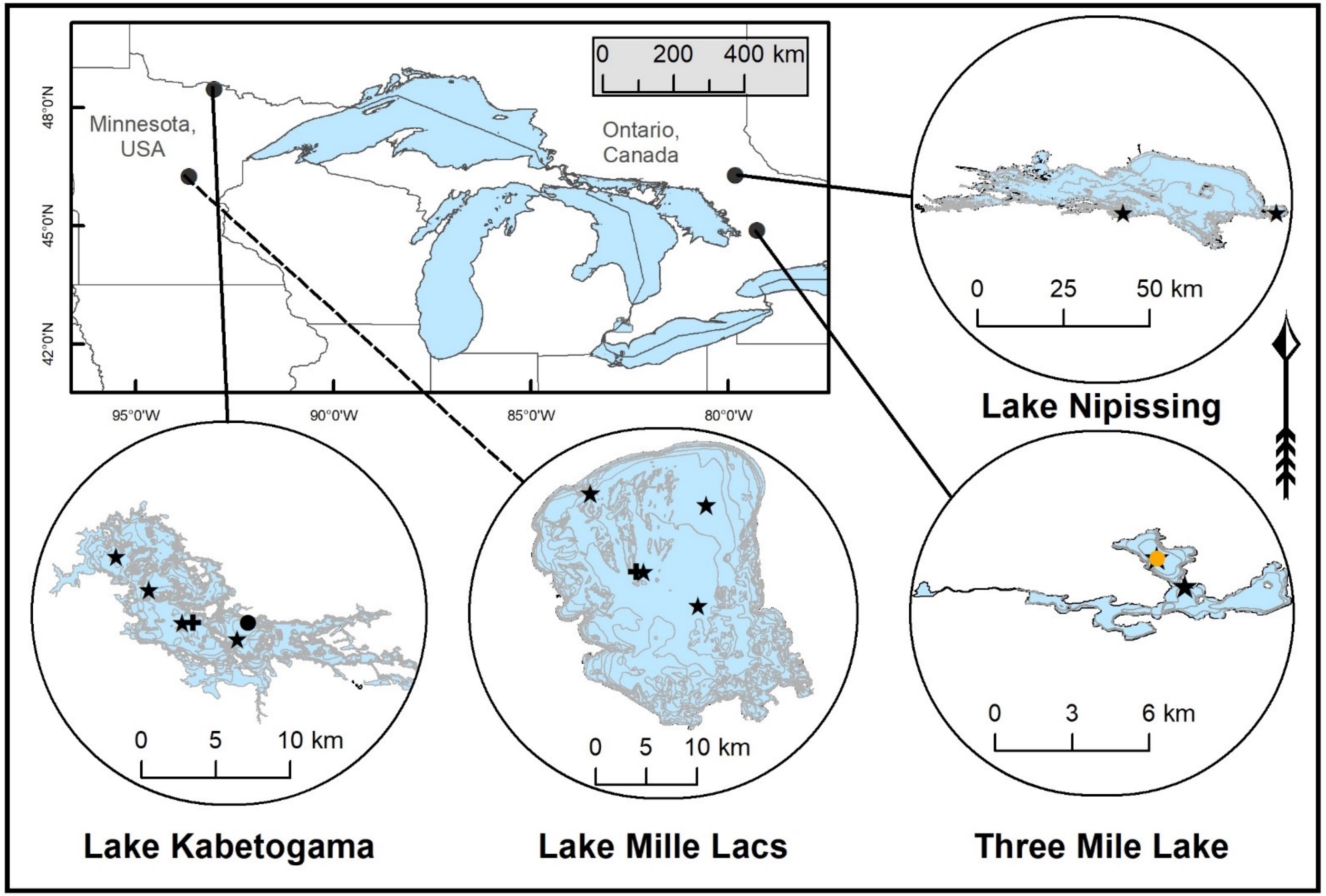

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Fig. 1. This map shows the general locations of the four lakes that we studied. Lake Kabetogama and Lake Mille Lacs were studied under this MAISRC project. The two Canadian lakes were studied under a companion project. The lake locations where sediment cores were collected for analysis of spiny water flea remains and dating by 210Pb are marked by black stars. The locations in Lake Kabetogama and Lake Mille Lacs where sediment cores were collected for 7Be analysis are marked by black crosses. The locations where historical sediment cores were collected in Lake Kabetogama for analysis of spiny water flea remains are marked by black circles.

Final Report – Visual Component

MAISRC Subproject 12.2: Historical analyses of spiny water flea invasion patterns

Dr. Donn Branstrator

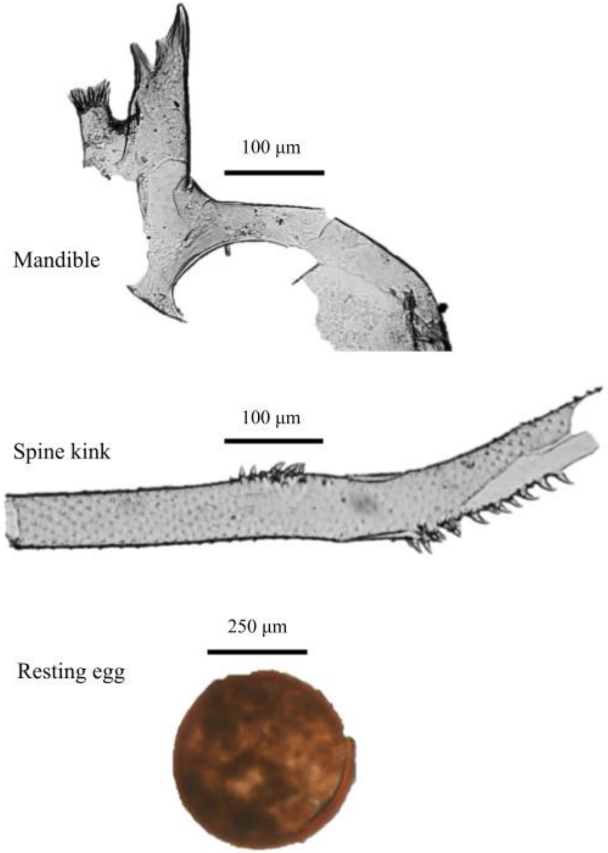

Fig. 2. These images with scale bars are examples of a mandible, tail spine kink, and resting egg of spiny water flea that we recovered from the sediment core material.

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Final Report – Visual Component

MAISRC Subproject 12.2: Historical analyses of spiny water flea invasion patterns
Dr. Donn Branstrator

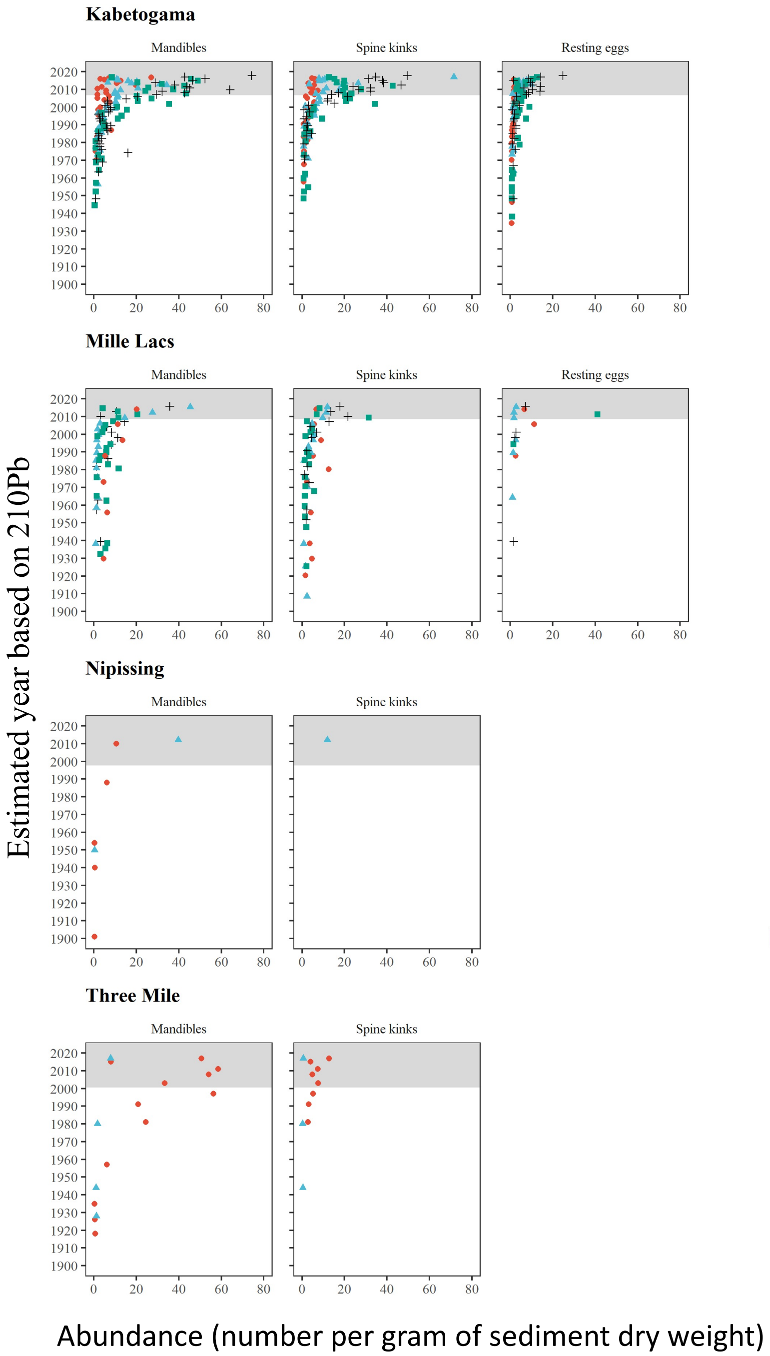

Fig. 3. These results show the abundances of mandibles, tails spine kinks, and resting eggs of spiny water flea that we recovered from 210Pb dated sediments (y axis) in the four study lakes. The different colors (symbols) correspond to different coring site locations within each lake. The abundances of remains are reported as the number per gram of sediment dry weight. In each panel, the youngest sediment is at the top of the core and the oldest sediment is at the bottom of the core. The gray shaded portion represents the reported invasion period based on net-tow collections. Only non-zero abundance values are reported.

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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
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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

Sound Bite of Project Outcomes and Results

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.

Overall Subproject Outcome and Results

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

ISSN: 1040-2381

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Lake and Reservoir Management
Volume 37 Number 4 December 2021

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Articles

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
https://doi.org/10.1080/10402381.2021.1941447

Lines snag spines! A field test of recreational angling gear ensnarement of *Bythotrephes*

Donn K. Branstrator[a], Joshua D. Dumke[b], Valerie J. Brady[b] and Holly A. Wellard Kelly[b]

[a]Department of Biology, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN, 55812; [b]Natural Resources Research Institute, University of Minnesota Duluth, 5013 Miller Trunk Hwy., Duluth, MN, 55812

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

$$\alpha = \left(r_i/p_i\right)/\Sigma\left(r_i/p_i\right), \text{ for i} = 1-3 \tag{1}$$

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:

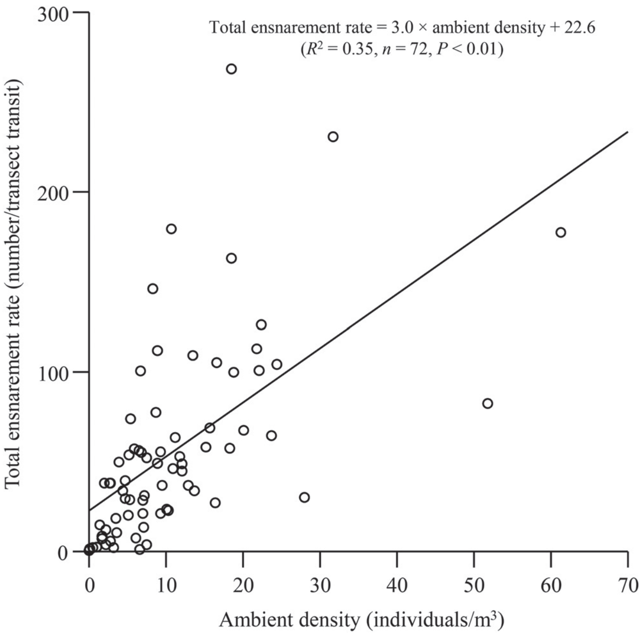

Figure 1. *Bythotrephes* total ensnarement rate as a function of ambient density as defined by a best-fit linear relationship for Island Lake Reservoir and Lake Mille Lacs.

$$\text{total ensnarement rate} = 3.0 \times \text{ambient density} + 22.6 \quad (2)$$

(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

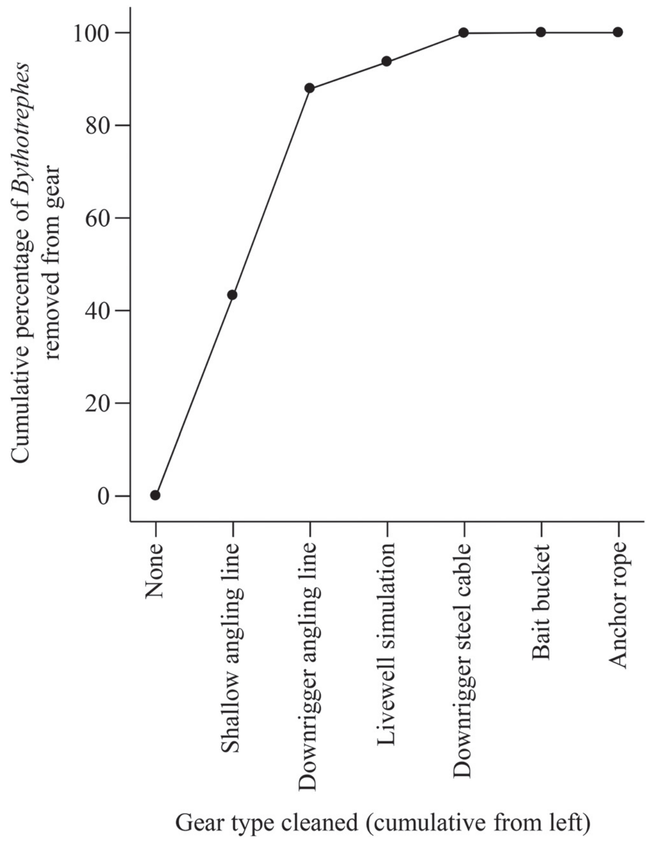

Figure 2. A projection of the cumulative percentage of ensnared *Bythotrephes* that would be removed from recreational angling gear if the gear (*x* axis) were cleaned in sequence from left to right. Numbers are based on the ensnarement rate results in Table 2.

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

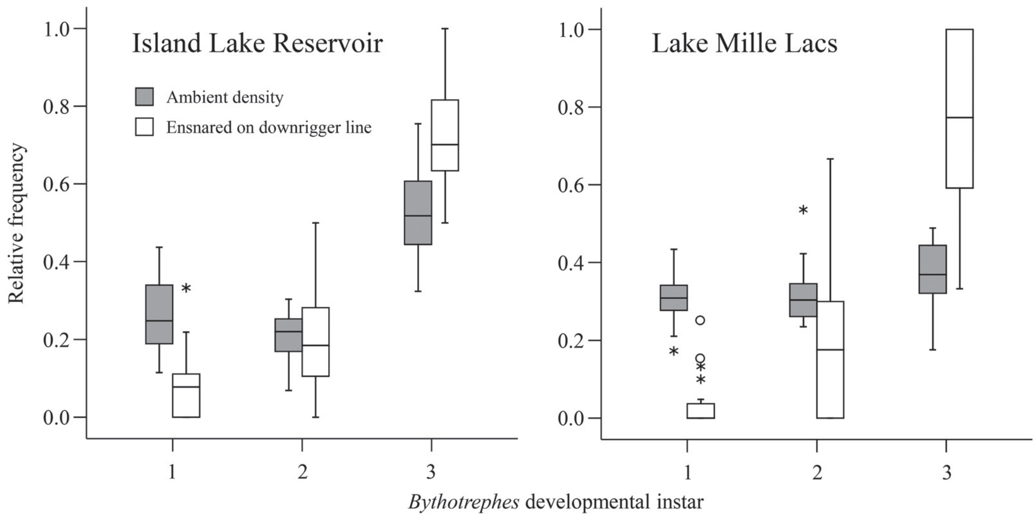

Figure 3. Box plots of the relative frequency (0–1) of *Bythotrephes* by developmental instar in ambient density (gray bar, left) and those ensnared on the downrigger angling line (open bar, right). Centerline = median, box edges = first and third quartiles, whisker ends = range, circles and asterisks = 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.

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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.

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Final Report – Map

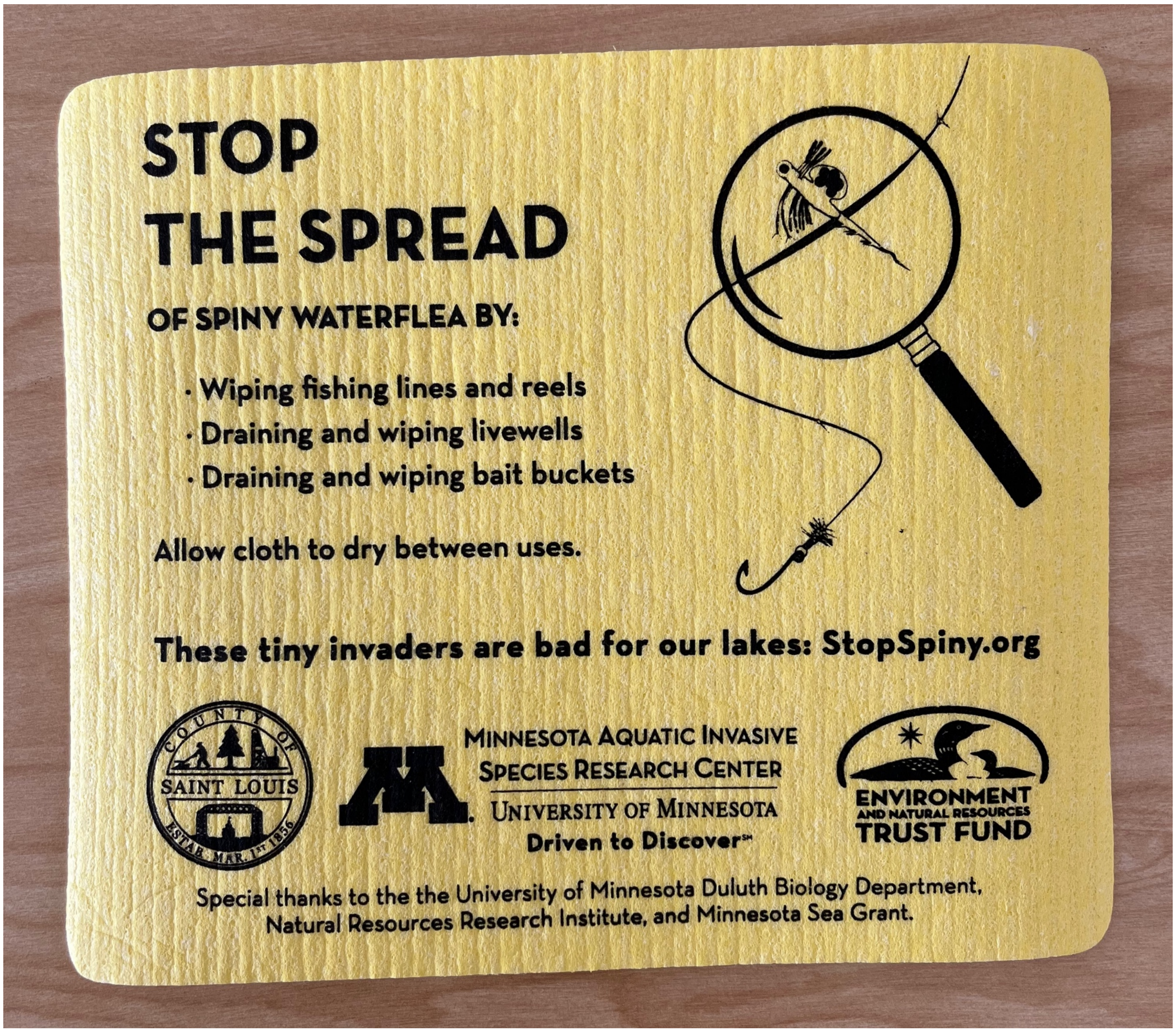
MAISRC Subproject 15: Determining Highest Risk Vectors of Spiny Water Flea Spread

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.



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2022

SUBPROJECT TITLE: MAISRC Subproject 16.2: AIS impacts on walleye populations and mercury concentrations
SUBPROJECT MANAGER: Dr. Gretchen Hansen
ORGANIZATION: University of Minnesota Twin Cities
COLLEGE/DEPARTMENT/DIVISION: Department of Fisheries, Wildlife, and Conservation Biology
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CITY/STATE/ZIP: Saint Paul, MN 55108
PHONE: 612-624-4228
E-MAIL: ghansen@umn.edu
WEBSITE: https://maisrc.umn.edu/walleye-ais; https://gretchenhansen.squarespace.com/
FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $199,853
AMOUNT SPENT: $199,853
AMOUNT REMAINING: $0

Sound Bite of Project Outcomes and Results

Invasive zebra mussels impact lakes supporting walleye in Minnesota. Our research demonstrates that zebra mussels are associated with lower recruitment (reproduction and survival) of walleye in their first year. Furthermore, walleye and yellow perch alter their feeding habits in lakes with zebra mussels, which is associated with increased mercury concentrations.

Overall Subproject Outcome and Results

Invasive zebra mussels profoundly affect lake ecosystems, but their impacts on walleye are not well understood. We used a multi-pronged approach to understanding zebra mussel impacts on walleye in Minnesota lakes. First, we evaluated how walleye recruitment (reproduction and survival) to their first fall was affected. We used statistical models applied to data collected by the Minnesota Department of Natural Resources to quantify changes in walleye recruitment. Walleye recruitment declined by ~41% following zebra mussel invasion. Additionally, lakes with zebra mussels supported the highest walleye recruitment prior to invasion, suggesting that zebra mussels invade high quality walleye lakes. Next, we evaluated how zebra mussels influence food webs supporting walleye and yellow perch, and how food web changes influence mercury concentrations in fish tissue. Using stable isotope analysis, we found that walleye and yellow perch in zebra mussel invaded lakes use 36-50% more nearshore food resources compared to those in uninvaded lakes. Mercury concentrations in fish were also influenced by zebra mussels; mercury in fish tissue was 66% higher for adult walleye and 91% higher for adult yellow perch in lakes containing zebra mussels compared to those in uninvaded lakes. On average, mercury concentrations in 16-inch walleye from lakes containing zebra mussels were 0.28 ppm, above the 0.2 ppm threshold triggering human consumption advisories by the Minnesota Department of Health. Zebra mussel-induced changes have important implications for walleye in Minnesota lakes. Lower walleye recruitment in invaded lakes may influence abundance at later life stages, which could influence harvest and stocking plans. Walleye were able to persist on nearshore food resources following zebra mussel invasions, but mercury concentrations were higher in these fish with important implications for human consumption. Given the significance of the impacts of zebra mussels documented in our study, preventing zebra mussel invasions into additional walleye lakes is critical.

Subproject Results Use and Dissemination

We have provided regular updates of our progress to scientists, managers, and the public via oral presentations and posters. PI Hansen and graduate student Kundel are members of the MN DNR Walleye-Zebra Mussel task force, and our research on the effects of zebra mussels on walleye recruitment has been critical for informing their approach to monitoring and managing for zebra mussel invasions. We have presented our results directly to MN DNR fisheries staff, at the MAISRC showcase, and at a national conference, as well as through several public virtual sessions. Progress on each objective was delayed due to the Covid-19 pandemic, and we are in the process of preparing manuscripts describing our results for peer-reviewed publications.

Presentation Recordings

- 2020 MAISRC Research & Management Showcase Presentation
  https://z.umn.edu/2020ShowcaseZMWalleye

Factsheets/Informational Documents

- Zebra mussel impacts on walleye populations and mercury concentrations: A collaborative project investigating the connections between zebra mussels, changes to lake food webs, and walleye success
  https://z.umn.edu/HansenWalleyeFactsheet

Zebra mussel impacts on walleye populations and mercury concentrations:

A collaborative project investigating the connections between zebra mussels, changes to lake food webs, and walleye success

Young sport fish, like walleye, can be negatively affected by zebra mussels. In lakes with and without zebra mussels, this project will compare the success of young-of-year walleye, compare the food sources of young-of-year and adult walleye, and measure mercury concentrations in walleye.

Our questions:

1. **Do zebra mussels reduce walleye habitat?** *Increased water clarity can negatively affect walleye habitat and abundance.*

2. **How are walleye food sources affected by zebra mussels?** *Food web changes caused by zebra mussels could potentially reduce walleye abundance*

3. **Do changes in the food web from zebra mussel presence affect mercury concentrations in walleye?** *Food web changes caused by zebra mussels can increase mercury in fish, with important implications for consumption.*

How will we answer these?

1. **Sample walleye, other fish, zooplankton, and aquatic insects in the summer months**
    - *Intensive sampling will take place in July and August on each lake*
    - *Samples will be sent to a lab to evaluate mercury concentrations*
    - *We will use stable isotope tracers to determine who is eating who in each lake and how zebra mussels influence walleye diets.*
2. **Analyze past and present data on walleye habitat and how it relates to abundance**
    - *We will use computer models to estimate how walleye habitat is affected by changes in water clarity*
    - *We will use historical walleye abundance to relate habitat to walleye population size in order to anticipate future changes caused by zebra mussels*

Where are we sampling?

Sampling will be conducted collaboratively by University of Minnesota researchers, students, and DNR biologists in the summers of 2021 and 2022.

- 16 small and medium sized lakes will be sampled in 2021 and 2022
- 2021 sampling lakes: Buffalo, Big Sandy, Chippewa, Shamineau, Alexander, North Lida, Steamboat, Little Boy
- 2022 sampling lakes may include: Big, Potato, Round (Aitkin), Round (Brainerd), Woman, Bemidji, and Reno

Initial results can be expected in 2021, and will help managers proactively manage walleye fisheries in lakes with zebra mussel invasions.

About zebra mussels

- Found in 409 water bodies in Minnesota
- Can become very abundant in lakes
- Increase water clarity
- Impacts on walleye and other fish can vary among lakes, and this project seeks to understand that variation.

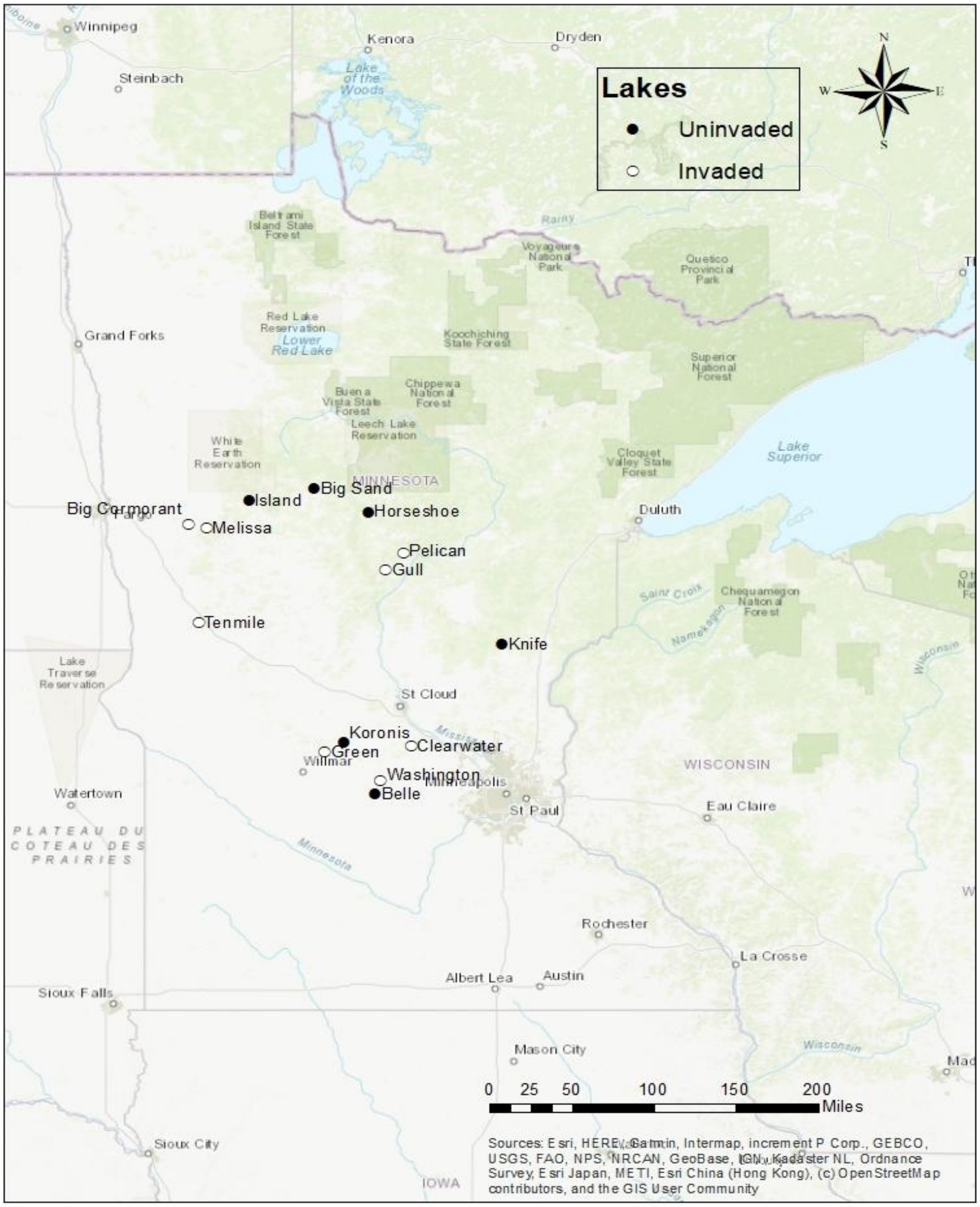
More details

This project is funded by the United States Geological Survey and the University of Minnesota Water Resources Center, with additional support from the Minnesota Department of Natural Resources and the Environment and Natural Resources Trust Fund through the Minnesota Aquatic Invasive Species Research Center.

For more information, visit https://gretchenhansen.squarespace.com/food-webs

Final Report – Map
MAISRC Subproject 16.2: AIS impacts on walleye populations and mercury concentrations
Dr. Gretchen Hansen

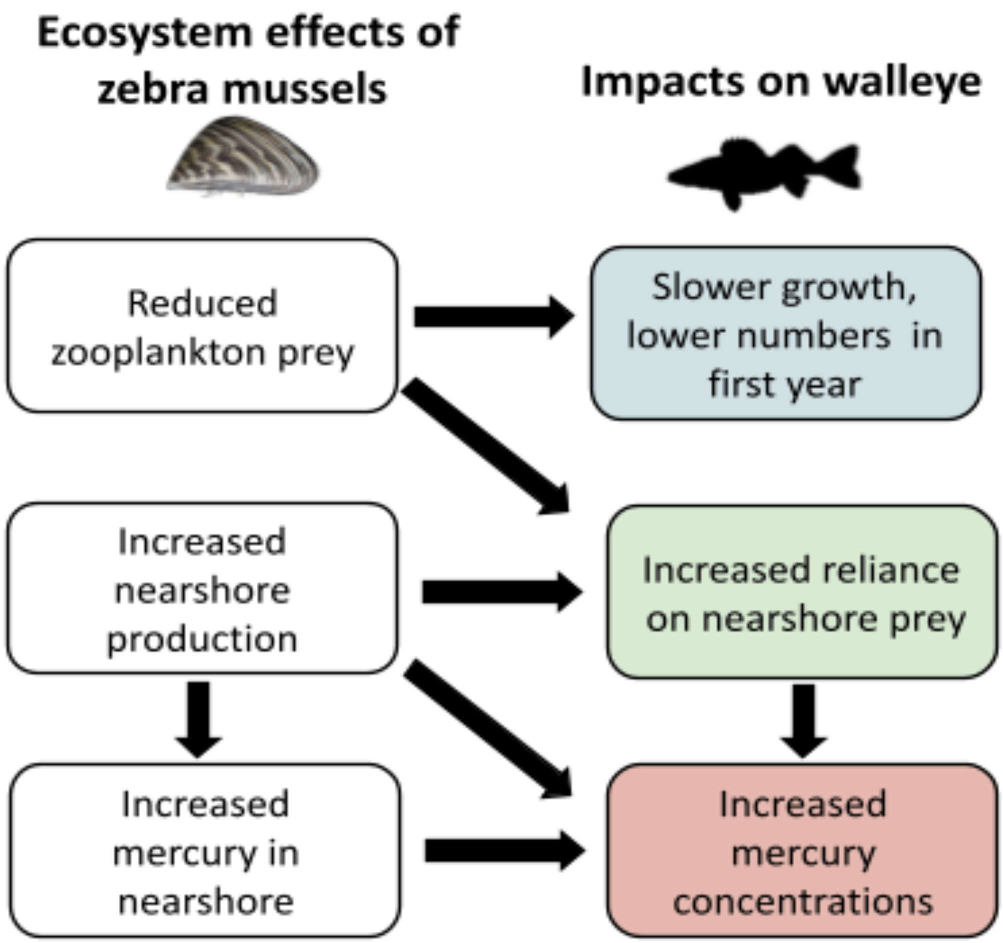
Map of study lakes for Subproject 16.2, Objective 2: Quantify the effects of zebra mussels on energy sources and mercury pathways and concentrations in walleye. Open circles represent lakes containing zebra mussels, and filled circles represent uninvaded lakes.



MINNESOTA AQUATIC INVASIVE SPECIES RESEARCH CENTER
UNIVERSITY OF MINNESOTA
Driven to Discover℠

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND

Final Report – Visual Component
MAISRC Subproject 16.2: AIS impacts on walleye populations and mercury concentrations
Dr. Gretchen Hansen



M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2021

SUBPROJECT TITLE: MAISRC Subproject 18.2: Genetics to improve hybrid and Eurasian watermilfoil management
SUBPROJECT MANAGER: Dr. Raymond Newman
ORGANIZATION: 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: 612-625-5704
E-MAIL: RNewman@umn.edu
WEBSITE: http://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: $236,423
AMOUNT SPENT: $236,423
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

Invasive Eurasian and native northern watermilfoil can hybridize and we identified hybrid watermilfoil in 39 lakes across the state. Hybrid watermilfoil is genetically more diverse than Eurasian watermilfoil and has potential to be more invasive and resistant to herbicides; several potentially problematic genotypes have been identified for further study.

Overall Subproject Outcome and Results

Invasive Eurasian and native northern watermilfoil can hybridize and some genotypes of hybrid watermilfoil have been shown to be more invasive or resistant to herbicidal control. Our aim was to determine the occurrence and distribution of hybrid watermilfoil in Minnesota, assess the response of different genotypes to herbicidal management, identify potentially problematic genotypes and assess the response of some of these genotypes to herbicide in controlled laboratory conditions. We assessed watermilfoil genetic composition in 81 waterbodies in Minnesota; 55 lakes had pure Eurasian, mostly one widespread genotype that was found in 52 lakes. Eight other Eurasian genotypes were found. We identified hybrid watermilfoil in 39 lakes across the state, mostly, but not entirely, in the Twin Cities Metro. Hybrid watermilfoil is genetically more diverse than Eurasian watermilfoil and 82 genotypes were found. Most lakes have one unique genotype of hybrid but multiple genotypes were found in several lakes and 26 have been identified in Lake Minnetonka. One hybrid genotype has been found in 10 lakes. No clearly problematic genotypes have been identified in Minnesota but we did find changes in genotype frequency with management in an assessment of 5 managed waterbodies and 3 reference waterbodies over 3 years. Several hybrid genotypes have expanded while Eurasian decreased and two hybrids from Lake Minnetonka have persistently rebounded after control. We also identified one genotype of northern watermilfoil that may be less affected by herbicide treatment. We conducted laboratory performance and herbicide challenge tests with the widespread Eurasian genotype and 4 hybrid genotypes. Additional experiments are needed but preliminary results suggest that two hybrid genotypes may be more tolerant of 2,4-D than the widespread Eurasian and two other hybrid genotypes. Continued identification of hybrid genotypes

and response to management will improve milfoil management by allowing manager to appropriate controls for their particular populations.

Subproject Results Use and Dissemination

We presented our insights and results and interacted with stakeholders at the MAISRC Showcase in 2019 and 2020 and held two in person and two virtual meetings with stakeholders to discuss observations and interest in genetic testing. We provided information to update the MAISRC website and hybrid watermilfoil fact sheet and developed a genotyping fact sheet for distribution by MAISRC and the DNR. We gave 8 presentations at regional and national scientific meetings and published three papers: Eltawely et al. 2020, Pashnick and Thum 2020, and Thum et al. 2020. In addition, two Masters projects, Eltawely 2019 and Gannon 2021 were completed.

We are in regular contact with the DNR, consultants and applicators about our results, which have been used to inform management actions.

Peer-reviewed publications:

Eltawely, J. A., R. M. Newman, and R. A. Thum. 2020. Factors Influencing the Distribution of Invasive Hybrid (Myriophyllum Spicatum x M. Sibiricum) Watermilfoil and Parental Taxa in Minnesota. Diversity 12(3):120. https://doi.org/10.3390/d12030120

Pashnick, J., and R. A. Thum. 2020. Comparison of molecular markers to distinguish genotypes of Eurasian watermilfoil, northern watermilfoil, and their hybrids. Journal of Aquatic Plant Management 58(1):61-71. http://www.apms.org/wp/wp-content/uploads/japm-58-01-61-full.pdf

Thum, R.A., Chorak, G.M., Newman, R.M., Eltawely, J.A., Latimore, J., Elgin, E., and Parks, S. 2020. Genetic diversity and differentiation in populations of invasive Eurasian (*Myriophyllum spicatum*) and hybrid (M*yriophyllum spicatum × Myriophyllum sibiricum*) watermilfoil. Invasive Plant Science and Management **13**(2): 59-67. https://doi.org/10.1017/inp.2020.12

Masters' theses:

Eltawely, J. A. 2019. Distribution of Eurasian and hybrid watermilfoil in Minnesota. Water Resources Science Masters Plan B Paper, University of Minnesota, St. Paul, MN. https://hdl.handle.net/11299/211341

Gannon, K. A. 2021. Integrating DNA fingerprinting of invasive watermilfoil strains into aquatic vegetation monitoring and assessment. Plant Sciences Masters of Science Thesis, Montana State University, Bozeman, MT.

MINNESOTA AQUATIC INVASIVE SPECIES RESEARCH CENTER

Hybrid Watermilfoil

How genetic analyses can inform management and support research

What is hybrid watermilfoil?

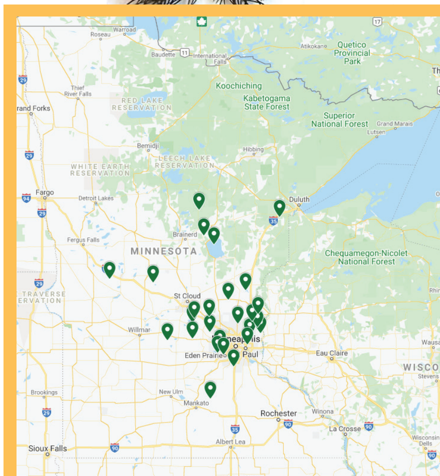
Hybrid watermilfoil (*Myriophyllum spicatum x Myriophyllum sibiricum*) is a hybrid of invasive Eurasian watermilfoil (*Myriophyllum spicatum*) and native, northern watermilfoil (*Myriophyllum sibiricum*). Though similar in appearance to Eurasian watermifoil, in terms of impacts, invasiveness, and difficulty of control, hybrid watermilfoil may be an even greater threat. Researchers at the Minnesota Aquatic Invasive Species Research Center (MAISRC) have found strong evidence of hybrid vigor, the term for when hybrid offspring exhibit more vigorous growth, increased fertility, and decreased sensitivity to stressors than its parents. The scientists documented greater surface matting, earlier and more abundant flower production, and greater tolerance to chemical control in hybrid milfoil compared to Eurasian watermilfoil.

High genetic variability can impact treatment efficacy

An added complexity to managing hybrid milfoil is its high genetic diversity, with some hybrid genotypes being less responsive to the herbicides and application rates used to treat invasive watermilfoils. Herbicide resistant strains of hybrid watermilfoil have already been found in Michigan and evolution of resistant strains locally is a serious concern. In Minnesota, researchers have found 66 different hybrid milfoil genotypes so far. Lakes may contain a single or multiple genotypes. And, though many lakes have unique genotypes of hybrid milfoil, some genotypes are found across multiple lakes. Research to assess how different genotypes respond to chemical treatments is on-going and depends on information-sharing from cooperators. In particular, knowledge sharing about treatment outcomes in lakes with common hybrid genotypes can help us advance research-based management recommendations.

Genotyping hybrid milfoil from your lake can help you make more informed management decisions and support research advances in invasive milfoils

Knowing how many, and which, genotypes of milfoil you have in your lake can assist with management planning and evaluation (e.g., interpreting results of herbicide treatments). Collecting milfoil samples and sending them to a lab for genetic analysis is the first step towards understanding the infestation at your lake and may be helpful in planning which herbicide to use. This analysis costs about $50 per sample and is currently done by MAISRC Co-investigator Dr. Ryan Thum at Montana State University. Staff from the Newman Lab at the University of Minnesota can provide technical support and assist with sample processing and shipment logistics to the Thum lab.



Learn about hybrid watermilfoil, including its known distribution in MN: www.MAISRC.umn.edu/hybrid-distribution

For more information, contact:

Hybrid milfoil genotyping
Ryan Thum
ryan.thum@montana.edu
www.montana.edu/thumlab/Genetic-Identifications

Sampling and sample processing
Ray Newman
rnewman@umn.edu
www.maisrc.umn.edu/team-newman

Learn more about invasive milfoil research at MAISRC:
www.MAISRC.umn.edu/eurasianwatermilfoil-research

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
CITY/STATE/ZIP: Saint Paul, MN 55108
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:

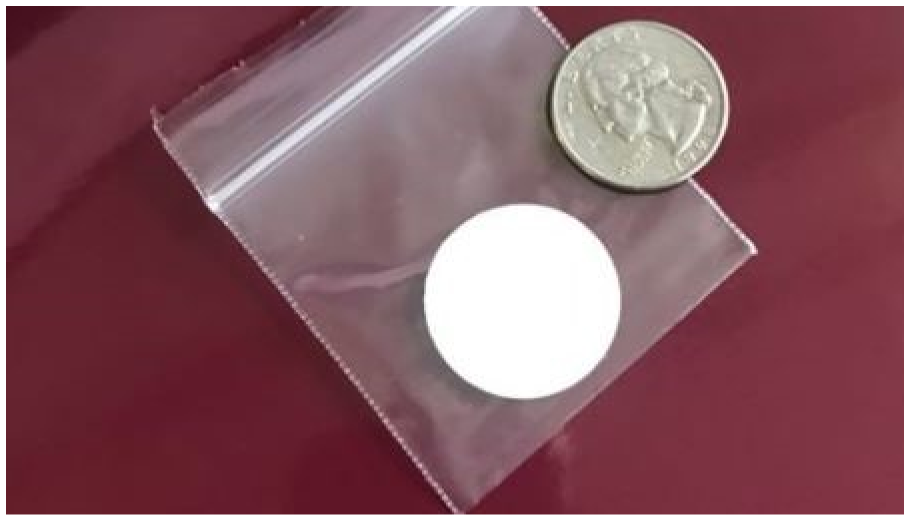
- Zarouri, A., A. Abbas. September 2019. Enhancing fish surveys: A novel technology for environmental DNA capture. MAISRC Research and Management Showcase. Saint Paul, MN.
- Quichen, D., A. Zarouri, A. Abbas. September 2019. A Novel Technology for Environmental DNA Collection and Concentration. American 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
E-MAIL: jluoma@usgs.gov
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:

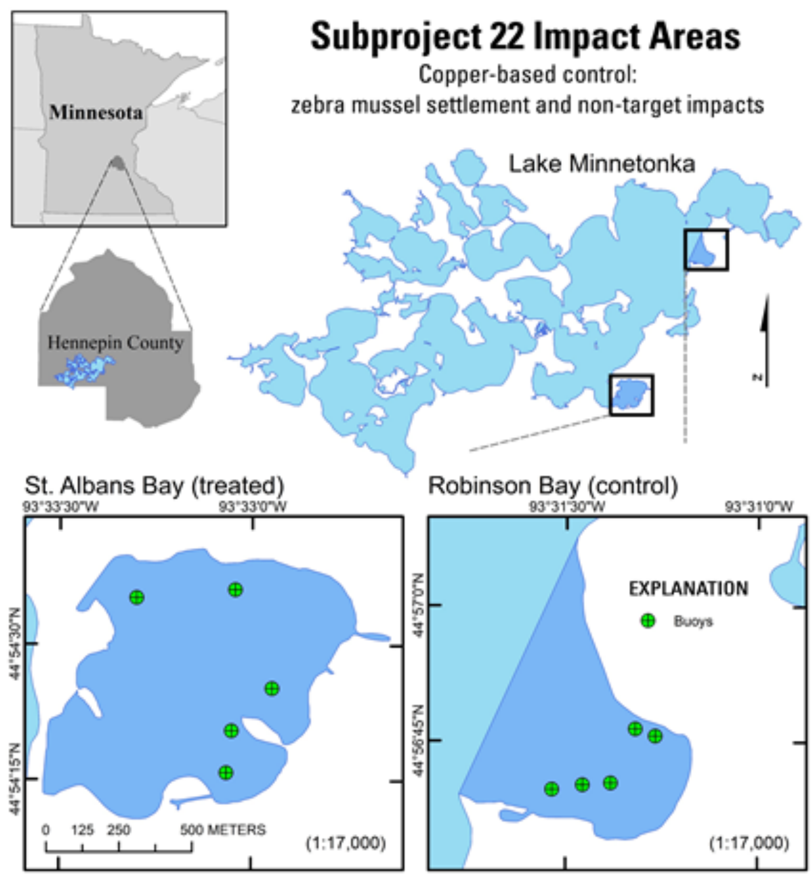
- UMN Driven to Discover video: Guardians of the Lake (2019). https://twin-cities.umn.edu/discover/guardians-lake
- Zebra mussels research project planned for Lake Minnetonka this summer. Melissa Turtinen, Southwest News Media. 23 April 2019. https://www.swnewsmedia.com/lakeshore_weekly/news/local/zebra-mussels-research-project-planned-forlake- 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
CITY/STATE/ZIP: Saint Paul, MN 55108
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.

Fig. 1

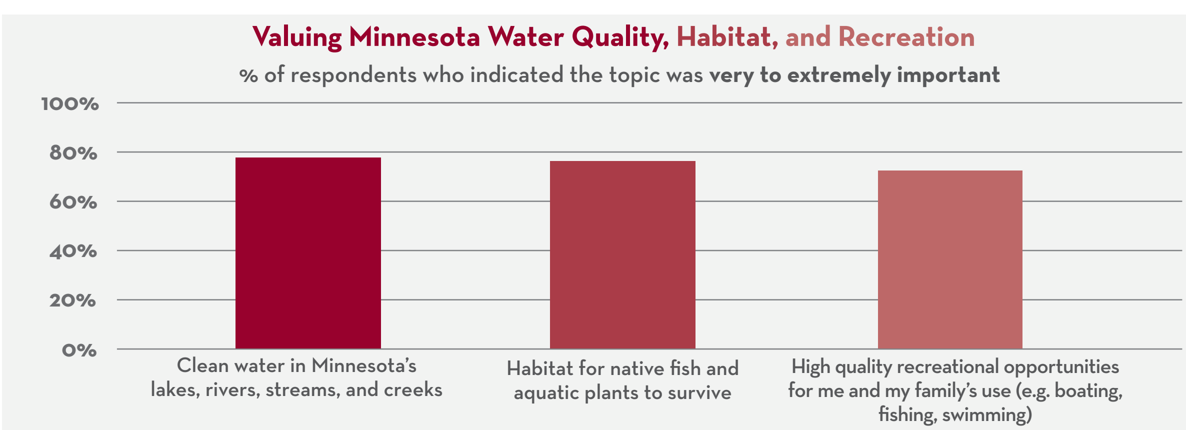
Polling lakeside users

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

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



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.

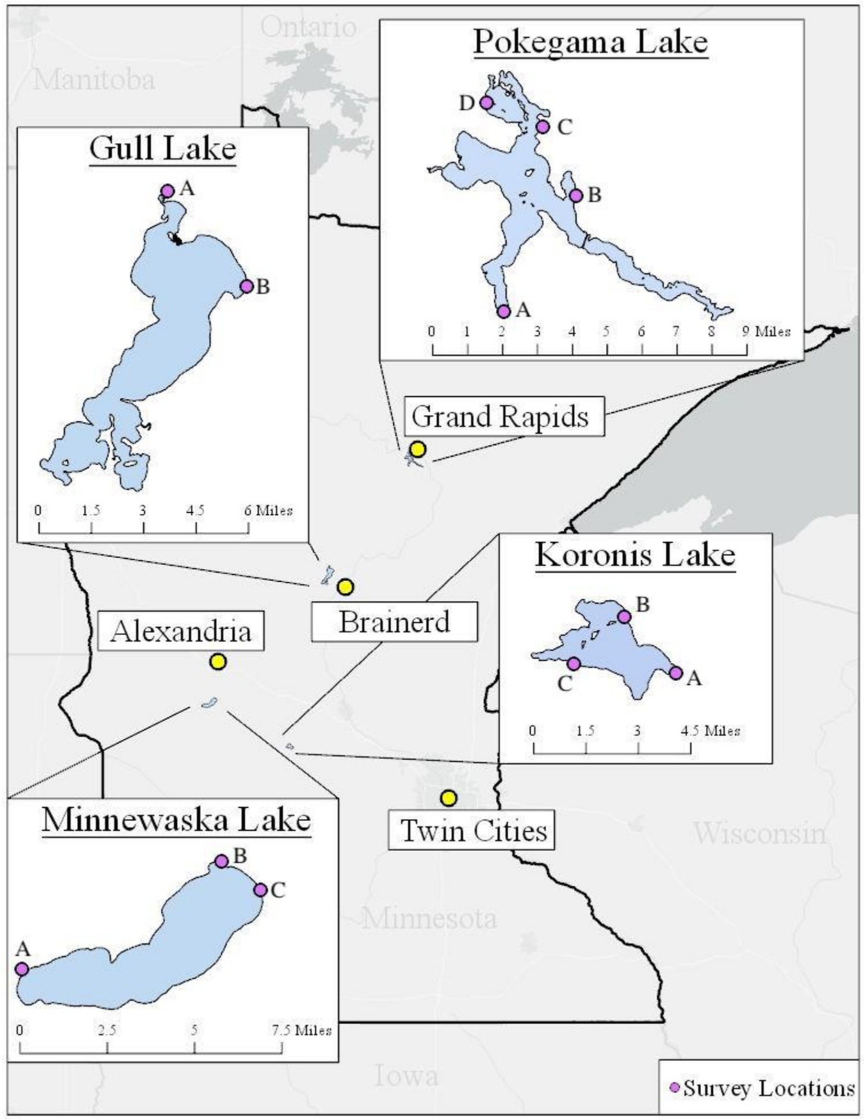
4 Survey design and statistical model

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.

https://doi.org/10.1371/journal.pone.0246860.g001 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

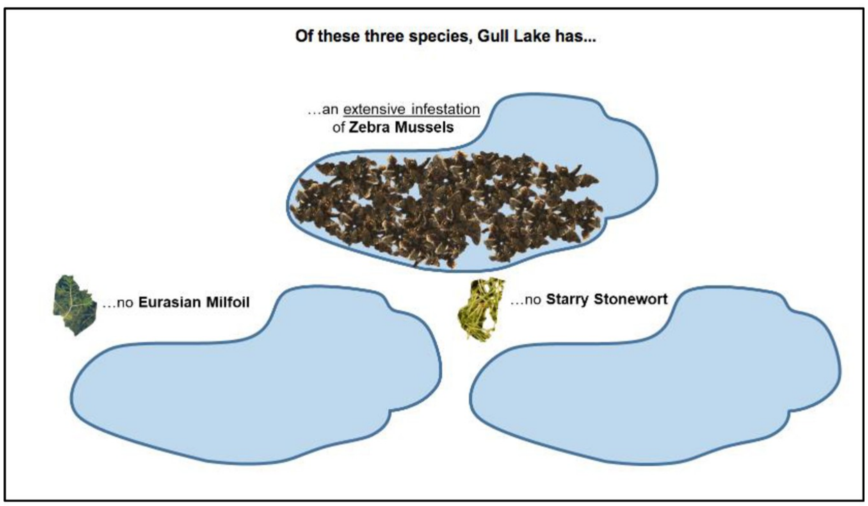
(S1 Appendix question). We also asked respondents to identify the invasive species that were present in the lake.

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)

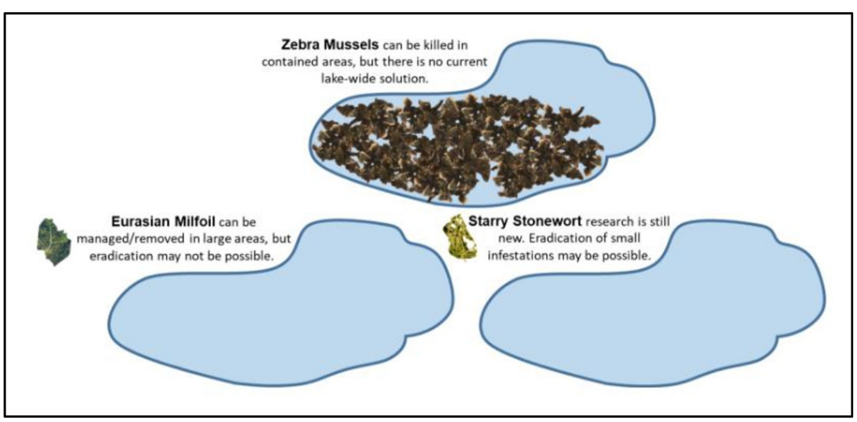


Fig 2. Question 1: Lake infestation.

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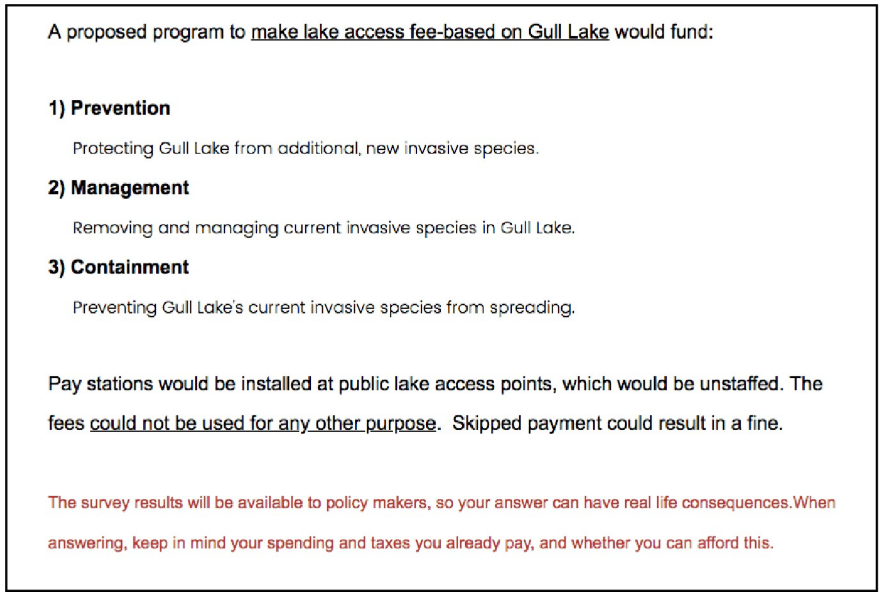
A proposed program to make lake access fee-based on Gull Lake would fund:

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:

$$WTP_i = \boldsymbol{x}_i\boldsymbol{\beta} + \boldsymbol{\varepsilon}_i \quad (1)$$

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:

$$Prob\ (Yes; Yes) = Prob[WTP_i \geq v_i^2] \\ = 1 - \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \tag{2}$$

The probability that respondent i will answer "Yes" to the first question, and "No" to the second:

$$Prob\ (Yes; No) = Prob[v_i^1 \leq WTP_i < v_i^2] \\ = \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] - \Phi\left[\frac{v_i^1 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \tag{3}$$

The probability that respondent i will answer "No" to the first question, and "Yes" to the second:

$$Prob\ (No; Yes) = Prob[v_i^2 \leq WTP_i < v_i^1] \\ = \Phi\left[\frac{v_i^1 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] - \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \tag{4}$$

And finally, the probability that respondent i will answer "No" to both is:

$$Prob\ (No; No) = Prob[WTP_i < v_i^2] \\ = \Phi\left[\frac{v_i^2 - \boldsymbol{x}_i\boldsymbol{\beta}}{\sigma}\right] \tag{5}$$

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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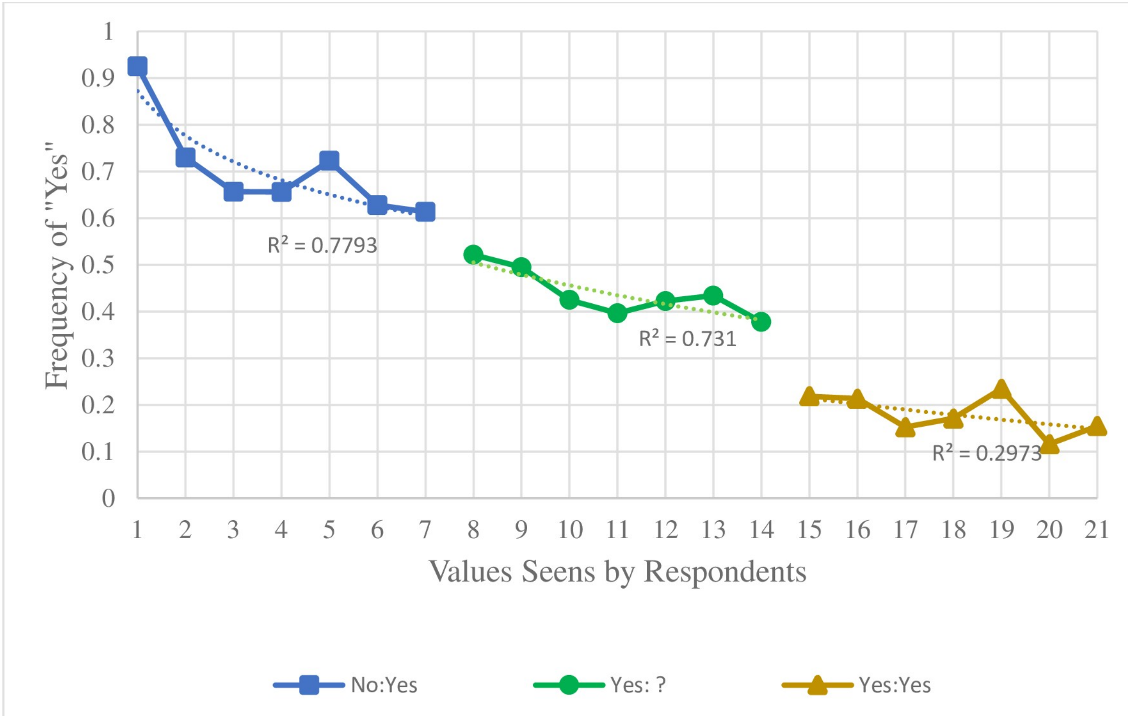

[1] [Yes: ?] values are the actual frequencies of "Yes" responses to the initial willingness to pay question (Appendix Question 3). [No: Yes] values are calculated with $\frac{\left(Y^1 + F_j^{1:No}(n - Y^1)\right)}{n}$ where Y^1 is the number of respondents who chose "Yes" to the first question, $F_j^{1:No}$ is the frequency of a "Yes" response to the second question with a value of j, $j \in \{1, \dots, 7\}$, 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, $k \in \{15, \dots, 21\}$, 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%).

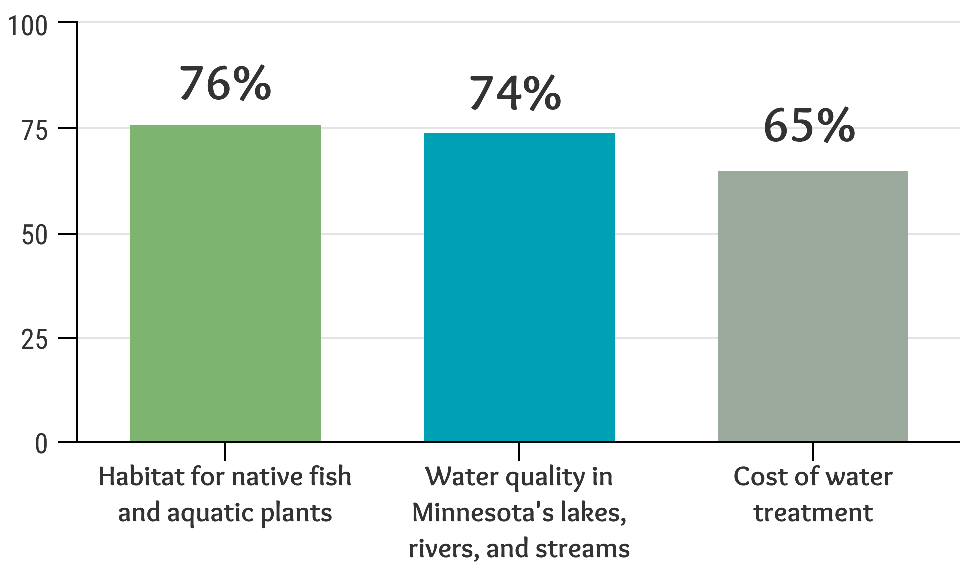
88% of respondents believed that AIS are a ***moderate to severe problem*** in Minnesota

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)*



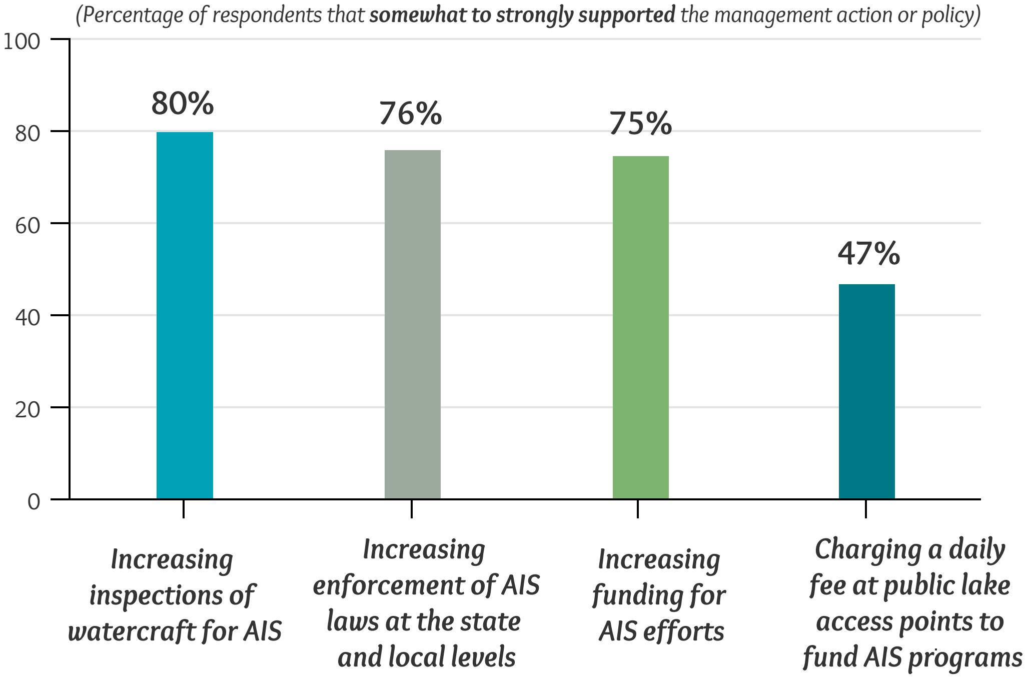
Respondents reported generally low engagement in AIS control and prevention

- 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)*



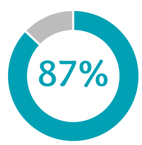
Only 25% of respondents were very to extremely confident that their personal actions can help reduce the spread of AIS in Minnesota.

57% of respondents somewhat to strongly agreed that they do *not* personally know enough about how to prevent the spread of AIS

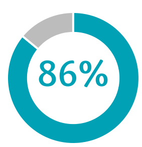
Only 19% of respondents reported that they were very to extremely confident that the residents in their community can solve the problem of AIS, and only 9% were very to extremely confident that everyone is working together when it comes to AIS control in their communities.

86% of respondents either disagreed or were unsure that their community has the financial resources it needs to manage AIS.

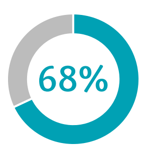
Where did respondents turn for information about AIS?



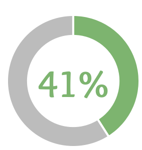
87% of respondents somewhat to strongly trust **MN Department of Natural Resources**



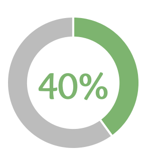
86% of respondents somewhat to strongly trust **university researchers/Extension**



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).

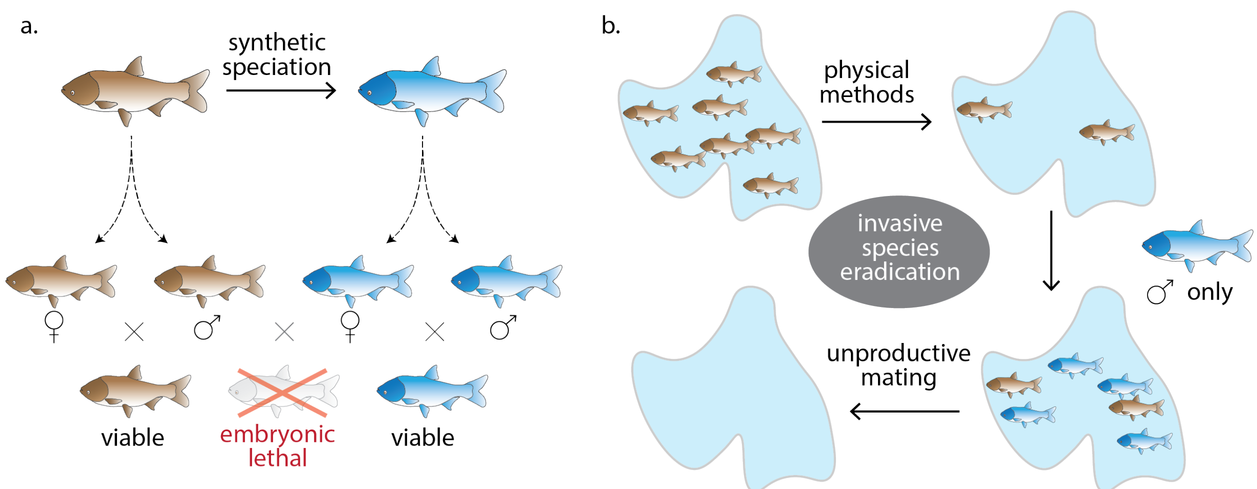
In addition to these primary research reports, one book chapter that describes the techniques developed under this subproject has already been published:

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

Sound Bite of Project Outcomes and Results

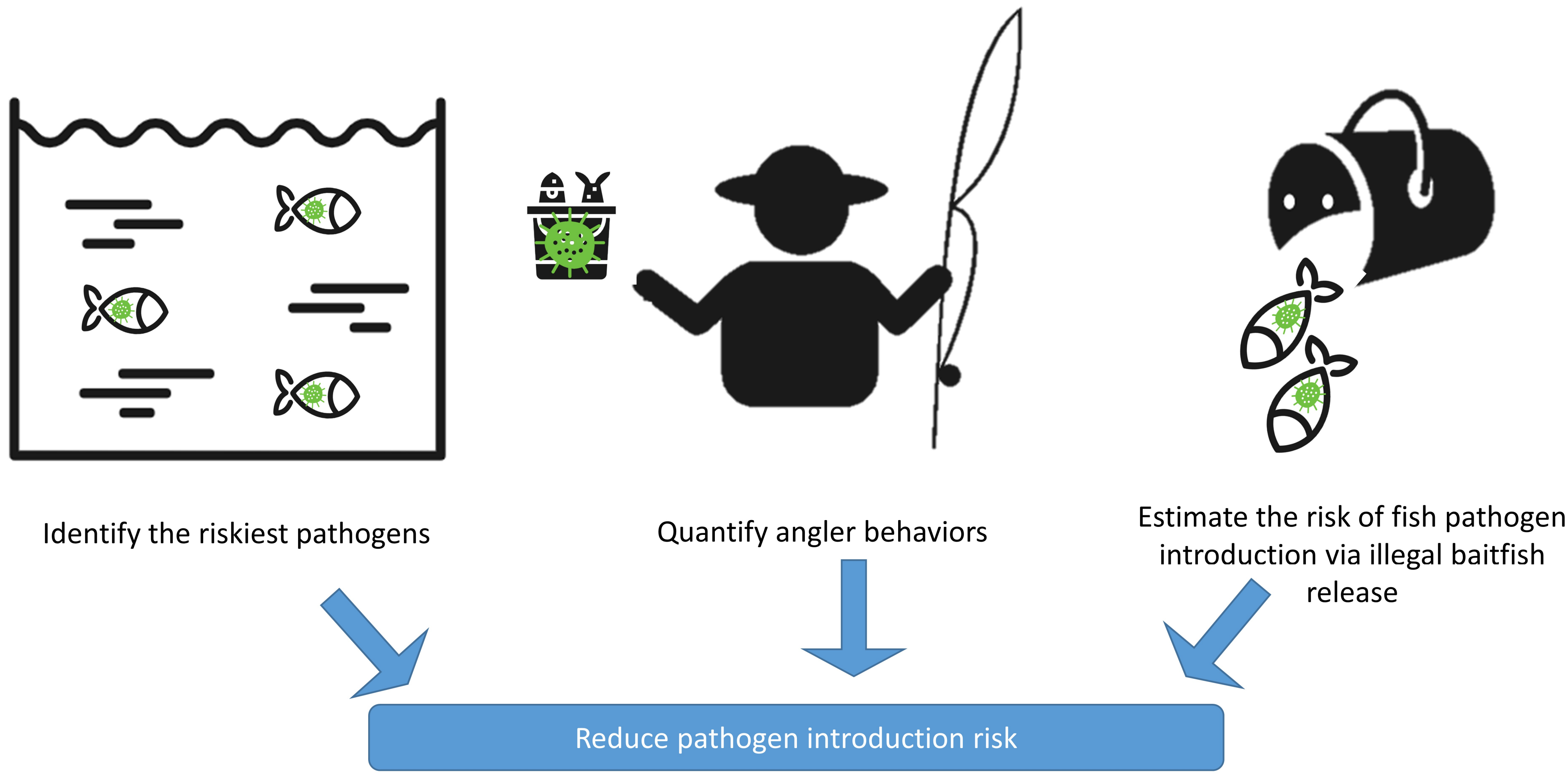
Live baitfish are popular among Minnesota anglers, but their illegal release is a known risk factor for spreading harmful diseases to wild fish populations. Our research identified high-risk pathogens in Minnesota, estimated the number of times anglers release an infected baitfish each year, and identified opportunities for strategic management intervention.

Overall Subproject Outcome and Results

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

M.L. 2017 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2020

SUBPROJECT TITLE: MAISRC Subproject 28: Evaluating Innovative Coatings to Suppress Priority AIS
SUBPROJECT MANAGER: Dr. Mikael Elias
ORGANIZATION: University of Minnesota
COLLEGE/DEPARTMENT/DIVISION: College of Biological Sciences; Department of Biochemistry, Molecular Biology, and Biophysics – Biotechnology Institute
MAILING ADDRESS: 1479 Gortner Avenue
CITY/STATE/ZIP: Saint Paul, MN 55108
PHONE: 612-626-1915
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WEBSITE: (1) https://www.eliaslab.org/ **(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: $84,940
AMOUNT SPENT: $51,234
AMOUNT REMAINING: $33,734

Sound bite of Project Outcomes and Results

Biofouling is a natural phenomenon that includes the adhesion of Zebra mussels to structures or boats and contribute to their spread in Minnesota waters. Current antifouling coatings are environmentally toxic. We demonstrate the efficacy of an eco-friendly coating technology that could help mitigate the spread of sessile invasive species, while minimizing non-target impacts.

Overall Subproject Outcome and Results

Biofouling is a natural phenomenon that sticks on structures or boats. It is a vector for the spread of numerous invasive species in Minnesota waters. A current way of fighting biofouling involves using metals that are harmful to the environment. We successfully evaluated a new generation of coatings containing a non-toxic, antifouling, biological molecule, and demonstrate that it reduces the adhesion of invasive species. These coatings could help mitigate the spread of sessile invasive species not only in coastal and inland waterways but also on recreational and industrial equipment surfaces, while minimizing non-target impacts.

Problem: Replace current toxic antifouling coatings with coatings containing a non-toxic, antifouling, biological molecule to mitigate the spread of sessile invasive species while minimizing non-target impacts.

Methodology: We took advantage of our unique technical and scientific edges to evaluate the potential of this technology to replace toxic biocides currently used to limit biofouling. Coated samples were submerged in the field in three different sites in Minnesota, including infested sites, and samples were analyzed using microscopies, organisms were quantified and measured, and surface microbial communities determined.

Results and Significance: Biofouling is a main vector for the spread of aquatic invasive species. Current antifouling solutions are both partly effective and highly toxic to the environment. In this proof-of-concept project, we demonstrate that our non-toxic enzyme technology can prevent the adhesion of AIS on submerged surfaces. We show that in three different Minnesotan field sites that enzymatic coatings can outperform coatings containing biocides, and prevent Zebra mussels adhesion to polycarbonate surface over the course of two summer months. This enzyme-based coatings could help mitigate the spread of sessile invasive species in Minnesota and beyond. These results evidence that this novel technology has the potential to replace toxic antifouling coatings and help mitigate the spread of AIS in Minnesota and beyond.

Subproject Results Use and Dissemination

We have disseminated our findings to stakeholders to increase awareness of our technology and allow us to learn about market landscape and end-users needs. In particular, we discussed with lake owner associations leaders at and representative of the Legislature at the AIS Research and Management Showcase. We also have communicated via seminars and presentation with other stakeholders, including Dupont, the MN DNR, the Bureau of Reclamation, and presented our results at the iPrime meeting, an academic-industrial meeting where key stakeholders were present, including 3M, BASF, Evonik and Ecolab. We also used communication services at the Biotechnology Institute to disseminate our results to the public in the form of a blog article and we are preparing two research articles to communicate to the scientific community.

Presentations:

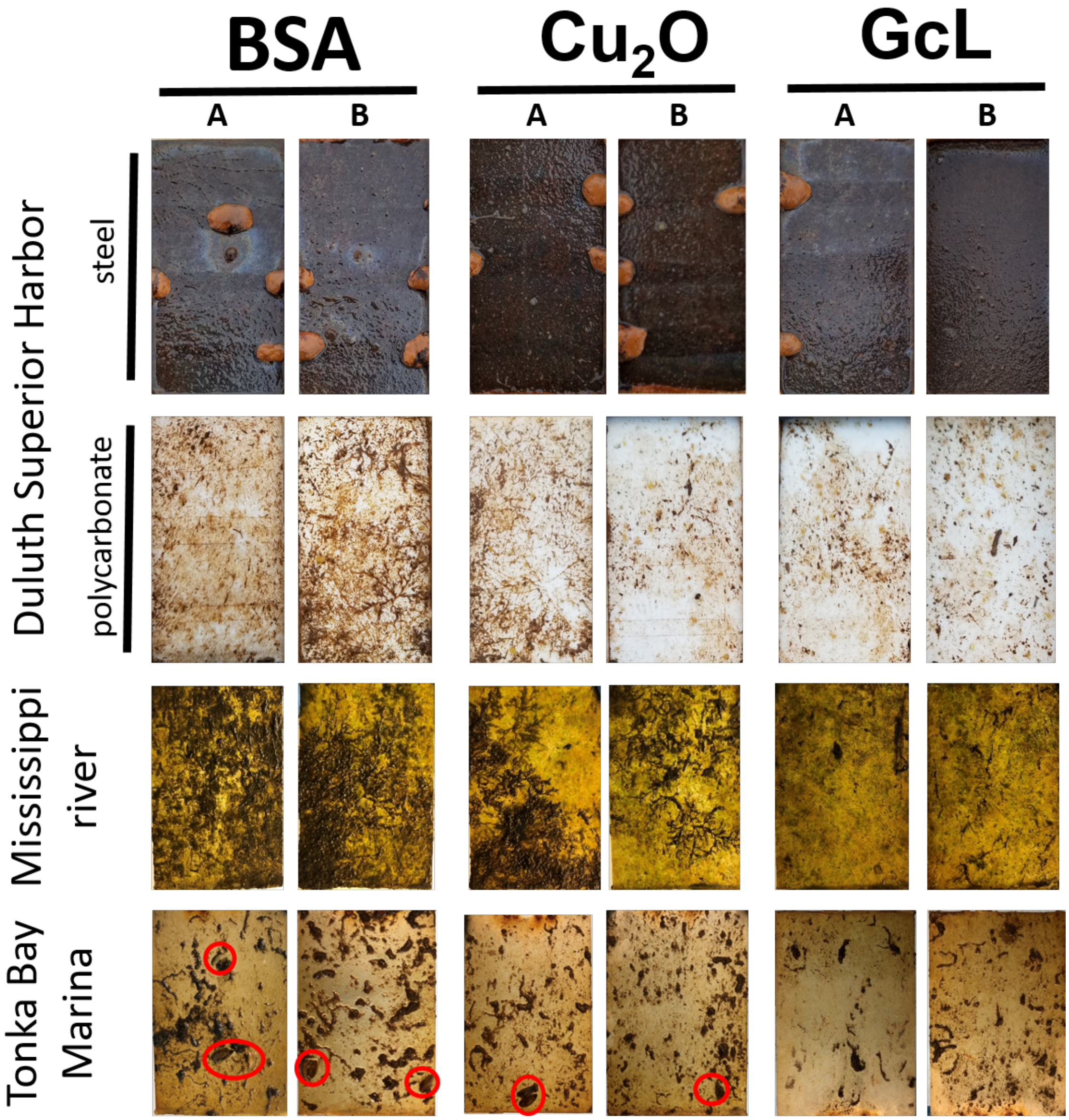
- Huang, Hicks, Elias. Suppressing Microbial Communication to Mitigate the Spread of AIS. 9/18/2019. MAISRC Research and Management Showcase.
- Elias. Interference in Microbial Signaling: a powerful way to control microbes and study their languages. 12/3/2019. Presentation to the bureau of Reclamation.
- Elias. Interference in Microbial Signaling: a powerful way to control microbes and study their languages. 11/18/2019. Presentation to Dupont.
- Elias. New Advances in Controlling Microbial Behaviors by Interfering in Microbial Speech. 8/6/2020. iPrime national meeting.

Media:

- Enzyme-based coatings developed at the University of Minnesota help protect port infrastructure by disrupting the signals underwater bacteria use to communicate. Nick Minor and Kristal Leebrick, Gateway: Signal and Noise. 18 May 2020. https://gateway.bti.umn.edu/2020/05/18/signal-and-noise/

Attachments:

- Figure of results of coupons coated with paint containing control protein.



Sample coupons coated with acrylic base, submerged at the Tonka Bay Marina (Lake Minnetonka), the UMN boathouse (Mississippi River) and the Duluth Superior Harbor. Coupons were coated with paint containing control protein (BSA; 200µg/mL), copper oxide (Copper; 200µg/mL) or our lactonase enzyme (GcL; 200µg/mL) and submerged for 45 days (July-August 2019). In the Duluth Superior harbor, steel and polycarbonate coupons were used. Polycarbonate was used for the other sites. The lactonase embedded coatings shows highest performance in reducing biofouling (the dark dots attached to the coupons) and the adhesion of zebra mussels (circled in red). Replicates (A and B) shows that lactonase-based coating significantly outperforms other testing coatings for biofouling and zebra mussel adhesion.