

2018 Project Abstract

For the Period Ending June 30, 2022

PROJECT TITLE: Restoring Wetland Invertebrates to Revive Wildlife Habitat

PROJECT MANAGER: Dr. Megan Fitzpatrick

AFFILIATION: Minnesota Department of Natural Resources

MAILING ADDRESS: 102 23rd St. NE

CITY/STATE/ZIP: Bemidji, MN 56601

PHONE: 218-308-2284

E-MAIL: megan.fitzpatrick@state.mn.us

WEBSITE: <https://www.dnr.state.mn.us/wildlife/research/index.html>

FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION:

M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

APPROPRIATION AMOUNT: \$400,000

AMOUNT SPENT: \$367,985

AMOUNT REMAINING: \$32,015

Sound bite of Project Outcomes and Results

Our research showed amphipods are particularly sensitive to their wetland environments. High abundances of amphipods useful as wildlife food requires wetlands with high plant diversity and abundance, low concentrations of pesticides, and few fishes. Our work suggests many ways to manage and protect amphipod populations and their high biodiversity habitats.

Overall Project Outcome and Results

Amphipods (shrimp-like aquatic invertebrates) are found in a variety of Minnesota's wetlands and are important food for many species of waterfowl, amphibians, and fish. Two species of prairie amphipods (*Gammarus lacustris* and *Hyalella azteca*) have declined in recent decades and have been linked to decline in the continental population of Lesser Scaup (*Aythya affinis*) ducks. Our project had two objectives: 1) to learn what wetland conditions support high abundances of amphipods, and 2) to assess whether "stocking" amphipods in seemingly high-quality wetlands was successful at establishing new populations.

We surveyed 66 wetlands across western Minnesota for amphipod abundance, fish, aquatic plants, water quality, and surrounding landcover. Key findings included a positive relationship between amphipod abundance and aquatic plant biodiversity, and negative relationships to pyrethroid pesticide levels and several fish species, especially black bullheads (*Ameiurus melas*).

We also collected *G. lacustris* amphipods and stocked them into wetlands at 19 sites. We surveyed amphipods before and up to three years after stocking to assess survival and reproduction. *G. lacustris* were detected in only one stocked site after stocking, suggesting that our stocking methods did not create sustained new populations.

Our results will inform management actions to support wetland quality and wildlife populations of interest to Minnesota's waterfowl hunters, birdwatchers, and other wetland enthusiasts. Our results suggest amphipods will benefit from actions that increase aquatic plant diversity, remove and prevent black bullhead and other fish invasions, and reduce impacts of high intensity agriculture. Management might include drawdowns, fish barriers, upland riparian buffers, and reduced agricultural pesticide use. Further, results from Objective 1 can be used to better target stocking wetlands where *G. lacustris* are likely to thrive and spread on the Minnesota landscape. We are sharing results with natural resource managers and the public via publications, presentations, and depositing data in publicly-accessible repositories.

Project Results Use and Dissemination

We trained, mentored, and provided paid work experience to two successful master's students and 23 undergraduates, producing two theses and five capstone projects. We shared information via MNDNR Research Summaries, two open-access scientific publications describing invertebrate sampling methods and amphipod-aquatic plant relationships, and 16 oral and poster presentations at professional conferences (see YouTube and ResearchGate). We have drafted a third paper and planned four more. Additional outreach included a MNDNR virtual presentation, Minnesota Conservation Volunteer article, Minnesota Public Radio podcast and article, KSTP-TV broadcast, and informal communication with curious members of the public in the field, site managers, and landowners.



Environment and Natural Resources Trust Fund (ENRTF)

M.L. 2018 ENRTF Work Plan Final Report (Main Document)

Date of Status Update Report: August 15, 2022

Final Report

Date of Work Plan Approval: 06/30/2019 and approved revisions on 07/31/2018

Project Completion Date: 06/30/2022

PROJECT TITLE: Restoring Wetland Invertebrates to Revive Wildlife Habitat

Project Manager: Dr. Megan Fitzpatrick

Organization: Minnesota Department of Natural Resources

College/Department/Division: Fish and Wildlife Division

Mailing Address: 102 23rd St NE

City/State/Zip Code: Bemidji, MN 56601

Telephone Number: 218-308-2284

Email Address: Megan.Fitzpatrick@state.mn.us

Web Address: <https://www.dnr.state.mn.us/wildlife/research/index.html>

Location: Statewide

Total ENRTF Project Budget: \$400,000

Amount Spent: \$367,985

Balance: \$32,015

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

Appropriation Language: \$400,000 the second year is from the trust fund to the commissioner of natural resources to assess invertebrate amphipods in wetlands and explore stocking them as a valuable food source for ducks and other wildlife in the Prairie Pothole Region of the state. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

I. PROJECT STATEMENT:

Amphipods are wetland invertebrates that are key food resources for salamanders, fish, water birds, ducks, and geese. Within the past 30 years, amphipods have substantially declined across the Prairie Pothole Region (PPR), and particularly in Minnesota, for reasons unknown. We will document the habitat characteristics that allow amphipods to thrive and assess the stocking of amphipods to help them successfully re-establish.

Amphipods (also called “scuds”) are critical wildlife food, biological indicators of water quality and ecosystem health, and cherished by duck hunters and anglers. Amphipod decline has been noted in Minnesota, and the loss of amphipods have been blamed as a primary reason for decline of duck harvests. Today, amphipods are unevenly distributed across the PPR and some species are absent from most of Minnesota’s wetlands, while few wetlands have an extremely high abundance (habitats we term “**super-wetlands**”). Amphipods are poor dispersers because they cannot fly, and the increasing distance between super-wetlands may make it difficult to establish in new wetlands. Despite the importance of amphipods, the factors that affect their distribution and abundance are poorly understood. Further, conservation groups and private land owners have been stocking amphipods to improve wetland habitats for 25+ years but the success of these efforts have not been well documented.

The overarching goals of this project are to:

- (1) Identify the habitat characteristics of super-wetlands that make them of great wildlife value. This information will outline why amphipods are in decline, why specific wetlands have naturally high abundance of amphipods, and determine how to restore and manage wetlands towards high-quality habitats that promote amphipod and duck use.
- (2) Document the effects of amphipod stocking to improve understanding of the habitat requirements and the utility of stocking. Experimental stockings will enable some control over factors that might influence success (e.g., size of wetland, fish presence) and further aid in understanding the habitat requirements.

Our diverse project team, which will include expert ecologists, two graduate students and many young-career technicians, will provide valuable information regarding wetland habitat quality and management. We have identified several super-wetlands to study and secured a proprietor to stock amphipods, and are requesting funds to study these sites and stocking practices in more detail.

II. OVERALL PROJECT STATUS UPDATES:

Revision July 23, 2018: The budget was reconfigured to reflect a transfer of travel funds to Bemidji State University per an interagency agreement. Nothing else has changed.

Project Status as of January 31, 2019:

Between July 1 2018 and January 15 2019, our research group has met weekly to work towards project goals. We typically meet in-person for two hours, and the agenda ranges from logistical planning to graduate theses development.

The MNDNR’s Contract Specialists were able to establish written contracts with the three contractors outlined in this work plan (Lincoln Bait LCC, Bemidji State University, and the U.S. Geological Survey).

Larson had hired two graduate students who started in summer 2018 (Breanna Keith and Jake Carleen), 3 undergraduate, paid technicians who started in September 2018 (Anna Maria Estes, Alexander Binsfeld, Alissa Chalberg), and mentored 3 volunteers who started in September 2018 (Brad Morris, Ali Chalberg, and Parker Vrolson). The students work daily under Larson’s supervision and mentorship at the Minnesota DNR’s Wetland Research Laboratory in Bemidji. We have made formal job offers and received acceptance to 4 student workers for full-time employment in summer 2019 (Anna Estes, Bemidji State Univ.; Demey Everett, Augsburg University; Kayla Cross, Augsburg University; and Michael Bieganeck, Augsburg University). Additionally, Larson was

promoted to a Research Supervisory role within the MNDNR in November 2018. The MNDNR supported the job transition by ensuring Larson will remain the Project Manager, as well as and by financially supporting and hiring a 3-year term position to assist Larson with the execution of this project and disseminating results quickly (e.g. conducting data analysis, writing papers, giving presentations). This 3-year position is advertised internationally, and we will recruit a full-time professional with an advanced degree for work in March 2019-March 2022.

We have delivered 5 oral presentations, prepared 3 student posters for presentation at professional societies, and had 2 popular media news releases regarding this project.

Amendment as of 06/06/2019: We request that Dr. Megan Fitzpatrick with the Minnesota Department of Natural Resources becomes the Project Manager. The current Project Manager, Danelle Larson, is leaving state service for a federal service Research position, and would remain as one of the primary investigators on the project. Please see details in the PROJECT PARTNERS section below.

Amendment Approved by LCCMR **7/02/2019**.

Project Status as of June 30, 2019

Our research group continues to meet in-person, over the phone, or email at least once weekly. We discuss action items, progress, sampling and data analysis plans. The graduate students interact daily with Larson, Fitzpatrick, and Isaacson.

We have recruited Dr. Emily Schilling from Augsburg University as a strong, new collaborator on the project. Dr. Schilling has expertise in aquatic invertebrate biology, and she is working with us to develop new research avenues and undergraduate coursework related to Activity 1. Dr. Schilling has also provided salary funds through Augsburg University for three undergraduates to engage in summer 2019 employment and new undergraduate research for years 2019-2020. The 2019 summer salary provided by Augsburg has allowed us to recruit additional summer help and freed salary funding for a MNDNR intern.

We added a waterfowl study component to this project, led by Dr. Jim Berdeen and Edmund Zlonis of the MNDNR. We will survey waterfowl at all sites for Activities 1 and 2 in springs 2019 and 2020. All field protocols were established and approved by the research group and external biometricians. Waterfowl surveys were completed for both Activities in spring 2019.

In April 2019, we successfully hired 5 new persons to the research group. Dr. Megan Fitzpatrick was hired as a 3-year term Natural Resource Specialist through the MNDNR to assist Larson with project planning, data analysis, and dissemination. The MNDNR committed full funding for her salary as new, in-kind match directed to this work plan. We also hired four summer technicians (Anna Estes, Bemidji State Univ.; Demey Everett, Augsburg University; Kayla Cross, Augsburg University; and Michael Bieganek, Augsburg University) who work 40 hours per week in the summer to assist with field sampling, data entry, laboratory analysis, and developing related undergraduate research projects. Larson, Fitzpatrick, Schilling, and Isaacson supervise these four technicians.

The two graduate students through Bemidji State University successfully defended their research proposals in March 2019. Jake Carleen's committee members consist of: Danelle Larson (co-advisor); Carl Isaacson (co-advisor); Michael Anteau (member); and Andrew Hafs (member). Breanna Keith's committee members consist of: Danelle Larson (co-advisor); Carl Isaacson (co-advisor); Michael Anteau (member); and Jeffrey Ueland (member). The proposals were oral defended in front of the committee and consisted of ~35 written pages and will be published on BSU's website. The graduate students were required to amend the proposals based on committee comments, and then were signed into approval by the committee.

Larson obtained all required permits to conduct the work (6 permits total).

Our team is on course for accomplishing the goals, which is further described in Activity 1 and 2 Project Status' and the Research Addendum. We continue to engage with the public through media and site visits that is described in the "DISSEMINATION" section below.

Project Status as of September 20, 2019

Our research group continues to meet in-person, over the phone, or email at least once weekly. We discuss action items, progress, sampling and data analysis plans. The graduate students interact daily with Fitzpatrick and Isaacson.

In September 2019, we hired two Bemidji State University undergraduates, Jarret Janu and Henry Egland, to assist with amphipod picking and identification during the fall semester. Anna Estes (summer technician) also remains hired for fall semester. Carleen and Keith have trained the new technicians in sample picking.

Amendment as of 09/20/19: We request three changes our budget.

- (1) We request to transfer travel (housing/hotel) funds from the Bemidji State University (BSU) interagency agreement to the travel funds outside of the interagency agreement.
 - The BSU interagency agreement travel funds would be reduced by \$8,500 to a revised budget of \$4,900.
 - The travel budget external to the BSU agreement would increase by \$8,500 to a revised budget of \$44,066.

We will still use these funds for their original purpose of paying for graduate and undergraduate student housing during field work. We wish to make this change because BSU does not have a good way to pay for hotels/housing during field work. The university requires information about the exact dates and locations where the students will be several months in advance. Providing exact dates this far in advance is difficult because field work is subject to weather and other unpredictable field conditions. If dates are not provided months in advance, students are required to pay for housing themselves and get reimbursed. To avoid asking this of our students, we have been paying for hotels/housing through the project manager's DNR purchasing card. The budget shift will leave \$4,900 for BSU student meals in the travel funds of the interagency agreement, which we will be sufficient based on meal costs so far in the project. Dr. Carl Isaacson, our BSU partner, supports this request.

- (2) We request to hire one full-time Minnesota DNR technician to assist with field and lab work instead of two DNR interns. We are making this request to better support our BSU graduate students. Specifically, our amphipod sampling starts immediately after ice-out, which is usually during the spring semester. Our students found it difficult to balance the demands of their classes and field work during spring semester last year, and they expect a busier class schedule this year. DNR interns (undergraduate students) cannot begin work before the end of their spring semesters, whereas a full-time technician could begin in time to assist with field work at ice-out. This change involves a requested budget shift, described in #3, below.

- (3) We request to shift funds from the supplies budget to personnel.
 - The supplies budget would decrease by \$6,380 to a revised budget of \$38,794.
 - The personnel budget would increase by \$6,380 to a revised budget of \$24,300.

We wish to make this change to support the hiring of one DNR technician instead of two DNR interns, as explained in #2 above. We require additional personnel funds because DNR technicians costs ~\$6/hr more than interns. To pay for these costs, we will use savings accomplished from being able to borrow supplies (e.g. boat

motors) from the Minnesota DNR and from finding that some supplies (plastic and glass sample bottles for dip net and soil samples, respectively) cost less than expected.

Amendment Approved by LCCMR **9/30/2019**.

Project Status as of January 31, 2020

Our team is on course for accomplishing Activity 1 and 2 goals, as further described in Activity 1 and 2 Project Status and the Research Addendum sections. In short, we have successfully completed our first Activity 1 field season. We have entered almost all data into our group Access database and are beginning preliminary data analysis while preparing for our upcoming second field season. We carried out data collection on Activity 2 wetlands in summer and fall. We are currently analyzing lab samples and preparing to stock our final set of basins in the next 1-2 months.

Four undergraduate student technicians completed a successful summer field season: Demey Everett, Kayla Cross, Mike Bieganek, and Anna Medina. Three of these students were an LCCMR match (free labor) provided by our Augsburg University collaborator Emily Schilling.

Our local research group (Fitzpatrick, Isaacson, Keith, Carleen) continues to meet once weekly to discuss action items, progress, and preliminary results, and plan for data collection and analysis. We communicate by e-mail or Skype with Larson at least twice per month.

We have hired former student technician Anna Medina (Estes) as a full-time Natural Resources Technician following her graduation from Bemidji State University. She will leverage her experience with our project methods to assist with leading field crews, training student technicians, and laboratory analysis, and data entry.

We have re-hired our two Bemidji State University undergraduate technicians, Jarrett Janu and Henry Eglund, to continue assisting with amphipod picking and identification during the spring semester.

Project Status as of June 30, 2020

We here give a brief overview of overall project status. More details follow in the Activity 1 and 2 and Dissemination update sections.

Our research group continues to meet in-person, over the phone, or email at least once weekly. We have met more frequently since March, developing plans for pursuing project goals while maintaining crew safety during the COVID-19 pandemic. We comply with safety guidelines established by DNR, Bemidji State University, and Augsburg University.

In January, DNR contributed \$10,000 to our project (LCCMR match) from mid-fiscal year funds in January, to help pay for travel in the remainder of fiscal year 2020.

Carleen, Keith, Medina, and Fitzpatrick presented a poster describing the project at the Minnesota Chapter of the Wildlife Society's Annual Meeting in February, where Carleen and Keith won the conference award for Best Graduate Student Poster.

Prior to the COVID-19 pandemic, we remained on track to achieve project goals for field and lab work. Eglund, Janu, and Medina continued to pick and identify amphipods from Activity 2 fall samples. We finished stocking Activity 2 wetlands with amphipod in late January. We continued data entry into our project databases and carried out preliminary data analysis. We obtained permits and access permissions for 61 new Activity 1 wetland sites in preparation for spring ice melt. We also recruited and hired 3 undergraduate summer research

technicians for Bemidji State University (Alaina Taylor, Megan Howard, and Carson Tembrock), with an anticipated start date of May 11, 2020.

Augsburg University (Emily Schilling) arranged to provide stipends and housing funds (LCCMR match) for two undergraduate summer student workers: Ciashia Shiongyaj and Michael Bieganek. Bieganek worked for us last year and began an undergraduate research project focused on Activity 2 fish surveys.

With the COVID-19 pandemic and Governor's Executive Orders in mid-March, our project progress was restricted. Bemidji State University closed following spring break (March 16) and did not reopen for student researchers until late May, such that we lost approximately 1.5 months of invertebrate picking work from undergraduates Janu and Eglund. However, Medina continued to pick and identify amphipods at the DNR Wetland Wildlife Population and Research Group lab until DNR employees were required to telework in accordance with the governor's Stay-at-Home order.

In accordance with anticipated bans to overnight travel, we selected and obtained access permissions for as many wetlands as possible within day-trip distance of Bemidji. We obtained permission to survey 29 basins for amphipods, waterfowl, fish, and aquatic vegetation for Activity 1. However, graduate students Jake Carleen and Breanna Keith were ultimately not authorized to begin field work on May 7, and Fitzpatrick and Medina (DNR employees) were not authorized to begin field work and lab work until May 27. With the reduced crew size and late start date, we worked to sample as many basins as possible for amphipods and waterfowl within the phenologically appropriate timeframe. We were able to survey 19 Activity 1 basins for amphipods and waterfowl, and we completed spring waterfowl surveys on all Activity 2 basins stocked in winter 2020. However, we did not see scaup (*Aythya affinis* and *A. marila*) at many basins in our surveys. We are particularly interested in assessing scaup relationships to amphipods because previous studies suggest that recent scaup population declines may be tied to amphipod declines. We are not sure whether we did not see many scaup because our surveys were pushed later than this year's peak scaup migration period, or because they were truly not using the wetlands we studied. We will assess our survey dates in relation to other avian sighting data (e.g. the eBird citizen science project) to assess whether our 2020 spring survey dates were appropriate to obtain a dataset truly representative of scaup wetland use.

With Fitzpatrick's return to field and lab work, we are onboarding our three Bemidji State University undergraduates. To date, all have been trained in picking procedures, and they have resumed picking of Activity 2 invertebrate samples in the Bemidji State University labs, where they can work >6 feet apart. We will begin training in field methods shortly. We have arranged Bemidji-area housing for Augsburg students, who will begin field and lab work shortly.

Remaining field tasks include fish sampling at our 19 Activity 1 sites and the remaining Activity 2 sites that we did not sample in summer 2019; as well as aquatic vegetation sampling, water chemistry measurements, and waterfowl brood surveys at our 19 Activity 1 wetlands and our Activity 2 sites. The need to maintain 6-foot social distancing in the field (per MNDNR and Bemidji State University safety protocols) will impact our ability to complete these tasks, as described in more detail for Activity 1 and Activity 2 below. Additionally, all travel costs are doubled due to the need for each member of our 2-person field crews to drive a separate vehicle. (Our field surveys require at least two people for completion.) We will aim to complete field tasks in as many sites as possible within phenologically appropriate timeframes, while maintaining worker safety.

Amendment Request as of October 27, 2020

We are requesting to shift \$15,100 from the U.S. Geological Survey contract line (Dr. Michael Anteau) to the Bemidji State University Interagency agreement to fund each of the project's two Master's students for an additional semester (Spring 2021; \$7,550 per student).

- The US Geological Survey contract line would be reduced from \$32,700 to \$17,600.
- The Bemidji State University Interagency Agreement line would increase from \$214,140 to \$229,240.

COVID-19 has impacted our project in such a way that an additional semester of work by our graduate students (Breanna Keith and Jake Carleen) will be key to helping us achieve our project outcomes. The students have not been able to complete project lab work within our planned timeline due to COVID-19 related issues: a late start to sample collections per Minnesota DNR and Bemidji State University's safety policies earlier in the pandemic, Bemidji State University's temporary lab closure, and child-care responsibilities in light of school/day-care closures and Bemidji State University's policy of not allowing children into laboratory buildings during the pandemic. The students contributed to other aspects of the project via remote, but will need an additional semester to complete responsibilities that require physical presence in the laboratory.

Money is available from our USGS contract line. We budgeted for a \$32,700 contract with amphipod expert Michael Anteau (USGS Northern Prairie Wildlife Research Center) for his work on the project (helping design the study, mentoring the two graduate students, and disseminating findings), with a \$32,700 match from USGS. However, the USGS Northern Prairie Wildlife Research Center is required to use part of the money for overhead (indirect costs), whereas ENRTF funds may not be used for overhead costs. Our DNR grants specialists have been working with USGS on this issue for some time and have not been able to find a solution. That is, USGS will not accept the money if it cannot be used partially for overhead. However, Dr. Anteau and USGS have agreed to continue providing services to the project via additional in-kind support from USGS.

\$17,600 remains in the USGS contract line. Given the reduced sample size of wetlands in the 2020 field season due to COVID-19 (described in our June 30, 2020 status update), we may need to conduct additional sampling in 2020 to achieve our project goals of identifying wetland characteristics that support large amphipod populations and, simultaneously, improving understanding of how amphipod density affects waterfowl use of wetlands. Completing data entry and preliminary data analysis will allow us to identify which wetland characteristics (if any) require further sampling. We are submitting this budget amendment now to ensure that funds are available in the Bemidji State University interagency agreement in time for the beginning of the Spring 2021 semester, pending LCCMR's decision. Following preliminary data analysis, we will assess the extent to which additional sampling is necessary, as informed by preliminary results.

Amendment approved by LCCMR 11/10/2020.

Project Status as of January 31, 2021

We here give a brief overview of overall project status. More details follow in the Activity 1 and 2 and Dissemination update sections.

With the exception of a reduced sample size of field sites due to COVID-19 restrictions (as detailed in the previous status update), our research progress remains on track. Our research group continues to meet at least once weekly via Zoom and communicate regularly via e-mail. All members are complying with COVID-19 safety guidelines established by DNR, Bemidji State University, and Augsburg University.

As of our last status update, we were able to conduct spring field work despite a later-than-expected start due to COVID-19 safety restrictions. After adjusting our methodology, we were able to survey 19 new Activity 1 sites for amphipods and waterfowl, and to conduct all 14 of our planned Activity 2 waterfowl surveys.

With creativity and hard work, we were able to safely complete fish, aquatic vegetation, and waterfowl brood surveys on most of our new Activity 1 basins this summer. We were also able to complete fish, aquatic vegetation, and waterfowl brood surveys on a number of Activity 2 wetlands, and to complete fall amphipod surveys at all but one site.

Our field crews included DNR technician Anna Medina, three undergraduate technicians from Bemidji State University (Alaina Taylor, Megan Howard, and Carson Tembrock) and two undergraduate technicians from Augsburg University (Michael Bieganek and Ciashia Shiongyaj). Medina completed her term with DNR and is now

interviewing for graduate school positions relating to wetlands and fisheries research. Taylor and Bieganek are carrying out undergraduate research projects studying aquatic vegetation and fish communities, respectively, in our stocked wetlands.

Following the field season, we are engaged in picking and identifying invertebrates from our fall field samples. Bemidji State University continues to provide support in the form of additional lab space and equipment, so that our undergraduate technicians can remain socially distanced while working. Our graduate students are making use of their extra semester of support (per our last budget amendment) to finish up laboratory analysis, conduct detailed data analysis and thesis writing (as detailed below). Larson led the writing of a paper that is undergoing internal review at USGS prior to submission for publication in a scientific journal. Several group members shared project methods and preliminary results at a national virtual conference, and we have additional presentations scheduled for February.

We look forward to hearing the legislature's decision about extending deadlines for LCCMR projects impacted by COVID-19.

Amendment Request as of March 2, 2021

As mentioned in previous status updates, COVID-19 safety restrictions reduced the number of basins we were able to survey in spring-summer 2020. We have been analyzing data this winter to determine which (if any) project goals are impacted. Our data analyses show that our reduced sample size of wetlands is such that we are not able to make conclusions about the relationship between waterfowl (particularly scaup) wetland use and amphipod abundance. Consequently, we would like to make two changes. First, we would like to carry out an additional set of amphipod and waterfowl surveys this spring-summer. Second, we would like to extend our graduate students' appointments through the summer semester. Their expert field skills will contribute to the efficiency of our work, and they will advance papers toward publication with further analysis and writing.

Due to COVID-19 impacts, LCCMR has submitted a request for a one-year extension of our grant to the legislature on our behalf. We understand that, if the legislature denies the request, our remaining grant funds will cease to be available after our original project end date of June 30, 2021. The field work and most of the lab work can be completed by June 30. DNR and USGS will provide in-kind support (Megan Fitzpatrick, Danelle Larson, and Michael Anteau's time) to complete lab work, analysis, and writing if the grant extension is denied. The graduate students have been informed that funding beyond June 30 is dependent on legislative decisions, and they will plan accordingly in consultation with their Bemidji State University advisor Carl Isaacson.

Our specific budget requests are as follows:

1. We request to shift \$9,160 from the U.S. Geological Survey contract line (Dr. Michael Anteau) to the travel line. This money is available because USGS agreed to provide Anteau's time as in-kind support. This money will fund travel to study sites for spring waterfowl and amphipod surveys.
2. We request to shift an additional \$458 from the supplies line to the travel line. This money will fund the remainder of our travel to study sites for spring waterfowl and amphipod surveys.
3. We request to shift the remaining \$8,440 from the U.S. Geological Survey contract line to the Bemidji State University Interagency Agreement to fund the project's two Master's students for the summer 2021 semester (\$9,345 per student).
4. The remainder of graduate student salary can be supplemented from the undergraduate salary line in the Bemidji State University Interagency Agreement. We have money available in the undergraduate salary line because we were not able to survey as many wetlands as expected in summer 2020, reducing overall undergraduate personnel time spent in the field and lab.

In consequence:

- The US Geological Survey contract line would be reduced from \$17,600 to \$0.
- The supplies line would be reduced from \$38,794 to \$38,336.
- The Bemidji State University Interagency Agreement line would increase from \$229,240 to \$237,680
- The travel line would increase from \$44,066 to \$53,684.

Amendment approved by LCCMR 3/29/21.

Project extended to June 30, 2022 by LCCMR 6/30/21 as a result of M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18, legislative extension criteria being met.

Project Status as of June 30, 2021:

We here give a brief overview of overall project status. More details follow in the Activity 1 and 2 and Dissemination update sections.

Our research progress remains on track following previously described adjustments for COVID-19. Regarding Activity 1, we successfully completed a third field season to improve our sample size of basins used by scaup, adding 20 basins to the dataset with waterfowl and amphipod surveys. We hired and trained two new Bemidji State University undergraduates (Trevor Loberg and Anna Wetrosky) to participate in this work. We are now engaged in associated lab work (invertebrate picking and identification). Larson, Isaacson, and Fitzpatrick are meeting weekly with graduate students (Jake Carleen and Breanna Keith) to provide comments on thesis drafts. We have also submitted two manuscripts for publication.

We completed lab work and data entry associated with our final season of Activity 2 field sampling for amphipods in stocked and control basins. Basins were stocked with the amphipod species *Gammarus lacustris*. Our results suggest that probability of *G. lacustris* population establishment following stocking is low. As planned, we are conducting data analysis of fish and aquatic vegetation surveys to learn about wetland characteristics supporting *G. lacustris* establishment. Additionally, in light of current results, we are comparing fish and aquatic vegetation in stocked basins to basins that support natural *G. lacustris* populations (Activity 1). One of our undergraduates (Mike Bieganeck, Augsburg University) presented a poster summarizing fish communities in our stocked and reference basins at the international Society for Freshwater Science meeting. Alaina Taylor (Bemidji State University) has submitted a term paper summarizing aquatic vegetation communities in a similar manner for an upper-level research course. Our next step will be combining the two datasets into a single analysis for an overall view of the relationship between amphipod establishment and wetland habitat. This analysis will allow us to decide whether additional management steps (e.g. wetland restoration actions) should be recommended prior to stocking amphipods in the future.

Additionally, DNR has agreed to provide support (\$27,251 plus staff time) for continued monitoring of stocked and control basins for another two years. This funding was provided in light of on-going results from a DNR-stocked lake in southern Minnesota (see Activity 2 update for details). We will produce reports based on the first three years of monitoring (LCCMR Project Outcomes) and refine the recommendations following DNR-funded monitoring.

Project Status as of January 31, 2022:

We here give a brief overview of overall project status. More details follow in the Activity 1 and 2 and Dissemination update sections.

Our research progress remains on track following completion of all LCCMR-funded field work. Highlights from this period include graduate student Breanna Keith's successful thesis defense and new job in the natural resources field. Additionally, Danelle Larson's manuscript describing relationships between amphipods and plant

communities has been accepted for publication in the scientific journal *Biodiversity and Conservation*. Keith continues to lead a manuscript describing our invertebrate sampling methods through the peer review process in pursuit of its publication as a USGS Open-File Report. We also hired four new Bemidji State University undergraduates to assist with timely completion of lab work, and Fitzpatrick has cleaned and summarized our 3-year Activity 2 amphipod dataset in preparation for final statistical analysis.

Megan Fitzpatrick and Anna Wetrosky (Bemidji State University undergraduate technician) made use of DNR in-kind funding (mentioned in our last status update) to conduct follow-up surveys on amphipod-stocked and control basins in August-October. Lab work (invertebrate picking) on these samples is on-going.

Project Status as of March 29, 2022:

Our research progress remains on track. We have nearly completed Activity 1 lab work (one sample remaining) and made substantial progress on our MN DNR-funded, Activity 2 extensions. Breanna Keith continues to lead a manuscript describing our invertebrate sampling methods through the peer review process in pursuit of its publication as a USGS Open-File Report. Fitzpatrick has begun formal statistical analysis of Activity 2 amphipod surveys with a before-after/control-impact model.

Amendment Request as of March 29, 2022

We are requesting funds be shifted from the travel line to fund registration fees and out-of-state travel costs for two group members (Breanna Keith and Danelle Larson) to present our project results at the international Joint Aquatic Sciences Meeting (Grand Rapids, MI, May 14-20, 2022; <https://jasm2022.aquaticsocieties.org/>).

- Travel would be reduced by \$4,924 to \$48,760.
- Two new budget lines would be Travel Expenses Outside of Minnesota at \$3,394 and Conference Costs Outside of Minnesota (i.e., conference registration fees) at \$1,530

We propose to present results of our project at an out-of-state scientific conference - the international Joint Aquatic Sciences Meeting (Grand Rapids, MI, May 14-20, 2022). This conference is a unique opportunity to reach an unusually large and diverse group of scientists and managers, because nine of North America's major aquatic science groups are combining their annual meetings into one large event this year. Groups of particular interest to our work include the American Fisheries Society, Association for the Sciences of Limnology and Oceanography, North American Lake Management Society, Phycological Society of America, Society for Freshwater Science, and Society of Wetland Scientists. Because the conference is being held in an adjacent state, travel costs are reasonable. Because this is an exceptionally large international conference, we will be able to share our results with, and obtain ideas and feedback from, scientists and natural resource professionals both within and outside of Minnesota. This benefits Minnesota (via our project) in two ways. First, it allows us to gather feedback on the project from a wide group of experts in freshwater invertebrate ecology. Ideas and discussion will improve our interpretation of our results, thereby improving our recommendations regarding amphipod management to Minnesota's resource managers. Given that our focal amphipod species range beyond Minnesota, and share characteristics with amphipod populations throughout North America and worldwide, experts from beyond Minnesota will have knowledgeable feedback. Second, we will be able to share project results and forge relationships with resource professionals who may be interested in collaborative amphipod research and management based on our results in the future. Given the migratory nature of avian species that rely on amphipods (such as Lesser Scaup), research and management that improves amphipod populations throughout the Upper Midwest and Canada will benefit Minnesota hunters and bird watchers.

Fitzpatrick has requested in-kind support from MN DNR to attend the conference with Larson and Keith.

Amendment Approved by LCCMR 4/18/2022.

Overall Project Outcomes and Results

Amphipods (shrimp-like aquatic invertebrates) are found in a variety of Minnesota’s wetlands and are important food for many species of waterfowl, amphipods, and fish. Two species of prairie amphipods (*Gammarus lacustris* and *Hyalella azteca*) have declined in recent decades and have been linked to decline in the continental population of Lesser Scaup (*Aythya affinis*) ducks. Our project had two objectives: (1) to learn what wetland conditions support high abundances of amphipods, and (2) to assess whether “stocking” amphipods in seemingly high-quality wetlands was successful at establishing new populations.

We surveyed 66 wetlands across western Minnesota for amphipod abundance, fish, aquatic plants, water quality, and surrounding landcover. Key findings included a positive relationship between amphipod abundance and aquatic plant biodiversity, and negative relationships to pyrethroid pesticide levels and several fish species, especially black bullheads (*Ameiurus melas*).

We also collected *G. lacustris* amphipods and stocked them into wetlands at 19 sites. We surveyed amphipods before and up to 3 years after stocking to assess survival and reproduction. *G. lacustris* were detected in only one stocked site after stocking, suggesting that our stocking methods did not create sustained new populations.

Our results will inform management actions to support wetland quality and wildlife populations of interest to Minnesota’s waterfowl hunters, birdwatchers, and other wetland enthusiasts. Our results suggest amphipods will benefit from actions that increase aquatic plant diversity, remove and prevent black bullhead and other fish invasions, and reduce impacts of high intensity agriculture. Management might include drawdowns, fish barriers, upland riparian buffers, and reduced agricultural pesticide use. Further, results from Objective 1 can be used to better target stocking wetlands where *G. lacustris* are likely to thrive and spread on the Minnesota landscape. We are sharing results with natural resource managers and the public via publications, presentations, and depositing data in publicly-accessible repositories.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Understand the habitat conditions of super-wetlands with amphipods.

Description: We will identify the factors that affect amphipod abundance to understand why wetlands either have high abundance or no or few amphipods present. Factors will include: Landscape variables (e.g., land use, proximity to other wetlands, etc.) and in-lake variables (e.g., water chemistry, plant abundance, predator abundance).

ENRTF BUDGET: \$206,487

Summary Budget Information for Activity 1:

ENRTF Budget: \$206,487
Amount Spent: \$189,960
Balance: \$16,527

Outcome	Completion Date	Actual Completion Date
1. Choose sites and refine field methods	March 2019	December 2018
2. Wetland habitat and amphipod sampling	October 2020	June 2021 (COVID-19 delay)
3. Data processing in laboratory (water chemistry, plant and invertebrate identification)	March 2021	June 2021 (COVID-19 delay)

4. Publications, reports, and presentations	June 2021	June 2022 (with additional publications on-going)
---	-----------	---

Activity 1 Status as of January 31, 2019:

In summer 2018, we visited 120 wetlands as potential field sites for this Activity. We sampled each wetland for amphipod density and made notes regarding other features that would influence site eligibility (e.g. access, land use, etc). As of December 2019, we have identified the wetland sites that we will use for Activity 1. The wetlands will span most of Minnesota, and include forest, prairie, forest/prairie transition, urban, and agricultural settings. Additionally, we refined some field methods, such as sweep netting techniques for the invertebrates.

We created a detailed, written “Standard Operating Procedure” for processing invertebrate samples in the laboratory. This procedure was designed specifically for Minnesota’s wetlands and achieving Activity 1.

Larson has delivered five oral presentations regarding the proposed work in this plan. The audiences ranged from professional societies to wildlife manager meetings. Larson, Carleen, Isaacson, and Keith delivered a Minnesota Public Radio podcast and news article, and a 4 minute KSTP-TV news broadcast. The two graduate students each prepared poster presentations for delivery in February 2019 and the MN American Fisheries Society conference and the MN Wildlife Society conference.

Two volunteers (Brad Norris and Aly Chalberg) had developed side-projects related to this work plan for their undergraduate research capstone project, where they will receive 3 credit hours of coursework. They predicted wetlands with high amphipods are indicators of wetland health because they will have high invertebrate diversity. Brad and Aly have field sampled and lab processed the entire invertebrate community from 8 wetland sites (totaling about 60 hours of volunteer time to-date). They have developed a poster presentation for delivery in February 2019 at the MN Wildlife Society conference.

Activity 1 Status as of June 30, 2019

We selected all sites for Activity 1 and worked closely with biometricians to determine the best ways to select reference sites; this is the same process outlined in the Research Addendum.

We refined and finalized all Standard Operating Procedures (SOP’s) for data collection, laboratory processing, and Quality Assurance/Quality Control measures.

Isaacson and Keith lead a laboratory experiment with Isaacson’s “Ecotoxicology” course at Bemidji State University in Spring 2019. They designed a controlled experiment that exposed amphipods to various concentrations of road salts, and measured mortality and growth over 28 days. The class assisted with the project implementation, was exposed to data analysis, and wrote final reports for grade. Breanna Keith will be repeating the experiment in summer 2019 and including the work in her Master’s thesis.

We sampled 76 basins for Activity 1 in spring 2019. We sampled amphipods and waterfowl at all 75 sites. We are currently returning to these 75 basins in summer 2019 to collect additional data outlined in the addendum, such as plants, fish, land use, sediments, and water quality.

Amendment Approved by LCCMR 9/30/2019

Activity 1 Status as of September 20, 2019

We completed sampling of plants, fish, land use, sediments, and water quality in our 76 basins.

Fitzpatrick created an MS Access database for group data management. Carleen, Keith, Fitzpatrick, and Everett have begun entering fish, amphipod, waterfowl, and vegetation data to the database.

Everett is working on an undergraduate research project, studying the relationship between vegetation and amphipod density in Activity 1 study basins. Schilling and Fitzpatrick are providing mentorship. This project will form the basis of Everett's undergraduate thesis.

Total phosphorous samples have been sent to the Minnesota Department of Agriculture for analysis. Carleen and Keith are measuring chlorophyll-a in water samples under the guidance of Isaacson.

Activity 1 Status as of January 31, 2020

All Activity 1 invertebrate samples have been processed, including picking of all macroinvertebrates and identification/counting of amphipods. We have entered nearly all data (including all amphipod, waterfowl, fish, and aquatic vegetation sampling data) to our group Access database. Preliminary calculations of amphipod densities show that we are finding an appropriately wide range in values to support our statistical analyses, with values ranging from no amphipods to >1,000 amphipods/m³ of water. Larson, Everett, and Schilling continue preliminary data analysis of aquatic vegetation data, while Carleen carries out preliminary analysis of fish data. Fitzpatrick is working with Zlonis to carry out preliminary analysis of waterfowl data. Keith is conducting preliminary analysis of landscape-level data. Keith and Isaacson are completing wetland chemistry analyses on the Bemidji State University campus.

We have identified all super-wetland sites for our second Activity 1 field season. Fitzpatrick is coordinating with USFWS, local MN DNR Wildlife and Fisheries Areas, and private landowners to obtain access permissions and updated permits for sampling this year.

Activity 1 Status as of June 30, 2020

Prior to the COVID-19 pandemic, we proceeded with project plans as described in the previous status update. We carried out preliminary data analysis and prepared abstracts for conference presentations. We also recruited and interviewed Bemidji State University students to work as summer technicians. We successfully hired three students in early March, with plans for them to begin field and lab work in mid-May. The students are Alaina Taylor, Megan Howard, and Carson Tembrock.

We identified 61 basins to sample in spring-summer 2020. Fitzpatrick obtained access permissions from public land managers and updated all sampling permits. Carleen, Keith, and Fitzpatrick obtained access permission from private landowners.

The COVID-19 pandemic constrained our research progress. Bemidji State University closed following spring break (March 16) and did not reopen for student researchers until late May. DNR employees were required to telework ~2 weeks later. Anticipating a DNR ban on overnight travel for employee safety, we obtained access permissions for a new set of 29 basins within ~2 hours of Bemidji, which we could visit during day trips. Graduate students Jake Carleen and Breanna Keith obtained permission to begin field work on May 7. Fitzpatrick and Medina (DNR employees) were not authorized by DNR to begin field work until May 27. Despite the late start to our sampling season and reduced crew size, we were able to sample 19 basins. Carleen and Keith's hard work in mid-May, including ~4 hours of driving each day, was crucial to this outcome. As described in the Project Status section, we will assess our survey dates in relation to eBird citizen science data to assess whether the mandated late start to our field season impacted our ability to accurately assess the relationship between scoup use of wetlands and amphipod density.

With Fitzpatrick's return to field and lab work, we have brought our three Bemidji State University undergraduates onboard. To date, all have been trained in invertebrate picking procedures. They have resumed picking of Activity 2 invertebrate samples in the Bemidji State University labs, where they can work >6 feet apart. We will begin training in field methods shortly.

Augsburg University (Emily Schilling) is providing stipends and housing funds for two undergraduate summer student workers: Ciashia Shiongyaj and Michael Bieganeck. Bieganeck worked for us last year and began an undergraduate research project focused on Activity 2 fish surveys. We have arranged Bemidji-area housing for Shiongyaj and Bieganeck, and they will join our lab and field work shortly.

Our remaining tasks for Activity 1 in this field season include fish surveys, aquatic vegetation surveys, and summer waterfowl brood surveys at our 19 basins in June-August, 2020. Required social 6-foot social distancing (per Bemidji State University and DNR) impacts these types of surveys to different degrees.

The greatest impact is to our fish survey methods. In particular, our large and heavy mini-fyke nets must be retrieved by a single person, rather than by two people grasping the net on either side. (Nets are ~3 feet wide.) The process is also challenging because we work from canoes, rather than larger/sturdier boats. (Canoes are necessary due to the lack of boat launches on the small, often isolated wetlands where we study amphipods.) We are in the process of determining whether net retrieval by a single crew member is physically possible without high risk of injury, and which crew members - undergraduates, or experienced crew members only - are able to participate in fish net retrieval. Unfortunately, we cannot make a change to smaller nets (e.g minnow traps) for this field season. Smaller nets would bias the types and numbers of fish we catch, making our data incomparable to data collected in 2019 and impeding our ability to draw conclusions about the effects of fish communities on amphipods. At present, we plan to collect data on as many wetlands as we safely can during appropriate phenological period for sampling.

Aquatic vegetation survey methods can be carried out with social distancing if we reduce our crew size to 2 people per canoe, instead of 3 people per canoe. The third person's tasks will be assigned to one of the other two crew members. This is likely to slow down our data collection process, but we are still aiming to visit all 19 Activity 1 basins.

On a positive note, the only impact of COVID-19 on waterfowl surveys is the doubled travel costs due to the need for crew members to drive separate vehicles. Our waterfowl methods do not require other alternation.

Activity 1 Status as of January 31, 2021

As of our previous research update, we were working to accomplish field data collection from our 2020 study sites while following COVID-19 safety protocols. As per our previous update, we adjusted our spring field plans in response to the pandemic. Though we were not authorized to begin field work until later in the season than planned, we were able to obtain access permission and sample 19 basins within ~2 hours of Bemidji (avoiding overnight travel) for amphipods and waterfowl within a phenologically appropriate timeframe. Remaining field tasks for summer included fish surveys, aquatic vegetation surveys, and summer waterfowl brood surveys.

Graduate student Jake Carleen and DNR technician Anna Medina, experienced fish surveyors, carried out summer fish surveys in Activity 1 basins while maintaining COVID-19 safety protocols. After careful trial, they found that nets could be retrieved by a single person while maintaining social distancing. This slowed the survey process; however, Carleen and Medina were able to complete surveys in 11 of our basins within an appropriate timeframe (i.e., with timing comparable to the 2019 field season to avoid seasonal bias in our data). Carleen, Medina, and Fitzpatrick also used these surveys to assess how undergraduates could participate in our later Activity 2 fish surveys while maintaining safety. (See Activity 2 status update below.)

We completed training in field and laboratory methods with all undergraduates hired for the season: Alaina Taylor, Megan Howard, and Carson Tembrock (Bemidji State University); and Michael Bieganek and Ciashia Shiongyaj (Augsburg University). Undergraduates picked invertebrates from spring dip net samples at Bemidji State University, where they could work >6 feet apart.

We carried out aquatic vegetation surveys, along with summer waterfowl brood surveys. As our aquatic vegetation survey methods were only slightly slowed by the need to reduce crew size to maintain social distancing, and we were able to involve undergraduate crew members right away, we achieved our goal of sampling nearly all (17) of our basins. Graduate student Breanna Keith provided important field mentorship and plant identification support to undergraduates.

Following the field season, we have completed data entry of spring waterfowl and amphipod surveys, fish surveys, and aquatic vegetation surveys into our shared project database. All dip net samples have been picked and amphipods have been identified, measured, and counted.

Our graduate students are taking advantage of their additional semester of support to finalize laboratory analysis and engage in detailed data analysis. Jake Carleen is engaged in fish survey data analysis and writing his first thesis chapter. Keith is engaged in landscape-level analysis of wetland characteristics, writing her first thesis chapter, and lab work analyzing sediment characteristics. She is also leading a write-up of our group's invertebrate sampling methods for submission as a USGS publication. USGS is contributing \$2,500 of in-kind support for this publication.

During summer-fall, Danelle Larson completed data analysis of aquatic vegetation data. Key new findings included a link between amphipod population density and aquatic plant diversity, along with relationships between amphipod species and plant species. She has written a paper describing the work, and it is currently undergoing internal review at USGS prior to submission to a scientific journal.

Activity 1 Status as of June 30, 2021:

As of our previous research update, we had completed invertebrate picking and data entry associated with the amphipod, fish, and aquatic vegetation surveys conducted in spring-summer 2021. Now, graduate students Jake Carleen and Breanna Keith have completed associated lab work and progressed with data analysis and thesis writing pertaining to the relationship of amphipods to fish communities (Carleen) and landscape-level and water chemistry characteristics (Keith). Larson, Isaacson, and Fitzpatrick are meeting weekly with the graduate students to provide feedback on thesis drafts. Additionally, as mentioned previously, Keith has been leading a write-up of our group's invertebrate sampling methods for submission as a USGS publication (USGS "Open-File Report"). She has completed the manuscript and submitted it to USGS, where it is undergoing review.

As of our last update, Danelle Larson had submitted a manuscript describing links between amphipod population density and aquatic plant communities for internal review within USGS. She has completed post-review revisions and submitted the manuscript to the journal *Conservation Biology*, where it is undergoing peer review.

As per our most recent amendment (3/29/21), we conducted an additional set of waterfowl and amphipod surveys in spring-summer 2021 to improve our sample size and ability to draw conclusions about the relationship between waterfowl (particularly scaup) wetland use and amphipod abundance. Though still restricted to day trips and separate vehicles as a COVID-19 safety precaution, we scouted ~110 basins (plus many more viewed along roadsides en route) in western Minnesota for scaup. We conducted waterfowl surveys on the 25 basins where scaup were observed. This left time to return to most (20) of the basins for amphipod surveys, tripling our sample size of basins with scaup and substantially increasing our sample size for waterfowl surveys in general. We are currently engaged in associated amphipod picking and identification/counting in the

lab. All field data sheets have been scanned and backed up electronically. We will shortly begin data entry to the project database, allowing analysis of the complete waterfowl-amphipod dataset.

We hired and trained two Bemidji State University undergraduates who assisted with spring-summer waterfowl surveys, amphipod surveys, and lab work: Trevor Loberg and Anna Wetrosky. Megan Howard (undergraduate hired last year) also assisted with waterfowl surveys until her graduation in May. Loberg and Wetrosky accepted other jobs for the remainder of the summer (post June 30) due to the delay in the legislature approving LCCMR COVID-19 extensions, but we are happy to report that both accepted offers to continue amphipod picking and identification with us when they return to campus the fall.

Activity 1 Status as of January 31, 2022:

We are happy to report that graduate student Breanna Keith graduated in December after completing lab work, data analysis, writing, and a successful thesis defense. She is now employed at the Anoka County Soil and Water Conservation district, where she is using the skills she developed on this project to manage healthy waters and shorelines in southern Minnesota. Project PIs and Keith continue to meet virtually to plan publication of results. As mentioned previously, Keith has also been leading a write-up of our group's invertebrate sampling methods for submission as a USGS publication (USGS "Open-File Report"). She is now editing the manuscript in response to USGS reviewer comments.

Graduate student Jake Carleen has progressed with data analysis regarding relationships between amphipods and fish communities. He is meeting weekly with Larson and Isaacson to receive comments on thesis drafts.

Our manuscript describing relationships between amphipod population density and aquatic plant communities, led by Larson, has been accepted for publication in the scientific journal *Biodiversity and Conservation*. Important findings include a link between amphipod population density and aquatic plant diversity, suggesting that managing wetlands for aquatic plant diversity would provide quality amphipod for amphipods. Similarly, amphipods could be treated as an "umbrella" species – a species whose protection will extend to a wide range of co-occurring wetland biota. We are excited to formally share these findings with scientists and managers.

As of our last update, we had completed Spring 2021 waterfowl and amphipod surveys (tripling our sample size of sites used by scaup) and were engaged in invertebrate picking from samples. We have completed invertebrate picking and made substantial progress in counting and identifying amphipod species. Undergraduate technicians and Fitzpatrick are continuing lab work.

Undergraduates Trevor Loberg and Anna Wetrosky continued to assist with project lab work in the fall 2021 semester. Loberg graduated in December and is interviewing for jobs in the natural resources field in Minnesota. Wetrosky is continuing lab work with us in spring semester. We also hired four new Bemidji State University undergraduates to ensure that project lab work is completed on schedule: Jacob Engel, Kathryn Cichy, Max Tostenson, and Kyle Schwartz.

Activity 1 Status as of March 29, 2022:

Recent MS graduate Breanna Keith has been leading a write-up of our group's invertebrate sampling methods for submission as a USGS publication (USGS "Open-File Report"). She continues to edit the manuscript in response to USGS reviewer comments, with Larson and Anteau (USGS) now assisting with final stages of editing. Graduate student Jake Carleen continues to meet weekly with Larson and Isaacson to receive comments on thesis drafts.

Undergraduates Anna Wetrosky, Jacob Engel, Max Tostenson, and Kyle Schwartz have continued laboratory work processing amphipod samples. As of our last update, we had completed invertebrate picking of our Spring 2021 samples and begun amphipod identification and counting. We have nearly completed amphipod identification (one sample remaining). Following data entry into our project database, the data from this

additional field season will substantially improve our ability to detect relationships between amphipod abundance and use of wetlands by migratory scoup.

Final Report Summary

Our objective in Activity 1 was to learn what wetland conditions support high abundances of amphipods in the Prairie Pothole Region of Minnesota, with the goal of informing future management and conservation actions. Toward this goal, we surveyed 66 basins across western Minnesota for amphipod abundance, fish and aquatic plant communities, water chemistry and quality (pH, turbidity, chlorophyll-a levels, and pyrethroid pesticide levels), along with GIS-derived measures of surrounding landcover and stream connectivity. Additionally, we explored waterfowl relationships to amphipod abundance by conducting waterfowl surveys while we visited basins to survey amphipod abundance. Field work was primarily completed in spring-summer of 2019 and 2020.

COVID-19 safety restrictions limited the number of basins that we could survey in 2020, reducing the number of sample points available for statistical analysis. We were still able to effectively analyze relationships between amphipods and fish, aquatic vegetation, water quality, and GIS-derived data. However, our waterfowl dataset was significantly impacted. In particular, we had a small sample size of basins used by Lesser Scaup, a species of particular interest because they specialize on amphipods as a food resource. LCCMR granted us a COVID-19 extension to collect and process more data. We surveyed 22 additional basins with Lesser Scaup in spring 2021, tripling our sample size of basins with Lesser Scaup and substantially increasing our sample size in general.

The two most common amphipod species found were *Hyaella azteca* (smaller, more common, higher densities) and *Gammarus lacustris* (larger, less common, lower densities). Some wetlands (<25%) also had amphipods of the *Crangonyx* genus, but their relatively low occurrence precluded statistical modelling. As outlined in our research proposal, approximately half of our study basins were known to have high amphipod abundances based on preliminary surveys, and half were randomly selected. The goal of this study design was to allow assessment of wetlands with very high amphipod densities, despite their relative rarity on the landscape. Our study design was successful. *G. lacustris*, in particular, were present in 54% of our study wetlands, despite being present at a rate of only 9% in randomly-selected basins on the landscape. We were therefore able to evaluate conditions of wetlands with amphipod densities ranging from 0 to 991 per cubic meter of water (*G. lacustris*) and 0 to 1996 per cubic meter of water (*H. azteca*).

Our Activity 1 results have been published as two Master of Science theses and two scientific papers, with additional results and publications in the works. Danelle Larson led publication of a paper in the journal *Biodiversity and Conservation*, which described relationships between amphipods and aquatic vegetation. Key new findings indicate that aquatic plant diversity directly supports high amphipod densities, or that wetland conditions favoring high amphipod densities also favor high plant diversity. The results suggest that amphipods may be used as ‘indicator’ species of high-quality habitats, and as ‘umbrella’ species (whose conservation focus can extend to other life, like aquatic biodiversity). Our aquatic vegetation work also composed an undergraduate capstone project for Demey (Everett) DeJong.

Jake Carleen successfully defended his master’s thesis describing relationships between fish communities and amphipods in June 2022. Most shallow prairie pothole wetlands were historically fishless, but fish have invaded many prairie potholes due to human activity and heightened water levels (increased wetland connectivity and reduced winterkill) associated with consolidation drainage in the 20th century. Important findings from fish community analysis include a strong negative association between black bullheads and both amphipod species (*G. lacustris* and *H. azteca*). In addition to preying directly on amphipods, black bullheads are benthivores that stir up bottom sediments while feeding. Their activity often increases water turbidity and decreases aquatic plant abundance and diversity. Fathead minnows, which can both consume amphipods and compete with them for food resources, were negatively related to *G. lacustris* abundance when they (fathead minnows) occurred at

high densities. Brook Stickleback (amphipod predators) were also negatively related to both amphipod species. Management to benefit amphipods might include protecting remaining basins with high amphipod density from fish invasion and removing fish from other basins. Carleen, Larson, Isaacson, Anteau, Keith, and Fitzpatrick met to discuss publication of Carleen's thesis work after his defense. We plan to develop the thesis into 1-2 scientific papers for publication.

Breanna Keith also successfully completed a master's thesis (October 2021) focused on relationships between amphipod abundance and several measures of wetland condition, including pyrethroid levels and GIS-derived measures of landcover and stream connectivity. Water quality analyses revealed that *G. lacustris* and *H. azteca* density were both negatively related to pyrethroid concentrations. This may indicate a direct lethal or sublethal impact of pyrethroid pesticides on amphipods, and/or an indirect effect (e.g., basins with higher pyrethroids may be likely to also contain additional pesticides or other disturbances stemming from intensive agricultural practices.) *G. lacustris* also exhibited a negative relationship with connectivity to stream systems, which may influence amphipods by facilitating fish invasion or increased agrochemical loading. We plan to develop Keith's thesis into 1-2 publications. Keith, Larson, Isaacson, Anteau, and Fitzpatrick have begun a series of regular virtual meetings to organize and collaborate on this effort, with support from MN DNR and USGS.

Together, our results suggest that amphipods in Minnesota's prairie potholes would benefit from management actions to increase aquatic plant diversity, remove fish, prevent fish invasion of remaining amphipod-rich basins, and reduce disturbances related to intensive agriculture. Actions might include drawdowns (benefiting aquatic plants and removing fish), removal of hydrological connections, installation of fish barriers, piscicide treatments, maintenance of riparian buffer zones of non-cultivated vegetation, and changing agricultural practices to reduce the need for pyrethroid use.

Our waterfowl data have been completely entered into the project database, but we have not yet analyzed the full dataset. Fitzpatrick will turn to that dataset after submission of the Activity 2 paper currently in preparation. (See Activity 2 Final Report.) MN DNR will fund Fitzpatrick's time to bring the analyses to publication.

Our research team plans to prepare data analysis and publication describing the relative impact of environmental factors impacting amphipods. 'Ranking' the most important environmental variables we know now are important from achieving Objective 1 (e.g., pyrethroids, plant diversity, fish abundance, landcover, etc.) will help resource managers prioritize actions to protect amphipods and their habitats.

ACTIVITY 2: Assess amphipods after stocking in natural and restored wetlands.

Description: We will experimentally stock amphipods in wetlands to determine if they will survive the transplant, reproduce, and become a sustaining population for at least 2 years. We will stock amphipods in two different years ("Trial stockings #1" in January 2018 and "Trial stockings #2" in January 2019) and sample for 2-3 years following the stockings. We will assess rates that amphipods naturally recolonize and reproduce in suitable wetlands relative to stocked wetlands.

ENRTF BUDGET: \$193,513

ENRTF Budget: \$193,513

Summary Budget Information for Activity 2:

Amount Spent: \$178,025

Balance: \$15,488

Outcome	Completion Date	Actual Completion Date

1.	Trial stockings #1 (in-kind MNDNR project secured FY 17-18)	February 2019	October 2018
2.	Trial stockings #2 (this proposal)	February 2020	January 2020
3.	Monitor amphipods populations for 2-3 years after stocking	January 2021	October 2020
4.	Data analyses	April 2021	May 2022
5.	Publications, reports, and presentations	June 2021	June 2022 (with additional publications on-going)

Activity 2 Status as of January 31, 2019:

In January-March 2018 (prior to this appropriation), we stocked 11 wetlands with amphipods across Minnesota following the procedures outlined in the Research Addendum. In September 2018, we re-visited and sampled these sites for amphipod density. Although these activities were funded through the MNDNR, we will use this data to further inform Activity 2 by increasing the number of wetlands we stock.

In fall 2018, we processed all the invertebrate samples for Objective 1, which was a total of 22 wetlands. We field sampled and lab processed invertebrate samples from 14 wetlands that will be stocking sites for Objective 2. We did laboratory processing for all the samples to-date, which was information from 36 wetlands and resulted in ~216 samples. To process all the samples took ~220 hours, which was executed by the paid undergraduate technicians. In order to reduce laboratory time while still achieving good results, we created a "Standard Operating Procedure" for processing invertebrate samples. The future lab processing will be more time efficient with the revised procedure and experienced technicians.

Activity 2 Status as of June 30, 2019

In January-March 2019, we stocked 8 wetlands with amphipods across Minnesota under contract with Lincoln Bait LLC. The amount of amphipods stocked totaled 200 gallons. We returned to the stocked sites to assess survival into spring 2019 and sample waterfowl use.

We are entering data for Activity 2 into databases and archiving, which will be complete by July 2019.

Activity 2 Status as of September 20, 2019

Fitzpatrick created an MS Access database for group data management.

Bieganek and Estes sampled fish in twelve Activity 2 basins during the summer. The remainder of Activity 2 basins will be sampled for fish next summer. Bieganek is now working on an undergraduate research project studying the relationship between amphipod density and fish in Activity 2 basins. Schilling and Fitzpatrick are providing mentorship.

Keith, Carleen, and Fitzpatrick have begun amphipod and water chemistry sampling in Activity 2 basins stocked in winter 2017-18 and winter 2019-20.

Fitzpatrick is communicating with federal and state land managers to compile a list of recommended wetlands for stocking in winter 2019-20.

Activity 2 Status as of January 31, 2020

Fitzpatrick, Keith, and Carleen completed resampling of amphipods and water chemistry in Activity 2 basins stocked in winter 2017-18 and winter 2019-20. Janu, Egland, and Medina are processing Activity 2 macroinvertebrate lab samples, including picking invertebrates and counting/identifying amphipods.

Fitzpatrick, Keith, and Carleen have identified seven basins to stock this winter, along with seven reference basins, and collected pre-stocking amphipod samples from all wetlands. Fitzpatrick obtained permits and access permissions for this year's stocking. Barry Thoele stocked our first six wetlands in mid-January, with Medina and Fitzpatrick collecting field data.

Activity 2 Status as of June 30, 2020

Prior to the COVID-19 pandemic, we proceeded with project plans as described in the previous status update. Barry Thoele successfully stocked our seventh and final wetland with amphipods, with Medina and Fitzpatrick recording field data. Janu, Egland, and Medina continued processing Activity 2 macroinvertebrate lab samples, including picking invertebrates and counting/identifying amphipods.

We recruited and interviewed Bemidji State University students to work as summer technicians. We successfully hired three students in early March, with plans for them to begin field and lab work in mid-May. The students are Alaina Taylor, Megan Howard, and Carson Tembrock.

The COVID-19 pandemic constrained our research progress. Bemidji State University closed following spring break (March 16) and did not reopen for student researchers until late May. Consequently, Janu and Egland ceased invertebrate picking for the remainder of the spring semester. Medina continued to pick and identify amphipods at the DNR Wetland Wildlife Population and Research Group lab until DNR employees were required to telework in keeping with the governor's Stay-at-Home order. Janu and Egland could not help during this time because there was insufficient lab space for workers in the DNR lab to remain 6 feet apart. Other DNR work spaces were not available outside our group office due to similar space constraints.

Graduate students Jake Carleen and Breanna Keith were authorized to begin field work on May 7. Fitzpatrick and Medina (DNR employees) were not authorized to begin field work and lab work until May 27. However, we completed spring waterfowl surveys on the remaining 14 Activity 2 wetlands by 6/2/2020.

With Fitzpatrick's return to field and lab work, we are onboarding our three Bemidji State University undergraduates. To date, all have been trained in picking procedures, and they have resumed picking of Activity 2 invertebrate samples in the Bemidji State University labs, where they can work 6 feet apart. We will begin training in field methods shortly.

In addition, Augsburg University (Emily Schilling) is providing stipends and housing funds for two undergraduate summer student workers: Ciashia Shiongyaj and Michael Bieganek. Bieganek worked for us last year and began an undergraduate research project focused on Activity 2 fish surveys.

Our remaining tasks for Activity 2 in the summer field season include fish surveys, aquatic vegetation surveys, and summer waterfowl brood surveys. Required social 6-foot social distancing (per Bemidji State University and DNR) impacts these types of surveys to different degrees.

As detailed in the Activity 1 status update, the greatest impact is to our fish survey methods. At present, we plan to collect data on as many Activity 2 wetlands as we safely can during the appropriate phenological period for sampling. Unfortunately, this is unlikely to be full remaining set of stocked/reference basins even if solo fish netting can be safely achieved, due to the need to wait for sampling days with low wind speeds. A single person cannot prevent the canoe from blowing across the water while keeping both hands on the fish net and processing the catch.

As detailed in the Activity 1 status update, aquatic vegetation survey methods can be carried out with social distancing via reducing crew size. Reduced crew size will slow our sampling and reduce the number of basins we

can sample per day. We are unlikely to be able to sample all Activity 2 basins for vegetation, but we will sample as many as possible within the phenological time constraints.

Activity 2 Status as of January 31, 2021

As of our previous research update, we were working to accomplish field data collection from our 2020 study sites while following COVID-19 safety protocols. As per our previous update, we accomplished our goal of completing spring waterfowl surveys on the remaining 14 Activity 2 wetlands as soon as possible after we were authorized to complete field work. Remaining field tasks for Activity 2 in the summer field season included fish surveys, aquatic vegetation surveys, and summer waterfowl brood surveys.

As mentioned in the Activity 1 status update, Jake Carleen and Anna Medina (experienced fish surveyors) and Megan Fitzpatrick used Activity 1 fish surveys to assess how undergraduates could participate in Activity 2 fish surveys while maintaining safety. In particular, we hoped to find a way for Michael Bieganeck (Augsburg University) to complete a second season of field data collection for his undergraduate research project studying fish communities in stocked wetlands. Carleen and Medina found that nets could be retrieved by a single person to maintain social distancing. After discussion with co-PIs, Fitzpatrick purchased canoe stabilizers to make this process easier for less experienced surveyors, and Emily Schilling purchased waist-high waders (in-kind support from Augsburg University) for improved mobility compared to chest waders used in past fish surveys. Bieganeck and Fitzpatrick worked as team to complete surveys at 11 Activity 2 basins, with one person pulling nets and the other providing support from a solo canoe. Carleen and Medina provided support in training and fish identification. Fitzpatrick was able to coordinate vegetation and fish surveys on Activity 2 basins such that each of the Bemidji State University undergraduates was able to participate in at least one fish survey, despite the more complicated field procedures.

As mentioned in the Activity 1 status update, aquatic vegetation survey methods were slightly slowed by reduced crew size to maintain social distancing. However, we were able to sample 19 basins for aquatic vegetation and summer waterfowl broods.

Undergraduate Alaina Taylor (Bemidji State University) began a research project studying aquatic vegetation communities in stocked wetlands. This project will fulfill her research requirement for an upper-level class at Bemidji State University, taught by Dr. Andrew Hafs. Her goal is to publish the work on Dr. Hafs's website. During summer, we worked with her to explore background information in the scientific literature, formalize research questions, and prepare a research proposal for Dr. Hafs.

Following the summer field season, Taylor has completed aquatic vegetation data entry into our shared project database. She is currently working with Fitzpatrick on data QA/QC and beginning data analysis. Bieganeck is currently engaged in entering fish survey data and is preparing to begin data analysis.

We accomplished our project goal of surveying all 41 of our Activity 2 basins for amphipods in fall 2020, with the exception of one site. To maintain safety, surveyors traveled in separate vehicles to all basins, maintained social distancing, and accomplished all surveys in day trips (no overnight travel). Bemidji State University undergraduates Taylor, Howard, and Tembrock took advantage of flexible online class scheduling to participate in fall field surveys.

With the large number of samples acquired through fall surveys, we recruited and hired two new Bemidji State University undergraduates to assist with lab work: Elizabeth Wilson and Alyssa Hauser. Taylor, Howard, Wilson, and Hauser have nearly completed invertebrate picking from samples. We recently trained Taylor and Howard in amphipod identification, measuring, and counting procedures, and they have begun collecting data to calculate amphipod density from this fall's samples.

Activity 2 Status as of June 30, 2021:

As of our previous update, we had completed fish and aquatic vegetation surveys in amphipod-stocked and control basins for summer 2020 and begun data analysis. Michael Bieganek (Augsburg University undergraduate) has since completed preliminary data analysis of fish communities, which he presented at the Society for Freshwater Science meeting in May. His results indicate that benthivorous fish (such as black bullheads) may have suppressed *G. lacustris* establishment in some stocked basins. However, additional factors (aquatic vegetation, water depth) will be necessary to understand stocking outcomes in other basins. Alaina Taylor (Bemidji State University undergraduate) has completed data entry and preliminary analysis of aquatic vegetation surveys of stocked basins, which she presented in a term paper for an upper-level class at Bemidji State University. Our next step will be to combine information from the two projects into a new analysis.

Megan Howard and Alaina Taylor (Bemidji State University undergraduates) completed amphipod identification, counting, and measuring from our fall 2020 dip net samples. Howard, along with undergraduates Trevor Loberg and Anna Wetrosky (hired as mentioned in Activity 1 update) completed amphipod data entry into the project database in May.

As of our final sampling, we have detected *G. lacustris* in 2 of our 22 stocked basins, and detection has been intermittent in the 2 basins with *G. lacustris* present due to low population density. This suggests that probability of *G. lacustris* population establishment following stocking is low. However, based on on-going amphipod monitoring in another lake stocked by DNR several years ago (Smith Lake in Wright County), *G. lacustris* may be persisting in low/undetectable numbers in some of our basins and simply require ~4 years to reproduce to detectable levels. Smith Lake was stocked in March 2016, with *G. lacustris* first detected at the stocked site in spring 2019. Consequently, DNR has agreed to provide support (\$27,251 plus staff time) for continued monitoring of stocked and control basins for another two years.

In the meantime, we will continue analysis of aquatic vegetation and fish surveys in relation to our Activity 1 surveys of basins supporting naturally-occurring *G. lacustris* populations. Wetland restoration activities prior to stocking may be required for stocking success, pending our results. We will share results based on our first three years of data (LCCMR Project Outcomes), and refine recommendations if necessary following our additional two years of DNR-funded amphipod surveys.

Activity 2 Status as of January 31, 2022:

As of last update, we had completed our final LCCMR-funded field sampling of Activity 2 basins, having found *G. lacustris* in 2 of our 22 stocked basins. We had also obtained DNR funding for continued monitoring of stocked and control basins for 2 years. We will report results based on our first three years of data (LCCMR Project Outcomes), and refine recommendations following our additional two years of DNR-funded amphipod surveys, if necessary.

Fitzpatrick and Anna Wetrosky (Bemidji State University undergraduate) conducted fall 2021 amphipod surveys. As mentioned in the Activity 1 status update, we hired four additional Bemidji State University undergraduates (Jacob Engel, Kathryn Cichy, Max Tostenson, and Kyle Schwartz) to assist with lab work. We are progressing with invertebrate picking from fall 2021 samples using our DNR funding.

Simultaneously, we have completed data quality control measures and calculated quantitative amphipod densities of *G. lacustris* and other amphipod species (*Hyaella*, *Crangonyx*) in all basins from the first three, LCCMR-funded years of the study. The clean dataset is now ready for formal statistical analysis. We will conduct our proposed before-after/control-impact model of amphipod stocking, as well as analyze stocked *G. lacustris* establishment vs. extinction in relation to fish communities, aquatic vegetation, and water chemistry. These analyses will incorporate fish and aquatic vegetation survey data collected by Mike Bieganek (Augsburg University undergraduate) and Alaina Taylor (Bemidji State University undergraduate) for their undergraduate research projects.

Activity 2 Status as of March 29, 2022:

As of our last update, undergraduates Anna Wetrowsky, Jacob Engel, Max Tostenson, and Kyle Schwartz were picking invertebrates from our Fall 2021 dip net surveys. Invertebrate picking is now complete. Amphipod identification and counting will begin following completion of the final Activity 1 sample mentioned above. (This extended work for longer-term monitoring of amphipod-stocked basins is funded by MN DNR.)

We continue to work toward analysis and publication of results based on our first three years of data (LCCMR Project Outcomes). Fitzpatrick, Anteau, Larson, and Isaacson have met to begin paper planning. Fitzpatrick has begun formal statistical analysis using the before-after/control-impact model described in the research proposal, along with a paper outline.

Final Report Summary

Our objective in Activity 2 was to assess the efficacy of transplanting amphipods from donor basins to create new populations in recipient basins ("stocking" amphipods). Toward this goal, we transplanted amphipods (species *Gammarus lacustris*) from local source populations into 19 recipient sites (22 individual basins) in western and central Minnesota. We stocked amphipods into 10 sites in 2018, 5 in 2019, and 7 in 2020. We evaluated transplant outcomes using a before-after/control-impact experimental design. That is, we surveyed amphipod abundance in each basin the year before stocking, and in every year thereafter to the end of the study. Each recipient site included a control basin, which we surveyed to account for any naturally-occurring variation in regional amphipod abundance during the project period.

We measured water chemistry (pH, turbidity, chlorophyll-a levels) in our study basins. We also surveyed fish and plant communities at a subset of the basins (fish at 14 stocked and 11 control basins; aquatic plants at 12 stocked and 8 control basins). COVID-19 restrictions to field work in 2020 prevented us from surveying the full set of Activity 2 basins for fish and aquatic plants. However, this subset provided us with an estimate of the range of basin conditions in our study. Additionally, the fish and aquatic plant community surveys served as undergraduate capstone research projects for undergraduates Mike Bieganek (Augsburg University) and Alaina Taylor (Bemidji State University).

G. lacustris were only detected in one site (two individual basins) following transplant, suggesting that transplants using our project methods rarely establish new populations. *G. lacustris* density in this basin increased through time but remained relatively low (<20 *G. lacustris* per cubic meter of water) within the first 1-3 years post-stocking.

One caveat to our results is that they are based on amphipod surveys conducted within three years following stocking. It is possible that *G. lacustris* exist in additional basins in low numbers that our dip nets did not capture and will reach detectable numbers through additional cycles of reproduction. Consequently, MN DNR is providing in-kind support for two additional years of surveys, with the oldest study basins surveyed in Fall 2021 (5 years post-stocking) and the remainder surveyed in Fall 2022 (4-5 years post-stocking). Fall 2021 surveys did not reveal *G. lacustris* in any additional basins. Fall 2022 surveys will be conducted in September-October 2022.

Though we found *G. lacustris* in only one site post-stocking, this project – the first thorough test of amphipod stocking, with trials at multiple sites and thorough quantitative assessment – provides valuable information for managers and an important baseline for future tests. We affirmed that >97% of *G. lacustris* were alive without visible injury immediately prior to release in recipient sites, and some (approximately 25%) maintained or commenced reproductive behavior (precopulatory amplexus with mates). Consequently, our results may indicate a need for a more targeted method of recipient basin selection, with ideal habitat conditions for *G. lacustris* to survive and reproduce. In this project, we selected recipient basins based on natural resource manager suggestions and private landowner requests fulfilling relatively generic criteria (water year-round with >1 m average depth to avoid amphipod desiccation or winterkill, absence of large predator fish

such as adult walleye or carp). However, information gained from Activity 1 (relationships of amphipod abundance to wetland plants, fish, stream connectivity and pyrethroid levels) could be used to target recipient basins where *G. lacustris* are predicted to thrive in the future. Together, our results from Activity 1 and Activity 2 are an important step toward improving our ability to manage and restore amphipods to the Minnesota landscape.

The unexpected nature of our results, and associated desire to organize and conduct longer-term amphipod sampling, slowed our progress toward publication. However, we have completed a draft paper describing our stocking study and will submit it for publication following within-group edits, to be completed as soon as possible. For our next step, we will explore the utility of a model predicting *G. lacustris* abundance based on combined Activity 1 results. MN DNR, BSU, and USGS will provide staff time to complete these analyses and publications.

IV. DISSEMINATION:

Description:

Danelle Larson will be responsible for data management throughout the project. The water samples will be stored at the Bemidji Wetland Research Station and not discarded until all data is published. Amphipod specimens will be preserved in 70% ethanol and permanently maintained at the Bemidji Wetland Research Station. The data will be permanently archived online using a service such as Dryad.

The Project Research Addendum will be added and updated 2x per year on Danelle Larson's profile at Research Gate (www.researchgate.net). All presentations and publications will be added to Research Gate when completed.

We will prepare at least 3 peer-reviewed publications in scientific journals with open-access (i.e., freely available to the public).

We will deliver at least 3 poster or oral presentations at professional conferences and MN DNR research and wildlife manager meetings.

Status as of January 31, 2019:

Larson has stored all amphipod and other invertebrate specimens in labeled jars with 70% ethanol in the Bemidji Wetland Research Lab. Metadata for the labels are stored with the specimens, as well as in the written Standard Operating Procedure.

Larson has photocopied all field data sheets twice (3 paper copies of the data). Larson preserves the original data sheets, the graduate students house a copy of the data sheets, and Larson has a paper copy stored off-site. Further, all electronic data is temporarily stored on Larson's computer and the graduate student computers, and is permanently archived on a MNDNR server and Larson's Google Drive.

On Research Gate, Larson has promoted the Research Addendum, oral presentations, and posters.

Larson has delivered five oral presentations, and the audiences ranged from professional societies to wildlife manager meetings. Larson, Carleen, Isaacson, and Keith delivered a Minnesota Public Radio podcast and news article, and a 4 minute KSTP-TV news broadcast. The two graduate students each prepared poster presentations for delivery in February 2019 and the MN American Fisheries Society conference and the MN Wildlife Society conference. Two undergraduate volunteers have developed a poster presentation for delivery in February 2019 at the MN Wildlife Society conference.

Status as of June 30, 2019

Larson has photocopied all field data sheets twice (3 paper copies of the data). Larson preserves the original data sheets, the graduate students house a copy of the data sheets, and Larson has a paper copy stored off-site. All electronic data is temporarily stored on Larson's computer and the graduate student computers, and is permanently archived on a MNDNR server. In addition, we now have a FTP website managed by MNDNR that all collaborators can access the data.

In spring 2019, the group gave many oral and poster presentations. Carleen presented a poster at the MN Chapter of American Fisheries Society. Keith delivered a poster presentation to the MN Chapter of the Wildlife Society. Brad Morris and Ali Chalberg (BSU undergraduates) delivered an oral presentation to BSU's Student Achievement conference, and a poster to the MN Chapter of the Wildlife Society.

Carleen and Keith published a 2 page "Amphipod Profile" in the Minnesota Conservation Volunteer magazine.

Larson had a podcast and news article published on the Minnesota Public Radio broadcast and website related to Activity 2.

Status as of September 20, 2019

Fitzpatrick, Keith, and Carleen continue to scan all field data sheets. Scanned copies are stored on a hard drive, Fitzpatrick's computer, and an MNDNR server which is backed up daily.

Status as of January 31, 2020

Fitzpatrick continues to store all amphipod and other invertebrate specimens in labeled jars with 70% ethanol in the Bemidji Wetland Research Lab. Data sheets are scanned and stored on Fitzpatrick's computer, a back-up hard drive, and the MNDNR server via the FTP site. Project Access databases are housed on a server backed up daily by MN DNR.

Fitzpatrick gave a field presentation to LCCMR staff during their summer site visits. Mike Bieganek delivered an oral presentation at the Augsburg University Biology symposium during the Fall 2019 semester. Demey Everett presented at poster at the Augsburg University Biology symposium as well as the Student Conference on Conservation Science at the American Museum of Natural History in New York, NY. Keith presented background information, objectives, and methods for landscape-level analyses to undergraduates in a Landscape Ecology course at Bemidji State University. Keith, Fitzpatrick, and Carleen are registered to present a project poster at The Wildlife Society (Minnesota Chapter) annual conference.

Status as of June 30, 2020

Keith, Carleen, Medina, and Fitzpatrick presented a poster describing our project and preliminary results at the Minnesota Chapter of the Wildlife Society annual conference Feb. 18-20 in Willmar, MN. Carleen and Keith won the award for Best Graduate Student Poster. We discussed the project one-on-one with a number of Minnesota wildlife managers and researchers.

Keith, Carleen, Larson, Fitzpatrick, and Bieganek (Augsburg University) each submitted an abstract for a poster or oral presentation at the Society for Freshwater Science annual meeting in June 2020. This conference was canceled due to COVID-19. We resubmitted abstracts to the North American Lake Management Society meeting, which was scheduled for Nov. 16-20, 2020. This conference has recently been canceled, but we will pursue the opportunity to present at a virtual version of the conference.

Status as of January 31, 2021

As of our last status update, several group members had submitted abstracts to the North American Lake Management Society's annual conference. The conference was canceled, but rescheduled as a virtual conference (Nov. 16-20, 2020). Keith presented a poster describing Activity 1 work and preliminary results, including amphipod surveys, landscape-scale analysis, and fish surveys. Bieganek (Augsburg University) presented a poster describing his undergraduate project studying Activity 2 fish communities. Larson gave an oral presentation describing her recent work relating aquatic plant communities to amphipod abundance. Fitzpatrick gave an oral presentation describing the amphipod stocking project. All presenters were able to virtually interact with lake managers and researchers throughout the meeting.

Fitzpatrick gave an oral (virtual) presentation of Activity 1 and Activity 2 research to DNR managers and cooperators on 1/27/21. 236 people attended the presentation. It generated much interest and on-going follow-up discussion with managers. Fitzpatrick also submitted an abstract for an oral presentation to the Midwest Fish and Wildlife Conference (virtual), which will be held in early February.

We created a [YouTube channel \("Minnesota Amphipods"\)](#) for the project, and both oral presentations from the North American Lake Management Society meeting are posted there.

As mentioned in the Activity 1 status update, Larson has submitted a paper describing relationships between amphipod abundance and aquatic plant communities for internal review at USGS, in preparation for submission to a scientific journal.

Status as of June 30, 2021:

Fitzpatrick gave an oral presentation of Activity 2 research at the annual Midwest Fish and Wildlife Conference (Feb. 1-5, 2021; virtual). She shared stocking methods and preliminary results and answered questions from regional fish and wildlife managers.

Larson submitted a manuscript describing links between amphipod population density and aquatic plant communities to the journal *Conservation Biology*, where it is undergoing peer review.

Breanna Keith has submitted a manuscript describing our invertebrate field and lab sampling methods to USGS for publication as a USGS "Open-File Report". If accepted, our methods will serve as a reference for future studies sampling amphipods and wetland invertebrates with dip nets.

Keith also delivered an oral presentation summarizing Activity 1 research ("Investigating the Common Characteristics of Minnesota's Amphipod-Rich Depressional Wetlands") to the Minnesota Space Grant Consortium in March.

Mike Bieganek (Augsburg University undergraduate) presented a poster describing Activity 2 fish surveys at the annual Society for Freshwater Science meeting (May 23-27; virtual). He communicated methods and preliminary results regarding relationships between fish communities and amphipod establishment to an international audience of freshwater science researchers.

Ciashia Shiongyaj (Augsburg University undergraduate) and Emily Schilling also presented a poster at the Society for Freshwater Science meeting. Their poster described a course-based undergraduate research experience (CURE) that Dr. Schilling led at Augsburg University over the past year. In the course, Shiongyaj and other students developed research skills by using our project's amphipod sampling methods to investigate spatial heterogeneity in amphipod populations at field sites near the Augsburg campus.

Status as of January 31, 2022:

Per our Activity 1 update, Danelle Larson's manuscript describing relationships between amphipod population density and aquatic plant communities has been accepted for publication in the scientific journal *Biodiversity and Conservation*.

Breanna Keith shared her thesis results with BSU colleagues and DNR staff in a virtual thesis defense talk on October 18.

Status as of March 29, 2022:

Keith, Larson, and Anteau continue to work toward publication of our invertebrate sampling methods as a USGS open-access publication (USGS "Open-File Report"). They are currently responding to USGS reviewer comments.

Larson and Keith applied to present at the 2022 Joint Aquatic Sciences Meeting, including submission of poster abstracts. (This did not require paying conference registration fees. We await LCCMR's decision regarding this budget amendment before registering.)

Final Report Summary

We have disseminated information about our project and its results through numerous outlets. We also trained, mentored, and provided paid work experience to two master's students (Breanna Keith and Jake Carleen, Bemidji State University) and 23 undergraduate students (Bemidji State University and Augsburg University) who participated in our field and lab work. The two master's students wrote theses focusing on the relationships between amphipods and fish (Carleen) and amphipods, water quality, and landscape characteristics (Keith). Both gave thesis defense presentations to university faculty and students, wildlife professionals, and the public. Aspects of our project composed undergraduate capstone research projects for three of our Bemidji State University students (Alyssa Chalberg, Brad Morris, and Alaina Taylor) and two of our Augsburg University students (Michael Bieganeck and Demey (Everett) DeJong). These students produced undergraduate theses (DeJong), research papers for upper-level courses (Chalberg, Morris, and Taylor), and posters (all students) describing their work. We were able to hire another of our students (Anna Medina) as a DNR technician to assist with project field and lab work after graduation. Additionally, Keith gave a guest lecture describing her landscape-level analyses to undergraduates in a Landscape Ecology course at Bemidji State University. Our project partner Emily Schilling (Augsburg University) taught a course-based undergraduate research experience (CURE) in which 20 undergraduate students developed research skills by using our project's amphipod sampling methods to investigate spatial heterogeneity in amphipod populations in urban wetlands. Dr. Schilling and Dr. Larson mentored an undergraduate student, Ciasha "Cookie" Shiongyaj, in deeper analysis of this CURE data and presented at the international conference, 2021 Society of Freshwater Science.

We have published two publicly-available scientific papers describing our invertebrate sampling [methods](#) (led by Keith) and [amphipod relationships to aquatic plants](#) (led by Larson). We have drafted a third paper (led by Fitzpatrick) describing our amphipod translocation experiment and will submit it for publication following edits. We have at least four more papers planned from the data that has been collected. Summaries of our work are also available as publicly available [DNR Annual Research Summaries](#). Additional outreach included 6 oral and 6 poster presentations to scientists and natural resource professionals at professional conferences by project leaders and graduate students and 7 additional presentations at university-level and national conferences by undergraduate students. [Two of our presentations are posted on YouTube](#). Our MN DNR ["Wildlife Research Wednesday" virtual presentation](#) in 2021 reached an audience of 236 scientists, natural resource practitioners, and members of the public. Additional public outreach included [an article in Minnesota Conservation Volunteer](#), two Minnesota Public Radio news articles with podcasts ([first Minnesota Public Radio first article/podcast](#) and

[second Minnesota Public radio article/podcast](#)), and a KSTP-TV news broadcast. We also often communicated informally about work with wildlife managers and members of the public. For example, we provided updates to MN DNR and USFWS managers and private landowners via phone, e-mail, and in-person after conducting amphipod and habitat surveys on their properties. We also discussed our project with interested members of the public, who often stopped to ask our field crews about our work.

DATA CURATION:

All the data sets collected under this work will be disseminated publicly and archived permanently as a DOI link following a formal data review by the U.S. Geological Survey. The review will consist of final quality assurance and quality control checks; ensure usability of the data by creating metadata that describes each variable, how it was collected, and expected values; and review of key analysis scripts for scientific integrity.

The peer-reviewed, analysis-ready dataset and analysis codes from Larson et al. (2022) are available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

The data are also archived with the MNDNR on their cloud server, and Project Manager Megan Fitzpatrick will be point of contact for data questions. Dr. Fitzpatrick will also maintain specimens and raw, paper datasheets for at least five years (until 2027) at the MN DNR Wetland Research Laboratory.

V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached budget spreadsheet

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours:	TOTAL FTE: 3.25
--	-----------------

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours:	TOTAL FTE: 3.00
--	-----------------

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State \$ To Be Applied To Project During Project Period:			
N/A	\$	\$	
Other State \$ To Be Applied To Project During Project Period:			

MN DNR Research Section funds for pilot work on Activities 1 and 2. Pilot work included site selection, field methodology trials in summer 2017 and trialing stocking experiments in winter 2018 and 2019.	\$ 90,000	\$ 90,000	Secured. June 2017 – June 2019.
Past and Current ENRTF Appropriation:			
N/A	\$	\$	
In-kind Services To Be Applied To Project During Project Period:			
MN DNR supplies computers, software, GPS, field tablets (\$5,000), as well as laboratory facilities, microscopes, trucks, boats and trailers (\$60,000). MN DNR supplies 3 years of salary for: 2 assistant managers for field assistance (50 hours*3 years=\$9,000); Danelle Larson (0.35 FTE*3 years=\$82,000); and Fred Bengtson (0.10 FTE*3 years=\$25,000). Bemidji State University supplies office space for students and the facilities and equipment for analytical chemistry (\$60,000) and Issacson's salary (0.15 FTE*3 years; \$27,000). U.S. Geological survey to match Michael Anteau salary (0.20 FTE; \$65,400) and travel to visit field sites and disseminate findings (\$6,000).	\$ 339,400	\$ 339,400	Secured. June 2017 – June 2021.

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Dr. Carl Isaacson	Assistant Professor	Bemidji State University	Co-advise students and lead water chemistry analyses.
Barry Thoele	Owner	Lincoln Bait LCC	Will provide consulting, sell and stock amphipods (Activity 2).

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role
Fred Bengtson	Wildlife Manager	MN Dept of Natural Resources	Will assist sampling and stocking and disseminate findings
Dr. Danelle Larson	Research Ecologist	U.S. Geological Survey	Will co-advise graduate students, disseminate findings through professional conferences

			and peer-reviewed journals.
Dr. Michael Anteau	Research Chief	U.S. Geological Survey	Will help design studies, serve on graduate committees, disseminate findings
Emilly Schilling	Professor	Augsburg University	Will mentor undergraduate students, disseminate findings through professional conferences and peer-reviewed journals.

VII. LONG-TERM- IMPLEMENTATION AND FUNDING: Studying super-wetlands in detail will provide new knowledge about the characteristics of wetlands that support key invertebrates as wildlife food. The stocking survey and experiments will bring awareness and engage citizen scientists to the issue of wetland degradation and restoration options. Our analyses will provide cost-benefit comparisons of wetland enhancement and amphipod stocking for interest groups such as Ducks Unlimited, MN Waterfowl Association, Audubon Society, the many Fishing and Angling groups, and MN DNR.

VIII. REPORTING REQUIREMENTS:

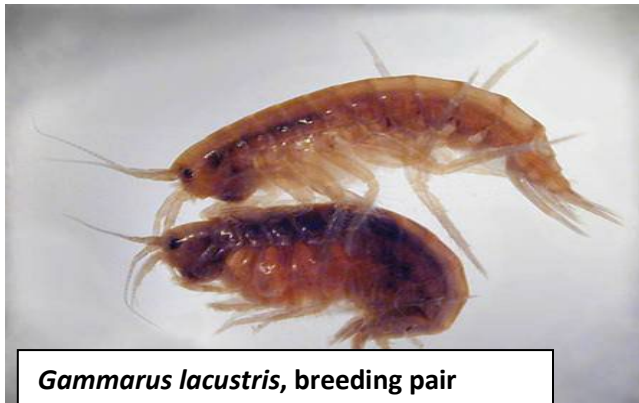
- The project is for 4 years, will begin on July 1 2018, and end on June 30 2022.
- Periodic project status update reports will be submitted January 31 and June 30 of each year.
- A final report and associated products will be submitted between June 30 and August 15, 2022.

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

- A. Budget Spreadsheet - separate document
- B. Visual Component - attached
- C. Research Addendum – separate document

Amphipods are vanishing from Prairie Pothole wetlands but ducks and salamanders are hungry!

Which habitat characteristics support amphipods in Minnesota's wetlands?



Gammarus lacustris, breeding pair



What is the efficacy of stocking amphipods?

FOR SALE



Gammarus Lacustris, freshwater shrimp, scuds for wetland restoration, forage or bait.
We are the first and only source for Gammarus in MN with over 25 years harvesting, handling and stocking.
Contact Barry Thoele 218-296-0446 p-13



Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet-Final



Project Title: Restoring Wetland Invertebrates to Revive Wildlife Habitat
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g
Project Manager: Megan Fitzpatrick
Organization: Minnesota Department of Natural Resources

College/Department/Division: Division of Fish and Wildlife

M.L. 2018 ENRTF Appropriation: \$400,000

Project Length and Completion Date: 4 years, June 30, 2022

Date of Report: August 15, 2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget 4/18/2022	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits) - Overall	\$24,300	\$24,131	\$169
MN DNR Technician. 52% FTE (27 weeks, beginning in early January). \$18.00/hr and 0.25% benefits. One person in position type. Spring and summer worker to assist with field work and invertebrate counting. (Total estimated amount \$24,300)			
Professional/Technical/Service Contracts	\$266,680	\$248,458	\$18,222
Michael Anteau, U.S. Geological Survey. 0.10% FTE. Summer 2018-2020. Scud expert to help design study, mentor two graduate students, and disseminate findings. (Total estimated amount \$0. In-kind support provided by U.S. Geological Survey)	\$0	\$0	\$0
Contract with Lincoln Bait to provide technical consultation regarding Activity 1 and 2. Lincoln Bait will provide ~400 gallons of scuds at rate of \$60/gallon. Only provider in Minnesota. (Total estimated amount \$29,000)	\$29,000	\$29,000	\$0
Interagency Agreement with Bemidji State University: Hire 4 Bemidji State University undergraduates to assist with field work and invertebrate counting. BSU has many trained students for invertebrates, and MNDNR has the facilities, microscopes, and invertebrate expertise for supervision. (\$29,120 each of 2 years - Total estimated amount \$47,990); salary for Carl Isaacson. 0.15% FTE. Summer 2018-2020. Co-advise two graduate students, provide office and laboratory space, and run analytical chemistry. (Total estimated amount \$27,000); Hire 2 graduate students through Bemidji State U. Environmental Studies Master's of Science Program. \$62,000 per student, which includes tuition, benefits, and salary for 2 years. (Total estimated amount \$157,790); meal expenses FY18-20 (Total estimated amount \$4,900)	\$237,680	\$219,458	\$18,222
Equipment/Tools/Supplies			
Activity 1 and 2: waders, temperature, oxygen, and pH meters and solutions; sampling bottles and nets; reagents for water chemistry	\$38,336	\$35,236	\$3,100
Travel expenses in Minnesota	\$48,760	\$46,440	\$2,320
Conferences to present per two activities (\$1333/conference)			
Fleet FY18-20 (25,000 miles @ 0.78/mi; includes loaner and fuel expenses) (Total estimated amount \$19,500)			
Hotels and meal expenses FY18-20 (MNDNR Personnel) (Total estimated amount \$21,900)			
Travel expenses outside of Minnesota: Purpose of travel is conference attendance for two presenters. Budget includes lodging (hotel at \$200/night for 5 nights for 2 people), meals (\$36/day for 2 people for 6 days), flight (lowest cost option for attendee based near Minneapolis/St. Paul) and mileage (lowest cost option for second attendee) at \$0.585/mile	\$3,394	\$1,441	\$1,953
Conference costs outside of Minnesota: Includes conference registration fee for two presenters, one early-career and one regular	\$1,530	\$1,110	\$420
Other			
Publications in peer-reviewed, open-access journals (4 pubs @ \$2,000 each)	\$8,000	\$3,738	\$4,262
DNR Direct and Necessary	\$9,000	\$7,431	\$1,569
COLUMN TOTAL	\$400,000	\$367,985	\$32,015

Amphipods: a declining wildlife food resource



Photo by Breanna Keith

Amphipods are small crustaceans that provide food for waterfowl, amphibians, and other wildlife. Studies show amphipod populations are declining in Minnesota's prairie pothole wetlands.



Photo by Glenn Bartolotti



Photo by Dick Daniels

RESEARCH QUESTIONS: What habitat characteristics support amphipods?
Can we transplant amphipods to restore populations?

Fish (-)

Fish have invaded many prairie potholes that were formerly fishless. Amphipod density was lower in wetlands with higher numbers of fathead minnows, brook stickleback, and especially black bullheads.



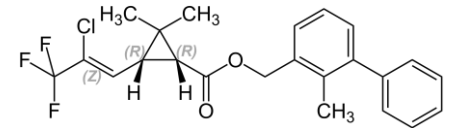
Aquatic plants (+)

Amphipod density was higher in wetlands with higher diversity of aquatic plant species.



Pesticides (-)

Amphipod density was lower in wetlands with higher levels of pyrethroid pesticides. Pyrethroids may directly affect amphipods or signal multiple impacts of intense agriculture.



Transplants

18 of 19 amphipod transplants failed to establish new populations. Future results might improve by targeting sites with high aquatic plant diversity, no fish, and minimal pesticides.

Conclusions - How to support amphipods and wildlife:

Control fish and improve aquatic plant diversity: drawdowns, fish barriers, restore hydrology

Reduce impacts of intensive agriculture: wetland buffer zones, support farming practices that reduce need for chemical pesticides





High abundance of a single taxon (amphipods) predicts aquatic macrophyte biodiversity in prairie wetlands

Danelle M. Larson¹ · Demey DeJong² · Michael J. Anteau³ · Megan J. Fitzpatrick⁴ · Breanna Keith⁵ · Emily G. Schilling² · Barry Thoele⁶

Received: 18 May 2021 / Revised: 3 January 2022 / Accepted: 25 January 2022

This is a U.S. government work and not under copyright protection in the U.S.; foreign copyright protection may apply 2022

Abstract

Conservation programs often aim to protect the abundance of individual species and biodiversity simultaneously. We quantified relations between amphipod densities and aquatic macrophyte (large plants and algae) diversity to test a hypothesis that biodiversity can support high abundance of a single taxonomic group. Amphipods (*Gammarus lacustris* and *Hyalella azteca*) are key forage for waterfowl and are declining in the Prairie Pothole Region of North America. We sampled a large gradient of amphipod densities (0–7050 amphipods/m³) in 49 semi-permanent wetlands, and 50% of the study wetlands had high amphipod densities (> 500 amphipods/m³). Generalized linear models revealed *G. lacustris* and *H. azteca* densities increased exponentially with macrophyte diversity indices. Further, *H. azteca* densities were greatest at moderate levels of submersed vegetation biomass. Community analyses showed both amphipod species were positively associated with diverse macrophyte assemblages and negatively associated with high coverage of cat-tails (*Typha* spp.), a taxon that creates monotypic stands, as well as bladderwort (*Utricularia* spp.), a carnivorous plant. Our results indicate that amphipods could be used as an umbrella species for protecting diverse macrophyte communities in semi-permanent and permanent wetlands of North America's Prairie Pothole Region.

Keywords Amphipoda · Aquatic vegetation · Systematic conservation planning · Umbrella species · Waterfowl

Introduction

Systematic conservation planning has focused on maintaining, protecting, and restoring biodiversity. The systematic conservation planning model is used worldwide to identify and prioritize landscape conservation networks (Margules and Pressey 2000). Planners

Communicated by Peter Bridgewater.

✉ Danelle M. Larson
dmlarson@usgs.gov

Extended author information available on the last page of the article

first choose which conservation proxies, such as ecosystem functions or species, will be used to represent biodiversity (Bal et al. 2018). Biodiversity proxies often include individual taxonomic groups that are easy to measure and are environmentally sensitive (“indicator” species) and taxa whose protection will extend to a wide range of co-occurring species (“umbrella” species). Decadal long debates continue as to whether a single-species focus can conserve biodiversity (Simberloff 1998; Runge et al. 2019). More scientific evidence linking single species abundance and biodiversity may provide opportunities to combine these conservation strategies (Lindenmayer et al. 2007).

The implied idea of managing the abundance of specific species for conserving biodiversity or vice versa is paradoxical. So, a key question remains: is the conservation of *biodiversity* at odds or compatible with the conservation of the *abundance of a single species*? We evaluated this question by examining the role of wetland macrophyte diversity in supporting abundant populations of amphipods, which are a key food resource for many waterfowl and other wetland wildlife (Olenick and Gee 1981; Afton and Hier 1991; Benoy et al. 2002). Similarly, we explored whether amphipods could be an *umbrella species* (i.e., whose protection would extend to a large number of other species, such as macrophyte diversity). Identifying more umbrella species can help to address global freshwater biodiversity loss (Kalinkat et al. 2017) by prioritizing conservation reserves and evaluating the effectiveness of restoration.

Amphipods (*Gammarus lacustris* and *Hyaella azteca*) are wetland crustaceans of known ecological significance to waterfowl (Afton et al. 1991) that are easy to detect when found in high densities (Fig. 1a–c). However, it remains unclear whether amphipod abundance indicates ecosystem health (such as high diversity) and whether a conservation focus on this taxonomic group might also enhance wetland biodiversity. Amphipods are an especially important forage base for some waterfowl species such as Lesser and Greater Scaup (*Aythya affinis* and *A. marila*, respectively), hereafter scaup (Afton et al. 1991). Brood-rearing and migrating scaup preferentially inhabit wetlands (Fast et al. 2004; Anteau and Afton 2009; Kahara and Chipps 2009), and scaup forage most efficiently in wetlands with high amphipod densities (Anteau and Afton 2009).

Several studies have shown amphipod abundances have declined across the Prairie Pothole Region (PPR) of North America in the past few decades (Afton and Hier 1991; Anteau and Afton 2008a, b; Strand et al. 2008), which likely cascaded to partially cause scaup population declines (Anteau and Afton, 2006, 2008b; Austin et al., 2000). Previous studies showed amphipod densities are negatively correlated with fish densities (Hanson and Butler 1994; Anteau et al. 2011) and high hydrologic connectivity that allows fish passage into wetlands (Wiltermuth 2014). Amphipod densities are also correlated with moderate concentrations of organic suspended sediments and the abundance of submersed macrophytes (Kahara and Chipps 2009; Anteau et al. 2011) but not with upland cultivation or vegetation buffer widths (Janke et al. 2019). This body of previous work was informative but limited because it included few study wetlands with high densities of amphipods (Anteau and Afton 2008a). Occasionally wetlands in the PPR still host high amphipod densities (> 500 amphipods/m³); however, little is known of the factors driving wetland habitat suitability for these high densities despite the importance of understanding those drivers to protect or enhance available habitat (Kahara and Chipps 2009; Larson et al. 2018).

Our research objective was to understand the association between macrophyte species diversity and high densities of amphipods (*G. lacustris* and *H. azteca*). We hypothesized that macrophyte diversity and amphipod abundance would be positively correlated because diversity may increase system productivity that could support amphipod forage

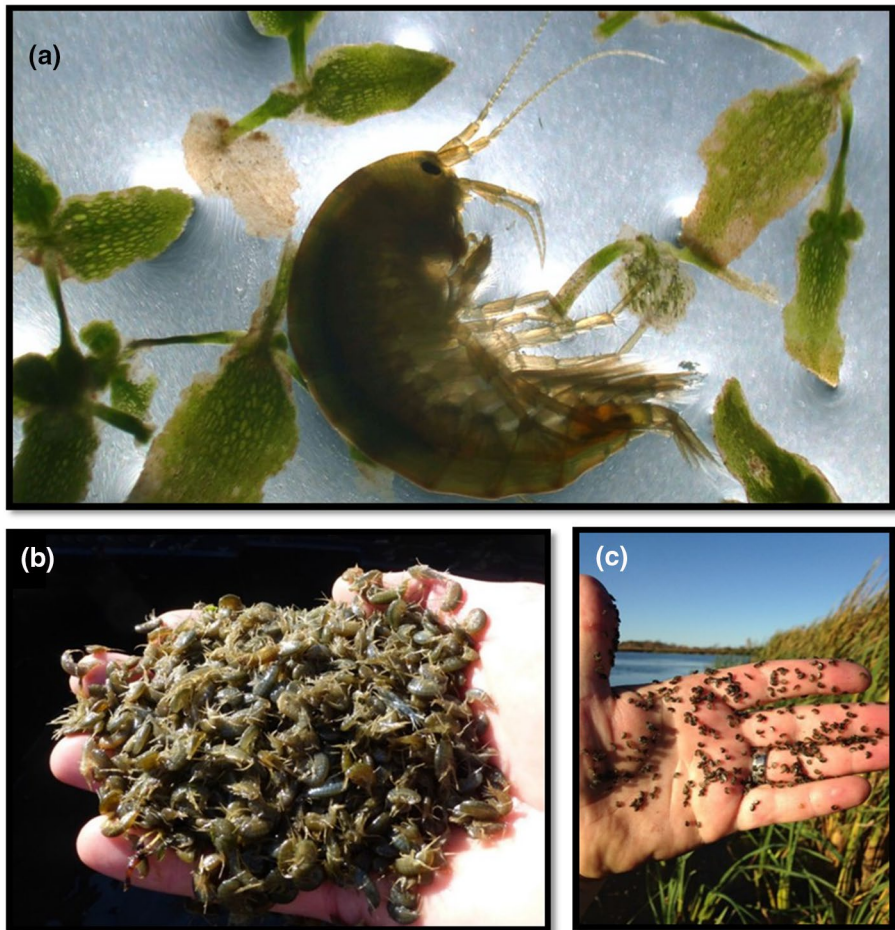


Fig. 1 Photographs of the amphipod *Gammarus lacustris* and the aquatic plant *Lemna trisulca* under 60×microscopy (panel **a**); a handful of *Gammarus lacustris* (panel **b**) and *Hyaella azteca* (panel **c**), both taken from natural wetlands with high amphipod densities (> 500 amphipods m^{-3}) in the Prairie Pothole Region, Minnesota, USA. Photographs taken by Breanna Keith (panel **a**), Danelle Larson (panel **b**), and Robert Baden (panel **c**), all used with permission

and because other factors such as water quality are interrelated. Further, we hypothesized that high amphipod abundance would be positively correlated to both submersed plant biomass and emergent cattail fringe because these plant resources could provide refuge from predation and a carbon-rich food source. We explored the idea that amphipods could be used as umbrella species because we hypothesized amphipods would have several characteristics of an umbrella species: (1) easy to detect in high densities, (2) are environmentally sensitive to factors such as water quality and macrophytes, and (3) amphipod conservation and management could likely extend to many other species (such as macrophyte diversity).

Methods

Study sites and design

We studied 49 small wetlands within the Prairie Pothole Region and forest-transition zone of Minnesota, U.S.A. Past research used random-sample approaches, which highlighted the lack of amphipod presence in most Minnesota wetlands and occasionally wetlands with very high densities (Anteau and Afton 2008a; Anteau et al. 2011). Those prior studies connected amphipod abundance with environmental conditions but contained few study sites with very high amphipod densities, thus limiting their inferential value for the environmental conditions of wetlands containing high amphipod densities. Therefore, our study design intentionally targeted wetlands with a range of amphipod densities, especially high densities not captured with previous studies. We randomly selected half of our study wetlands, and the other half were targeted to have high densities of any amphipod species. The high-density wetlands were identified through personal communications with eight resource managers in our study region, as well as the published reports by Anteau and Afton (2008a, 2009) and Janke (2016). A subsequent rapid assessment of amphipod density in spring 2018 at 111 candidate basins confirmed that 24 wetlands had > 500 amphipods/m³, which we included in this study as high-density wetlands based on expert opinion by the authors and resource managers. The other 25 study wetlands with unknown amphipod presence and density were randomly selected with ArcGIS software the following criteria: (1) located within a 24 km radius of high-density wetland; (2) a semi-permanent or permanent water body where amphipods can persist (Anteau et al. 2011); and (3) similar ($\pm 25\%$) wetland surface area as the selected high-density wetlands. The intention of this selection criteria was to increase the likelihood of finding amphipods in suitable hydrology (semi-permanent yet shallow) and ease of sampling (e.g., not driving long distances between sites). The design was not a “paired design” because the wetlands were independent of one from another and not meaningfully matched pairs.

The 49 wetlands represented a variety of amphipod densities (0–7050 amphipods/m³), land uses (including forested, native prairie, and agricultural cultivation), land ownership (including private wetlands, state lands, federal lands, and mixtures of ownership), wetland sizes (0.4–77 ha), average water depths (72–164 cm, excluding one outlier of 604 cm), water quality conditions, and macrophyte metrics and communities (see ‘Results’ below and the complete dataset [Larson et al. 2021]).

Field data collection

Amphipods were sampled in April and May 2019. We targeted adult amphipods and this sampling time because adults are easy to capture and identify as compared to juveniles that can be small and inconspicuous and to avoid detection error from high macrophyte biomass in summer. We sampled a total of eight stratified random sampling locations at each wetland. The strata included near-shore points (within ~5 m of shore or the outermost ring of impassable emerged vegetation) and deeper points (~50 m from shore) to capture a range of habitats. Using ArcMap (release 10; ESRI, Redlands, California, USA), we generated eight equally spaced transects that ran perpendicular to the longest axis of the wetland. Each transect contained one sample point, alternating between near-shore points and deeper points. The sample points were preprogrammed into a Garmin 76 GPS unit for field navigation (~3-m positional accuracy). Sampling was limited to points with a maximum

depth of 1.4 m due to sweep net limitations. Alternate points along the transect were sampled when water depth was > 1.4 m or the near-shore point was > 5 m from shore.

Separate water column and benthic samples were collected using sweep nets (mesh size: 1200 microns). Benthic samples were obtained by sweeping a D-frame net (net diameter: 0.0647 m^2) for 0.5 m along the wetland bottom to capture the top ~ 3 cm of sediment and organisms. Water column samples were collected with a D-frame or circular sweep net (net diameter: 0.0452 m^2) that was bent 90° so the net opening was perpendicular to the handle. We plunged the sweep net to a depth of 30 cm (the benthic net height) above the benthos in an area undisturbed by the benthic sweep, moved the net horizontally to undisturbed water, and raised the net vertically out of water. During the plunge the net was inverted, so a sample was only collected during the vertical retrieve. Samples were drained of excess water and preserved in 95% ethanol. Samples were transported to the Minnesota Department of Natural Resources' Wetland laboratory and processed within four months of collection.

We conducted comprehensive wetland habitat surveys once in July 2019 to capture representative wetland conditions and peak macrophyte species richness. Water samples were collected near the center of each wetland in 50-mL polypropylene sterile tubes, stored on ice in a cooler, and shipped on ice to the Minnesota Department of Agriculture Laboratory Services for analyses of total phosphorus (P). Wetland pH (Hach® HQ40d, Loveland, Colorado, USA) and turbidity (Hach® 2100P, Loveland, Colorado, USA) were also measured near the center of each wetland.

We sampled water depth and aquatic macrophytes (submersed aquatic vegetation [SAV], emerged, rooted-floating, and free-floating life forms) at evenly spaced, random sampling points. These sample points differed from where amphipods were collected, and so the depth and macrophyte data represent the entire wetland and not a sampling point. The entire wetland was gridded in ArcMap, and each grid provided a sampling point for water depth and vegetation. Grid size varied by wetland size; wetlands < 4 ha had 10 sampling points, and we increased sampling by one point for each additional 0.5 ha. The number of sampling points per wetland ranged from 10 to 67 (average: 25 points). We navigated via canoe to each sampling point using a Garmin 76 GPS unit. At each point, we measured water depth and dragged one plant rake of 35-cm circular diameter along the bottom of the wetland for 150 cm. We used a relative biomass index to estimate the total amount of submersed plants and filamentous algae on the rake (ordinal score ranging from 0 to 4; Online Appendix 1) and then identified each species present using identification keys (Skawinski 2018). We quantified emerged vegetation prevalence along shorelines using visual field surveys in which we recorded the presence of emerged vegetation guilds (including cattail, bulrush, and sedge/rush) at the end of each vegetation sampling transect line. Data from the individual points were compiled for each wetland to calculate average biomass and species-specific prevalence. We acknowledge that high wetland plant diversity occurs in the emergent and wet meadows on the upland periphery from the permanent water, but our assessments did not include species-level identification of shallow marsh plants principally because these are not the common habitats that amphipods reside in. Although fish abundance is known to be negatively correlated to amphipods, this study focused on the relations between macrophytes, water quality, and amphipods and not fish. Fish were not sampled as part of this study but may have been present.

Laboratory analyses

Sample contents were washed in a 500- μm rectangular sieve to remove fine sediments. All material on the sieve was suspended gently in a bucket of water to evenly disperse the contents. A grid was placed over the sieve to allow for sub-sampling. We picked a minimum of 25% of the sample on the sieve and added more grids until at least 400 invertebrates were picked or the entire sample was processed. All picked invertebrates were stored in 70% ethanol for identification and archiving.

We enumerated and identified all amphipods to the species-level using taxonomic keys (Merritt et al. 2008; Glazier 2014) by using a lighted trinocular microscope with 110 $\times$ magnification potential. Amphipod counts were scaled to the entire sample if they had been subsampled. Amphipod abundance was the total number of individuals counted at each wetland ($n=8$ standardized sweeps). Average amphipod density (units: $\#/\text{m}^3$) was calculated for each basin by dividing the basin-level abundance by the volume of water passed through the sweep net.

Data analyses

We averaged amphipod counts and density by wetland as response variables because the wetland was the experimental unit where samples were truly independent. Therefore, the inferences of amphipods and habitat associations were at the wetland-scale and not at the point of amphipod sampling. We calculated a variety of α -biodiversity facets, including Simpson diversity index, Shannon diversity index, Pielou's evenness index, and species richness using all vegetation species or guilds collected.

Three species of amphipods were collected in our study wetlands: *G. lacustris*, *H. azteca*, and *Crangonyx* spp. We chose to model *G. lacustris* and *H. azteca* separately because each species may respond differently to environmental predictors. We did not analyze *Crangonyx* spp. due to infrequent occurrences (<15%) and low densities (Table 1, Fig. 2).

We used two complementary analyses to infer biologically and statistically significant factors that influenced amphipod abundance: (1) generalized linear models to test our hypotheses that amphipod density would relate positively to SAV biomass, macrophyte diversity, and emerged vegetation prevalence along shorelines, and (2) a redundancy analysis (RDA) of amphipod abundance and macrophyte assemblages to test species affiliations. We reported the p -value with degrees of freedom and relative magnitude of differences to assess 'statistical significance' of model outputs. The statistical analysis outputs, graphical trends, and previous literature were used to conclude what constitutes 'biologically significant' results and where uncertainty remains (Wasserstein et al. 2019).

All models and graphs were produced using software R 4.0.5 (R Foundation for Statistical Computing 2018). We modeled species-specific amphipod counts to our predictor variables using a negative binomial distribution with a log link function using the 'MASS' package (Ripley et al. 2020); prior models with Poisson distribution of transformed data were over dispersed and thus not reported. We used the natural log of water-volume swept (m^3 ; summed per wetland) as an offset variable. The predictor variables included the three primary hypotheses about the influence of macrophytes on amphipods: (1) macrophyte diversity (all life forms); (2) SAV biomass; and (3) the percentage of shoreline rimmed by emerged vegetation. We also included several covariates to account for wetland-to-wetland

Table 1 The summary statistics of densities of each amphipod genus detected in the study ($n=49$ wetlands), which includes randomly selected wetlands, intentionally selected wetlands with known high densities (>500 amphipods m^{-3}) of at least one species of amphipod, and the combined summaries of both wetland selection types

Genus	Minimum	1st quartile	Median	Arithmetic mean	3rd quartile	Maximum
Randomly selected wetlands ($n=25$)						
Cragonyx	0	0	0	1	0	23
Gammarus	0	0	0	12	0	200
Hyalrella	0	0	19	189	102	1329
Intentionally selected, 'high density' wetlands ($n=24$)						
Cragonyx	0	0	0	16	15	124
Gammarus	0	58	422	681	1050	2736
Hyalrella	0	70	321	1001	1259	7050
All study wetlands ($n=49$)						
Cragonyx	0	0	0	9	3	124
Gammarus	0	0	15	353	422	2736
Hyalrella	0	13	77	620	734	7050

variability due to factors other than macrophytes. Our approach was to have a model for each amphipod species that contained at least three variables that represented each of the macrophyte hypotheses and then interpreted the strength of their support by examining the parameter estimates, 95% confidence limits, and p -values.

We took several steps to select the variables used in the final models. These steps included examining plots of the raw data, transforming data as necessary, and then performing a single-variable elimination. We assessed relative goodness of fit and parsimony by comparing the Type III likelihood ratio statistic, and single variables were eliminated if its p -value was >0.05 . We plotted species' density against each potential variable to identify non-linear relations and any needs for transformations. The *H. azteca* densities graphically appeared to increase with SAV biomass up to a point then decreased, so we included a quadratic effect. The covariates we initially considered were maximum depth, wetland area, pH, total P, turbidity, and an index of suspended sediments. There were low correlations ($r<0.4$) among these covariates and our three hypothesized variables, with exception of turbidity and total P ($r=0.49$). We suspected that our turbidity measurement was composed of phytoplankton and suspended sediments, which could be an important covariate based on Anteau et al. (2011). So, like Anteau et al. (2011), we ran a regression using total P to predict turbidity ($\text{Turbidity} = 0.94 + 75.48 \times \text{total P}$; $r^2=0.23$) and used the residuals of that regression to index suspended sediments. The resulting suspended-sediment index was orthogonal with total P. For *G. lacustris*, the plots indicated total P should be transformed with a natural log, and there were threshold effects of maximum depth (~ 150 cm) and wetland area (15 ha); so, we indexed maximum depths >150 cm as "1" and ≤ 150 as "0" and wetland areas >15 ha as "1" and ≤ 15 as "0." For *H. azteca*, we did not consider maximum depth, wetland area, total P, and suspended-sediment index because preliminary analyses indicated no pattern with their densities. Lastly, we chose a single, most informative variable to represent the three hypotheses related to macrophyte diversity, biomass,

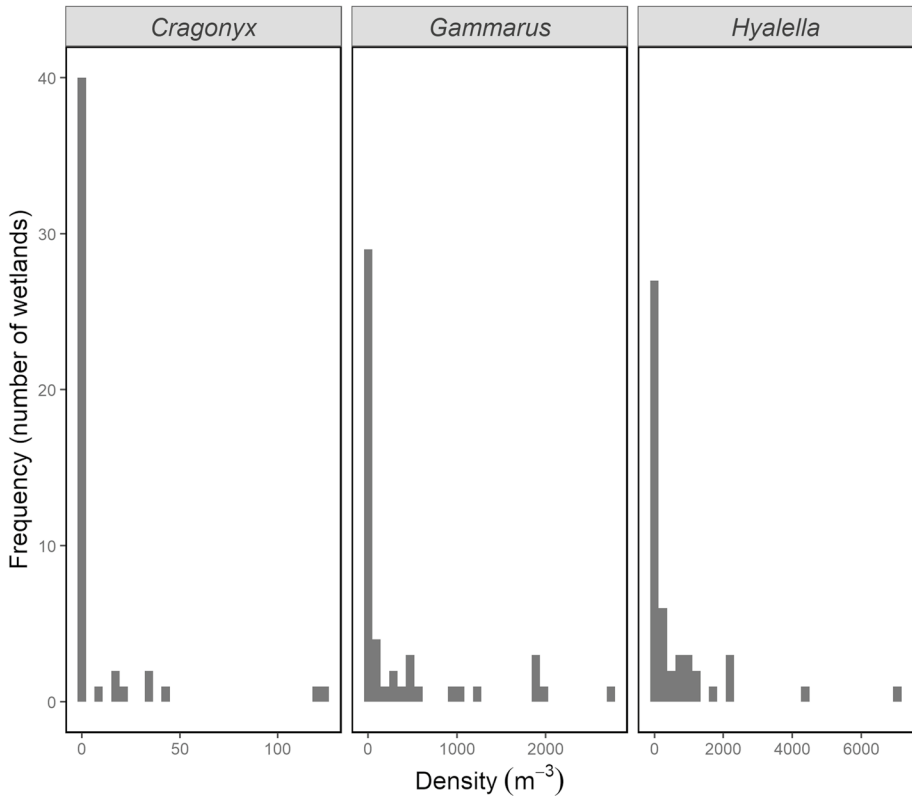


Fig. 2 Histograms showing the frequency of amphipod densities measured in 49 wetlands in the Prairie Pothole Region, Minnesota USA in spring 2019

and emergent vegetation in the models. For both amphipod species, the SAV biomass index was more informative than SAV prevalence. Simpson's diversity index was more informative than Shannon's diversity index, species richness, or Pielou's evenness. Shoreline emerged prevalence was a better predictor than total wetland emerged prevalence.

We reported results of full models that included the three hypothesized macrophyte variables and informative covariates (see Tables 2 and 3). To assess model fits, we used marginal model plots (Online Appendix 2) that compare the data and model fits. We calculated the predicted values of amphipod density of our hypothesized variables while holding the other covariates constant and provided graphs of each model's predictions (Figs. 3 and 4).

In addition, we conducted a redundancy analysis to examine relations among species-specific amphipod counts and macrophyte assemblages using the 'vegan' package in software R (Oksanen et al. 2019). This analysis is a multivariate extension of multiple regression where the ecological community is constrained by linear combinations of the predictor variables. The two amphipod species were used as separate response variables. Predictor variables included prevalence for the eight most common SAV species, one prevalent emerged species (*Typha* spp.), and filamentous algae (not speciated). The aquatic vegetation species matrix did not have ecological transformations, but we ln-transformed the amphipod count data to fit linear assumptions. We conducted a permuted, multivariate

Table 2 Analysis results of maximum likelihood parameter estimates for *Gammarus lacustris*

Parameter	DF _{num} , DF _{den}	Estimate	Standard error	Wald 95% confidence limits		Wald χ^2	p-value
Intercept	1, 40	1.54	2.97	−4.28	7.36	0.27	0.604
Simpson diversity	1, 40	13.16	3.31	6.68	19.63	15.85	<0.001
SAV biomass	1, 40	−1.45	0.45	−2.34	−0.56	10.13	0.002
Shoreline emergent prevalence	1, 40	−0.02	0.02	−0.06	0.02	1.25	0.265
Maximum water depth	1, 40	1.77	0.88	0.04	3.49	4.02	0.045
Wetland size	1, 40	−4.33	1.11	−6.51	−2.15	15.15	<0.001
Total P	1, 40	1.05	0.53	0.00	2.09	3.87	0.049
Suspended sediment	1, 40	0.14	0.06	0.02	0.26	4.89	0.027
Dispersion	1, 40	3.71	0.95	2.25	6.11	NA	NA

The parameters included in this table were of primary interest with hypotheses of positive associations of *G. lacustris* and aquatic vegetation. Parameters in bold were primary hypothesized variables of interest related to aquatic vegetation and the others were covariates of secondary interest and included to improve parameter estimates

Table 3 Analysis results of maximum likelihood parameter estimates for *Hyalella azteca*

Parameter	DF _{num} , DF _{den}	Estimate	Standard error	Wald 95% confidence limits		Wald χ^2	p-value
Intercept	1, 42	−18.87	4.90	−28.48	−9.25	14.79	<0.001
Simpson diversity	1, 42	3.20	1.32	0.59	5.79	5.80	0.016
SAV biomass	1, 42	2.90	0.96	1.00	4.79	8.97	0.003
SAV biomass × SAV biomass	1, 42	−0.80	0.23	−1.24	−0.36	12.74	<0.001
Shoreline emergent prevalence	1, 42	0.01	0.01	−0.01	0.03	0.54	0.464
pH	1, 42	2.23	0.54	1.18	3.28	17.30	<0.001
Dispersion	1, 42	2.28	0.55	1.93	4.15	NA	NA

The parameters included in this table were of primary interest with hypotheses. Parameters in bold were primary hypothesized variables of interest related to aquatic vegetation and the others were covariates of secondary interest and included to improve parameter estimates

analysis of variance ($perm = 9999$) to assess the statistical significance of the overall RDA model, axes, and terms.

Results

Amphipod densities and species co-occurrences

Our surveyed wetlands comprised a large range of amphipod densities (0–7050 amphipods/m³). Several wetlands included exceptionally high density (Table 1, Figs. 1 and 2); half of the wetlands had >500 amphipods/m³ (per the study design). Of the 25 randomly selected

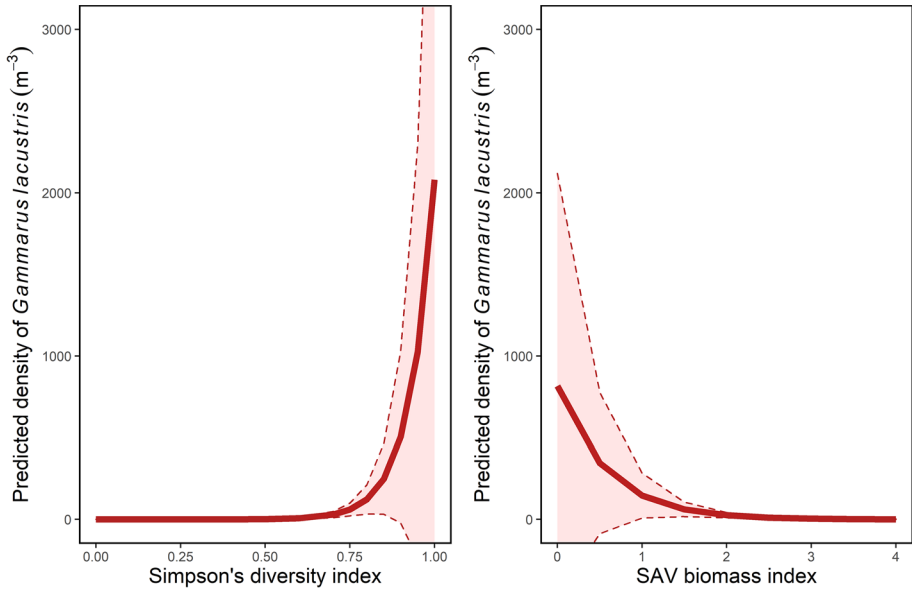


Fig. 3 The model-predicted densities of *Gammarus lacustris* in response to wetland vegetation diversity (Simpson diversity index) and submersed aquatic vegetation biomass (SAV biomass index). Shaded areas correspond to the prediction's 95% confidence intervals while other model covariates were held constant at their means

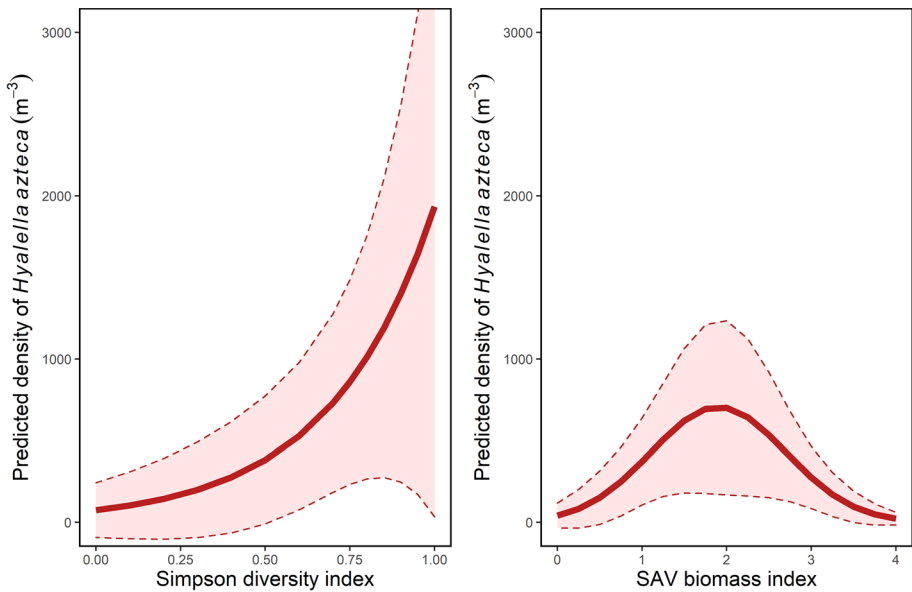


Fig. 4 The model-predicted densities of *Hyalella azteca* in response to wetland vegetation diversity (Simpson diversity index) and submersed aquatic vegetation biomass (SAV biomass index). Shaded areas correspond to the prediction's 95% confidence intervals while other model covariates were held constant at their means

wetlands, 8% had *G. lacustris* present but with relatively low densities (geometric mean: 37 *G. lacustris*/m³). The *H. azteca* were present in 70% of the 25 randomly selected wetlands (geometric mean: 63 *H. azteca*/m³), and 12% of those wetlands had relatively high density (i.e., > 500 *H. azteca*/m³).

The three amphipod species (*G. lacustris*, *H. azteca*, and *Crangonyx* spp.) typically co-occurred in wetlands, but only one species was dominant. *Gammarus lacustris* densities were not correlated with either *Crangonyx* or *H. azteca* densities ($r = -0.06$, $r = -0.02$, respectively). *Crangonyx* and *H. azteca* densities were positively correlated ($r = 0.43$).

Relationships among *Gammarus lacustris*, macrophytes, and wetland characteristics

The *G. lacustris* model was predicted by the independent variables of Simpson diversity, SAV biomass, shoreline emergent prevalence, maximum water depth, wetland size, total P, and suspended sediment (Table 2). The *G. lacustris* model predictions fit the observed data well ($r = 0.54$, Online Appendix 2A). The most informative macrophyte diversity metric (Simpson's *D*) had a strong, positive correlation with *G. lacustris* densities (Table 2, Fig. 3, Online Appendix 3B). The high-density *G. lacustris* wetlands always had a Simpson's *D* > 0.80. Although we hypothesized SAV biomass and shoreline emerged vegetation would be positively correlated with *G. lacustris* densities, the model indicated a negatively weak correlation and no correlation, respectively. The covariates showed that *G. lacustris* responded positively to wetland maximum water depth and productivity metrics but negatively to wetland size (Table 2).

Relationships among *Hyalella azteca*, macrophytes, and wetland characteristics

The *H. azteca* model was predicted by the independent variables of Simpson diversity, SAV biomass, a quadratic term for SAV biomass, shoreline emergent prevalence, and pH (Table 3). The *H. azteca* regression model fit the observed data less well than the *G. lacustris* model, but the predicted values were correlated with the observed data ($r = 0.37$). *H. azteca* were positively correlated to Simpson's diversity (Table 3, Fig. 4, Online Appendix 3D), but not as strongly as *G. lacustris* based on the parameter estimates. The high-density *H. azteca* wetlands had diversity-index values from *D* = 0.5 to 1.0. The *H. azteca* densities displayed a significant quadratic relationship with SAV biomass whereby densities were maximized at moderate SAV biomass values. Shoreline emerged prevalence was not a significant predictor variable. Only one informative covariate (pH) was included in the model, which was positively correlated with *H. azteca* densities (Table 3).

Community analysis of amphipods and macrophytes

We used redundancy analysis to examine the community composition of macrophytes to explore which species were most closely associated with amphipod abundance. The full community model was significant ($F_{1,38} = 1.82$, $p = 0.040$) as was the first axis ($F_{1,46} = 15.80$, $p = 0.043$). *Lemna trisulca* (LETR) along the first axis was a predictor of *G. lacustris* abundance ($F_{1,38} = 5.09$, $R^2 = 0.19$, $p = 0.001$) and *Myriophyllum sibiricum* (MYSI) was a predictor of *H. azteca* abundance ($F_{1,38} = 5.55$, $R^2 = 0.21$, $p = 0.005$).

Graphically, the redundancy analysis showed biologically significant relationships between macrophyte assemblages and amphipod abundance (Fig. 5). Most wetlands were

spread across ordination space (i.e., not clumped together on the graph), indicating that they had differing plant-community composition and amphipod abundances. The two amphipod species were not correlated and fell along two different ordination axes, indicating that the species often did not co-occur in the same wetlands and that each amphipod species were associated with different macrophyte assemblages. The *G. lacustris* was strongly associated with *L. trisulca* (LETR), which was positively correlated with SAV diversity and species richness but not evenness (Online Appendix 3B). The *H. azteca* was associated with macrophyte assemblages composed of predominately *Stuckenia pectinata* (STPE15), filamentous algae, *Potamogeton friesii* (POFR3), *Potamogeton zosteriformis* (POZO), and *M. sibiricum* (MYSI); these macrophyte species were also prevalent and had moderately high SAV biomass. Both amphipod species were negatively associated with high prevalence of *Typha* spp. (TYPHA) and the carnivorous plant, *Utricularia macrorhiza* (UTMA). *Chara* spp. (CHAR) and sometimes *Ceratophyllum demersum* (CEDE4) were

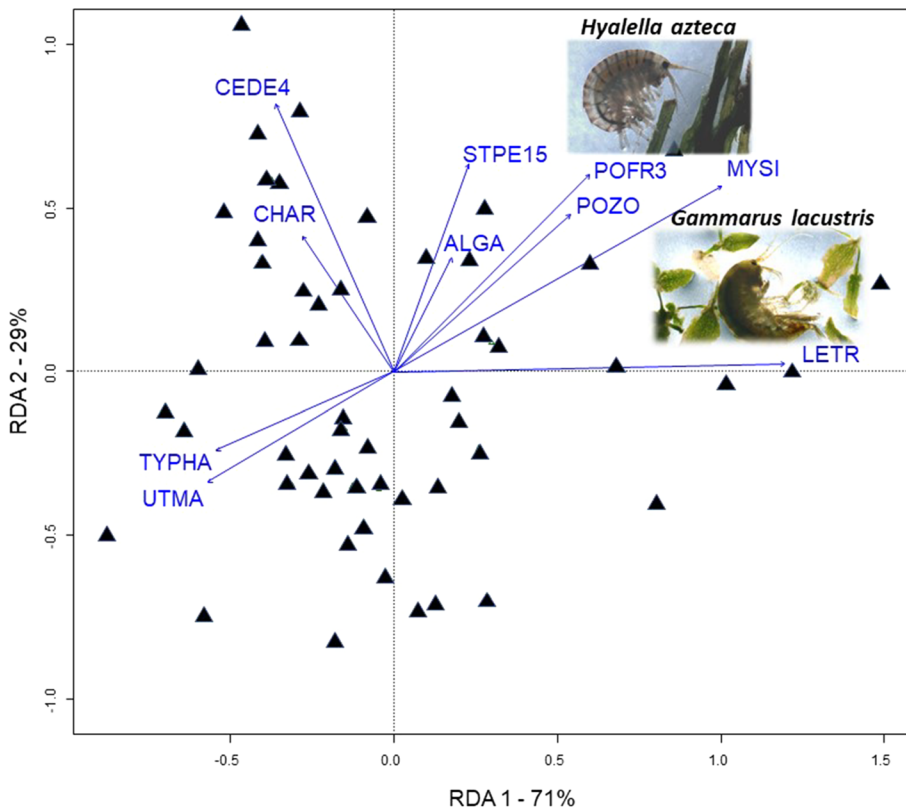


Fig. 5 A triplot of the amphipod communities and aquatic vegetation communities at 49 wetlands (triangles) in the Prairie Pothole Region, Minnesota, USA. The amphipod community in this analysis included two species: *Hyalella azteca* and *Gammarus lacustris*. The species included in the ordination included the ten most prevalent macrophytes: *Typha* spp. (TYPHA), *Utricularia macrorhiza* (UTMA), *Lemna trisulca* (LETR), *Potamogeton zosteriformis* (POZO), *Myriophyllum sibiricum* (MYSI), *Potamogeton friesii* (POFR3), *Stuckenia pectinata* (STPE15), filamentous algae (ALGA; not speciated), *Ceratophyllum demersum* (CEDE4), and *Chara* spp. (CHAR). The ordination scaling and interpretations are based on correlations; therefore, the vectors at $< 90^\circ$ angles are positively correlated, vectors at 90° angles are uncorrelated/orthogonal, and vectors at 180° are negatively correlated

dominant plants (SAV species richness = 1; biomass scores = 3–4), and neither amphipod species thrived in those conditions. The results of the redundancy analysis complemented the univariate regression models (Tables 2 and 3) without conflicting results, except the redundancy analysis indicated a negative association of *Typha* spp. (TYPHA) and amphipod densities (Fig. 5).

Discussion

We found that high densities of *G. lacustris* and *H. azteca* were positively associated with macrophyte taxonomic diversity, and *G. lacustris* were especially sensitive to macrophyte diversity and other wetland characteristics. The greatest amphipod densities were found in macrophyte assemblages composed of both cosmopolitan macrophytes (e.g., *S. pectinata* and filamentous algae) and less common macrophytes (e.g., *L. trisulca*). Both amphipod species had lower densities in wetlands with high cattail (*Typha* spp.) prevalence. Our results clearly showed strong, positive correlations among amphipods and several macrophyte metrics, but the causative relationships could only be speculated. The results indicated that amphipods could be an indicator and umbrella species for wetland plant diversity; macrophyte diversity provides habitat conditions that favor high amphipod densities; macrophytes and amphipods simply require similar wetland conditions (e.g., moderate water depths and semi-permanent hydrology); and that loss of macrophyte diversity could cascade to affect amphipod abundance due to habitat loss. Regardless of which causal mechanism(s) relate amphipods and macrophyte diversity, conservation actions that support either amphipod abundance or macrophyte diversity will likely aid the other taxa (with possible caveats described further herein). Specifically, amphipods as an umbrella species may also help conserve wetland biodiversity in semi-permanent and permanent wetlands. Likewise, a conservation strategy with a focus on aquatic macrophyte communities might concomitantly result in greater amphipod abundance.

Amphipod species abundances and wetland relations differed

Hyaella azteca was much more common and abundant than *G. lacustris*, which was also the case in other Prairie Pothole Region wetlands in recent past (Anteau and Afton 2008a). Densities of *H. azteca* were often three times greater than that of *G. lacustris*, which may be partially explained by the smaller body size of *H. azteca* that allows more occupation in the same space (Thomaz and Cunha 2010). *Hyaella* also have advantageous life-history traits such as greater reproductive capacity than *Gammarus* (Glazier 2014). Although *H. azteca* were more abundant, *G. lacustris* are also ecologically significant given their high density and large body size and biomass that is important for foraging water birds (Anteau and Afton 2006).

Hyaella azteca were tolerant to a wide range of wetland conditions, whereas *G. lacustris* had a narrower and more defined ecological niche (as indicated by the number of informative covariates in the models and model fits). This result corroborates findings of Anteau and Afton (2008a, b) that *H. azteca* were generally more widespread on the landscape. In our study, *G. lacustris* were more common in small wetlands (< 20 ha), were not found in average water depths < 100 cm and were positively related to wetland productivity measures like total P and turbidity. Previous work demonstrated fish are more common in large, permanent Prairie Pothole Region wetlands and that fish can negatively affect

amphipod abundance (Hanson and Butler 1994; Wiltermuth 2014; Janke 2016). Therefore, our significant results on wetland size and depth may either indicate increased carrying capacity for amphipods, or reflect the unmeasured, interactive effects of fish abundance more so than wetland size or depth independently. Note our study wetlands had typically 4× lower productivity (total P and turbidity) compared to other Prairie Pothole Region wetlands in Minnesota that were classified as a degraded “turbid state” (Larson et al. 2020b), so we do not know if *G. lacustris* declines after certain productivity thresholds of eutrophication. Our findings refined niches of *H. azteca* and *G. lacustris* in relation to macrophyte communities and showed *G. lacustris* had a narrow niche breadth and is likely an environmentally sensitive species to wetland factors such as macrophyte diversity and biomass, as well as wetland size, water depth, total P, and suspended sediments.

Macrophyte biodiversity shapes the wetland that supports amphipods

Our results showed that both amphipod species were affiliated with macrophyte diversity. We suspect macrophyte diversity is indicative of overall wetland condition and supports the “biodiversity ecosystem functioning” concept (Engelhardt and Ritchie 2002; Brisson et al. 2020). Ecosystem functions provided by macrophytes to amphipods may include nutrient turnover, carbon availability, structural habitat, and water purification (Carpenter and Lodge 1986). A previous study indicated positive associations of amphipod density and stands of *Potamogeton* spp. and *L. trisulca*, but not *Chara* spp. (Hanson 1990). Our results from 49 wetlands concurred (Fig. 5) with findings from Hanson 1990 and may hint at niche-differentiation or other mechanisms linking biodiversity and amphipods.

SAV biomass

Hyaella azteca densities were maximized at moderately high macrophyte biomass whereas *G. lacustris* densities were negatively associated with SAV biomass. Total macrophyte leaf surface area and complexity from leaf branching presumably support amphipods by providing places of attachment for shelter and feeding (Thomaz and Cunha 2010). More space provided by macrophyte abundance and leaf branching is a plausible reason that the smaller-bodied *H. azteca* were often found at higher densities than the larger-bodied *G. lacustris*. Although fish can also be attracted to macrophyte beds, and the SAV biomass and complexity can reduce fish foraging effectiveness (Thomaz and Cunha 2010) and could reduce predation. We suspect *G. lacustris* were negatively associated with SAV biomass because *G. lacustris* were more common in deep wetlands, which can have less SAV due to light limitations.

Monocultures

Chara spp. and *C. demersum* were species that often dominated in high biomass, and amphipods were not typically detected in those conditions (Fig. 5). These plant species were typically found in wetlands with species richness < 2 and high SAV biomass scores from 3 to 4. We speculate the negative associations among amphipods, *Chara* spp., and *C. demersum* are related to the strong affinity of amphipods to macrophyte diversity (Figs. 3 and 4) more than the plant biomass itself. First, amphipods consume a broad array of

aquatic plants, whereas most other herbivorous aquatic invertebrates consume only one or two plant species (Newman 1991). In near monocultures, the plants senesce at the same time of year, which could reduce the availability of detritus over a long overwintering season. In contrast, a staggered timing in macrophyte senescence and varying plant decay rates could provide an ongoing refuge and detrital food base for amphipods. Further, *Chara* spp. are often encrusted with ‘marl,’ which are carbonate compounds from hard water that are slow to decompose and release carbon for detritivores. Non-native species like *Potamogeton crispus* or *Myriophyllum spicatum* can reduce macrophyte diversity and can have exceptionally high biomass, so we speculate these common invasive plants could negatively affect amphipods.

Typha × *glauca*

The emerged vegetation in this study was predominately cattails (presumably the invasive hybrid *Typha* × *glauca*), which are ecologically important but can also encroach shallow wetlands which have strong, negative ecological effects (Bansal et al. 2019). We found a weak, negative association among *Typha*, macrophyte diversity, and amphipods (Fig. 5), but our sweep-net sampling technique via canoe was insufficient for capturing amphipods in the interior of dense *Typha* beds where amphipods may reside in high density (Christensen and Crumpton 2010). Wetlands with low to moderate *Typha* prevalence are usually larger basins with enough water depth to support *G. lacustris* and dynamic water levels that prevent *Typha* dominance. *Typha* dominance is often a symptom of multiple issues, including consolidation drainage and nutrient loading (Wiltermuth and Anteau 2016; Bansal et al. 2019). The ‘hemi-marsh’ of interspersed *Typha* and open water are also beneficial to an array of water birds that feed on *G. lacustris* (Murkin et al. 1997). Management of *Typha* dominance remains a high conservation priority and challenge in North America (Bansal et al. 2019; Larson et al. 2020a, b), but may benefit *H. azteca* that resides in shallow waters where *Typha* can dominate.

Conservation strategy: using amphipods as indicators and umbrella species to protect diversity

Gammarus lacustris and possibly *H. azteca* could be indicators and umbrella species in semi-permanent and permanent wetlands in the Prairie Pothole Region because (1) both species are easy to detect and enumerate (Fig. 1a–c), (2) *G. lacustris* are particularly sensitive to environmental conditions (Table 2; Anteau and Afton 2011), and (3) their conservation might extend to a suite of other species, like macrophyte biodiversity (Tables 2 and 3). However, amphipods would not be a suitable umbrella species for naturally ephemeral wetlands that frequently dry. An important trait in an umbrella species is that species conservation can improve management decisions. A separate structured decision making approach would be warranted to fully determine the ability for management to influence amphipod populations and protect macrophyte diversity (Bal et al. 2018). Here, we provide two examples of how amphipods could be used for systematic conservation planning and watershed restoration.

Amphipod distribution mapping could be a tool for conservation planning. The ease of detecting high densities of amphipods during early spring after ice-out (Fig. 1a–c) could allow for a feasible, landscape sampling campaign. Sampling amphipods is currently a

more efficient approach to indexing wetland quality than sampling macrophyte diversity or relative fish abundance. Amphipods are easy to identify and rapidly quantify in the field, thereby negating the need for costly sample preservation and laboratory quantification. In contrast, macrophyte surveys took our team about 2–5× longer in the field and required knowledge to identify many species. However, advances in remote sensing of submersed macrophyte diversity could reduce field sampling costs and increase access to larger landscapes in the future. Previous work showed that macroinvertebrates are indicator species because they are easy to measure compared to fish and can indicate fish absence (Schilling et al. 2009). A limitation of this study is we did not sample the wet meadow vegetation diversity, which can host many species; therefore, amphipods may or may not capture diversity alterations to the shallow marsh and wet meadow plant communities. Regardless, mapping the distribution of amphipods across the Prairie Pothole Region could help managers prioritize and conserve the remaining diverse, semi-permanent wetlands in this highly degraded landscape.

Amphipods could be an indicative metric for assessing conditions of the catchment and its terminal basins, which tend to have semi-permanent and permanent hydroperiods that amphipods favor (Anteau and Afton 2011, this study). Sustained amphipod populations are limited to basins with semi-permanent and permanent hydroperiods for overwinter survival, and such basins are typically found at the catchment terminal. Managing directly for amphipod success may be difficult, but restoration of suitable wetland types specific for amphipods could extend to improving catchment health. Hydrology of these basin types is strongly affected by land use and wetland drainage throughout the catchment (McCauley et al. 2015), and in turn, the hydrology affects connectivity and macroinvertebrate communities within these terminal basins (Wiltermuth 2014). Restoration of all wetland types and wetland complexes throughout a catchment can restore natural hydroperiods of the terminal basin (Anteau 2012), and thus support amphipods. Consolidation drainage also provides more permanent water that might be favorable to amphipods, but consolidated basins are also likely to support high abundances of fish and cattails that negatively affect amphipods (Wiltermuth 2014; Wiltermuth and Anteau 2016).

Conservation strategy: managing aquatic plant diversity to provide habitat for high amphipod abundance

Our results indicated that restoring and managing wetlands for macrophyte diversity can produce habitat suitable for amphipods to thrive in high densities. Wetland restorations are often successful at simultaneously restoring hydrology, vegetation cover, and biodiversity (Galatowitsch and van der Valk 1996a; Larson et al. 2020b). Prairie wetland restorations are particularly successful at restoring the diversity of the submergent and emergent plant communities (Galatowitsch and van der Valk 1996b), and our results showed amphipod densities were strongly correlated with diversity in these plant community types. In degraded or recently restored Prairie Pothole Region wetlands, management of related wetland factors such as nutrient loading, water depth, and fish abundance is sometimes feasible and often successful for promoting macrophyte abundance and diversity (Larson et al. 2020a). Wetlands with varying water levels over space and time promote water depth requirements for amphipods and predictable, diverse macrophyte communities (Larson et al. 2020b). Major changes to the Prairie Pothole Region landscape over the past century have stabilized the water levels of many wetlands and adversely affected water clarity, macrophytes, and macroinvertebrates (McCauley et al. 2015; Wiltermuth and Anteau 2016;

McLean et al. 2019). Water level management (through intentional drawdowns) represents a strategy to mimic ecosystem processes that would otherwise remove nuisance fish, increase water quality, increase macrophyte diversity and abundance, and attract waterfowl (Hanson and Butler 1994; Larson et al. 2020a).

Our results indicated low water depths of < 100 cm during a drawdown may preclude high densities of *G. lacustris*, although the survival, growth, reproduction, and recruitment of amphipods following drawdown would warrant more study. Given the sheer number and small size of these wetlands, a possible approach for promoting variable water levels and deeper refuges for amphipod overwintering may be restoration of wetland complexes that include several hydrologic regimes. The careful application of an ecosystem-functioning based approach may preserve or restore dynamic wetland hydrology (Euliss et al. 2008), remove nuisance fish, increase macrophyte diversity, and benefit amphipods in this heavily modified landscape.

A habitat conservation strategy assumes the amphipods are not dispersal limited; if the correct habitat conditions are available (such as semi-permanent water and diverse macrophyte communities), then amphipods would relocate and establish successfully. Amphipods are likely dispersal limited because they do not have a life stage capable of flight, unlike most wetland invertebrates. Instead, amphipod dispersal is limited to transport by other organisms or flowing water and therefore dispersal depends on wetland complexes that have adequate proximity and some hydrologic connectivity. Careful evaluation of purposeful reintroduction by translocating amphipods to restored wetlands may be considered (Larson et al. 2018), and our results suggest suitable wetland habitat conditions for amphipod translocation and stocking.

Conclusions

A probable cause of amphipod and scap abundance declines may be attributed to a loss of wetland quantity and quality (Austin et al. 2000; Anteau and Afton 2006). Using amphipods as an umbrella species may be useful for managers in identifying and prioritizing conservation for biodiversity across the Prairie Pothole Region. Likewise, the preservation and restoration of amphipod abundance may be achieved through explicit conservation and management of macrophyte diversity. The seemingly disparate strategies of conserving a single taxon (e.g., amphipods) and biodiversity (e.g., macrophyte communities) can be compatible and complementary.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10531-022-02379-9>.

Acknowledgements We thank N. Hansel-Welch, J. Boucher, P. Borash, and D. Perleberg for suggestions on protocol development and teaching plant identification skills. We are especially grateful to C. Isaacson and J. Carleen for protocol development, and to A. Medina, K. Cross, and M. Bieganek for helping collect and process data. Thank you to our reviewers J. Delaney, M. Anderson, J. Sauer, M. Gaikowski, and two anonymous journal reviewers for improving this manuscript.

Author contributions All authors have made significant contributions to this work. DML designed research, analyzed data, and wrote the manuscript; DD designed research, collected data, analyzed data; MA designed research, analyzed data, wrote the manuscript; BK designed research, collected data, analyzed data; MF designed research, analyzed data; ES designed research, edited manuscript; BT designed research, wrote the manuscript.

Funding Funding was provided by the Minnesota Environment and Natural Resources Trust Fund, the Minnesota Department of Natural Resources, U.S. Geological Survey, and Augsburg University.

Data availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Code availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Material availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Declarations

Conflict of interest The author declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Afton A, Hier R (1991) Diets of lesser scaup breeding in Manitoba. *J Field Ornithol* 62:325–334
- Afton A, Hier R, Paulus S (1991) Lesser scaup diets during migration and winter in the Mississippi flyway. *Can J Zool* 69:328–333
- Anteau MJ (2012) Do interactions of land use and climate affect productivity of waterbirds and prairie-pothole wetlands? *Wetlands* 32:1–9. <https://doi.org/10.1007/s13157-011-0206-3>
- Anteau M, Afton A (2006) Diet shifts of lesser scaup are consistent with the Spring Condition Hypothesis. *Can J Zool* 84:779–186
- Anteau MJ, Afton AD (2008a) Amphipod densities and indices of wetland quality across the upper-Midwest, USA. *Wetlands* 28:184–196. <https://doi.org/10.1672/07-53.1>
- Anteau MJ, Afton AD (2008b) Diets of Lesser Scaup during Spring Migration throughout the Upper-Midwest are Consistent with the Spring Condition Hypothesis. *Waterbirds* 31:97–106. [https://doi.org/10.1675/1524-4695\(2008\)31\[97:dolsds\]2.0.co;2](https://doi.org/10.1675/1524-4695(2008)31[97:dolsds]2.0.co;2)

- Anteau M, Afton A (2009) Wetland use and feeding by lesser scaup during spring migration across the upper Midwest, USA. *Wetl* 29:704–712
- Anteau MJ, Afton AD (2011) Lipid catabolism of invertebrate predator indicates widespread wetland ecosystem degradation. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0016029>
- Anteau MJ, Afton AD, Anteau ACE, Moser EB (2011) Fish and land use influence *Gammarus lacustris* and *Hyalella azteca* (Amphipoda) densities in large wetlands across the upper Midwest. *Hydrobiologia* 664:69–80. <https://doi.org/10.1007/s10750-010-0583-2>
- Austin JE, Afton AD, Anderson MG et al (2000) Declining scaup populations: issues, hypotheses, and research needs. *Wildl Soc Bull* 28:254–263
- Bal P, Tulloch AIT, Addison PFE et al (2018) Selecting indicator species for biodiversity management. *Front Ecol Environ* 16:589–598. <https://doi.org/10.1002/fee.1972>
- Bansal S, Lishawa SC, Newman S et al (2019) Typha (Cattail) invasion in North American Wetlands: biology, regional problems, impacts, ecosystem services, and management. *Wetlands* 39:645–684. <https://doi.org/10.1007/s13157-019-01174-7>
- Benoy GA, Nudds TD, Dunlop E (2002) Patterns of habitat and invertebrate diet overlap between tiger salamanders and ducks in prairie potholes. *Hydrobiologia* 481:47–59. <https://doi.org/10.1023/A:1021260920095>
- Brisson J, Rodriguez M, Martin CA, Proulx R (2020) Plant diversity effect on water quality in wetlands: a meta-analysis based on experimental systems. *Ecol Appl*. <https://doi.org/10.1002/eap.2074>
- Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. *Aquat Bot* 26:341–370. [https://doi.org/10.1016/0304-3770\(86\)90031-8](https://doi.org/10.1016/0304-3770(86)90031-8)
- Christensen JR, Crumpton WG (2010) Wetland invertebrate community responses to varying emergent litter in a prairie pothole emergent marsh. *Wetlands* 30:1031–1043. <https://doi.org/10.1007/s13157-010-0109-8>
- Engelhardt KAM, Ritchie ME (2002) The effect of aquatic plant species richness on wetland ecosystem processes. *Ecology* 83:2911–2924
- Euliss NH, Smith LM, Wilcox DA, Browne BA (2008) Linking ecosystem processes with wetland management goals: charting a course for a sustainable future. *Wetlands* 28:553–562
- Fast PLF, Clark RG, Brook RW, Hines JE (2004) Patterns of wetland use by brood-rearing lesser scaup in Northern Boreal forest of Canada. *Waterbirds* 27:177–182. [https://doi.org/10.1675/1524-4695\(2004\)027\[0177:POWUBB\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2004)027[0177:POWUBB]2.0.CO;2)
- Galatowitsch SM, van der Valk AG (1996a) Characteristics of recently restored wetlands in the prairie pothole region. *Wetlands* 16:75–83. <https://doi.org/10.1007/BF03160647>
- Galatowitsch SM, van der Valk AG (1996b) The vegetation of restored and Natural Prairie Wetlands. *Ecol Appl* 6:102–112. <https://doi.org/10.2307/2269557>
- Glazier DS (2014) Amphipoda. In: Reference Module in Earth Systems and Environmental Sciences. Elsevier
- Hanson JM (1990) Macroinvertebrate size-distributions of two contrasting freshwater macrophyte communities. *Freshw Biol* 24:481–491
- Hanson MA, Butler MG (1994) Responses to food web manipulation in a shallow waterfowl lake. *Hydrobiologia* 279–280:457–466. <https://doi.org/10.1007/BF00027877>
- Janke AK (2016) A Physiological Assessment of Wetland Habitats for Spring-migrating Ducks in the Agricultural Landscapes of the Southern Prairie Pothole Region. South Dakota State University
- Janke AK, Anteau MJ, Stafford JD (2019) Prairie wetlands confer consistent migrant refueling conditions across a gradient of agricultural land use intensities. *Biol Conserv* 229:99–112. <https://doi.org/10.1016/j.biocon.2018.11.021>
- Kahara SN, Chipps SR (2009) Predicting lesser scaup wetland use during spring migration in eastern South Dakota. *Gt Plains Res* 19:157–167
- Kalinkat G, Cabral JS, Darwall W et al (2017) Flagship umbrella species needed for the conservation of overlooked aquatic biodiversity. *Conserv Biol* 31:481–485. <https://doi.org/10.1111/cobi.12813>
- Larson DM, Anteau M, Isaacson C et al (2018) Restoring Wetland Invertebrates to Revive Wetland Habitats. Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g
- Larson DM, Cordts SD, Hansel-Welch N (2020a) Shallow lake management enhanced habitat and attracted waterbirds during fall migration. *Hydrobiologia* 846:3364–3379. <https://doi.org/10.1007/s10750-020-04342-7>
- Larson DM, Riens J, Myerchin S et al (2020b) Sediment excavation as a wetland restoration technique had early effects on the developing vegetation community. *Wetl Ecol Manag* 28:1–18. <https://doi.org/10.1007/s11273-019-09690-3>

- Larson DM, DeJong D, Anteau MJ, Fitzpatrick M, Keith B, Schilling EG, Thoele B (2021) Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019. U.S. Geological Survey data release. <https://doi.org/10.5066/P9INBET3>.
- Lindenmayer DB, Fischer J, Felton A et al (2007) The complementarity of single-species and ecosystem-oriented research in conservation research. *Oikos* 116:1220–1226. <https://doi.org/10.1111/j.2007.0030-1299.15683.x>
- Margules C, Pressey R (2000) A framework for systematic conservation planning. *Nature* 405:243–253
- McCauley LA, Anteau MJ, Van Der Burg MP, Wiltermuth MT (2015) Land use and wetland drainage affect water levels and dynamics of remaining wetlands. *Ecosphere* 6:1–22. <https://doi.org/10.1890/ES14-00494.1>
- McLean KI, Mushet DM, Sweetman JN et al (2019) Invertebrate communities of Prairie-Pothole wetlands in the age of the aquatic Homogenocene. *Hydrobiologia* 847:3773–3793. <https://doi.org/10.1007/s10750-019-04154-4>
- Merritt R, Cummins K, Resh V, Batzer D (2008) An introduction to the aquatic insects of North America, 4th edn. Kenall/Hunt Publishing Company, Dubuque
- Murkin H, Murkin E, Ball J (1997) Avian habitat selection and prairie wetland dynamics: a 10-year experiment. *Ecol Appl* 7:1144–1159
- Newman RM (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. *J North Am Benthol Soc* 10:89–114
- Oksanen J, Blanchet FG, Friendly M, et al (2019) Package “vegan” Community Ecology Package
- Olenick RJ, Gee JH (1981) Tiger salamanders (*Ambystoma tigrinum*) and stocked rainbow trout (*Salmo gairdneri*): potential competitors for food in Manitoa prairie pothole lakes. *Can Field-Naturalist* 95:129–132
- Ripley B, Venables B, Bates DM et al (2020) Package “MASS”
- Runge CA, Withey JC, Naugle DE et al (2019) Single species conservation as an umbrella for management of landscape threats. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0209619>
- Schilling EG, Loftin CS, Huryn AD (2009) Macroinvertebrates as indicators of fish absence in naturally fishless lakes. *Freshw Biol* 54:181–202. <https://doi.org/10.1111/j.1365-2427.2008.02096.x>
- Simberloff D (1998) Flagships, Umbrellas, and Keystones: Is single-species management passe in the landscape era? *Biol Conserv* 83:247–257
- Skawinski PM (2018) Aquatic Plants of the Upper Midwest: A photographic field guide to our underwater forests, 3rd edn.
- Strand K, Chipps S, Kahara S et al (2008) Patterns of prey use by lesser scaup *Aythya affinis* (Aves) and diet overlap with fishes during spring migration. *Hydrobiologia* 598:389–398
- Thomaz SM, Cunha ER (2010) The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages’ composition and biodiversity. *Acta Limnol Bras* 22:218–236. <https://doi.org/10.4322/actalb.02202011>
- Wasserstein RL, Schirm AL, Lazar NA (2019) Moving to a World Beyond “ $p < 0.05$.” *Am Stat* 73:1–19. <https://doi.org/10.1080/00031305.2019.1583913>
- Wiltermuth M (2014) Influences of climate variability and landscape modifications on water dynamics, community structure, and amphipod populations in large prairie wetlands. North Dakota State University
- Wiltermuth MT, Anteau MJ (2016) Is consolidation drainage an indirect mechanism for increased abundance of cattail in northern prairie wetlands? *Wetl Ecol Manag* 24:533–544. <https://doi.org/10.1007/s11273-016-9485-z>

Authors and Affiliations

Danelle M. Larson¹  · **Demey DeJong²** · **Michael J. Anteau³**  · **Megan J. Fitzpatrick⁴** · **Breanna Keith⁵** · **Emily G. Schilling²** · **Barry Thoele⁶**

Demey DeJong
demey.everett@gmail.com

Michael J. Anteau
manteau@usgs.gov

Megan J. Fitzpatrick
megan.fitzpatrick@state.mn.us

Breanna Keith
breanna.keith@my.smsu.edu

Emily G. Schilling
schillin@augsborg.edu

Barry Thoele
liveaqua@msn.com

¹ U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, USA

² Augsburg University, 2211 Riverside Avenue, Minneapolis, MN 55454, USA

³ U.S. Geological Survey, Northern Prairie Wildlife Research Center, 8711 37th Street SE, Jamestown, ND 58401, USA

⁴ Wetland Wildlife Population and Research Group, Minnesota DNR, 102 23rd St. NE, Bemidji, MN 56601, USA

⁵ Bemidji State University, 1500 Birchmont Dr. NE, Bemidji, MN 56601, USA

⁶ Lincoln Bait, LCC, 48301 County 21, Staples, MN, USA

**Prepared in collaboration with Minnesota Department of Natural Resources and
Bemidji State University**

Protocols for Collecting and Processing Macroinvertebrates from the Benthos and Water Column in Depressional Wetlands

Open-File Report 2022–1029

Protocols for Collecting and Processing Macroinvertebrates from the Benthos and Water Column in Depressional Wetlands

By Breanna R. Keith, Jake D. Carleen, Danelle M. Larson, Michael J. Anteau, and
Megan J. Fitzpatrick

Prepared in collaboration with Minnesota Department of Natural Resources and
Bemidji State University

Open-File Report 2022–1029

U.S. Department of the Interior
U.S. Geological Survey

U.S. Geological Survey, Reston, Virginia: 2022

For more information on the USGS—the Federal source for science about the Earth, its natural and living resources, natural hazards, and the environment—visit <https://www.usgs.gov> or call 1–888–ASK–USGS.

For an overview of USGS information products, including maps, imagery, and publications, visit <https://store.usgs.gov/>.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this information product, for the most part, is in the public domain, it also may contain copyrighted materials as noted in the text. Permission to reproduce copyrighted items must be secured from the copyright owner.

Suggested citation:

Keith, B.R., Carleen, J.D., Larson, D.M., Anteau, M.J., and Fitzpatrick, M.J., 2022, Protocols for collecting and processing macroinvertebrates from the benthos and water column in depressional wetlands: U.S. Geological Survey Open-File Report 2022–1029, 22 p., <https://doi.org/10.3133/ofr20221029>.

ISSN 2331-1258 (online)

Acknowledgments

All authors have made substantial contributions to this work. These protocols have been adapted and improved over the past 15 years. We thank Adam Janke, Alan Afton, Mark Hanson, Ray Norgaard, David Mushet, Allen Rutherford, and Carl Isaacson for their contributions to protocol development.

These protocols were funded by the Minnesota Environment and Natural Resources Trust Fund, the U.S. Geological Survey, and the Minnesota Department of Natural Resources.

This report was kindly peer reviewed and improved by Brian Tangen and Jennifer Sauer from the U.S. Geological Survey.

Contents

Acknowledgments	iii
Abstract	1
Introduction	1
Field Protocols	2
Selecting Survey Stations	2
Invertebrate Sampling	2
Laboratory Protocols	5
Sample Organization and Preservation During Storage	5
Sample Picking	5
Identifying Macroinvertebrates	5
Calculating Macroinvertebrate Densities	5
Summary	8
References Cited	8
Glossary	9
Appendix 1. Field Supplies List	10
Appendix 2. Acquiring a Modified Dip Net	11
Appendix 3. Detailed Instructions for Generating Survey Stations	12
Appendix 4. Macroinvertebrate Sample Collection and Preservation Protocols	13
Appendix 5. Field Data Sheets	14
Appendix 6. Laboratory Supply List	16
Appendix 7. Laboratory Data Sheets	17
Appendix 8. Directions for Building a Subsampling Apparatus	21
Appendix 9. Detailed Instructions for Macroinvertebrate Picking	22

Figures

1. Map showing an example of a macroinvertebrate survey using a stratified random sampling design for depressional wetlands.....3
2. Photograph showing a technician collecting a macroinvertebrate sample from the water column using a modified dip net where the frame was bent at a 90-degree angle relative to the net handle 4
3. Diagram showing benthic and water column sweeping techniques using dip nets in depressional wetlands.....4
4. Photographs showing the four subsampling steps in processing macroinvertebrates in the laboratory 6
5. Diagram showing the steps for calculating benthic and water column macroinvertebrate densities collected from dip nets in depressional wetlands 7

Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
Area		
square meter (m ²)	0.0002471	acre
square meter (m ²)	10.76	square foot (ft ²)
hectare (ha)	2.471	acre
hectare (ha)	0.003861	square mile (mi ²)
Volume		
cubic meter (m ³)	6.290	barrel (petroleum, 1 barrel = 42 gal)
cubic meter (m ³)	264.2	gallon (gal)
cubic meter (m ³)	0.0002642	million gallons (Mgal)
cubic meter (m ³)	35.31	cubic foot (ft ³)
cubic meter (m ³)	1.308	cubic yard (yd ³)
cubic meter (m ³)	0.0008107	acre-foot (acre-ft)
milliliter (mL)	0.03381402	ounce, fluid (fl. oz)

Supplemental Information

Mesh and sieve sizes are given in micrometers (μm).

Abbreviations

GPS	Global Positioning System
SOP	standard operating procedure
>	greater than
<	less than

Protocols for Collecting and Processing Macroinvertebrates from the Benthos and Water Column in Depressional Wetlands

By Breanna R. Keith,¹ Jake D. Carleen,¹ Danelle M. Larson,² Michael J. Anteau,² and Megan J. Fitzpatrick³

Abstract

Freshwater aquatic macroinvertebrates are key links in food webs and nutrient cycles, and thus often serve as biological indicators of ecosystem health. Macroinvertebrate investigations in research and monitoring require consistent and reliable field and laboratory procedures. Comprehensive standard operating procedures for sampling macroinvertebrates from depressional wetlands, which can range from riverine floodplain lakes to wetlands of any size and hydrologic regime, remain relatively sparse. This report provides step-by-step protocols for efficient use of time and resources while collecting and processing aquatic macroinvertebrate samples; for example, a single wetland can typically be field surveyed in less than 1 hour, and the samples can be processed in the laboratory in less than 2 hours. Samples can be collected from inside a motorboat or canoe or while wading. This procedures manual describes dip netting to collect macroinvertebrates from the wetland bottom and water column separately to facilitate investigations of habitat use by species occupying different areas of the wetland. This report also provides descriptive supplemental materials and data sheets to assist with the preparation of survey maps, the acquisition of field and laboratory equipment, and the calculation of macroinvertebrate densities from the wetland bottom and water column. These procedures can be applied to most macroinvertebrate species and communities that inhabit a variety of wetland sizes and types. Uses and applications can range from elementary and secondary environmental education to rigorous scientific evaluations of community abundance, diversity, distribution, or species-habitat relations.

Introduction

The purpose of this standard operating procedure (SOP) is to outline and describe step-by-step instructions for efficiently collecting, preserving, and processing aquatic macroinvertebrate samples, and for subsequently calculating species counts and densities. Although several related field and laboratory manuals are available (Barbour and others, 1999; Moulton and others, 2002; DiFranco, 2014; Minnesota Pollution Control Agency, 2014), this SOP was developed to provide comprehensive and adaptable procedures that can quantify macroinvertebrates in depressional wetlands. Depressional wetlands form in topographic depressions with closed contours on at least three sides that collect standing water for various hydroperiods. Depressional wetlands occur globally across all biomes in a variety of wetland types, such as but not limited to vernal pools, freshwater marshes, semi-permanent wetlands, permanent wetlands, riverine floodplain wetlands, and shallow lakes.

Related SOPs often target wadable streams and rivers with little organic matter in the benthos (the bottom of the aquatic system), resulting in methods that are commonly ineffective in systems with standing water and soft, organic sediments. Other SOPs describe passive sampling techniques, such as passive activity traps suspended in the wetland overnight (Hanson and others, 2000). Activity traps provide relative abundance estimates and minimize sample debris, but also restrict quantifying macroinvertebrate abundance and increase sample time and travel costs, and can be biased by time of day, weather, water temperature, and predators. Some procedures detail intensive, active sampling techniques that collect large quantities of organic matter (for example, using dredge samplers or extensive dip-netting surveys), which require substantial resources for sample preservation and laboratory processing.

Several factors should be considered before the selection and use of an SOP, including the type of data required (that is, quantitative versus qualitative), the project's purpose (for example, environmental education versus rigorous research), the project's scope (for example, sampling one system intensively versus less intensive sampling for multisystem

¹Bemidji State University, Center for Sustainability Studies.

²U.S. Geological Survey.

³Minnesota Department of Natural Resources, Wetland Wildlife Populations and Research Group.

comparison), and the availability of resources (such as time, personnel, storage space for samples, and preservation chemicals). For further guidance on selecting procedures for macroinvertebrate investigations, see Turner and Trexler (1997), Anderson and others (2013), and Meyer and others (2013); however, this SOP is highly adaptable for the user's needs regarding all data types, project purpose and scope, and limited resources. As an example, the SOP has been effectively applied to rigorous scientific research on invertebrate and habitat associations (Larson and others, 2022). Many steps in this SOP instruct the user to choose between options to best suit their circumstances.

This SOP improves upon several published procedures for the sampling of amphipods and other macroinvertebrates in depressional wetlands of the United States (Anteau, 2006; Anteau and Afton, 2008; Anteau and others, 2011; Janke, 2016). Revisions were derived from the experiences of the authors, who trialed the various methods within several hundred depressional wetland systems throughout the Prairie Pothole Region of the United States. Major modifications from previous studies that were adopted within this SOP include (1) an optional stratified random sampling design to capture near-shore and offshore habitats, (2) instructions for locating parallel sample transects instead of radial transects to evenly disperse survey stations throughout the wetland, (3) instructions for differing sweep net techniques for improved quantification of invertebrates, (4) an optional vertical sweep to capture and quantify macroinvertebrates occupying the water column only, and (5) instructions for sampling a smaller and standardized benthos area (0.5 meter [m]) to increase reproducibility of sample technique, as well as substantially reduce the amount of organic material to store and preserve.

Field Protocols

This section of the SOP describes processes for selecting survey stations, sampling aquatic macroinvertebrates in wetlands, and preserving samples containing specimens and wetland debris. For field supply information, see appendixes 1 and 2. Descriptive data sheets and step-by-step protocols coinciding with field processes are provided in appendixes 3–5.

Selecting Survey Stations

At each wetland, samples are collected from several stratified-random survey stations along transects within near-shore zones (less than [$<$] 5 m) and likely open-water zones ($<$ 50 m from shore). This stratified random sampling design is optimal if the wetland is greater than ($>$) 1 hectare and $>$ 1.5 m deep because there will be zones of vegetation and open water to target. If wetlands are small with little change in water depth or vegetation community, samples could simply be taken randomly, ignoring stratum designations.

Following methodology from Anteau and Afton (2008) and Anteau and others (2011), macroinvertebrates are collected from eight locations dispersed throughout the wetland

(fig. 1); however, the SOP user can choose the number of survey stations for their work dependent on wetland size or study objectives. Survey stations are predetermined to ensure randomization of selection. Stations use the emergent vegetation zone as the spatial reference for survey point spacing along transects, but details in appendix 3 describe solutions when emergent cover is lacking or covering the wetland. The stations are uniquely identified and mapped using ArcMap software (Esri, Redlands, California) and programmed into a Global Positioning System device for field navigation. This selection process can be completed manually using step-by-step instructions (appendix 3).

Invertebrate Sampling

Most surveys should be completed from inside a boat because depressional wetlands frequently contain soft benthic zones where sediments are easily disturbed and depths that cannot be sampled via wading. Wetlands with hard bottoms and shallow depths (less than or equal to 1 m) throughout could be sampled via wading if care is taken to not disturb sediments before collecting samples. Samples are collected using D-frame dip nets constructed with a mesh that is small enough to contain macroinvertebrates but large enough to allow some organic matter and sediment to filter out. Depending on the body size of targeted macroinvertebrates, suitable mesh size can range from 500 to 1,200 micrometers. Sampling depths should be limited to the length of the dip-net handle to reduce error to invertebrate quantification from unknown sweep length. Handle extensions could be added to the dip net to sample deeper waters.

Samples from the benthic and water column zones can be collected and stored separately, which requires sweeps using two nets at each survey station. Both nets should be the same size (handle length of at least 1.5-m and frame dimensions of about a 0.3-m width and 0.3-m height), but the water column net (also referred to as the “modified” net) contains a frame bent at a 90-degree angle relative to the net handle (figs. 2 and 3). This modification allows for the collection of macroinvertebrates from a vertical path extending throughout the entire water column zone. Both net handles must be marked with depth increments so that water depth can be measured while sampling. Samples from each survey station can be stored in separate containers or composited into one bottle, depending on whether the research objectives are at the survey station level or the wetland level. Samples should be immediately preserved with 95-percent ethanol to minimize predation of specimens and decay during storage.

Step-by-step procedures coinciding with the field sampling and preservation techniques listed previously are described in appendix 4. Field personnel should complete two data sheets while at the wetland: (1) a site information data sheet for key information about the wetland and (2) a macroinvertebrate sampling data sheet to be filled out while sampling at each survey station (appendix 5).

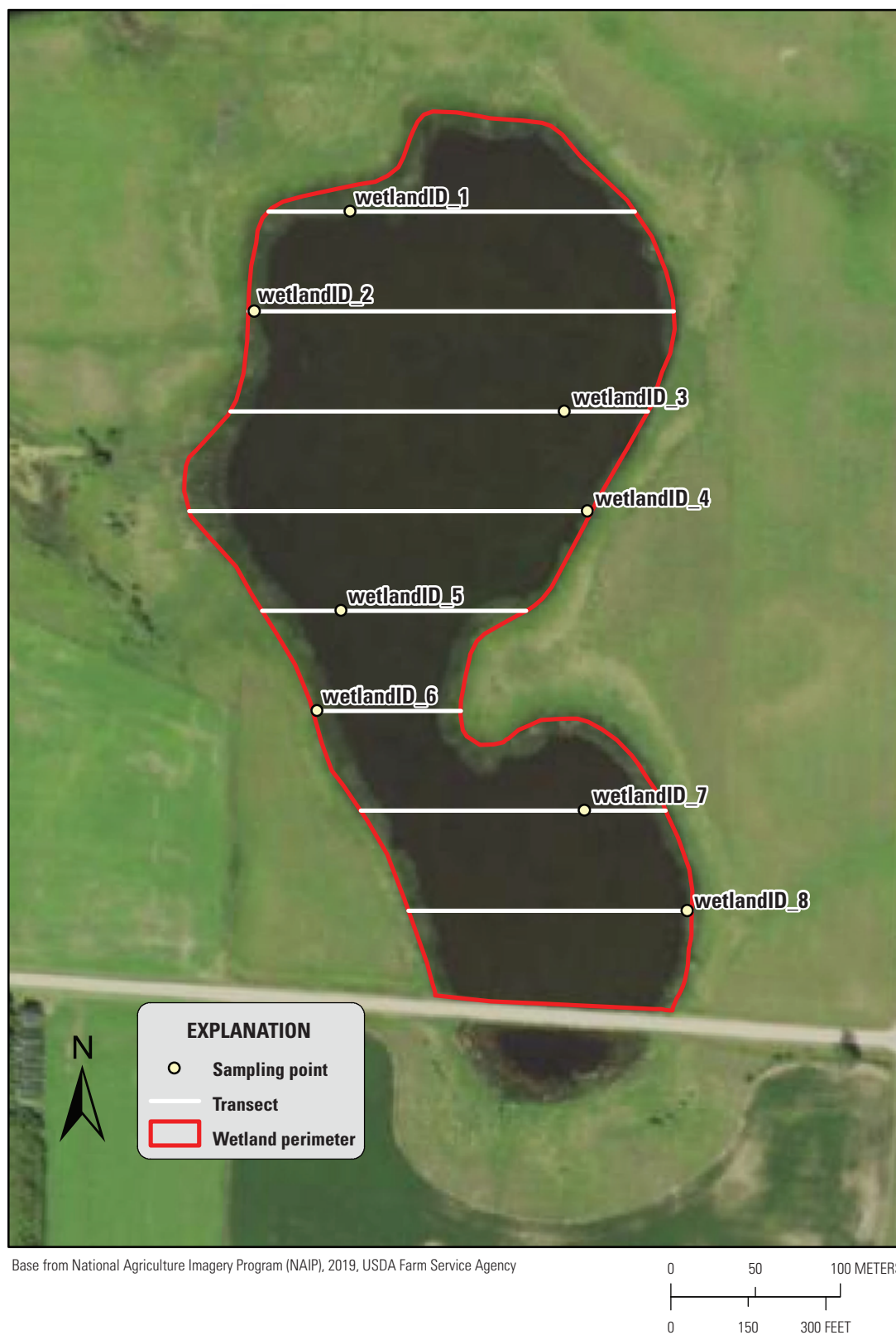


Figure 1. An example of a macroinvertebrate survey using a stratified random sampling design for depressional wetlands. Survey points (uniquely labeled with wetland name and survey number; for example, “wetlandID_1”) fall along the transects within a stratum.



Figure 2. Technician collecting a macroinvertebrate sample from the water column using a modified dip net where the frame was bent at a 90-degree angle relative to the net handle.

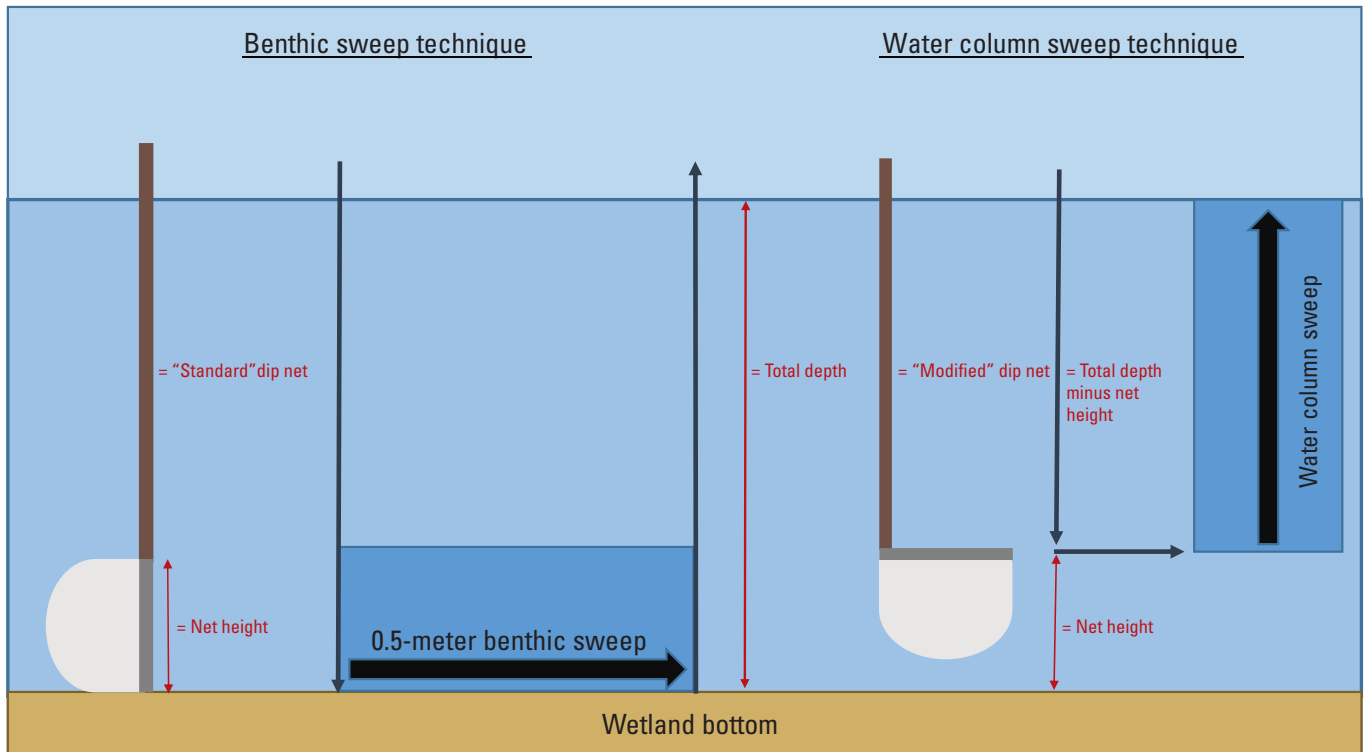


Figure 3. Benthic and water column sweeping techniques using dip nets in depressional wetlands. In a benthic sweep, the net is plunged vertically into the top layers of wetland benthos, swept for a standard 0.5-meter distance, and then pulled up vertically to sample only the benthos. In a water column sweep, a modified dip net (where the net is angled 90 degrees from the handle) is plunged vertically to the benthos, minus the distance of the net height, and slowly towed vertically to the surface to capture free-floating macroinvertebrates within the water column.

Laboratory Protocols

This section of the SOP describes the process for organizing samples, decanting samples to prevent decay, picking macroinvertebrates, and preserving specimens for long-term (>1 year) archiving. Laboratory personnel should consult the laboratory supply list (appendix 6) and complete three data sheets to assist with these processes (appendix 7).

Sample Organization and Preservation During Storage

Upon returning to the laboratory, check field data sheets to ensure all sample bottles are accounted for. Then, record all samples on the macroinvertebrate sample inventory log (appendix 7). All samples should be stored in a flammable safety cabinet because the ethanol used for preservation is flammable. Organize sample bottles in the cabinet according to the date they were collected to ensure the oldest samples are processed first.

To prevent decomposition, samples must be monitored and occasionally decanted by carefully removing old ethanol and refreshing the sample with new 95-percent ethanol. All samples should be inspected and decanted (only if necessary) within 72 hours of field collection and every 2–3 weeks thereafter until processed. First, put on gloves and safety goggles, carefully open the sample bottle, and waft the sample's smells towards your nose. If the sample smells like alcohol, no decanting is required. If the sample smells slightly organic, it must be decanted. If the smell of decaying organic matter overtakes the smell of alcohol, the sample may be compromised. If decanting is needed, carefully decant the ethanol through a 500-micrometer sieve held over a labeled ethanol waste container. Backwash the sieve over the sample bottle with 95-percent ethanol to collect any organisms or debris on the sieve. Inspect the sieve with a 3× magnifying light to ensure no macroinvertebrates remain. Pour fresh 95-percent ethanol into the sample bottle following a 1 part sample to 2 parts ethanol ratio. Label the bottle with decant date and fill out the macroinvertebrate sample decanting form (appendix 7) for organization.

Sample Picking

Macroinvertebrates are ideally “picked” from the sample containing debris under good lighting, ventilation, and microscopy in the laboratory to maximize detection of all

species. Alternatively, macroinvertebrates could be processed at the wetland in sunlight with a hand magnifier, especially for environmental education applications or rapid assessments of select species. The samples could be picked in entirety or subsampled, depending on user preference for time allotted per sample. For example, if benthic samples or water column samples are large and have a picking time greater than 2 hours, macroinvertebrates could be quantitatively subsampled using a randomized fixed count of 25 percent of the entire sample volume or a fixed number of macroinvertebrate specimens per sample (determined by the SOP user). A minimum of 25 percent of the sample volume must be picked to increase the likelihood that the subsample is representative of the overall sample.

Guidance for building a subsampling apparatus is provided in appendix 8, and all subsampling and picking is completed using detailed instructions described in appendix 9 and depicted in [figure 4A–D](#). Macroinvertebrates can be identified and quantified during the picking process or preserved in 70-percent ethanol and processed later.

Identifying Macroinvertebrates

Invertebrates may be identified to any level (family, genus, or species) using an appropriate microscope and identification key. If desired, body length measurements can be collected simultaneously for the approximation of organism biomass, as described in Benke and others (1999). All identification and length data should be recorded in the macroinvertebrate identification data sheet (appendix 5).

Calculating Macroinvertebrate Densities

Invertebrate counts (the number of individuals) and volumetric densities (the number of individuals per cubic meter) can be calculated for the benthos and (or) water column because samples are collected using a dip net with known net and sweep areas. These calculations can be applied in Microsoft Excel, R, or similar software. The steps to calculate volumetric and areal densities for the benthos and water column are shown in [figure 5](#).

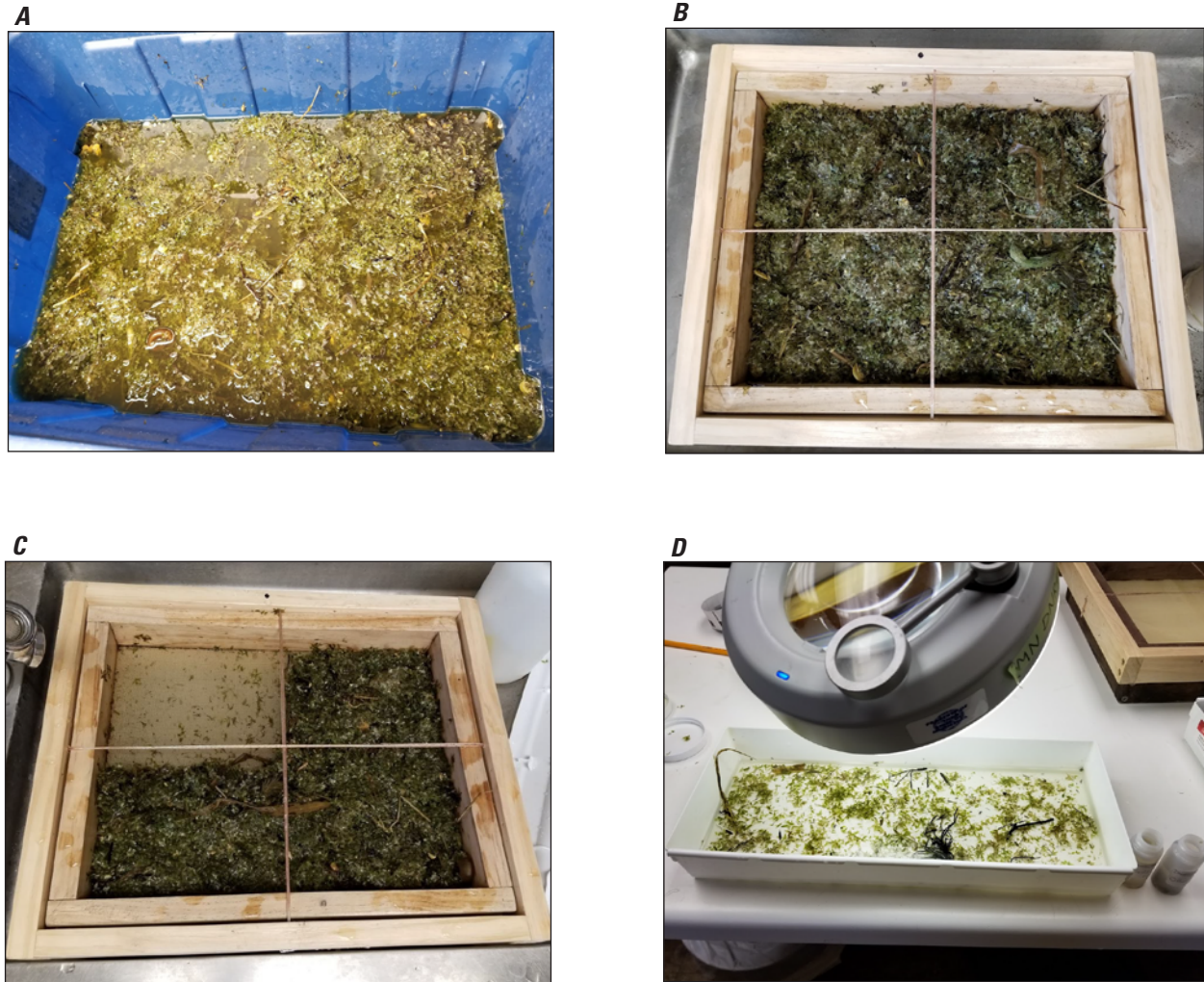


Figure 4. The four subsampling steps in processing macroinvertebrates in the laboratory. *A*, the composited invertebrate sample, which has been evenly mixed and poured over a squared subsampling apparatus (see appendix 8 for details on building this apparatus); *B*, a gridded frame overlaying the subsampling apparatus to divide the sample into four equal parts; *C*, one grid (or 25 percent of the sample) processed in entirety for macroinvertebrates. The user may choose to sample a minimum of one grid or as many as four grids, depending on time and research objectives; *D*, a part of contents from one grid, which are placed in a white tray under high lighting and 3× magnification for picking invertebrates from the detritus.

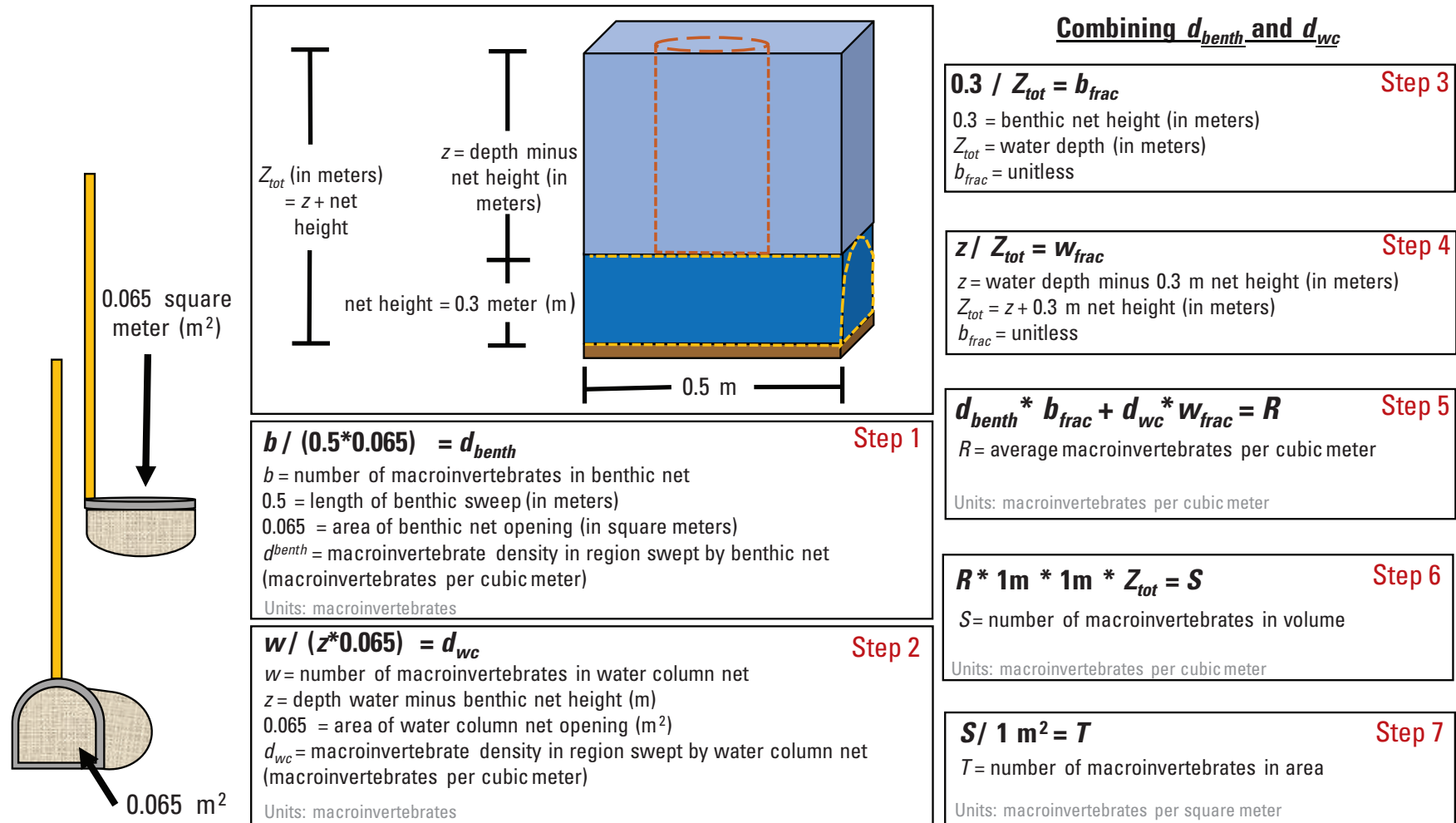


Figure 5. The steps for calculating benthic (d_{benth}) and water column (d_{wc}) macroinvertebrate densities collected from dip nets in depressional wetlands. To calculate volumetric density, proceed from steps 1 to 6. To calculate areal density, proceed from steps 1 to 7.

Summary

Aquatic macroinvertebrate investigations are often integrated into wetland research and monitoring and require consistent and reliable field and laboratory procedures. This SOP was developed to meet a need for comprehensive and standardized procedures that can be applied to a wide range of depressional wetland types. These procedures improved upon previously published works and are designed to be descriptive but adaptable to accommodate a wide range of project objectives. Following these procedures, a wetland can typically be field surveyed at the wetland and processed in the laboratory in a matter of hours. The use of a two different dip nets allows for investigations within either the benthos or the water column zones. The supplemental materials assist with the preparation of field sampling, laboratory processing, and the calculation of macroinvertebrate abundances. Collectively, these procedures can be applied to a wide variety of macroinvertebrate investigations in both educational and research-based settings.

References Cited

- Anderson, J.T., Zilli, F.L., Montalto, L., Marchese, M.R., McKinney, M., and Park, Y.L., 2013, Sampling and processing aquatic and terrestrial invertebrates in wetlands: Wetland Techniques, v. 2, p. 143–195. [Also available at https://doi.org/10.1007/978-94-007-6931-1_5.]
- Anteau, M.J., 2006, Ecology of lesser scaup and amphipods in the upper-Midwest—Scope and mechanisms of the Spring Condition Hypothesis and implications for migration habitat conservation: Baton Rouge, Louisiana, Louisiana State University, Ph.D. dissertation, 213 p.
- Anteau, M.J., and Afton, A.D., 2008, Amphipod densities and indices of wetland quality across the upper-Midwest, USA: Wetlands, v. 28, no. 1, p. 184–196. [Also available at <https://doi.org/10.1672/07-53.1>.]
- Anteau, M.J., Afton, A.D., Anteau, A.C.E., and Moser, E.B., 2011, Fish and land use influence *Gammarus lacustris* and *Hyalella azteca* (Amphipoda) densities in large wetlands across the upper Midwest: Hydrobiologia, v. 664, no. 1, p. 69–80. [Also available at <https://doi.org/10.1007/s10750-010-0583-2>.]
- Barbour, M.T., Gerritsen, J., Snyder, B.D., and Stribling, J.B., 1999, Rapid bioassessment protocols for use in streams and wadable rivers—Periphyton, benthic macroinvertebrates, and fish (2d ed.): Washington, D.C., U.S. Environmental Protection Agency, EPA 841–B–99–002, [variously paged].
- Benke, A.C., Huryn, A.D., Smock, L.A., and Wallace, J.B., 1999, Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States: Freshwater Science, v. 18, no. 3, p. 308–343. [Also available at <https://doi.org/10.2307/1468447>.]
- DiFranco, J.L., 2014, Protocols for sampling aquatic macroinvertebrates in freshwater wetlands: Portland, Maine, Maine Department of Environmental Protection, DEPLW0640A–2014, 10 p.
- Hanson, M.A., Roy, C.C., Euliss, N.H., Zimmer, K.D., Riggs, M.R., and Butler, M.G., 2000, A surface-associated activity trap for capturing water-surface and aquatic invertebrates in wetlands: Wetlands, v. 20, no. 1, p. 205–212. [Also available at [https://doi.org/10.1672/0277-5212\(2000\)020\[0205:ASAATF\]2.0.CO;2](https://doi.org/10.1672/0277-5212(2000)020[0205:ASAATF]2.0.CO;2).]
- Janke, A.K., 2016, A physiological assessment of wetland habitats for spring-migrating ducks in the agricultural landscapes of the southern Prairie Pothole Region: Brookings, South Dakota, South Dakota State University, Ph.D. dissertation, 217 p.
- Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2022, High abundance of a single taxon (amphipods) predicts aquatic macrophyte biodiversity in prairie wetlands. Biodiversity and Conservation [original paper], 22 p., accessed February 2022 at <https://doi.org/10.1007/s10531-022-02379-9>.]
- Meyer, M.D., Davis, C.A., and Bidwell, J.R., 2013, Assessment of two methods for sampling invertebrates in shallow vegetated wetlands: Wetlands, v. 33, no. 6, p. 1063–1073. [Also available at <https://doi.org/10.1007/s13157-013-0462-5>.]
- Minnesota Pollution Control Agency, 2014, Macroinvertebrate community sampling protocol for depressional wetland monitoring sites: Minnesota Pollution Control Agency, Biological Monitoring Program, wq-bwm5–02, 7 p.
- Moulton, S.R., II, Kennen, J.G., Goldstein, R.M., and Hambrook, J.A., 2002, Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 2002–150. [Also available at <https://doi.org/10.3133/ofr2002150>.]
- Turner, A.M., and Trexler, J.C., 1997, Sampling aquatic invertebrates from marshes—Evaluating the options: Journal of the North American Benthological Society, v. 16, no. 3, p. 694–709. [Also available at <https://doi.org/10.2307/1468154>.]

Glossary

amphipod Aquatic crustacean in the order Amphipoda.

benthic/benthos Refers to the bottom; for example, the benthic zone in a wetland system is at or near the sediment surface.

decant Slowly pour liquid from container without disturbing the underlying solids.

macroinvertebrates Animals lacking a backbone that can be seen with the naked eye, such as insects, mollusks, worms, and crustaceans.

picking The process of removing macroinvertebrates from sample debris.

sieve Apparatus consisting of a wire mesh and solid frame that allows for the separation of smaller particles (for example, sediment) from larger particles (for example, macroinvertebrates).

survey station Predetermined location in wetland at which the macroinvertebrate sample is collected.

sweep A steady, smooth movement; in this report, referring to the movement of passing a dip net across the wetland bottom or through the water column to collect invertebrates.

transect Straight line extending across the lake or wetland surface, along which survey stations are positioned for collecting invertebrates.

wetland Topographic depression containing a wetland system.

Appendix 1. Field Supplies List

This appendix contains a list of the necessary supplies that should be gathered before sample collection and preservation.

- 1 or 2 500–1,200-micrometer dip nets (see appendix 2 on acquiring a “modified” net, as shown in [fig. 2](#))
- Field data sheets (appendix 5)
- 225–1,000-milliliter sample bottles (small bottles for individual survey station samples; large bottles for composite samples)
- Storage tub(s)
- 95-percent ethanol (for sample preservation)
- Internal labels
- External labels
- Clear packing tape
- Gloves
- Global Positioning System with preprogrammed survey station points
- Wetland map
- Clipboard
- Ethanol-resistant markers
- Pencils
- Flagging tape
- Waders
- U.S. Coast Guard-approved inherently buoyant personal flotation devices (life jackets)
- Canoe/sport boat/motor (if needed)
- Paddles

Appendix 2. Acquiring a Modified Dip Net

Modified D-frame dip nets referenced in this report for water column sweeping (that is, those with the net frame bent at a 90-degree angle from the net handle, as shown in [fig. 2](#)) are generally not readily available through major suppliers; however, these nets can be custom made or constructed from traditional dip nets.

Option 1—Ordering custom nets.—Some scientific supply companies will accept custom orders for dip nets. Although convenient, this option is typically more expensive and requires a longer wait time to receive the product.

Option 2—Manually bending the metal frame.—Some dip nets are manufactured with a durable yet malleable metal frame that can be easily bent. To bend the net frame at a 90-degree angle, lay the net on the ground with the net opening facing up, stand on the metal D frame, and slowly pull up on the wooden handle until it is perpendicular to the ground.

Option 3—Modifying the net frame's connection.—Some dip nets contain a D frame that can be detached from the net handle, often by simply unscrewing it. This allows for the insertion of an elbow joint pipe fitting, which positions the D-frame net at a 90-degree angle in relation to the handle.

Appendix 3. Detailed Instructions for Generating Survey Stations

These instructions are based on ArcMap version 10.6 (Esri, Redlands, California).

1. Using ArcMap software, extract or construct the open-water shapefile corresponding to the targeted study wetland. This shapefile may be extracted from existing publicly accessible wetlands data such as the National Wetlands Inventory data layer (U.S. Fish and Wildlife Service, 2020), or it may be created manually by constructing a polygon in reference to aerial imagery. For either method, select data or imagery collected as close to your sampling time as possible to ensure desktop-sourced wetland boundaries are representative of current conditions.
2. Navigate to the “create fishnet” tool (“Toolboxes” → “Data Management Tools” → “Sampling” → “Create Fishnet”). Using the measuring tool, determine whether or not the distance between the northern and southern shoreline is longer than the distance between the eastern and western shoreline; if longer, transects should run in an east–west direction (n rows = 9, n columns = 1, where n is number), and if shorter should run in a north–south direction (n rows = 1, n columns = 9).
3. Use the “clip” tool (“Toolboxes” → “Analysis Tools” → “Extract” → “Clip”) to clip the transects to the wetland outline.
4. Each transect will contain one survey station. The survey stations are in two strata: either less than 5 meters (m) of the innermost ring of emergent vegetation near shore or 50 m from the innermost ring of emergent vegetation (see example in [fig. 1](#)). If the wetland is small, it is possible to modify the strata according to proportional distances between the vegetation zone and wetland center. Use a random-selection technique (such as a coin flip or a random number generator) to select the starting transect and shoreline to alternate between strata.
5. Create survey point shapefiles by creating a new feature class for point features and manually placing them in the appropriate positions along the transects.
6. Assign a unique identifier to each survey station that includes the wetland name and station number.
7. Print a copy of the map, and load survey station shapefiles onto a Global Positioning System as waypoints, ensuring survey points are clearly labeled with a unique identifier on both.

Reference Cited

U.S. Fish and Wildlife Service, 2020, National Wetlands Inventory: U.S. Fish and Wildlife Service web page, accessed July 18, 2021, at <https://www.fws.gov/wetlands/>.

Appendix 4. Macroinvertebrate Sample Collection and Preservation Protocols

This appendix contains the steps for collecting and preserving macroinvertebrate samples.

1. After loading the boat with necessary supplies (appendix 1), use the field Global Positioning System (GPS) device to navigate to a preprogrammed survey station.
2. Upon arriving at the survey station, prepare to take a benthic sweep. To collect the benthic sample, use the unmodified dip net. Plunge the net to the wetland bottom in a vertical position to avoid capturing invertebrates on the way down. Note the water depth by using depth increments marked on the handle and record it on the macroinvertebrate sampling data sheet (appendix 5). If water depth exceeds the length of the dip-net handle, either attach an extension to the handle or move along the transect toward shore until reaching an appropriate depth. If the GPS coordinates changed from the predetermined point because of depth or accessibility limitations, record the actual GPS coordinates for repeating samples or spatial analyses.
3. While keeping the net perpendicular to the wetland bottom, sweep horizontally for 0.5 meter (m), which can be referenced on your boat for improved accuracy. Skim the net along the top layer of sediment to the depth at which target organisms reside (generally a few centimeters deep) for 0.5 m. Take care to keep the handle vertical (that is, straight). Then, raise the net out of the water while keeping it in a vertical position to avoid capturing water column invertebrates.
4. Once the net emerges from the water, rotate it into a horizontal position. If vegetation and other organic debris are hanging over the net's rim, place 50 percent of the vegetation/debris into the net and discard the rest. While keeping the dip-net rim out of the water, rinse sediment and fine organic matter from the sample by gently dipping the bell of the net in and out of the water, or by moving it from side to side slightly below the water's surface. Splash the edges with water to flush all materials to the bottom, allow water to drain out, and place the contents into an appropriately labeled benthic sample bottle. Benthic samples can either be stored in separate bottles for each survey station or composited into the same bottle(s) for wetland-level estimates. Each bottle should contain an external and internal label. Internal labels must be written on an ethanol-resistant paper with an ethanol-resistant writing utensil (such as a graphite pencil), and external labels should be secured with clear packing tape. Immediately pour 95-percent ethanol into the bottle to an approximate volumetric ratio of 1 part sample to 2 parts ethanol.
5. An optional step for wetlands with sufficient water depth and depending on research objectives is to prepare to take a water column sweep. Shift the boat slightly (1–2 m) away from the disturbed area. To collect the water column sample, plunge the modified dip net to a depth equal to the total water depth minus the benthic net height. Shake out the net to ensure the mesh is free of the frame. Move the net sideways to undisturbed water and raise it vertically through the water column. Once the net emerges, while keeping the rim out of the water, splash the edges with water to flush all materials to the bottom and place the contents into an appropriately labeled water column sample bottle; like the benthic samples, water column samples can either be stored in separate bottles for each survey station or composited into the same bottle(s). Immediately pour 95-percent ethanol into the bottle to an approximate volumetric ratio of 1 part sample to 2 parts ethanol.
6. Repeat steps 2–5 for each survey station and ensure both field data sheets (appendix 5) have been completed before leaving the wetland.

Appendix 5. Field Data Sheets

This appendix contains two field data sheets, one for macroinvertebrate sampling and one for site information (figs. 5.1 and 5.2).

[Initials, initials of the person(s) completing field work and certifying that all data were collected before leaving the wetland; ID, identifier; cm, centimeter; no., number; B, indicates benthic sample bottles; WC, indicates water column sample bottles]

	Data complete	Initials:		
	Data entry	Initials: Date:		
Macroinvertebrate sampling				
Wetland name:	Date:	Crew:		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Survey station ID:	Depth (cm):	No. of bottles (B WC):		
Benthic sweeps	Label name:			
Total no. of benthic ("B") bottles: 0 1 2 3 4 5 6 7 8 ____				
Water column sweeps	Label name:			
Total no. of water column ("WC") bottles: 0 1 2 3 4 5 6 7 8 ____				
Notes				

Figure 5.1. Data sheet for macroinvertebrate sampling.

[Initials, initials of the person(s) completing field work and certifying that all data were collected before leaving the wetland]

	Data complete	Initials:
	Data entry	Initials: Date:
Site information		
Wetland name:	Date(s):	Crew:
Wetland coordinates:		
Site access notes (parking, approaches, watercraft used, and so on)		
Time of day		
Sample start time:	Sample end time:	
Site checklist		
☐ Macroinvertebrate Sampling	☐	
☐	☐	
Notes		

Figure 5.2. Data sheet for site information.

Appendix 6. Laboratory Supply List

This appendix contains a list of the supplies that are needed for organizing samples, decanting samples to prevent decay, picking macroinvertebrates, and preserving specimens for long-term archiving.

- Macroinvertebrate laboratory data sheets (appendix 7)
- Standardized subsampling apparatus with 500-micrometer sieve and gridded frame (appendix 8)
- White plastic or enamel pan for sorting
- Small 500-micrometer sieve for decanting ethanol
- 3× lighted magnifier
- Forceps
- Stainless steel spatula
- Ethanol waste container
- Funnel
- Gloves
- Specimen vials with screw caps (for example, 20-milliliter scintillation vials or 125-milliliter Nalgene bottles)
- Sample labels
- Ethanol-resistant pen
- 70-percent ethanol for long-term preservation of specimens (after picked from sediments and organic matter)
- Wash bottles with tap water
- Hand tally counter
- Scissors
- Spray bottle

Appendix 7. Laboratory Data Sheets

This appendix contains three laboratory data sheets, one for sample inventory, one for sample decanting, and one for sample logging (figs. 7.1–7.3).

[ID, identifier; no., number]

Macroinvertebrate sample inventory form					
Survey station ID (unique)	Date collected	No. of benthic sample bottles	No. of water column sample bottles	Date and initials of laboratory picking	Date and initials of laboratory identification/quantification

Figure 7.1. Data sheet for macroinvertebrate sample inventory.

[ID, identifier; Y/N, yes/no]

Macroinvertebrate sample decanting form				
Survey station ID (unique)	Sample type (benthic versus water column)	Date checked	Decanted (Y/N)	Worker initials

Figure 7.2. Data sheet for macroinvertebrate sample decanting.

[ID, identifier; no., number; MMDDYYYY, month, day, year]

Macroinvertebrate sample log form									
Survey station ID (unique)	Sample type (benthic versus water column)	No. of bottles	Worker initials	Date picked (MMDDYYYY)	Time started (military time)	Time finished (military time)	No. of grids sorted	No. of invertebrate specimen vials	Notes

Figure 7.3. Data sheet for macroinvertebrate sample logging.

Appendix 8. Directions for Building a Subsampling Apparatus

The subsampling apparatus used in this standard operating procedure consists of two components: a sieve on which the sample is deposited and a gridded frame that lies on top of the sieve. This apparatus can be easily constructed using materials commonly found at most hardware stores. The use of sealed wood and stainless-steel mesh produces a durable subsampling sieve that can withstand heavy use and large sample volumes.

1. Supplies

- Screw gun with screws
- Staple gun with staples
- 500-micrometer mesh (preferably stainless steel)
- 2–4-centimeter (cm) wood board
- Wood sealer

- Thick wire
- Saw

2. Construction of sieve

The sieve is composed of a stainless-steel mesh secured with staples between two wooden frames; this provides depth above and below the mesh to contain the sample and allow water to rinse through. Wood should be treated with a sealer to prevent water damage. The sieve shown in [figure 8.1A–C](#) measures about 29 cm long by 34 cm wide by 7.5 cm high.

3. Construction of grid

The overlying, removable grid is composed of a wooden frame containing a simple 2 x 2 wire grid to define the subsampling areas. The grid frame shown in [figure 8.1D–F](#) measures about 33 cm long by 38 cm wide.

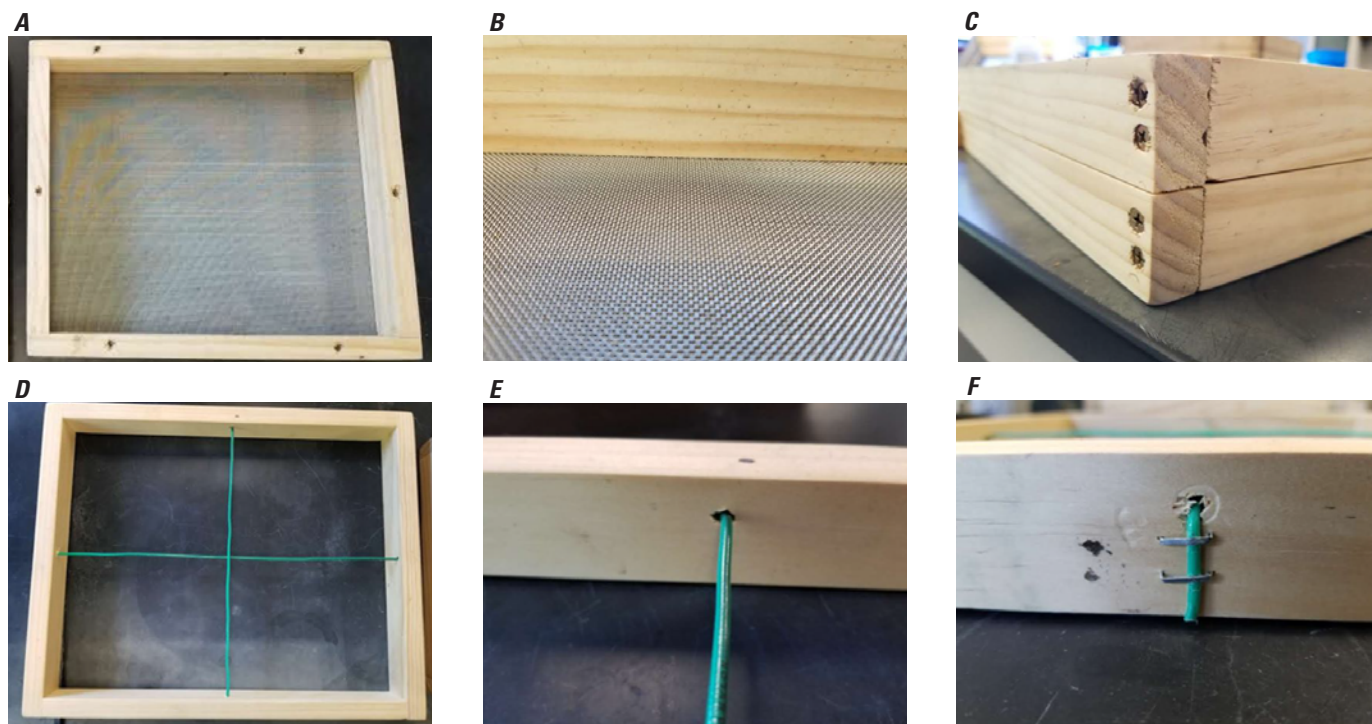


Figure 8.1. The equipment and process for constructing the subsampling sieve (A–C) and overlying gridded frame (D–F). A, subsampling sieve; B, stainless steel mesh; C, wooden frames that secure the mesh; D, overlying removable grid; E, wire of removable grid; F, wire of removable grid secured to wooden frame. Descriptive laboratory processing protocols are provided in appendix 9.

Appendix 9. Detailed Instructions for Macroinvertebrate Picking

This appendix contains instructions for picking macroinvertebrate samples in their entirety or following a subsampling technique.

1. Put on gloves and safety goggles, open the sample bottle, and carefully decant ethanol from the sample by pouring the fluid through a small 500-micrometer sieve held inside a funnel over a labeled ethanol waste container. Repeat this step for the remaining bottles of the same sample. Thoroughly backwash/rinse the small sieve into the sample bottle to remove any organisms or debris that may have been collected on the sieve mesh while decanting.
2. If the contents of one sample are stored in more than one sample bottle, pour the contents of all bottles into a single appropriately sized tub. Rinse the inside of the sample bottles over the tub using water to collect any organisms or debris remaining inside. Pull the internal label out of the sample using forceps. Rinse the label over the tub using a water wash bottle, and set the label aside. Add enough water to the tub to completely cover all sample materials and thoroughly but gently mix them with a spoon or spatula (fig. 4A).
3. Once the sample has been mixed, pour its contents onto the subsampling sieve, which contains 500-micrometer stainless steel mesh. Next, gently rinse the sample with water to remove remaining ethanol and fine sediment. Rinse any large rocks or organic materials (whole leaves, twigs, macrophyte mats, and so on) over the sieve, visually inspect them under a lighted 3× microscope to remove remaining attached macroinvertebrates and discard them.
4. Place the subsampling sieve inside a tub filled with water to a level slightly above that of the sample materials; this allows the macroinvertebrates and debris to become slightly suspended in the water while remaining contained within the sieve frame. Use a spoon, spatula, or long forceps to gently spread the sample material over the sieve mesh as evenly as possible. Once the sample is evenly dispersed across the mesh, lift the sieve out of the water-filled container and place the gridded subsampling frame (four grids) over the sieve's frame (fig. 4B).
5. Record the sample identifier information from the sample labels on a macroinvertebrate sample log form (appendix 7).
6. Use a random number generator to determine the order in which grids will be processed from the subsampling apparatus. Using a plastic spoon, spatula, paintbrush, and scissors (if needed to cut through vegetation), remove all materials (organisms and debris) from the randomly

selected grid and place them into a separate container (fig. 4C). Any organism that is lying over a line separating two grids is considered to be on the grid containing its head. If it is not possible to determine the location of the head, the organism is in the grid containing most of its body. Cover the remaining sample in the apparatus with the tub lid or tin foil to prevent the desiccation of remaining macroinvertebrates, and occasionally mist the sample with water to keep it moist.

7. To maximize the visibility of macroinvertebrates mixed with sample debris, pick the subsampled contents gradually. Transfer 2–3 heaping tablespoons of subsampled materials into a shallow white tray partially filled with water (fig. 4D). Visually inspect the contents once with the naked eye and then again with the 3× lighted magnifier while using forceps to gently remove all macroinvertebrates. Place all macroinvertebrates or select taxonomic groups directly into prelabeled vials with 70-percent ethanol; a single vial may be filled as much as halfway with specimens. Keep track of total individuals collected by using a hand tally counter. Once the tray's contents have been thoroughly examined and all macroinvertebrates have been removed, remaining materials in the tray may be discarded. Repeat these steps until the subsampled grid's contents have been processed entirely.
8. Once processed, if the cumulative analysis of the first grid (25 percent of the sample) yields a representative quantity of macroinvertebrates (this may vary based on project objectives and needs), then subsampling is complete. In some cases, project investigators may wish to incorporate an additional subsampling target, such as a minimum number of macroinvertebrates (for example, 400 macroinvertebrates per composited, wetland-level sample) to ensure representativeness. If so, the grids must be processed, one at a time, until the target is reached. Once you begin processing a grid, that grid must be picked entirely to ensure macroinvertebrate counts are scaled up properly. Record the total number of grids processed and total number of specimen vials used in the macroinvertebrate sample log form (appendix 7) and label the vials with the following information: contents, sample identifier, and number of grids processed.
9. Once picking is completed and all data have been recorded in the macroinvertebrate sample log form (appendix 7), remaining sample debris may be discarded, and sample bottles can be cleaned. Pour excess water into the sink with a strainer to prevent debris from entering the drain. Dispose of ethanol according to local regulations.

For more information about this publication, contact:
Director, USGS Northern Prairie Wildlife Research Center
8711 37th Street Southeast
Jamestown, ND 58401
701-253-5500

For additional information, visit: <https://www.usgs.gov/centers/npwrc>

Publishing support provided by the
Rolla and Sacramento Publishing Service Centers



Fish Invasion of Prairie Pothole Wetlands Reduces Amphipod Abundance, A Key Vertebrate Forage

Jake D. Carleen¹ · Danelle M. Larson²  · Michael J. Anteau³  · Megan J. Fitzpatrick⁴  · Andrew W. Hafs⁵ · Carl W. Isaacson¹ · Breanna R. Keith¹

Received: 30 August 2023 / Accepted: 16 April 2024

© Jake D. Carleen, Megan J. Fitzpatrick, Andrew W. Hafs, Carl Isaacson, Breanna R. Keith. Parts of this work were authored by US Federal Government authors and are not under copyright protection in the US; foreign copyright protection may apply 2024

Abstract

Fishes have spread into previously fishless wetlands, likely affecting other species. In the Prairie Pothole Region of North America, the invasion of fish into wetlands is facilitated by interactions of altered land use, climate, and hydrology. We aimed to understand the effects of fishes on amphipods, which are macroinvertebrates that vertebrates rely on as forage. We hypothesized the presence and abundance of fish, particularly benthivores, would have detrimental effects on amphipod abundance. Our study design targeted a large gradient of amphipod abundances among wetlands, including very high abundances of two amphipod species: *Gammarus lacustris* and *Hyalella azteca*. We found that fishless basins had twice as many amphipods as those with fish, on average. *Gammarus lacustris* were not detected in the presence of Black Bullhead *Ameiurus melas*. The abundance of both amphipod species had negative associations with the most common fishes, Fathead Minnow *Pimephales promelas* and Brook Stickleback *Culaea inconstans*. A multivariate community analysis showed the benthivore-fish functional feeding guild was negatively associated with the amphipod community, as hypothesized. However, our study design captured several wetlands with anomalies of high abundances of both fish and amphipods, obscuring their relationships. Our results aid resource managers by confirming several fish guilds and species are associated with lower abundances of amphipods. These findings can inform resource managers who make decisions about managing for fish and wildlife; for example, they may choose to manage existing fish populations or protect existing wetlands with high amphipod densities from new fish invasions.

Keywords Amphipoda · Benthivore fishes · Fish invasion effects · *Gammarus lacustris* · *Hyalella azteca* · Prairie Pothole Region wetlands

Introduction

Many wetland species provide services to humankind, and so the factors diminishing biodiversity need to be identified. Aquatic invertebrates are essential for many ecosystem functions but are vulnerable to habitat alterations, especially if the invertebrates have low dispersal abilities or are endemic to specific regions (Strayer 2006). Amphipods (crustaceans in the taxonomic order Amphipoda) are considered an “umbrella” species whose protection may extend to benefit many species. For example, amphipod abundance is positively associated with biodiversity and overall community structure of the wetland plant community (Larson et al 2022).

Amphipods are prominent and important organisms in the Prairie Pothole Region (PPR) of North America. The PPR

✉ Danelle M. Larson
dmlarson@usgs.gov

¹ Bemidji State University, Department of Environmental Studies, Sattgast Hall 109, Bemidji, MN 56601, USA

² U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, USA

³ U.S. Geological Survey, Northern Prairie Wildlife Research Center, 8711 27th St SE, Jamestown, ND 58401, USA

⁴ Minnesota Department of Natural Resources, Wetland Wildlife Population and Research Group, 102 23rd St. NE, Bemidji, MN 56601, USA

⁵ Bemidji State University, Aquatic Biology Program, Department of Biology, 1500 Birchmont Drive Northeast, Bemidji, MN 56601, USA

is an expansive mosaic of grasslands and depressional wetlands, covering approximately 800,000 square kilometers. The PPR is considered one of the most productive habitats for waterfowl in the world, and amphipods are key forage for species of conservation concern like lesser scaup *Aythya affinis* (Anteau and Afton 2008a; Anteau et al 2014). Lesser scaup primarily feed on amphipods and seek wetlands with high amphipod abundance (Lindeman & Clark 1999; Anteau and Afton 2009). Amphipod availability in this region is likely declining, as indicated by studies showing the diets from lesser scaup in 2000 contained 94% less amphipods when compared to diets from the same location in the 1980s (Anteau and Afton 2006, 2008a). Amphipod abundance is known to be affected by aquatic plants, insecticide concentrations, watershed land use, and fish (Anteau et al 2011; Hanson et al 2012; Janke et al 2019; Keith 2021; Larson et al 2022).

Observational and experimental studies have shown increased fish presence negatively affects amphipods, yet sometimes fish effects were strong and other times were moderate (Bouffard and Hanson 1997; Anteau et al 2011). However, the relationships between fish and amphipods across a large gradient of amphipod density have not been studied, particularly for wetlands with a very high abundance of amphipods. Prior studies surveyed a random sample of wetlands that yielded few wetlands with high amphipod densities, which limited knowledge of the magnitude to which various fish species and functional feeding guilds influence amphipod abundance (Anteau and Afton 2008b; Anteau et al 2011).

Over the past few decades, the lesser scaup population has declined concurrently with amphipods on scaup's important spring staging habitats in the PPR (Anteau et al 2014), and the scaup declines seemingly coincide with an increase in fish abundance in this region (Afton et al 1991; Anteau and Afton 2008b). Historically, prairie pothole wetlands underwent frequent drying and freezing that limited fish establishment. However, recent landscape-level hydrological changes like consolidation drainage and changes in precipitation events have synergistically resulted in greatly increased runoff into extant wetlands (Anteau et al 2016; McKenna et al 2019). Such changes have resulted in increased water levels and connectivity among wetland potholes (Anteau 2012; McCauley et al 2015), allowing for invasion and persistence of fish into previously fishless wetlands. Fish are now present in about 80% of semi-permanent and permanent wetlands and shallow lakes in the PPR (Anteau and Afton 2008b; Hanson et al 2012; McLean et al 2016), and one study documented a notable increase in the abundance of fishes in the PPR from 2004 to 2011 (Wiltermuth 2014).

Amphipod abundance likely declines in association with increases in fish abundance directly through predation and indirectly through fish-induced wetland alterations

(Friederichs et al 2011; Maurer et al 2014). In the PPR, Fathead Minnow *Pimephales promelas* are very abundant and detrimental to macroinvertebrate densities by predation (Friederichs et al 2011), competition for detritus as food (Anteau et al 2011), and causing bioturbation that affects wetland quality (Maurer et al. 2014). In addition, other common PPR fishes like Brook Stickleback *Culaea inconstans* readily consume amphipods when available (Tompkins & Gee 1983). Benthivorous fish, like the invasive Common Carp *Cyprinus carpio* and native Black Bullhead *Ameriurus melas*, reduce submerged aquatic vegetation (SAV; Fischer et al. 2013; Mork et al. 2009) that is beneficial to amphipods (Hanson et al 2012; Larson et al 2022).

In this study, we evaluated how fish abundance, species composition, and functional feeding guilds affected amphipod abundance in PPR wetlands. We deliberately incorporated wetlands with high amphipod densities to better ascertain the effects of fish on amphipods compared to previous studies using a random selection of wetlands (Anteau & Afton 2008a, b; Janke et al. 2019). We hypothesized that amphipod abundance was negatively associated with the presence and abundance of fish because of the direct and indirect effects fish can have on invertebrates. We also hypothesized the benthivore-fish functional feeding guild would have the greatest effects on amphipods because both organisms would co-occur on the benthos (i.e., wetland bottom), where predation and bioturbation would negatively affect amphipods.

Methods

Study Area & Design

The study area includes several large ecoregions (Lake Agassiz Plain, Northern Glaciated Plains, Northern Lakes and Forests, and Western Corn Belt Plains) from across Minnesota that represent the eastern portion of the PPR. We sampled 59 semi-permanent wetlands and shallow lakes (hereafter, wetlands) during the spring and summer of 2019 and 2020. Western Minnesota and specifically the PPR are known to have wetlands with conditions that help amphipods survive and sometimes thrive (Anteau et al 2011; Larson et al 2022).

We aimed to better understand the associations between fish and amphipods, so we designed an observational study that intentionally sampled two types of wetlands: wetlands with very high amphipod densities (> 500 individuals/m³) and wetlands with a gradient of densities (randomly selected). Prior to this study, we used a rapid assessment protocol (Carleen 2022) to design and conduct rapid assessments on 111 wetlands in May 2018 to identify those with relatively high densities of

amphipods. Roughly half of the 59 wetlands sampled for this study were selected based on the greatest amphipod densities detected during the rapid assessments (Figure S1, Table S1). The other half of sampled wetlands were randomly selected from the National Wetlands Inventory Database 2020 shapefiles to capture a gradient of amphipod densities. Candidates for selection included wetlands with semi-permanent or permanent hydroperiods, which helped ensure that selected wetlands were able to support amphipods without desiccation or winterkill and had similar wetland size distributions to the high amphipod density wetlands. We further constrained the selection of random wetlands, so they were within 24 km of a high amphipod abundance wetland to facilitate travel efficiency.

Amphipod Field Sampling and Laboratory Processing

Crews began amphipod sampling in the southern portion of the study area and moved north following the timing of ice-out (March–April 2019 and 2020) to target adult amphipods, similar to (Anteau and Afton 2008b). We crafted novel amphipod sampling techniques most suitable for depressional, mucky wetlands as described by (Keith et al 2022). In summary, we sampled amphipods at eight random sampling locations along evenly spaced transects. Two sampling locations occurred along each transect, specifically at 2 m from the shoreline (shallow water) and 50 m from the shoreline (open, deeper water). Sampling was restricted to water depths between 0.3 m and 1.2 m to ensure accurate density estimates. We collected samples with two D-shaped sweep nets (1,200 μ m mesh) from the benthos and water column, and all sweep net contents (including debris and invertebrates) were immediately preserved with 95% ethanol in containers.

We processed sweep net samples using a standard operating procedure in the laboratory (Keith et al 2022). In summary, we emptied the sample contents into a 500 μ m sieve and rinsed to remove fine particles. We then placed a grid with four equal-sized openings over the sieve and processed the contents of one randomly selected grid at a time, removing all macroinvertebrates from the benthic debris under a 3 $\times$ lighted desk magnifier. We continued processing grids until at least 25% of the grids or 400 macroinvertebrates were picked, whichever came first. We identified amphipods under 110 $\times$ microscopy and identified individuals to either the genus-level (*Crangonyx spp.*) or to the species-level (*Gammarus lacustris* and *Hyaella azteca*). We reported speciated

amphipod densities from the benthos and water column as individuals/m³.

Fish Sampling

We sampled the fish community in April–June in 2019 and 2020, which was a few weeks after the amphipod sampling. The differences in sampling timing between organisms was based on sampling efficacy (e.g., adult amphipods were best detected in spring) and secondarily for logistics (e.g., we had larger field crews to sample fish in early summer). We also timed fish sampling to occur before potentially dense aquatic vegetation would hinder the setting, retrieval, and efficacy of fish gear in late summer. We surveyed fish count and biomass (both the total biomass of all fish and each fish species) in each wetland using one gill net and three mini fyke nets. We used a short gill net (21 m $\times$ 2 m composed of 3 m panels, ranging in mesh size from 1.9–7.6 cm) due to the small size of study wetlands (often < 1 hectare). In contrast to (Anteau et al 2011), we did not use minnow traps due to low effectiveness of capturing fish in wetlands with low fish abundances during our pilot studies (Carleen 2022). Instead, we used mini fyke nets (6.5 mm bar mesh with 4 hoops, 1 throat, 7.62 m lead, and a 0.69 $\times$ 0.99 m rectangular frame opening; Duluth Trading Company, Mt. Horeb, WI) to capture many fish species and sizes. Each net was placed along a randomly selected amphipod sampling transect. All fish gear were left for an overnight soak. Most gear were deployed for approximately 19 h. A few nets were deployed for shorter or longer periods (16–22 h) because of typical logistical constraints.

Crews processed catch on-site immediately after retrieving gear. All catch were identified, weighed, measured for length, and enumerated by species. Crews sub-sampled catch (minimum of 20% by weight) if the estimated time to process a net would be over 30 min. We calculated a variety of metrics, including overall total number of fish caught, total fish biomass, catch per unit effort (CPUE; defined as the number of fish caught per unit time and effort, which was 19-h overnight set using the three nets), and species-specific CPUE and biomass.

Wetland Characteristics as Key Covariates

Crews sampled SAV biomass because SAV biomass might represent habitat structure for both fish and amphipods. We sampled SAV using the methods described in Larson et al (2022). Briefly, we sampled SAV in July–August 2019 and 2020 from boats with the number of sampling locations scaled to wetland size. Crews dragged a plant rake alongside the boat for 1.5 m and identified aquatic plants species collected and their relative biomass. Crews used a Hach HQ30D

multimeter to measure pH. We calculated wetland area using National Wetland Inventory Database 2020 shapefiles in ArcGIS.

Data Analyses

All data are publicly archived online (Carleen et al 2024). For aquatic vegetation and fish data entries into spreadsheets, we used double data entry and then compared entries with the R package ‘arsenal’ to minimize data entry errors. Any data entry errors were reconciled and corrected via referring to the original field data sheets. We periodically detected the amphipod *Crangonyx spp.* in study wetlands but omitted it from our analyses due to low relative abundance.

Two types of statistical analyses were used to test the hypotheses: (1) generalized linear models to test hypotheses that individual fish species had significant, negative associations with amphipods, and (2) redundancy analysis (RDA) to statistically test the fish functional feeding guild associations with amphipod abundance.

We modeled *G. lacustris* and *H. azteca* data separately using generalized linear models using R 4.1.3 (2022, R Development Core Team, Vienna, Austria) and the ‘glm-TMB’ package (Brooks et al 2017). Initially, a Poisson distribution was tested but the resulting models were over-dispersed, so we used a negative binomial distribution and log link due to right skew and improved model fits, similar to the methods of related studies (Anteau and Afton 2008b; Larson et al 2022). Conceptually, the response variable was the density of amphipod species per wetland (m^3). Model inputs were counts of amphipods per wetland with the natural log of the total water volume swept per wetland included as an offset variable. The offset variable controlled for the differing volumes of water swept according to wetland depths and allowed the model to compute density. We chose to report amphipod density rather than counts because it is more comparable to prior studies with different study designs and accounts for the water volume swept, which changed with the number of sample locations and water depths. Two wetlands were removed from the *G. lacustris* negative binomial analyses due to extreme data outliers (defined as 5 times greater than the interquartile range for Fathead Minnow counts).

We used CPUE as the predictor variables in the negative binomial models. We selected the primary predictor variables as CPUE of Fathead Minnow, Brook Stickleback, and Black Bullhead to match hypotheses and because these species were caught with high frequency and variable abundances. Black Bullhead and *G. lacustris* were nearly mutually exclusive, co-occurring in only one wetland, and therefore the relationship between them could not be modeled with regression. We also reported the results from the

negative binomial analyses with the two outliers retained for full transparency (Supporting Information Table S2). For the *G. lacustris* model, the CPUE of Fathead Minnow and Brook Stickleback were square root transformed to improve the model’s predictive power and meet model assumptions of linearity and equal variance.

We included amphipod habitat covariates to improve the model fit and obtain the best parameter estimates for the primary predictors of fish. Potential covariates initially included SAV biomass, average depth, wetland pH, total phosphorus, and wetland size. We graphically observed a threshold effect of wetland area in relation to the abundance of both amphipod species, so we created a binary variable that classified wetlands as either > 15 ha or < 15 ha. The primary predictors, which were the CPUE of select fish species and habitat covariates, were weakly correlated ($r < 0.35$).

We constructed a global model for each amphipod species that included the three primary fish predictors and potential covariates. Then, we manually used a backwards stepwise selection approach to remove environmental covariates with a p -value > 0.10 but did not remove our primary fish covariates regardless of statistical significance. We reported the relative magnitude of fish effects on amphipods, $\pm 90\%$ confidence intervals from the model coefficients, and the p -value with degrees of freedom to assess ecological significance of fish associations with amphipods (Wasserstein et al 2019).

We examined the role of fish functional feeding guild on amphipod abundance using redundancy analysis (RDA). The RDA is an extension of multiple regression where the ecological community is constrained by linear combinations of the predictor variables (Legendre 2018). The response variables (*G. lacustris* and *H. azteca*) were amphipod count data that were log-transformed. The predictor variables included the CPUE of fish that we assigned into three functional feeding guilds (benthivore, piscivore, and planktivore; Table 1). The variance inflation factors found no redundancy in the model ($\text{VIF} < 10$), so all predictor variables were included. We conducted a permuted ($\text{perm} = 9,999$) multivariate analysis of variance (PERMANOVA) to assess the overall RDA models, RDA axes, and RDA terms.

Results

Amphipod Densities Were Greater in Fishless Wetlands

Among our surveyed wetlands, 19% were fishless, while the other 81% had fish (Fig. 1). The average densities of amphipod species were nearly double in fishless wetlands compared to those with fish (Welch’s t -test: $p > 0.10$ for both amphipod species). Wetlands with fish had uniformly low

Table 1 A summary of 23 species of fish captured in 59 wetlands in western Minnesota, USA during springs and summers of 2019–2020. The names (American Fisheries Society abbreviation, common name, and scientific name), functional feeding guild (“FFG”; PLK=planktivore, BEN=benthivore, PIS=piscivore), prevalence (percent of the

59 sampled wetlands where species was detected), total counts captured from 59 wetlands (the capture per unit effort was a 19-h overnight soak for one gill net and two fyke nets), and mean counts in a wetland (including wetlands with no detections)

Abbreviation	Common Name	Scientific Name	FFG	Prevalence (%)	Count (total)	Count (mean)
FHM	Fathead Minnow	<i>Pimephales promelas</i>	PLK	68	21,418	363.00
BSB	Brook Stickleback	<i>Culaea inconstans</i>	PLK	51	961	16.29
BLB	Black Bullhead	<i>Ameiurus melas</i>	BEN	29	1,318	22.34
GSF	Green Sunfish	<i>Lepomis cyanellus</i>	PLK	12	132	2.24
DCE	Dace spp.	<i>Chrosomus spp.</i>	PLK	10	2,596	44.00
WAE	Walleye	<i>Sander vitreus</i>	PIS	10	159	2.70
YEP	Yellow Perch	<i>Perca flavescens</i>	PLK	8	138	2.34
CMM	Central Mudminnow	<i>Umbra limi</i>	PLK	8	140	2.37
CAP	Common Carp	<i>Cyprinus carpio</i>	BEN	8	22	0.37
NOP	Northern Pike	<i>Esox lucius</i>	PIS	8	19	0.32
OSS	Orange-Spotted Sunfish	<i>Lepomis humilis</i>	PLK	7	98	1.66
PMK	Pumpkinseed	<i>Lepomis gibbosus</i>	PLK	7	16	0.27
WTS	White Sucker	<i>Catostomus commersonii</i>	BEN	5	206	3.49
GSH	Golden Shiner	<i>Notemigonus crysoleucas</i>	PLK	5	13	0.22
LMB	Largemouth Bass	<i>Micropterus salmoides</i>	PIS	5	6	0.10
BLC	Black Crappie	<i>Pomoxis nigromaculatus</i>	PLK	5	11	0.18
BLG	Bluegill	<i>Lepomis macrochirus</i>	PLK	5	165	2.80
BMB	Bigmouth Buffalo	<i>Ictiobus cyprinellus</i>	BEN	3	10	0.17
BOW	Bowfin	<i>Amia calva</i>	PIS	3	6	0.10
MSK	Muskellunge	<i>Esox masquinongy</i>	PIS	2	1	0.02
TPM	Tadpole Madtom	<i>Noturus gyrinus</i>	BEN	2	10	0.17
YLB	Yellow Bullhead	<i>Ameiurus natalis</i>	BEN	2	7	0.12

amphipod density while amphipod density varied widely among fishless wetlands, indicating that wetlands with fish were likely to lack amphipods and that fishless wetlands were less predictable for amphipod density. The greater variability in fishless wetlands could also arise from minor differences in fish or amphipod sampling or the differences of habitats between fish- and fishless wetlands. Several wetlands had anomalies of very high abundances of either amphipod species in the presence of fish.

Amphipod Densities were Lower with Greater Fish Abundances

Catch of fish varied considerably, with total fish catch ranging from 0–2,631 fish/19 h, with the median value being 185 fish/19 h. Total fish biomass also varied widely, ranging from 0–41 kg/19 h. The three most encountered fish species were Fathead Minnow, Brook Stickleback, and Black Bullhead (Table 1). Black Bullhead and Fathead Minnows were the primary contributors to total fish biomass, accounting for 26% and 24% of biomass, respectively. Twenty-two species of fish were sampled and varied widely with respect

to prevalence and CPUE (Table 1). The most prevalent species was Fathead Minnow (68% of wetlands), with CPUE up to 2,621 fish/19 h in a single wetland. Brook Stickleback occurred in 51% of wetlands with CPUE up to 265 fish/19 h. Black Bullhead were present in 29% of wetlands with CPUE towards 400 fish/19 h. Dace *Chrosomus spp.* had only 10% prevalence but were often in high abundance, if present.

Amphipods Were Negatively Associated with Several Common Fish Species

Gammarus lacustris were negatively associated with the three dominant fish species: Black Bullhead, Fathead Minnow, and Brook Stickleback (after accounting for other environmental covariates). *Gammarus lacustris* were not detected in the presence of the Black Bullhead, a benthivore, except for one basin with co-occurrence of very low density of *Gammarus* (Fig. 2). The perfect separation of *G. lacustris* and Black Bullhead detections prohibited modeling their relationship further. As hypothesized, *G. lacustris* counts were also negatively correlated with Fathead Minnow and Brook Stickleback CPUE (Table 2, Fig. 2, Fig. 3).

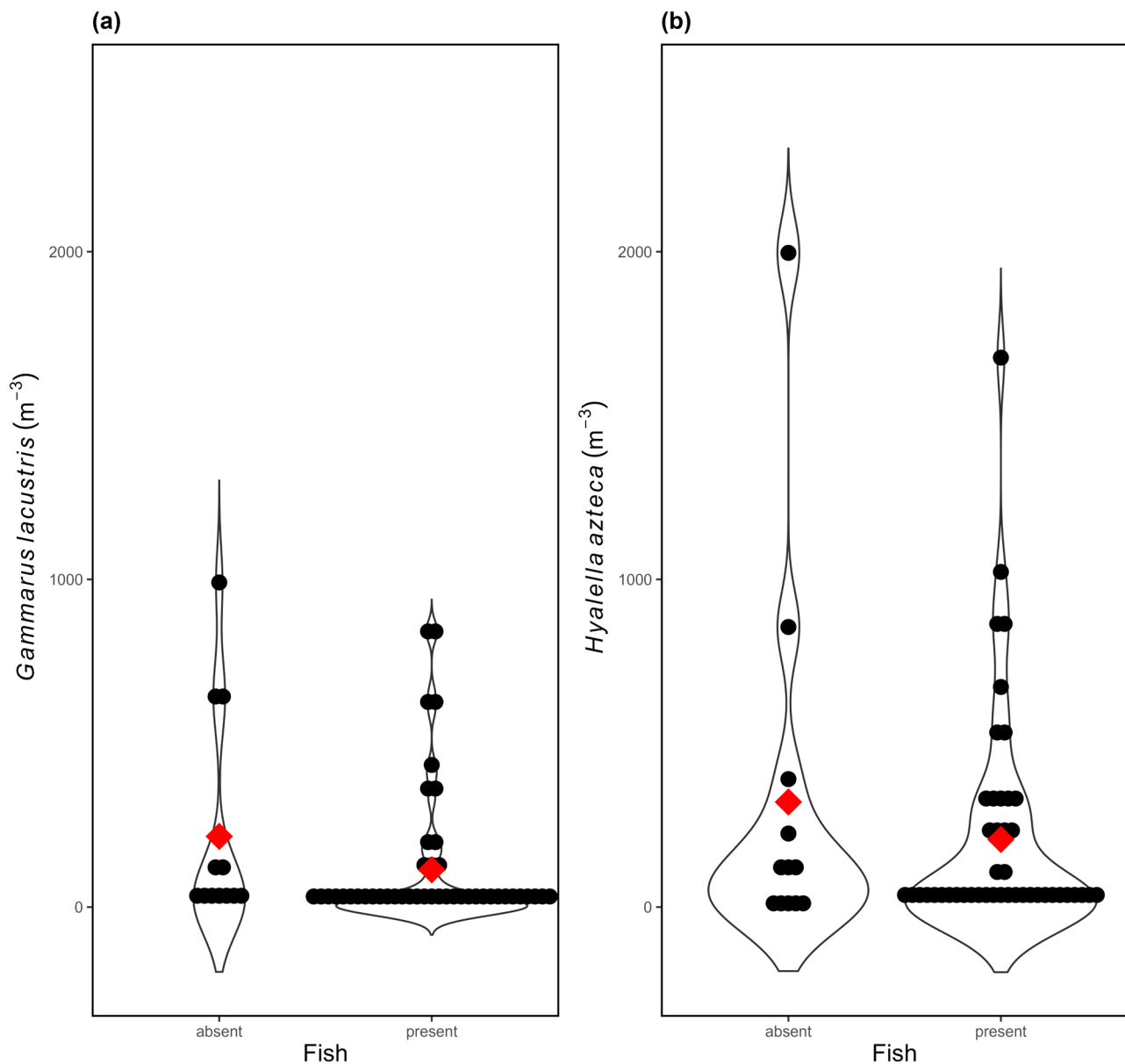


Fig. 1 The densities of *Gammarus lacustris* (panel a) and *Hyalella azteca* (panel b) in 59 prairie pothole wetlands of Minnesota, USA in years 2019–2020. The violin plots contrast the wetlands that are fishless (fish not detected) to those with any number and species of fish (fish present), and the impacts to amphipod densities. The catch

per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The black dots are amphipod densities from 59 wetlands, the red diamond indicates the mean amphipod density, and the violin plots show the data distributions

Gammarus had a non-linear response to Brook Stickleback and were predicted to be absent when Brook Stickleback counts exceeded 100 CPUE. The covariates indicated a positive correlation of *G. lacustris* with wetland pH and negative correlations to SAV biomass and wetland size over 15 hectares, analogous to previous studies guiding the use of these covariates (Anteau et al 2011; Larson et al 2022). The negative binomial regression results were similar when removing or retaining the two outliers (Table 2, Table S2).

Hyalella azteca varied in its responses to various fish species (Table 2, Fig. 2, Fig. 4). *Hyalella azteca* was relatively abundant in wetlands with low to moderate CPUE of Fathead Minnow; however, *Hyalella*'s response to high CPUE of Fathead Minnow was either positive or negative and had large confidence intervals encompassing 0 m⁻³. In contrast, *H. azteca* were negatively associated with CPUE of Brook Stickleback and Black Bullhead. The covariate of SAV biomass indicated positive correlation with *H. azteca*, like Larson et al. (2022). The negative binomial

Fig. 2 The raw (unmodeled) associations between three common fish species and two amphipod species (*Gammarus lacustris* and *Hyaella azteca*) in 59 wetlands sampled in 2019–2020 from prairie pothole wetlands of Minnesota, USA. The three fish species include Black Bullhead *Ameiurus melas*, Fathead Minnow *Pimephales promelas*, and Brook Stickleback *Culaea inconstans*. The catch per unit effort (CPUE) was a 19-h overnight soak for one gill net and two fyke nets. The two extreme outliers (defined as greater than 5 times the interquartile range) of Fathead Minnow CPUE (> 2,500 Fathead Minnow) were retained in panel (b) and panel (e)

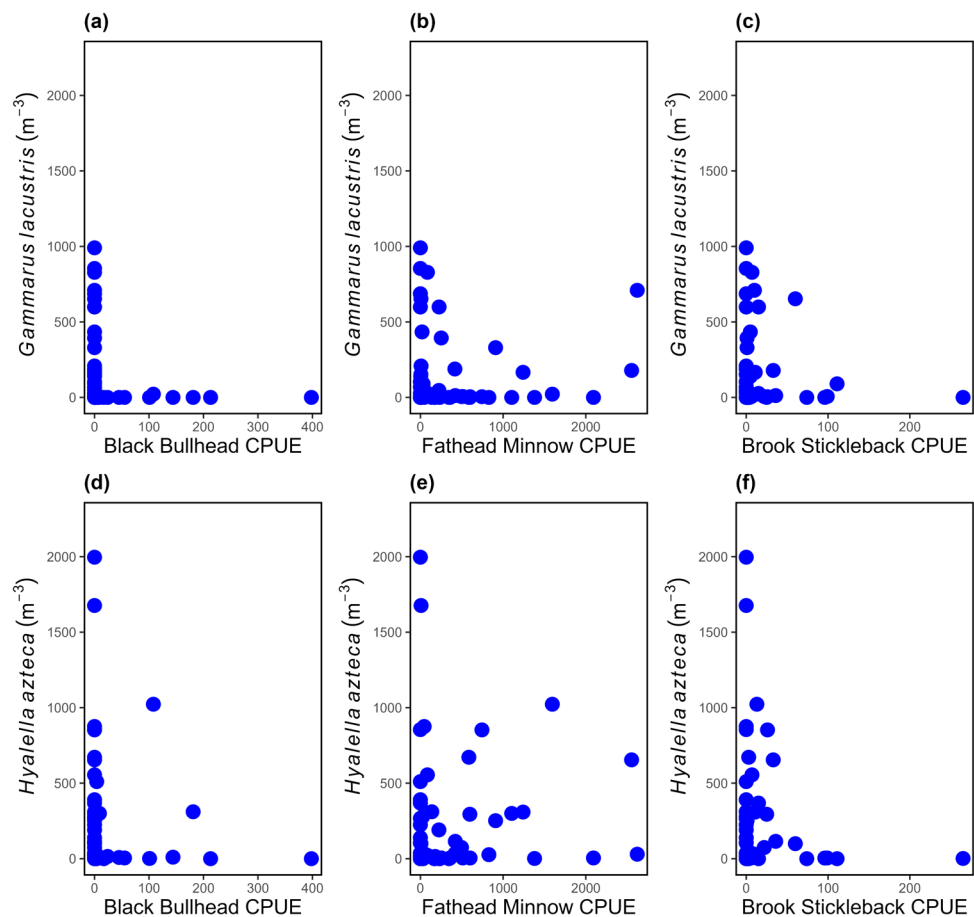


Table 2 Results of final models using negative binomial regression analysis for *Gammarus lacustris* and *Hyaella azteca* ($n=57$ wetlands; two extreme statistical outliers were removed) in prairie pothole wetlands of Minnesota, USA in years 2019–2020. The fish terms are primary hypothesized variables, and the other variables (plants, pH, and wetland size) are covariates included to improve

parameter estimates for fish effects. The counts of Fathead Minnow and Brook Stickleback are square root transformed ($\sqrt{\cdot}$) in the *Gammarus lacustris* model to improve the model's predictive power and meet model assumptions. Wetland size threshold is a binary variable used to classify wetlands either as > 15 ha and < 15 ha

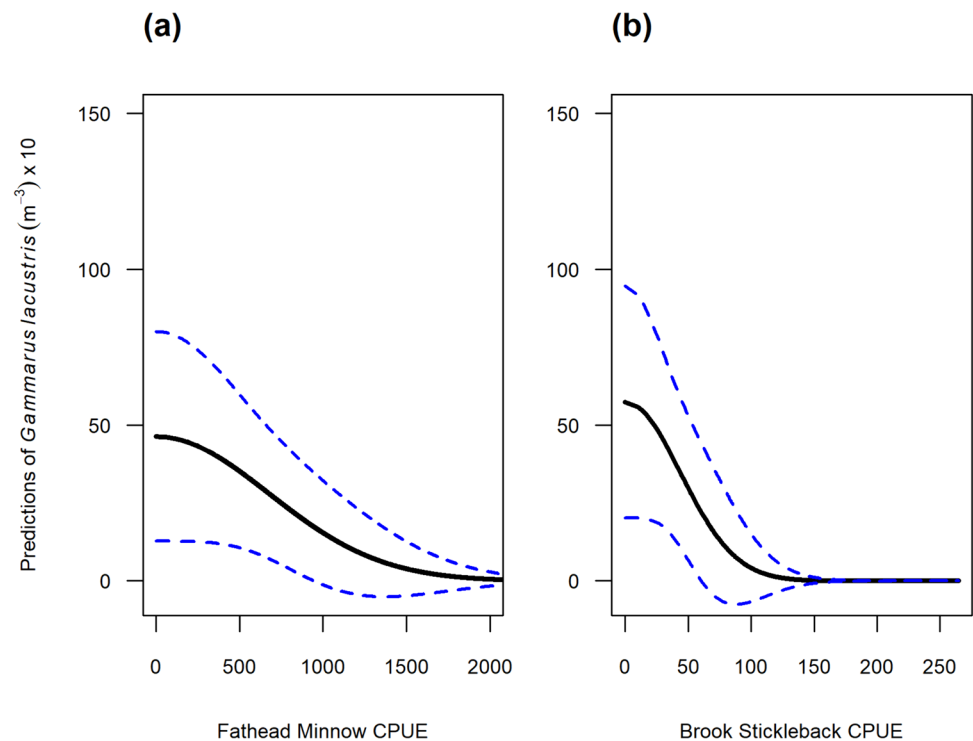
Effect on <i>Gammarus lacustris</i>	<i>E</i>	SE	Z-score	<i>P</i>
Fathead Minnow count ²	-1.10e-06	6.29e-07	-1.75	0.08
Brook Stickleback count ²	-2.61e-04	1.66e-04	-1.58	0.11
Submerged aquatic plant (SAV) biomass	-1.89	0.60	-3.15	<0.01
pH	2.63	1.03	2.55	0.01
Wetland size threshold	-2.55	0.97	-2.63	<0.01
Effect on <i>Hyaella azteca</i>	<i>E</i>	SE	Z-score	<i>P</i>
Fathead Minnow count	1.19e-04	6.25e-04	1.91	0.05
Brook Stickleback count	-2.32e-02	7.05e-03	-3.29	<0.01
Black Bullhead count	-1.12e-02	-5.89e-03	-2.06	0.04
Submerged aquatic plant (SAV) biomass	0.62	0.33	1.89	0.06

regression results were similar regardless of removing or retaining the two outliers with high Fathead Minnow biomass (Table 2, Table S2).

Fish Functional Feeding Guilds Affected Amphipods

Amphipods were negatively associated with the fish community and fish functional feeding guilds (PERMANOVA: $F_{3,55} = 2.63$, $p = 0.013$). Amphipods were strongly and

Fig. 3 Model-predicted densities of *Gammarus lacustris* in response to counts of Fathead Minnow *Pimephales promelas* (panel a) and Brook Stickleback *Culaea inconstans* (panel b) in 59 wetlands sampled in 2019–2020 from prairie pothole wetlands of Minnesota, USA. The catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The other model covariates in Table 2 were held constant at their mean values. Blue dashed lines represent 90% confidence intervals



negatively associated with benthivore fishes along the RDA1 axis ($F_{1,56}=7.53$, $p=0.010$). The Black Bullhead were the most prevalent benthivore (29% prevalence), followed by Common Carp (8% prevalence). The RDA2 axis and the associated functional feeding guilds of planktivore and piscivore were not significantly associated with amphipod community abundance ($F_{1,56}=0.51$, $p=0.922$).

Graphically, the RDA revealed further relationships between the fish and amphipod communities (Fig. 5). Most wetlands were clustered in ordination space, indicating similar fish assemblages among wetlands. Although fish assemblages were often similar, the fish guild abundances were along a strong gradient of few fish to very high abundances. The planktivores and piscivores were positively correlated, but not correlated to benthivores. Both amphipod species had slight positive correlations with each other, indicating that wetlands can house both amphipod species.

Discussion

High amphipod densities are essential forage for certain wildlife like scaup and tiger salamander, *Ambystoma tigrinum* (Olenick and Gee 1981; Strand et al 2008). Our results and others (Hanson and Butler 1994; Anteau et al 2011) show that fishless wetlands often support greater amphipod densities than wetlands with fish. Amphipod densities decline non-linearly with greater CPUE of the three most

common fishes. The PPR has been extensively modified in ways that support greater fish abundances and movements among wetlands. Resource managers are faced with the decisions and challenges of fish management for bolstering amphipod densities, and we discuss tools that managers may consider implementing in the altered PPR landscape that wildlife continually depend on.

Fish Presence and Abundance Negatively Affected Amphipod Densities

Our results, along with other observational and experimental studies (Hanson and Butler 1994; Anteau et al 2011), show that fish presence and abundance are not generally observed with high densities of amphipods. The PPR was extensively modified in favor of fish movement and high abundances, yet our study and others show that ~20% of wetlands remain fishless (Anteau and Afton 2008b; Anteau et al 2011; Hanson et al 2012). Considering the marked effects that fish likely have on amphipods and other invertebrate communities, a landscape survey and predictive model that can identify semi-permanent and permanent wetlands that remain fishless would be a valuable tool for conservation because it could be used to prioritize conservation towards preventing the spread of fish. In wetlands with existing fish populations, resource managers need to weigh multiple tradeoffs to make decisions on whether to manage either fish or amphipods (Hanson and Butler 1994; Potthoff et al 2008).

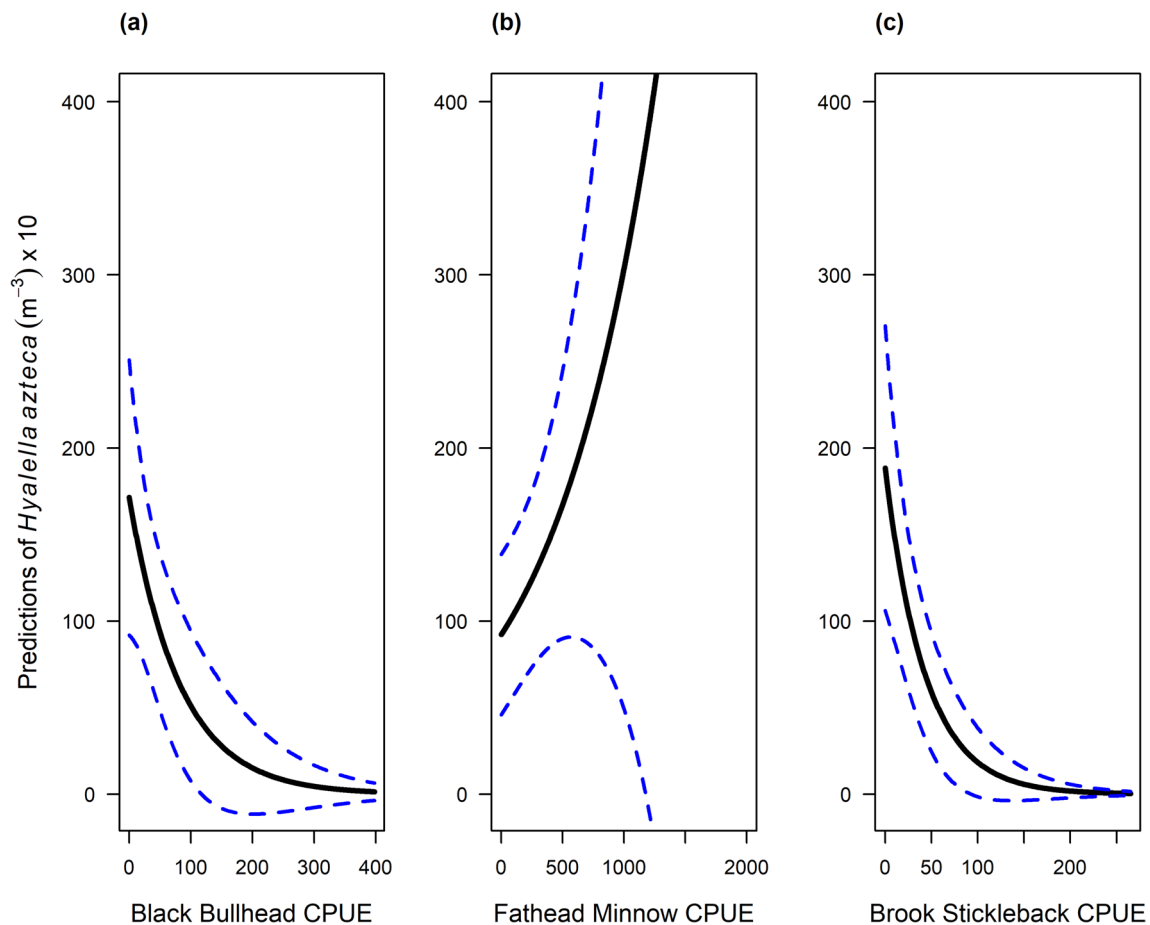


Fig. 4 Model-predicted densities of *Hyalella azteca* in response to counts of Black Bullhead *Ameiurus melas* (panel a), Fathead Minnow *Pimephales promelas* (panel b), and Brook Stickleback *Culaea inconstans* (panel c) in 59 prairie pothole wetlands of Minnesota, USA in

years 2019–2020. The catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The other model covariates in Table 2 were held constant at their mean values. Blue dashed lines represent 90% confidence intervals

In our study, amphipods were not detected in the presence of Black Bullheads. Black Bullhead are one of the most common native fish species found in Minnesota wetlands and are known to be tolerant to wider environmental conditions than many fish (Mork et al 2009). This species can reach high densities and may have increased due to anthropogenic influences like increased wetland connectivity and consolidation. Diet studies from other regions indicated Black Bullhead are highly opportunistic feeders and will readily consume amphipods (Repsys et al 1976; Strand et al 2008). Black Bullhead and other benthivores like Common Carp may also indirectly affect the quality of wetland habitats that amphipods rely on by increasing turbidity and decreasing aquatic vegetation (Zimmer et al 2002; Fischer et al 2013; Kowal et al 2022). Therefore, we hypothesize that Black Bullheads are excluding amphipods, but it is also possible that the two organisms rely on different wetland types.

Amphipods had a strong, negative association with Brook Stickleback. The Brook Stickleback are efficient

at colonizing wetlands (Wieker et al 2016) and tolerant to a broad range of environmental conditions (McLean et al 2016). Although Brook Stickleback has a small gape (mouth), they may be consuming amphipods by picking them apart or eating *Gammarus* in their early life stage when small. In Manitoba prairie wetlands, *G. lacustris* was the most consumed food item by weight for Brook Stickleback (Moodie 1986). Further, Brook Stickleback preferentially consumed ovigerous female amphipods in a controlled experiment (Lewis and Loch-Mally 2010). We hypothesize that the mechanism for amphipod reduction is direct predation because we found no support in the literature for the notion that Brook Stickleback are responsible for major habitat degradation.

The effects of Fathead Minnow on amphipod densities remain unclear. Our study shows Fathead Minnow had a negative effect on *G. lacustris* densities, similar to previous work (Zimmer et al 2002; Anteau et al 2011). The effects of the planktivorous Fathead Minnow on *G. lacustris* are

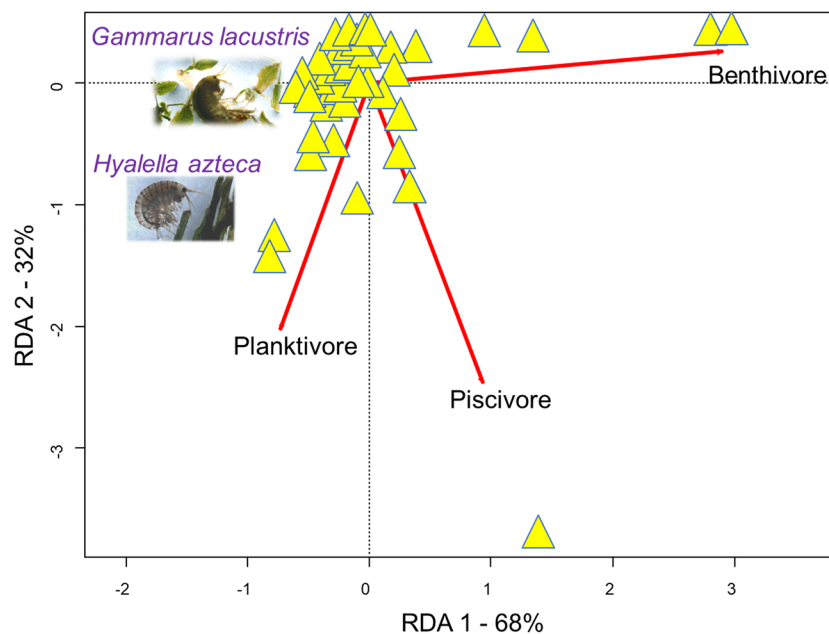


Fig. 5 Redundancy analysis (RDA) biplot of the amphipod community (*Gammarus lacustris* and *Hyalella azteca* counts) in relation to the catches of fish within three fish functional feeding guilds (benthivore, planktivore, and piscivore) from 59 wetlands in western Minnesota, USA. The counts of amphipods and fish were standardized for sampling effort. The fishes catch per unit effort (CPUE) for this study

was a 19-h overnight soak for one gill net and two fyke nets. The yellow triangles represent individual wetlands, and the red lines are vectors showing the abundance gradients of fishes within each functional feeding guild. Vectors at $<90^\circ$ angles are positively correlated, vectors at 90° angles are uncorrelated, and vectors at 180° angle are negatively correlated

likely to be both direct and indirect, though this study did not elucidate these effects due to insufficient sample sizes for such a model. Fathead Minnow could readily consume amphipods and likely compete with amphipods for detritus as food, and cause bioturbation (Zimmer et al 2002). Interestingly, the tiger salamander can affect trophic structure of prairie potholes similarly to planktivorous fish like the Fathead Minnow; yet, tiger salamander gut contents showed high amphipod consumption but not a strong effect on overall wetland amphipod densities (Benoy 2008; Wiltermuth 2014).

Contrary to our hypothesis, we did not detect a negative association between Fathead Minnows and *H. azteca*; however, this result had a weak, positive coefficient and a marginal p -value. Our finding contrasts with results found by Anteau et al. (2011), in which *H. azteca* showed greater decline in response to low Fathead Minnow abundances compared to *G. lacustris*. Based on Anteau et al. (2011) and our observations, Fathead Minnows and amphipods thrive in similar ecological niches. For example, a wetland must be deep enough to support overwinter survival of each species, but shallow enough not to support large abundances of large, predatory fish. This habitat-driven co-occurrence makes discerning cause and effect mechanisms of species interactions impossible in this observational study. However, it is also possible that some wetlands have conditions that can support

productivity of both species. Two wetlands were excluded from our models due to having high populations of both *G. lacustris* and Fathead Minnows, and future research that targets wetlands with high co-occurrence may reveal what wetland characteristics support this anomaly. Further, we are aware of two wetlands in North Dakota with long-term data that also supported sustained populations of Fathead Minnows and amphipods (National Ecological Observatory Network 2023a; National Ecological Observatory Network 2023b). We believe further study would provide valuable insights into the mechanisms driving amphipod productivity.

The Interactions Among Aquatic Vegetation, Fish, Waterfowl, and Amphipods Remain Unknown

In both *G. lacustris* and *H. azteca* models, SAV biomass was an informative covariate. Interestingly, *G. lacustris* responded negatively to SAV biomass while *H. azteca* responded positively in this study and associated work (Larson et al 2022). We postulated reasons that amphipods respond differently to SAV, including use as a food resource, structural habitat, water purification, water-depth associations (e.g., lacustrine versus palustrine habitats), and mediating fish predation. Future research using more data than we currently have could explore interactions to learn whether

plant biomass and plant diversity negates fish predation on amphipods.

The effects of predation on amphipods from either waterfowl or fish remain unclear from this study and the literature. Following ice-out when we sampled amphipods (April–May), millions of waterfowl would be using the PPR under high energetic demands for migration and reproduction. For example, scaup feed extensively on amphipods during spring migration (Anteau et al 2014) and will target wetlands with high densities of amphipods (Lindeman & Clark 1999). Therefore, waterfowl may or may not negatively affect amphipod densities in this study and could be a confounding factor not controlled for in our study design. Future work could sample waterfowl use days, fish, and amphipods concurrently to better investigate their relationships or model out the effects of waterfowl to better understand the fish effects.

Managing Fish to Increase Amphipods for Wildlife Forage

Throughout the PPR, high amphipod densities are sometimes a management goal to support wildlife (Bouffard and Hanson 1997; Larson et al 2018). High amphipod densities can support migrating and breeding scaup (Lindeman and Clark 1999; Anteau and Afton 2006; Kahara and Chipps 2009), tiger salamanders (Benoy et al 2002; Benoy 2008), and likely other species that eat macroinvertebrates. Our results indicated that complete fish removal or reduced abundances may allow for greater densities of amphipods to serve as vertebrate food. A case study of Lake Christina, Minnesota, USA, suggested fish removal markedly increased amphipod densities until fish reinvaded (Hanson & Butler 1994). Exclusion of Common Carp from Delta Marsh, Manitoba, Canada, resulted in improved wetland conditions, SAV, and increased use of the marsh by migrating waterfowl (Kowal et al 2022; Bortolotti et al 2023).

Chemicals and water level drawdowns are two tools for reducing fish abundance in many wetland types. Rotenone is a non-selective piscicide that inhibits cellular respiration and leads to fish death. Non-target organisms, including amphipods, can be affected by Rotenone treatments but macroinvertebrate communities had quick recovery (Skaar et al 2017). Different species of fish require different concentrations of Rotenone; therefore, the target fish species should be identified before treatments for maximum effectiveness. Wetland drawdowns can also be an effective method of removing nuisance fish while increasing SAV that benefit amphipods (Larson et al 2020), but severe dewatering would likely harm amphipods as they cannot survive desiccation and are poor colonizers. Admittedly, removing fish from wetlands can be challenging, expensive, and often unsuccessful.

Stocking piscivorous fish to induce a trophic cascade may help reduce small nuisance fish (like Fathead Minnow). Biomanipulation via stocking Walleye *Sander vitreus* and Northern Pike *Esox lucius* successfully suppresses Fathead Minnow (Potthoff et al 2008). In Minnesota's wetlands stocked with Walleye fry, the amphipods responded positively although the effect was short-lived; therefore, repeated fry stocking might be needed for lasting impacts (Potthoff et al 2008). In our study, the wetland named 'Cuba' has very high densities of both amphipod species (> 5,000 individuals per m³) yet is commonly used as a Walleye rearing pond by Minnesota Department of Natural Resources. However, there is uncertainty whether piscivorous fish would preferentially consume amphipods if smaller fish were not available or if the stocked fry began to age and had greater energy demands.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13157-024-01808-5>.

Acknowledgements We thank three anonymous peer reviewers who provided suggestions that improved our research proposal prior to funding. We thank Fred Bengtson and Ricky Lien for study conceptualization and funding acquisition, Brian Herwig for sharing fish gear and advice, students from Bemidji State University and Augsburg University for help sampling, and to Dr. Jeff Ueland and Dr. William Sea for helpful feedback on early versions of the manuscript. This study was performed under the authority of the Minnesota Department of Natural Resources fisheries permit (special permit # 24,192 to Danelle Larson and designees) and prohibited invasive species, aquatic plants, and water permit (permit #418 to Danelle Larson and designees). Fish were handled in accordance with American Fisheries Society guidelines for the use of fishes in research: <https://fisheries.org/policy-media/science-guidelines/guidelines-for-the-use-of-fishes-in-research/>. Research on U.S. Fish and Wildlife Service wetlands was supported by a research and monitoring special use permit (permit #SUP-18-01R to Danelle Larson and designees). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Funding Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR) as cited in Larson et al 2018. Additional funding and equipment were provided by the Minnesota Department of Natural Resources. The U.S. Geological Survey Northern Prairie Wildlife Research Center, U.S. Geological Survey Upper Midwest Environmental Science Center, and Minnesota Space Grant Consortium provided in-kind support for the project.

References

- Afton AD, Hier RH, Paulus SL (1991) Lesser Scaup diets during migration and winter in the Mississippi flyway. *Canadian Journal of Zoology* 69:328–333. <https://doi.org/10.1139/z91-053>
- Anteau MJ (2012) Do interactions of land use and climate affect productivity of waterbirds and prairie-pothole wetlands? *Wetlands* 32:1–9. <https://doi.org/10.1007/s13157-011-0206-3>

- Anteau M, Afton A (2006) Diet shifts of lesser scaup are consistent with the Spring Condition Hypothesis. *Canadian Journal of Zoology* 84:779–186
- Anteau MJ, Afton AD (2008) Diets of Lesser Scaup during Spring Migration throughout the Upper-Midwest are Consistent with the Spring Condition Hypothesis. *Waterbirds* 31:97–106. [https://doi.org/10.1675/1524-4695\(2008\)31\[97:doldsj\]2.0.co;2](https://doi.org/10.1675/1524-4695(2008)31[97:doldsj]2.0.co;2)
- Anteau MJ, Afton AD (2008) Amphipod densities and indices of wetland quality across the upper-Midwest, USA. *Wetlands* 28:184–196. <https://doi.org/10.1672/07-53.1>
- Anteau M, Afton A (2009) Wetland use and feeding by lesser scaup during spring migration across the upper Midwest, USA. *Wetlands* 29:704–712
- Anteau MJ, Afton AD, Anteau ACE, Moser EB (2011) Fish and land use influence *Gammarus lacustris* and *Hyaella azteca* (Amphipoda) densities in large wetlands across the upper Midwest. *Hydrobiologia* 664:69–80. <https://doi.org/10.1007/s10750-010-0583-2>
- Anteau MJ, DeVink J-M, Koons DN et al (2014) Lesser Scaup (*Aythya affinis*). *The Birds of North America Online*. <https://doi.org/10.2173/bna.338>
- Anteau MJ, Wiltermuth MT, van der Burg MP, Pearse AT (2016) Prerequisites for Understanding Climate-Change Impacts on Northern Prairie Wetlands. *Wetlands*. <https://doi.org/10.1007/s13157-016-0811-2>
- Bajer PG, Beck MW, Hundt PJ (2018) Effect of non-native versus native invaders on macrophyte richness: are carp and bullheads ecological proxies? *Hydrobiologia* 817:379–391. <https://doi.org/10.1007/s10750-018-3592-1>
- Becker GC (1984) Fishes of Wisconsin. *Copeia*. <https://doi.org/10.2307/1445079>
- Benoy GA (2008) Tiger Salamanders in prairie potholes: a “fish in amphibian’s garments?” *Wetlands* 28:464–472. <https://doi.org/10.1672/07-07.1>
- Benoy GA, Nudds TD, Dunlop E (2002) Patterns of habitat and invertebrate diet overlap between tiger salamanders and ducks in prairie potholes. *Hydrobiologia* 481:47–59. <https://doi.org/10.1023/A:1021260920095>
- Bortolotti LE, Emery RB, Kowal PD et al (2023) Migrating Ducks and Submersed Aquatic Vegetation Respond Positively After Invasive Common Carp (*Cyprinus carpio*) Exclusion from a Freshwater Coastal Marsh. *Wetlands* 43:25. <https://doi.org/10.1007/s13157-023-01670-x>
- Bouffard SH, Hanson MA (1997) Fish in Waterfowl Marshes : Waterfowl Managers’ Perspective. *Wildlife Society Bulletin* 25:146–157
- Brooks ME, Kristensen K, van Benthem KJ, et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal*. <https://doi.org/10.32614/rj-2017-066>
- Carleen JD (2022) The effects of fish on amphipods in wetlands of Western Minnesota. Bemidji State University, Thesis
- Carleen JD, Larson DM, Anteau MJ, et al (2024) Abundance, biomass, and density data for fishes and amphipods in prairie pothole region wetlands in 2019 and 2020: U.S. Geological Survey data release at <https://doi.org/10.5066/P1Y9GBJK>
- Fischer JR, Krogman RM, Quist MC (2013) Influences of native and non-native benthivorous fishes on aquatic ecosystem degradation. *Hydrobiologia* 711:187–199. <https://doi.org/10.1007/s10750-013-1483-z>
- Friederichs SJ, Zimmer KD, Herwig BR et al (2011) Total phosphorus and piscivore mass as drivers of food web characteristics in shallow lakes. *Oikos* 120:756–765. <https://doi.org/10.1111/j.1600-0706.2010.19244.x>
- Hanson MA, Butler MG (1994) Responses to food web manipulation in a shallow waterfowl lake. *Hydrobiologia* 279–280:457–466. <https://doi.org/10.1007/BF00027877>
- Hanson MA, Herwig BR, Zimmer KD et al (2012) Comparing Effects of Lake- and Watershed-Scale Influences on Communities of Aquatic Invertebrates in Shallow Lakes. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0044644>
- Janke AK, Anteau MJ, Stafford JD (2019) Prairie wetlands confer consistent migrant refueling conditions across a gradient of agricultural land use intensities. *Biological Conservation* 229:99–112. <https://doi.org/10.1016/j.biocon.2018.11.021>
- Kahara SN, Chipps SR (2009) Predicting lesser scaup wetland use during spring migration in eastern South Dakota. *Great Plains Research* 19:157–167
- Keith B (2021) Interactions between wetland condition and amphipod abundance across Western Minnesota’s diverse landscape. Bemidji State University, thesis
- Keith BR, Carleen JD, Larson DM, et al (2022) Protocols for Collecting and Processing Macroinvertebrates from the Benthos and Water Column in Depressional Wetlands. Open File Report 2022–1029, U.S. Geological Survey 1–32. <https://doi.org/10.3133/ofr20221029>
- Kowal PD, Badiou P, Emery RB, et al (2022) Improvements in water clarity and submersed aquatic vegetation cover after exclusion of invasive common carp from a large freshwater coastal wetland, Delta Marsh, Manitoba. *Frontiers in Environmental Science*. <https://doi.org/10.3389/fenvs.2022.992690>
- Larson DM, Cordts SD, Hansel-Welch N (2020) Shallow lake management enhanced habitat and attracted waterbirds during fall migration. *Hydrobiologia* 846:3364–3379. <https://doi.org/10.1007/s10750-020-04342-7>
- Larson DM, DeJong D, Anteau MJ et al (2022) High abundance of a single taxon (amphipods) predicts aquatic macrophyte biodiversity in prairie wetlands. *Biodiversity and Conservation* 31:1073–1093. <https://doi.org/10.1007/s10531-022-02379-9>
- Larson DM, Anteau M, Isaacson C, et al (2018) Restoring Wetland Invertebrates to Revive Wetland Habitats. Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g
- Legendre P (2018) Numerical ecology. *Encyclopedia of Ecology*. <https://doi.org/10.1016/B978-0-12-409548-9.10595-0>
- Lewis SE, Loch-Mally AM (2010) Ovigerous female amphipods (*Gammarus pseudolimnaeus*) face increased risks from vertebrate and invertebrate predators. *Journal of Freshwater Ecology* 25:395–402. <https://doi.org/10.1080/02705060.2010.9664382>
- Lindeman DH, Clark RG (1999) Amphipods, Land-Use Impacts, and Lesser Scaup (*Aythya affinis*) Distribution in Saskatchewan Wetlands. 19:627–638
- Maurer KM, Stewart TW, Lorenz FO (2014) Direct and indirect effects of fish on invertebrates and tiger salamanders in prairie pothole wetlands. *Wetlands* 34:735–745. <https://doi.org/10.1007/s13157-014-0538-x>
- McCauley LA, Anteau MJ, Van Der Burg MP, Wiltermuth MT (2015) Land use and wetland drainage affect water levels and dynamics of remaining wetlands. *Ecosphere* 6:1–22. <https://doi.org/10.1890/ES14-00494.1>
- McKenna OP, Kucia SR, Mushet DM et al (2019) Synergistic interaction of climate and land-use drivers alter the function of North American, prairie-pothole wetlands. *Sustainability*. <https://doi.org/10.3390/su11236581>
- McLean KI, Mushet DM, Stockwell CA (2016) From “Duck Factory” to “Fish Factory”: Climate Induced Changes in Vertebrate Communities of Prairie Pothole Wetlands and Small Lakes. *Wetlands* 36:407–421. <https://doi.org/10.1007/s13157-016-0766-3>
- Moodie GEE (1986) The population biology of *Culaea inconstans*, the brook stickleback, in a small prairie lake. *Canadian Journal of Zoology* 64:1709–1717. <https://doi.org/10.1139/z86-258>
- Mork MD, Bisping SM, Fischer JR, Quist MC (2009) Population characteristics of black bullhead (*Ameiurus melas*) in Iowa natural

- lakes. *Journal of Freshwater Ecology* 24:635–644. <https://doi.org/10.1080/02705060.2009.9664341>
- National Ecological Observatory Network (2023a) Macroinvertebrate collection (DP1.20120.001). In: <https://data.neonscience.org>
- National Ecological Observatory Network (2023b) Fish electrofishing, gill netting, and fyke netting counts (DP1.20107.001). In: <https://data.neonscience.org>
- National Wetlands Inventory Database (2020) U.S. Fish and Wildlife Service. <https://www.fws.gov/program/national-wetlands-inventory/data-download>
- Olenick RJ, Gee JH (1981) Tiger salamanders (*Ambystoma tigrinum*) and stocked rainbow trout (*Salmo gairdneri*): potential competitors for food in Manitoba prairie pothole lakes. *The Canadian Field-Naturalist* 95:129–132
- Potthoff AJ, Herwig BR, Hanson MA et al (2008) Cascading food-web effects of piscivore introductions in shallow lakes. *Journal of Applied Ecology* 45:1170–1179. <https://doi.org/10.1111/j.1365-2664.2008.01493.x>
- Repsys AJ, Applegate RL, Hales DC (1976) Food and Food Selectivity of the Black Bullhead, *Ictalurus melas*, in Lake Poinsett, South Dakota. *Journal of the Fisheries Research Board of Canada* 33:768–775. <https://doi.org/10.1139/f76-094>
- Skaar DR, Arnold JL, Koel TM et al (2017) Effects of Rotenone on Amphibians and Macroinvertebrates in Yellowstone. *Yellowstone Science* 25:28–34
- Strand KA, Chipps SR, Kahara SN et al (2008) Patterns of prey use by lesser scaup *Aythya affinis* (Aves) and diet overlap with fishes during spring migration. *Hydrobiologia* 598:389–398. <https://doi.org/10.1007/s10750-007-9178-y>
- Strayer DL (2006) Challenges for freshwater invertebrate conservation. *Journal of the North American Benthological Society* 25:271–287
- Tompkins AM, Gee JH (1983) Foraging behavior of brook stickleback, *Culaea inconstans* (Kirtland): optimization of time, space, and diet. *Canadian Journal of Zoology*. <https://doi.org/10.1139/z83-329>
- Wasserstein RL, Schirm AL, Lazar NA (2019) Moving to a World Beyond “p < 0.05.” *American Statistician* 73:1–19. <https://doi.org/10.1080/00031305.2019.1583913>
- Wieker JE, Schoonover CM, Gaines RK et al (2016) Effects of Introduced Brook Stickleback (*Culaea inconstans*) on Benthic Macroinvertebrate Communities in the Nearshore Area of Lentic Systems in Turnbull National Wildlife Refuge, Washington. *Northwest Science* 90:278–289. <https://doi.org/10.3955/046.090.0306>
- Wiltermuth M (2014) Influences of climate variability and landscape modifications on water dynamics, community structure, and amphipod populations in large prairie wetlands. North Dakota State University, thesis
- Zimmer KD, Hanson MA, Butler MG (2000) Factors influencing invertebrate communities in prairie wetlands: a multivariate approach. *Canadian Journal of Fisheries and Aquatic Sciences* 57:76–85. <https://doi.org/10.1139/f99-180>
- Zimmer KD, Hanson MA, Butler MG (2002) Effects of fathead minnows and restoration on prairie wetland ecosystems. *Freshwater Biology* 47:2071–2086. <https://doi.org/10.1046/j.1365-2427.2002.00952.x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.



High abundance of a single taxon (amphipods) predicts aquatic macrophyte biodiversity in prairie wetlands

Danelle M. Larson¹ · Demey DeJong² · Michael J. Anteau³ · Megan J. Fitzpatrick⁴ · Breanna Keith⁵ · Emily G. Schilling² · Barry Thoele⁶

Received: 18 May 2021 / Revised: 3 January 2022 / Accepted: 25 January 2022 /

Published online: 15 February 2022

This is a U.S. government work and not under copyright protection in the U.S.; foreign copyright protection may apply 2022

Abstract

Conservation programs often aim to protect the abundance of individual species and biodiversity simultaneously. We quantified relations between amphipod densities and aquatic macrophyte (large plants and algae) diversity to test a hypothesis that biodiversity can support high abundance of a single taxonomic group. Amphipods (*Gammarus lacustris* and *Hyalella azteca*) are key forage for waterfowl and are declining in the Prairie Pothole Region of North America. We sampled a large gradient of amphipod densities (0–7050 amphipods/m³) in 49 semi-permanent wetlands, and 50% of the study wetlands had high amphipod densities (> 500 amphipods/m³). Generalized linear models revealed *G. lacustris* and *H. azteca* densities increased exponentially with macrophyte diversity indices. Further, *H. azteca* densities were greatest at moderate levels of submersed vegetation biomass. Community analyses showed both amphipod species were positively associated with diverse macrophyte assemblages and negatively associated with high coverage of cat-tails (*Typha* spp.), a taxon that creates monotypic stands, as well as bladderwort (*Utricularia* spp.), a carnivorous plant. Our results indicate that amphipods could be used as an umbrella species for protecting diverse macrophyte communities in semi-permanent and permanent wetlands of North America's Prairie Pothole Region.

Keywords Amphipoda · Aquatic vegetation · Systematic conservation planning · Umbrella species · Waterfowl

Introduction

Systematic conservation planning has focused on maintaining, protecting, and restoring biodiversity. The systematic conservation planning model is used worldwide to identify and prioritize landscape conservation networks (Margules and Pressey 2000). Planners

Communicated by Peter Bridgewater.

✉ Danelle M. Larson
dmlarson@usgs.gov

Extended author information available on the last page of the article

first choose which conservation proxies, such as ecosystem functions or species, will be used to represent biodiversity (Bal et al. 2018). Biodiversity proxies often include individual taxonomic groups that are easy to measure and are environmentally sensitive (“indicator” species) and taxa whose protection will extend to a wide range of co-occurring species (“umbrella” species). Decadal long debates continue as to whether a single-species focus can conserve biodiversity (Simberloff 1998; Runge et al. 2019). More scientific evidence linking single species abundance and biodiversity may provide opportunities to combine these conservation strategies (Lindenmayer et al. 2007).

The implied idea of managing the abundance of specific species for conserving biodiversity or vice versa is paradoxical. So, a key question remains: is the conservation of *biodiversity* at odds or compatible with the conservation of the *abundance of a single species*? We evaluated this question by examining the role of wetland macrophyte diversity in supporting abundant populations of amphipods, which are a key food resource for many waterfowl and other wetland wildlife (Olenick and Gee 1981; Afton and Hier 1991; Benoy et al. 2002). Similarly, we explored whether amphipods could be an *umbrella species* (i.e., whose protection would extend to a large number of other species, such as macrophyte diversity). Identifying more umbrella species can help to address global freshwater biodiversity loss (Kalinkat et al. 2017) by prioritizing conservation reserves and evaluating the effectiveness of restoration.

Amphipods (*Gammarus lacustris* and *Hyaella azteca*) are wetland crustaceans of known ecological significance to waterfowl (Afton et al. 1991) that are easy to detect when found in high densities (Fig. 1a–c). However, it remains unclear whether amphipod abundance indicates ecosystem health (such as high diversity) and whether a conservation focus on this taxonomic group might also enhance wetland biodiversity. Amphipods are an especially important forage base for some waterfowl species such as Lesser and Greater Scaup (*Aythya affinis* and *A. marila*, respectively), hereafter scaup (Afton et al. 1991). Brood-rearing and migrating scaup preferentially inhabit wetlands (Fast et al. 2004; Anteau and Afton 2009; Kahara and Chipps 2009), and scaup forage most efficiently in wetlands with high amphipod densities (Anteau and Afton 2009).

Several studies have shown amphipod abundances have declined across the Prairie Pothole Region (PPR) of North America in the past few decades (Afton and Hier 1991; Anteau and Afton 2008a, b; Strand et al. 2008), which likely cascaded to partially cause scaup population declines (Anteau and Afton, 2006, 2008b; Austin et al., 2000). Previous studies showed amphipod densities are negatively correlated with fish densities (Hanson and Butler 1994; Anteau et al. 2011) and high hydrologic connectivity that allows fish passage into wetlands (Wiltermuth 2014). Amphipod densities are also correlated with moderate concentrations of organic suspended sediments and the abundance of submersed macrophytes (Kahara and Chipps 2009; Anteau et al. 2011) but not with upland cultivation or vegetation buffer widths (Janke et al. 2019). This body of previous work was informative but limited because it included few study wetlands with high densities of amphipods (Anteau and Afton 2008a). Occasionally wetlands in the PPR still host high amphipod densities (> 500 amphipods/m³); however, little is known of the factors driving wetland habitat suitability for these high densities despite the importance of understanding those drivers to protect or enhance available habitat (Kahara and Chipps 2009; Larson et al. 2018).

Our research objective was to understand the association between macrophyte species diversity and high densities of amphipods (*G. lacustris* and *H. azteca*). We hypothesized that macrophyte diversity and amphipod abundance would be positively correlated because diversity may increase system productivity that could support amphipod forage

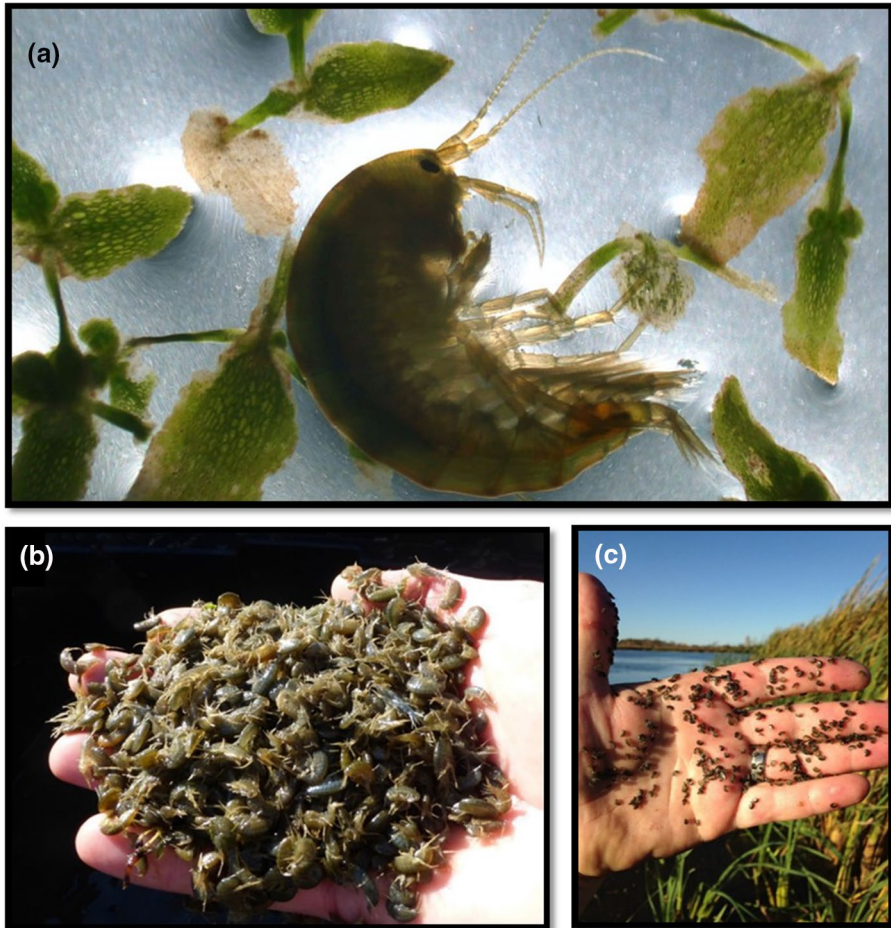


Fig. 1 Photographs of the amphipod *Gammarus lacustris* and the aquatic plant *Lemna trisulca* under 60×microscopy (panel **a**); a handful of *Gammarus lacustris* (panel **b**) and *Hyaella azteca* (panel **c**), both taken from natural wetlands with high amphipod densities (> 500 amphipods m^{-3}) in the Prairie Pothole Region, Minnesota, USA. Photographs taken by Breanna Keith (panel **a**), Danelle Larson (panel **b**), and Robert Baden (panel **c**), all used with permission

and because other factors such as water quality are interrelated. Further, we hypothesized that high amphipod abundance would be positively correlated to both submersed plant biomass and emergent cattail fringe because these plant resources could provide refuge from predation and a carbon-rich food source. We explored the idea that amphipods could be used as umbrella species because we hypothesized amphipods would have several characteristics of an umbrella species: (1) easy to detect in high densities, (2) are environmentally sensitive to factors such as water quality and macrophytes, and (3) amphipod conservation and management could likely extend to many other species (such as macrophyte diversity).

Methods

Study sites and design

We studied 49 small wetlands within the Prairie Pothole Region and forest-transition zone of Minnesota, U.S.A. Past research used random-sample approaches, which highlighted the lack of amphipod presence in most Minnesota wetlands and occasionally wetlands with very high densities (Anteau and Afton 2008a; Anteau et al. 2011). Those prior studies connected amphipod abundance with environmental conditions but contained few study sites with very high amphipod densities, thus limiting their inferential value for the environmental conditions of wetlands containing high amphipod densities. Therefore, our study design intentionally targeted wetlands with a range of amphipod densities, especially high densities not captured with previous studies. We randomly selected half of our study wetlands, and the other half were targeted to have high densities of any amphipod species. The high-density wetlands were identified through personal communications with eight resource managers in our study region, as well as the published reports by Anteau and Afton (2008a, 2009) and Janke (2016). A subsequent rapid assessment of amphipod density in spring 2018 at 111 candidate basins confirmed that 24 wetlands had > 500 amphipods/m³, which we included in this study as high-density wetlands based on expert opinion by the authors and resource managers. The other 25 study wetlands with unknown amphipod presence and density were randomly selected with ArcGIS software the following criteria: (1) located within a 24 km radius of high-density wetland; (2) a semi-permanent or permanent water body where amphipods can persist (Anteau et al. 2011); and (3) similar ($\pm 25\%$) wetland surface area as the selected high-density wetlands. The intention of this selection criteria was to increase the likelihood of finding amphipods in suitable hydrology (semi-permanent yet shallow) and ease of sampling (e.g., not driving long distances between sites). The design was not a “paired design” because the wetlands were independent of one from another and not meaningfully matched pairs.

The 49 wetlands represented a variety of amphipod densities (0–7050 amphipods/m³), land uses (including forested, native prairie, and agricultural cultivation), land ownership (including private wetlands, state lands, federal lands, and mixtures of ownership), wetland sizes (0.4–77 ha), average water depths (72–164 cm, excluding one outlier of 604 cm), water quality conditions, and macrophyte metrics and communities (see ‘Results’ below and the complete dataset [Larson et al. 2021]).

Field data collection

Amphipods were sampled in April and May 2019. We targeted adult amphipods and this sampling time because adults are easy to capture and identify as compared to juveniles that can be small and inconspicuous and to avoid detection error from high macrophyte biomass in summer. We sampled a total of eight stratified random sampling locations at each wetland. The strata included near-shore points (within ~5 m of shore or the outermost ring of impassable emerged vegetation) and deeper points (~50 m from shore) to capture a range of habitats. Using ArcMap (release 10; ESRI, Redlands, California, USA), we generated eight equally spaced transects that ran perpendicular to the longest axis of the wetland. Each transect contained one sample point, alternating between near-shore points and deeper points. The sample points were preprogrammed into a Garmin 76 GPS unit for field navigation (~3-m positional accuracy). Sampling was limited to points with a maximum

depth of 1.4 m due to sweep net limitations. Alternate points along the transect were sampled when water depth was > 1.4 m or the near-shore point was > 5 m from shore.

Separate water column and benthic samples were collected using sweep nets (mesh size: 1200 microns). Benthic samples were obtained by sweeping a D-frame net (net diameter: 0.0647 m^2) for 0.5 m along the wetland bottom to capture the top ~ 3 cm of sediment and organisms. Water column samples were collected with a D-frame or circular sweep net (net diameter: 0.0452 m^2) that was bent 90° so the net opening was perpendicular to the handle. We plunged the sweep net to a depth of 30 cm (the benthic net height) above the benthos in an area undisturbed by the benthic sweep, moved the net horizontally to undisturbed water, and raised the net vertically out of water. During the plunge the net was inverted, so a sample was only collected during the vertical retrieve. Samples were drained of excess water and preserved in 95% ethanol. Samples were transported to the Minnesota Department of Natural Resources' Wetland laboratory and processed within four months of collection.

We conducted comprehensive wetland habitat surveys once in July 2019 to capture representative wetland conditions and peak macrophyte species richness. Water samples were collected near the center of each wetland in 50-mL polypropylene sterile tubes, stored on ice in a cooler, and shipped on ice to the Minnesota Department of Agriculture Laboratory Services for analyses of total phosphorus (P). Wetland pH (Hach® HQ40d, Loveland, Colorado, USA) and turbidity (Hach® 2100P, Loveland, Colorado, USA) were also measured near the center of each wetland.

We sampled water depth and aquatic macrophytes (submersed aquatic vegetation [SAV], emerged, rooted-floating, and free-floating life forms) at evenly spaced, random sampling points. These sample points differed from where amphipods were collected, and so the depth and macrophyte data represent the entire wetland and not a sampling point. The entire wetland was gridded in ArcMap, and each grid provided a sampling point for water depth and vegetation. Grid size varied by wetland size; wetlands < 4 ha had 10 sampling points, and we increased sampling by one point for each additional 0.5 ha. The number of sampling points per wetland ranged from 10 to 67 (average: 25 points). We navigated via canoe to each sampling point using a Garmin 76 GPS unit. At each point, we measured water depth and dragged one plant rake of 35-cm circular diameter along the bottom of the wetland for 150 cm. We used a relative biomass index to estimate the total amount of submersed plants and filamentous algae on the rake (ordinal score ranging from 0 to 4; Online Appendix 1) and then identified each species present using identification keys (Skawinski 2018). We quantified emerged vegetation prevalence along shorelines using visual field surveys in which we recorded the presence of emerged vegetation guilds (including cattail, bulrush, and sedge/rush) at the end of each vegetation sampling transect line. Data from the individual points were compiled for each wetland to calculate average biomass and species-specific prevalence. We acknowledge that high wetland plant diversity occurs in the emergent and wet meadows on the upland periphery from the permanent water, but our assessments did not include species-level identification of shallow marsh plants principally because these are not the common habitats that amphipods reside in. Although fish abundance is known to be negatively correlated to amphipods, this study focused on the relations between macrophytes, water quality, and amphipods and not fish. Fish were not sampled as part of this study but may have been present.

Laboratory analyses

Sample contents were washed in a 500- μm rectangular sieve to remove fine sediments. All material on the sieve was suspended gently in a bucket of water to evenly disperse the contents. A grid was placed over the sieve to allow for sub-sampling. We picked a minimum of 25% of the sample on the sieve and added more grids until at least 400 invertebrates were picked or the entire sample was processed. All picked invertebrates were stored in 70% ethanol for identification and archiving.

We enumerated and identified all amphipods to the species-level using taxonomic keys (Merritt et al. 2008; Glazier 2014) by using a lighted trinocular microscope with 110 $\times$ magnification potential. Amphipod counts were scaled to the entire sample if they had been subsampled. Amphipod abundance was the total number of individuals counted at each wetland ($n=8$ standardized sweeps). Average amphipod density (units: $\#/\text{m}^3$) was calculated for each basin by dividing the basin-level abundance by the volume of water passed through the sweep net.

Data analyses

We averaged amphipod counts and density by wetland as response variables because the wetland was the experimental unit where samples were truly independent. Therefore, the inferences of amphipods and habitat associations were at the wetland-scale and not at the point of amphipod sampling. We calculated a variety of α -biodiversity facets, including Simpson diversity index, Shannon diversity index, Pielou's evenness index, and species richness using all vegetation species or guilds collected.

Three species of amphipods were collected in our study wetlands: *G. lacustris*, *H. azteca*, and *Crangonyx* spp. We chose to model *G. lacustris* and *H. azteca* separately because each species may respond differently to environmental predictors. We did not analyze *Crangonyx* spp. due to infrequent occurrences ($<15\%$) and low densities (Table 1, Fig. 2).

We used two complementary analyses to infer biologically and statistically significant factors that influenced amphipod abundance: (1) generalized linear models to test our hypotheses that amphipod density would relate positively to SAV biomass, macrophyte diversity, and emerged vegetation prevalence along shorelines, and (2) a redundancy analysis (RDA) of amphipod abundance and macrophyte assemblages to test species affiliations. We reported the p -value with degrees of freedom and relative magnitude of differences to assess 'statistical significance' of model outputs. The statistical analysis outputs, graphical trends, and previous literature were used to conclude what constitutes 'biologically significant' results and where uncertainty remains (Wasserstein et al. 2019).

All models and graphs were produced using software R 4.0.5 (R Foundation for Statistical Computing 2018). We modeled species-specific amphipod counts to our predictor variables using a negative binomial distribution with a log link function using the 'MASS' package (Ripley et al. 2020); prior models with Poisson distribution of transformed data were over dispersed and thus not reported. We used the natural log of water-volume swept (m^3 ; summed per wetland) as an offset variable. The predictor variables included the three primary hypotheses about the influence of macrophytes on amphipods: (1) macrophyte diversity (all life forms); (2) SAV biomass; and (3) the percentage of shoreline rimmed by emerged vegetation. We also included several covariates to account for wetland-to-wetland

Table 1 The summary statistics of densities of each amphipod genus detected in the study ($n=49$ wetlands), which includes randomly selected wetlands, intentionally selected wetlands with known high densities (>500 amphipods m^{-3}) of at least one species of amphipod, and the combined summaries of both wetland selection types

Genus	Minimum	1st quartile	Median	Arithmetic mean	3rd quartile	Maximum
Randomly selected wetlands ($n=25$)						
Cragonyx	0	0	0	1	0	23
Gammarus	0	0	0	12	0	200
Hyalrella	0	0	19	189	102	1329
Intentionally selected, 'high density' wetlands ($n=24$)						
Cragonyx	0	0	0	16	15	124
Gammarus	0	58	422	681	1050	2736
Hyalrella	0	70	321	1001	1259	7050
All study wetlands ($n=49$)						
Cragonyx	0	0	0	9	3	124
Gammarus	0	0	15	353	422	2736
Hyalrella	0	13	77	620	734	7050

variability due to factors other than macrophytes. Our approach was to have a model for each amphipod species that contained at least three variables that represented each of the macrophyte hypotheses and then interpreted the strength of their support by examining the parameter estimates, 95% confidence limits, and p -values.

We took several steps to select the variables used in the final models. These steps included examining plots of the raw data, transforming data as necessary, and then performing a single-variable elimination. We assessed relative goodness of fit and parsimony by comparing the Type III likelihood ratio statistic, and single variables were eliminated if its p -value was >0.05 . We plotted species' density against each potential variable to identify non-linear relations and any needs for transformations. The *H. azteca* densities graphically appeared to increase with SAV biomass up to a point then decreased, so we included a quadratic effect. The covariates we initially considered were maximum depth, wetland area, pH, total P, turbidity, and an index of suspended sediments. There were low correlations ($r < 0.4$) among these covariates and our three hypothesized variables, with exception of turbidity and total P ($r = 0.49$). We suspected that our turbidity measurement was composed of phytoplankton and suspended sediments, which could be an important covariate based on Anteau et al. (2011). So, like Anteau et al. (2011), we ran a regression using total P to predict turbidity ($\text{Turbidity} = 0.94 + 75.48 \times \text{total P}$; $r^2 = 0.23$) and used the residuals of that regression to index suspended sediments. The resulting suspended-sediment index was orthogonal with total P. For *G. lacustris*, the plots indicated total P should be transformed with a natural log, and there were threshold effects of maximum depth (~ 150 cm) and wetland area (15 ha); so, we indexed maximum depths > 150 cm as "1" and ≤ 150 as "0" and wetland areas > 15 ha as "1" and ≤ 15 as "0." For *H. azteca*, we did not consider maximum depth, wetland area, total P, and suspended-sediment index because preliminary analyses indicated no pattern with their densities. Lastly, we chose a single, most informative variable to represent the three hypotheses related to macrophyte diversity, biomass,

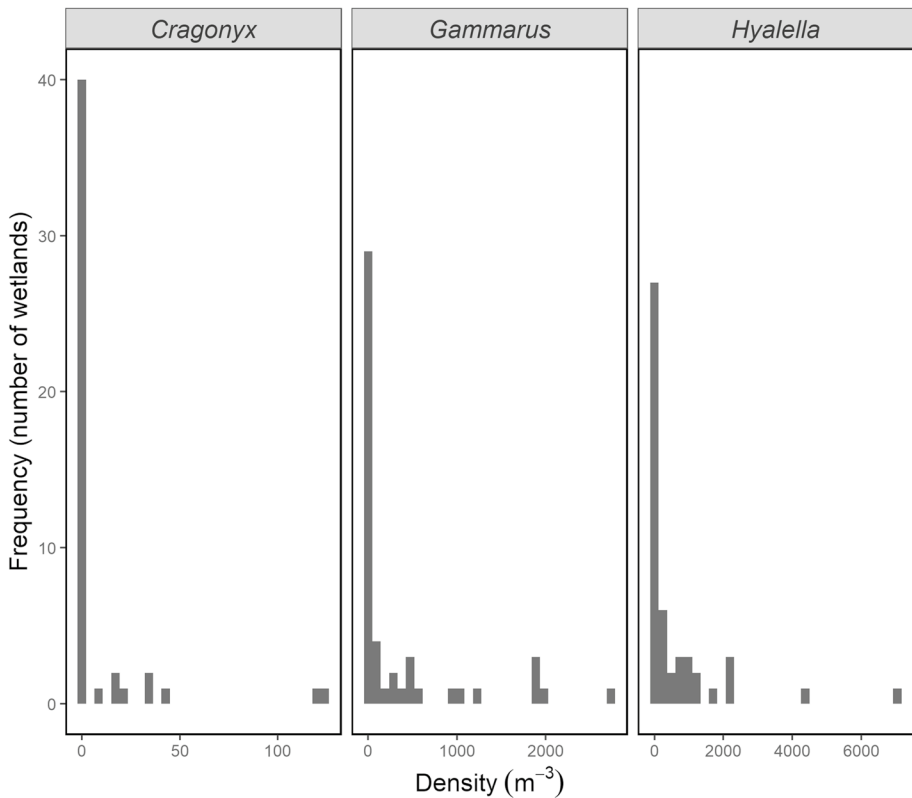


Fig. 2 Histograms showing the frequency of amphipod densities measured in 49 wetlands in the Prairie Pothole Region, Minnesota USA in spring 2019

and emergent vegetation in the models. For both amphipod species, the SAV biomass index was more informative than SAV prevalence. Simpson's diversity index was more informative than Shannon's diversity index, species richness, or Pielou's evenness. Shoreline emerged prevalence was a better predictor than total wetland emerged prevalence.

We reported results of full models that included the three hypothesized macrophyte variables and informative covariates (see Tables 2 and 3). To assess model fits, we used marginal model plots (Online Appendix 2) that compare the data and model fits. We calculated the predicted values of amphipod density of our hypothesized variables while holding the other covariates constant and provided graphs of each model's predictions (Figs. 3 and 4).

In addition, we conducted a redundancy analysis to examine relations among species-specific amphipod counts and macrophyte assemblages using the 'vegan' package in software R (Oksanen et al. 2019). This analysis is a multivariate extension of multiple regression where the ecological community is constrained by linear combinations of the predictor variables. The two amphipod species were used as separate response variables. Predictor variables included prevalence for the eight most common SAV species, one prevalent emerged species (*Typha* spp.), and filamentous algae (not speciated). The aquatic vegetation species matrix did not have ecological transformations, but we ln-transformed the amphipod count data to fit linear assumptions. We conducted a permuted, multivariate

Table 2 Analysis results of maximum likelihood parameter estimates for *Gammarus lacustris*

Parameter	DF _{num} , DF _{den}	Estimate	Standard error	Wald 95% confidence limits		Wald χ^2	p-value
Intercept	1, 40	1.54	2.97	−4.28	7.36	0.27	0.604
Simpson diversity	1, 40	13.16	3.31	6.68	19.63	15.85	<0.001
SAV biomass	1, 40	−1.45	0.45	−2.34	−0.56	10.13	0.002
Shoreline emergent prevalence	1, 40	−0.02	0.02	−0.06	0.02	1.25	0.265
Maximum water depth	1, 40	1.77	0.88	0.04	3.49	4.02	0.045
Wetland size	1, 40	−4.33	1.11	−6.51	−2.15	15.15	<0.001
Total P	1, 40	1.05	0.53	0.00	2.09	3.87	0.049
Suspended sediment	1, 40	0.14	0.06	0.02	0.26	4.89	0.027
Dispersion	1, 40	3.71	0.95	2.25	6.11	NA	NA

The parameters included in this table were of primary interest with hypotheses of positive associations of *G. lacustris* and aquatic vegetation. Parameters in bold were primary hypothesized variables of interest related to aquatic vegetation and the others were covariates of secondary interest and included to improve parameter estimates

Table 3 Analysis results of maximum likelihood parameter estimates for *Hyalella azteca*

Parameter	DF _{num} , DF _{den}	Estimate	Standard error	Wald 95% confidence limits		Wald χ^2	p-value
Intercept	1, 42	−18.87	4.90	−28.48	−9.25	14.79	<0.001
Simpson diversity	1, 42	3.20	1.32	0.59	5.79	5.80	0.016
SAV biomass	1, 42	2.90	0.96	1.00	4.79	8.97	0.003
SAV biomass × SAV biomass	1, 42	−0.80	0.23	−1.24	−0.36	12.74	<0.001
Shoreline emergent prevalence	1, 42	0.01	0.01	−0.01	0.03	0.54	0.464
pH	1, 42	2.23	0.54	1.18	3.28	17.30	<0.001
Dispersion	1, 42	2.28	0.55	1.93	4.15	NA	NA

The parameters included in this table were of primary interest with hypotheses. Parameters in bold were primary hypothesized variables of interest related to aquatic vegetation and the others were covariates of secondary interest and included to improve parameter estimates

analysis of variance ($perm = 9999$) to assess the statistical significance of the overall RDA model, axes, and terms.

Results

Amphipod densities and species co-occurrences

Our surveyed wetlands comprised a large range of amphipod densities (0–7050 amphipods/m³). Several wetlands included exceptionally high density (Table 1, Figs. 1 and 2); half of the wetlands had >500 amphipods/m³ (per the study design). Of the 25 randomly selected

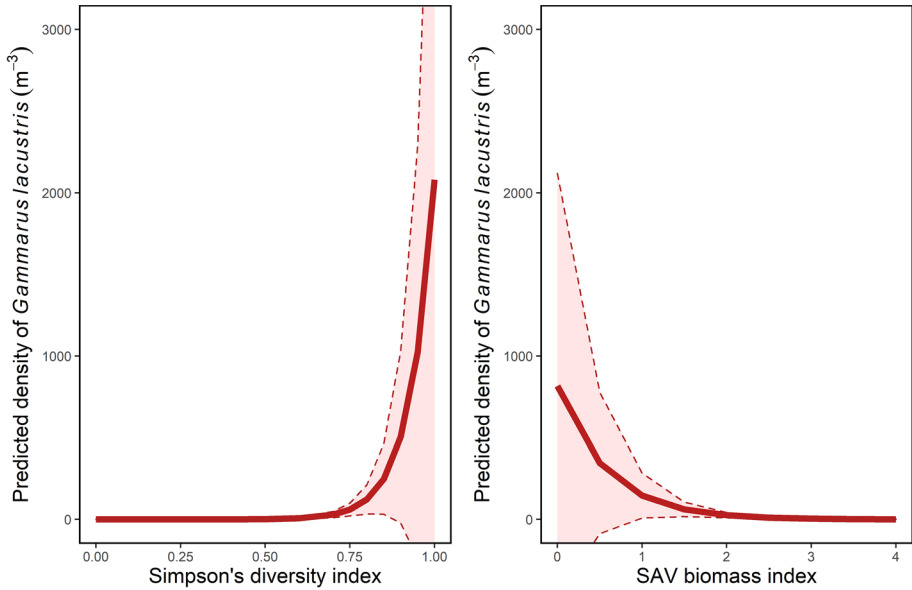


Fig. 3 The model-predicted densities of *Gammarus lacustris* in response to wetland vegetation diversity (Simpson diversity index) and submersed aquatic vegetation biomass (SAV biomass index). Shaded areas correspond to the prediction's 95% confidence intervals while other model covariates were held constant at their means

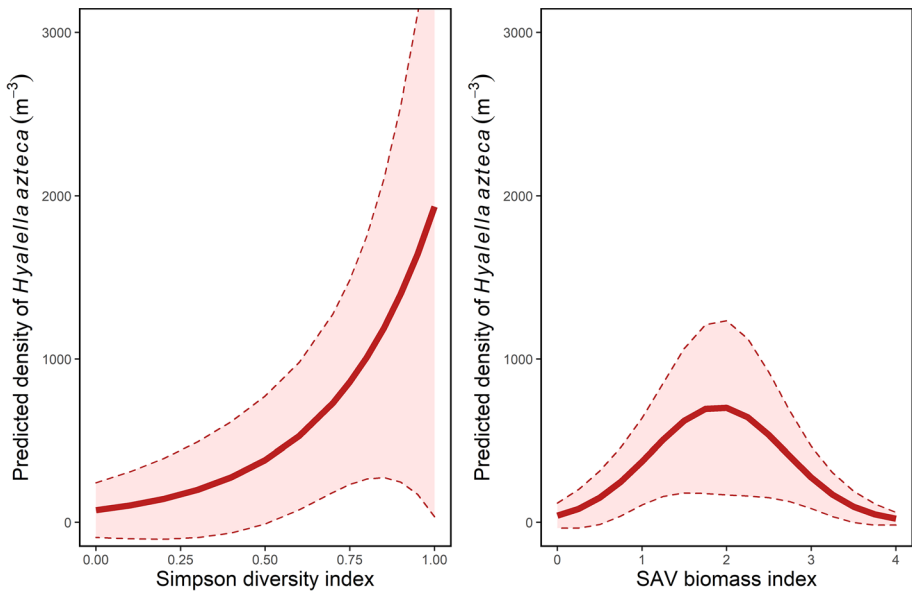


Fig. 4 The model-predicted densities of *Hyalella azteca* in response to wetland vegetation diversity (Simpson diversity index) and submersed aquatic vegetation biomass (SAV biomass index). Shaded areas correspond to the prediction's 95% confidence intervals while other model covariates were held constant at their means

wetlands, 8% had *G. lacustris* present but with relatively low densities (geometric mean: 37 *G. lacustris*/m³). The *H. azteca* were present in 70% of the 25 randomly selected wetlands (geometric mean: 63 *H. azteca*/m³), and 12% of those wetlands had relatively high density (i.e., > 500 *H. azteca*/m³).

The three amphipod species (*G. lacustris*, *H. azteca*, and *Crangonyx* spp.) typically co-occurred in wetlands, but only one species was dominant. *Gammarus lacustris* densities were not correlated with either *Crangonyx* or *H. azteca* densities ($r = -0.06$, $r = -0.02$, respectively). *Crangonyx* and *H. azteca* densities were positively correlated ($r = 0.43$).

Relationships among *Gammarus lacustris*, macrophytes, and wetland characteristics

The *G. lacustris* model was predicted by the independent variables of Simpson diversity, SAV biomass, shoreline emergent prevalence, maximum water depth, wetland size, total P, and suspended sediment (Table 2). The *G. lacustris* model predictions fit the observed data well ($r = 0.54$, Online Appendix 2A). The most informative macrophyte diversity metric (Simpson's *D*) had a strong, positive correlation with *G. lacustris* densities (Table 2, Fig. 3, Online Appendix 3B). The high-density *G. lacustris* wetlands always had a Simpson's *D* > 0.80. Although we hypothesized SAV biomass and shoreline emerged vegetation would be positively correlated with *G. lacustris* densities, the model indicated a negatively weak correlation and no correlation, respectively. The covariates showed that *G. lacustris* responded positively to wetland maximum water depth and productivity metrics but negatively to wetland size (Table 2).

Relationships among *Hyalella azteca*, macrophytes, and wetland characteristics

The *H. azteca* model was predicted by the independent variables of Simpson diversity, SAV biomass, a quadratic term for SAV biomass, shoreline emergent prevalence, and pH (Table 3). The *H. azteca* regression model fit the observed data less well than the *G. lacustris* model, but the predicted values were correlated with the observed data ($r = 0.37$). *H. azteca* were positively correlated to Simpson's diversity (Table 3, Fig. 4, Online Appendix 3D), but not as strongly as *G. lacustris* based on the parameter estimates. The high-density *H. azteca* wetlands had diversity-index values from $D = 0.5$ to 1.0. The *H. azteca* densities displayed a significant quadratic relationship with SAV biomass whereby densities were maximized at moderate SAV biomass values. Shoreline emerged prevalence was not a significant predictor variable. Only one informative covariate (pH) was included in the model, which was positively correlated with *H. azteca* densities (Table 3).

Community analysis of amphipods and macrophytes

We used redundancy analysis to examine the community composition of macrophytes to explore which species were most closely associated with amphipod abundance. The full community model was significant ($F_{1,38} = 1.82$, $p = 0.040$) as was the first axis ($F_{1,46} = 15.80$, $p = 0.043$). *Lemna trisulca* (LETR) along the first axis was a predictor of *G. lacustris* abundance ($F_{1,38} = 5.09$, $R^2 = 0.19$, $p = 0.001$) and *Myriophyllum sibiricum* (MYSI) was a predictor of *H. azteca* abundance ($F_{1,38} = 5.55$, $R^2 = 0.21$, $p = 0.005$).

Graphically, the redundancy analysis showed biologically significant relationships between macrophyte assemblages and amphipod abundance (Fig. 5). Most wetlands were

spread across ordination space (i.e., not clumped together on the graph), indicating that they had differing plant-community composition and amphipod abundances. The two amphipod species were not correlated and fell along two different ordination axes, indicating that the species often did not co-occur in the same wetlands and that each amphipod species were associated with different macrophyte assemblages. The *G. lacustris* was strongly associated with *L. trisulca* (LETR), which was positively correlated with SAV diversity and species richness but not evenness (Online Appendix 3B). The *H. azteca* was associated with macrophyte assemblages composed of predominately *Stuckenia pectinata* (STPE15), filamentous algae, *Potamogeton friesii* (POFR3), *Potamogeton zosteriformis* (POZO), and *M. sibiricum* (MYSI); these macrophyte species were also prevalent and had moderately high SAV biomass. Both amphipod species were negatively associated with high prevalence of *Typha* spp. (TYPHA) and the carnivorous plant, *Utricularia macrorhiza* (UTMA). *Chara* spp. (CHAR) and sometimes *Ceratophyllum demersum* (CEDE4) were

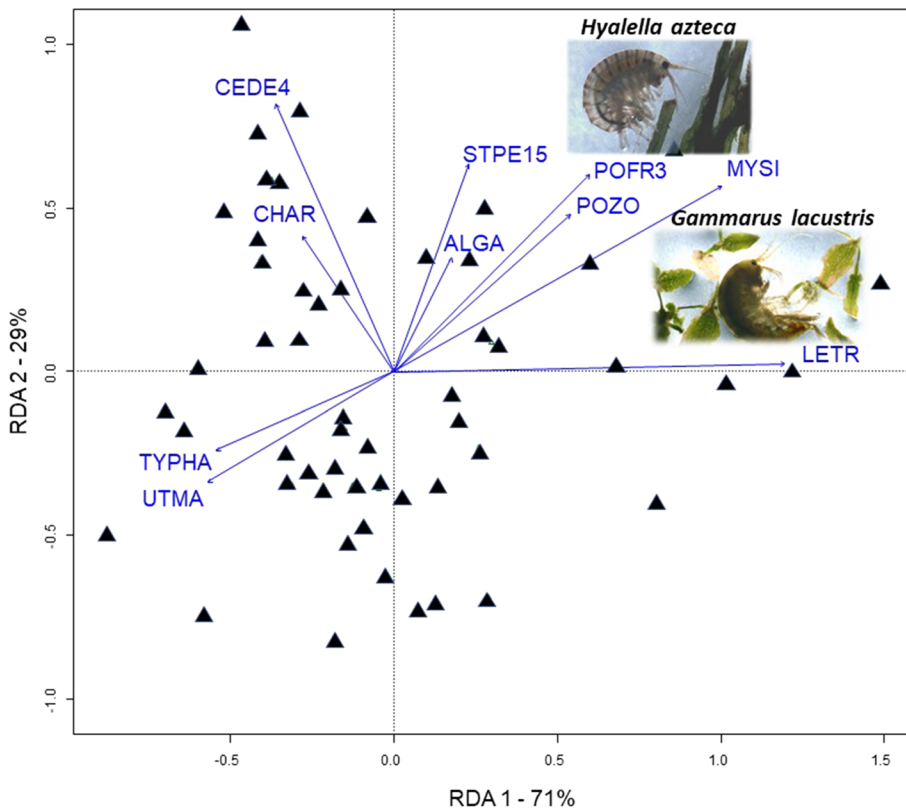


Fig. 5 A triplot of the amphipod communities and aquatic vegetation communities at 49 wetlands (triangles) in the Prairie Pothole Region, Minnesota, USA. The amphipod community in this analysis included two species: *Hyalella azteca* and *Gammarus lacustris*. The species included in the ordination included the ten most prevalent macrophytes: *Typha* spp. (TYPHA), *Utricularia macrorhiza* (UTMA), *Lemna trisulca* (LETR), *Potamogeton zosteriformis* (POZO), *Myriophyllum sibiricum* (MYSI), *Potamogeton friesii* (POFR3), *Stuckenia pectinata* (STPE15), filamentous algae (ALGA; not speciated), *Ceratophyllum demersum* (CEDE4), and *Chara* spp. (CHAR). The ordination scaling and interpretations are based on correlations; therefore, the vectors at $< 90^\circ$ angles are positively correlated, vectors at 90° angles are uncorrelated/orthogonal, and vectors at 180° are negatively correlated

dominant plants (SAV species richness = 1; biomass scores = 3–4), and neither amphipod species thrived in those conditions. The results of the redundancy analysis complemented the univariate regression models (Tables 2 and 3) without conflicting results, except the redundancy analysis indicated a negative association of *Typha* spp. (TYPHA) and amphipod densities (Fig. 5).

Discussion

We found that high densities of *G. lacustris* and *H. azteca* were positively associated with macrophyte taxonomic diversity, and *G. lacustris* were especially sensitive to macrophyte diversity and other wetland characteristics. The greatest amphipod densities were found in macrophyte assemblages composed of both cosmopolitan macrophytes (e.g., *S. pectinata* and filamentous algae) and less common macrophytes (e.g., *L. trisulca*). Both amphipod species had lower densities in wetlands with high cattail (*Typha* spp.) prevalence. Our results clearly showed strong, positive correlations among amphipods and several macrophyte metrics, but the causative relationships could only be speculated. The results indicated that amphipods could be an indicator and umbrella species for wetland plant diversity; macrophyte diversity provides habitat conditions that favor high amphipod densities; macrophytes and amphipods simply require similar wetland conditions (e.g., moderate water depths and semi-permanent hydrology); and that loss of macrophyte diversity could cascade to affect amphipod abundance due to habitat loss. Regardless of which causal mechanism(s) relate amphipods and macrophyte diversity, conservation actions that support either amphipod abundance or macrophyte diversity will likely aid the other taxa (with possible caveats described further herein). Specifically, amphipods as an umbrella species may also help conserve wetland biodiversity in semi-permanent and permanent wetlands. Likewise, a conservation strategy with a focus on aquatic macrophyte communities might concomitantly result in greater amphipod abundance.

Amphipod species abundances and wetland relations differed

Hyaella azteca was much more common and abundant than *G. lacustris*, which was also the case in other Prairie Pothole Region wetlands in recent past (Anteau and Afton 2008a). Densities of *H. azteca* were often three times greater than that of *G. lacustris*, which may be partially explained by the smaller body size of *H. azteca* that allows more occupation in the same space (Thomaz and Cunha 2010). *Hyaella* also have advantageous life-history traits such as greater reproductive capacity than *Gammarus* (Glazier 2014). Although *H. azteca* were more abundant, *G. lacustris* are also ecologically significant given their high density and large body size and biomass that is important for foraging water birds (Anteau and Afton 2006).

Hyaella azteca were tolerant to a wide range of wetland conditions, whereas *G. lacustris* had a narrower and more defined ecological niche (as indicated by the number of informative covariates in the models and model fits). This result corroborates findings of Anteau and Afton (2008a, b) that *H. azteca* were generally more widespread on the landscape. In our study, *G. lacustris* were more common in small wetlands (< 20 ha), were not found in average water depths < 100 cm and were positively related to wetland productivity measures like total P and turbidity. Previous work demonstrated fish are more common in large, permanent Prairie Pothole Region wetlands and that fish can negatively affect

amphipod abundance (Hanson and Butler 1994; Wiltermuth 2014; Janke 2016). Therefore, our significant results on wetland size and depth may either indicate increased carrying capacity for amphipods, or reflect the unmeasured, interactive effects of fish abundance more so than wetland size or depth independently. Note our study wetlands had typically 4× lower productivity (total P and turbidity) compared to other Prairie Pothole Region wetlands in Minnesota that were classified as a degraded “turbid state” (Larson et al. 2020b), so we do not know if *G. lacustris* declines after certain productivity thresholds of eutrophication. Our findings refined niches of *H. azteca* and *G. lacustris* in relation to macrophyte communities and showed *G. lacustris* had a narrow niche breadth and is likely an environmentally sensitive species to wetland factors such as macrophyte diversity and biomass, as well as wetland size, water depth, total P, and suspended sediments.

Macrophyte biodiversity shapes the wetland that supports amphipods

Our results showed that both amphipod species were affiliated with macrophyte diversity. We suspect macrophyte diversity is indicative of overall wetland condition and supports the “biodiversity ecosystem functioning” concept (Engelhardt and Ritchie 2002; Brisson et al. 2020). Ecosystem functions provided by macrophytes to amphipods may include nutrient turnover, carbon availability, structural habitat, and water purification (Carpenter and Lodge 1986). A previous study indicated positive associations of amphipod density and stands of *Potamogeton* spp. and *L. trisulca*, but not *Chara* spp. (Hanson 1990). Our results from 49 wetlands concurred (Fig. 5) with findings from Hanson 1990 and may hint at niche-differentiation or other mechanisms linking biodiversity and amphipods.

SAV biomass

Hyaella azteca densities were maximized at moderately high macrophyte biomass whereas *G. lacustris* densities were negatively associated with SAV biomass. Total macrophyte leaf surface area and complexity from leaf branching presumably support amphipods by providing places of attachment for shelter and feeding (Thomaz and Cunha 2010). More space provided by macrophyte abundance and leaf branching is a plausible reason that the smaller-bodied *H. azteca* were often found at higher densities than the larger-bodied *G. lacustris*. Although fish can also be attracted to macrophyte beds, and the SAV biomass and complexity can reduce fish foraging effectiveness (Thomaz and Cunha 2010) and could reduce predation. We suspect *G. lacustris* were negatively associated with SAV biomass because *G. lacustris* were more common in deep wetlands, which can have less SAV due to light limitations.

Monocultures

Chara spp. and *C. demersum* were species that often dominated in high biomass, and amphipods were not typically detected in those conditions (Fig. 5). These plant species were typically found in wetlands with species richness <2 and high SAV biomass scores from 3 to 4. We speculate the negative associations among amphipods, *Chara* spp., and *C. demersum* are related to the strong affinity of amphipods to macrophyte diversity (Figs. 3 and 4) more than the plant biomass itself. First, amphipods consume a broad array of

aquatic plants, whereas most other herbivorous aquatic invertebrates consume only one or two plant species (Newman 1991). In near monocultures, the plants senesce at the same time of year, which could reduce the availability of detritus over a long overwintering season. In contrast, a staggered timing in macrophyte senescence and varying plant decay rates could provide an ongoing refuge and detrital food base for amphipods. Further, *Chara* spp. are often encrusted with ‘marl,’ which are carbonate compounds from hard water that are slow to decompose and release carbon for detritivores. Non-native species like *Potamogeton crispus* or *Myriophyllum spicatum* can reduce macrophyte diversity and can have exceptionally high biomass, so we speculate these common invasive plants could negatively affect amphipods.

Typha × *glauca*

The emerged vegetation in this study was predominately cattails (presumably the invasive hybrid *Typha* × *glauca*), which are ecologically important but can also encroach shallow wetlands which have strong, negative ecological effects (Bansal et al. 2019). We found a weak, negative association among *Typha*, macrophyte diversity, and amphipods (Fig. 5), but our sweep-net sampling technique via canoe was insufficient for capturing amphipods in the interior of dense *Typha* beds where amphipods may reside in high density (Christensen and Crumpton 2010). Wetlands with low to moderate *Typha* prevalence are usually larger basins with enough water depth to support *G. lacustris* and dynamic water levels that prevent *Typha* dominance. *Typha* dominance is often a symptom of multiple issues, including consolidation drainage and nutrient loading (Wiltermuth and Anteau 2016; Bansal et al. 2019). The ‘hemi-marsh’ of interspersed *Typha* and open water are also beneficial to an array of water birds that feed on *G. lacustris* (Murkin et al. 1997). Management of *Typha* dominance remains a high conservation priority and challenge in North America (Bansal et al. 2019; Larson et al. 2020a, b), but may benefit *H. azteca* that resides in shallow waters where *Typha* can dominate.

Conservation strategy: using amphipods as indicators and umbrella species to protect diversity

Gammarus lacustris and possibly *H. azteca* could be indicators and umbrella species in semi-permanent and permanent wetlands in the Prairie Pothole Region because (1) both species are easy to detect and enumerate (Fig. 1a–c), (2) *G. lacustris* are particularly sensitive to environmental conditions (Table 2; Anteau and Afton 2011), and (3) their conservation might extend to a suite of other species, like macrophyte biodiversity (Tables 2 and 3). However, amphipods would not be a suitable umbrella species for naturally ephemeral wetlands that frequently dry. An important trait in an umbrella species is that species conservation can improve management decisions. A separate structured decision making approach would be warranted to fully determine the ability for management to influence amphipod populations and protect macrophyte diversity (Bal et al. 2018). Here, we provide two examples of how amphipods could be used for systematic conservation planning and watershed restoration.

Amphipod distribution mapping could be a tool for conservation planning. The ease of detecting high densities of amphipods during early spring after ice-out (Fig. 1a–c) could allow for a feasible, landscape sampling campaign. Sampling amphipods is currently a

more efficient approach to indexing wetland quality than sampling macrophyte diversity or relative fish abundance. Amphipods are easy to identify and rapidly quantify in the field, thereby negating the need for costly sample preservation and laboratory quantification. In contrast, macrophyte surveys took our team about 2–5× longer in the field and required knowledge to identify many species. However, advances in remote sensing of submersed macrophyte diversity could reduce field sampling costs and increase access to larger landscapes in the future. Previous work showed that macroinvertebrates are indicator species because they are easy to measure compared to fish and can indicate fish absence (Schilling et al. 2009). A limitation of this study is we did not sample the wet meadow vegetation diversity, which can host many species; therefore, amphipods may or may not capture diversity alterations to the shallow marsh and wet meadow plant communities. Regardless, mapping the distribution of amphipods across the Prairie Pothole Region could help managers prioritize and conserve the remaining diverse, semi-permanent wetlands in this highly degraded landscape.

Amphipods could be an indicative metric for assessing conditions of the catchment and its terminal basins, which tend to have semi-permanent and permanent hydroperiods that amphipods favor (Anteau and Afton 2011, this study). Sustained amphipod populations are limited to basins with semi-permanent and permanent hydroperiods for overwinter survival, and such basins are typically found at the catchment terminal. Managing directly for amphipod success may be difficult, but restoration of suitable wetland types specific for amphipods could extend to improving catchment health. Hydrology of these basin types is strongly affected by land use and wetland drainage throughout the catchment (McCauley et al. 2015), and in turn, the hydrology affects connectivity and macroinvertebrate communities within these terminal basins (Wiltermuth 2014). Restoration of all wetland types and wetland complexes throughout a catchment can restore natural hydroperiods of the terminal basin (Anteau 2012), and thus support amphipods. Consolidation drainage also provides more permanent water that might be favorable to amphipods, but consolidated basins are also likely to support high abundances of fish and cattails that negatively affect amphipods (Wiltermuth 2014; Wiltermuth and Anteau 2016).

Conservation strategy: managing aquatic plant diversity to provide habitat for high amphipod abundance

Our results indicated that restoring and managing wetlands for macrophyte diversity can produce habitat suitable for amphipods to thrive in high densities. Wetland restorations are often successful at simultaneously restoring hydrology, vegetation cover, and biodiversity (Galatowitsch and van der Valk 1996a; Larson et al. 2020b). Prairie wetland restorations are particularly successful at restoring the diversity of the submergent and emergent plant communities (Galatowitsch and van der Valk 1996b), and our results showed amphipod densities were strongly correlated with diversity in these plant community types. In degraded or recently restored Prairie Pothole Region wetlands, management of related wetland factors such as nutrient loading, water depth, and fish abundance is sometimes feasible and often successful for promoting macrophyte abundance and diversity (Larson et al. 2020a). Wetlands with varying water levels over space and time promote water depth requirements for amphipods and predictable, diverse macrophyte communities (Larson et al. 2020b). Major changes to the Prairie Pothole Region landscape over the past century have stabilized the water levels of many wetlands and adversely affected water clarity, macrophytes, and macroinvertebrates (McCauley et al. 2015; Wiltermuth and Anteau 2016;

McLean et al. 2019). Water level management (through intentional drawdowns) represents a strategy to mimic ecosystem processes that would otherwise remove nuisance fish, increase water quality, increase macrophyte diversity and abundance, and attract waterfowl (Hanson and Butler 1994; Larson et al. 2020a).

Our results indicated low water depths of < 100 cm during a drawdown may preclude high densities of *G. lacustris*, although the survival, growth, reproduction, and recruitment of amphipods following drawdown would warrant more study. Given the sheer number and small size of these wetlands, a possible approach for promoting variable water levels and deeper refuges for amphipod overwintering may be restoration of wetland complexes that include several hydrologic regimes. The careful application of an ecosystem-functioning based approach may preserve or restore dynamic wetland hydrology (Euliss et al. 2008), remove nuisance fish, increase macrophyte diversity, and benefit amphipods in this heavily modified landscape.

A habitat conservation strategy assumes the amphipods are not dispersal limited; if the correct habitat conditions are available (such as semi-permanent water and diverse macrophyte communities), then amphipods would relocate and establish successfully. Amphipods are likely dispersal limited because they do not have a life stage capable of flight, unlike most wetland invertebrates. Instead, amphipod dispersal is limited to transport by other organisms or flowing water and therefore dispersal depends on wetland complexes that have adequate proximity and some hydrologic connectivity. Careful evaluation of purposeful reintroduction by translocating amphipods to restored wetlands may be considered (Larson et al. 2018), and our results suggest suitable wetland habitat conditions for amphipod translocation and stocking.

Conclusions

A probable cause of amphipod and scaup abundance declines may be attributed to a loss of wetland quantity and quality (Austin et al. 2000; Anteau and Afton 2006). Using amphipods as an umbrella species may be useful for managers in identifying and prioritizing conservation for biodiversity across the Prairie Pothole Region. Likewise, the preservation and restoration of amphipod abundance may be achieved through explicit conservation and management of macrophyte diversity. The seemingly disparate strategies of conserving a single taxon (e.g., amphipods) and biodiversity (e.g., macrophyte communities) can be compatible and complementary.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10531-022-02379-9>.

Acknowledgements We thank N. Hansel-Welch, J. Boucher, P. Borash, and D. Perleberg for suggestions on protocol development and teaching plant identification skills. We are especially grateful to C. Isaacson and J. Carleen for protocol development, and to A. Medina, K. Cross, and M. Bieganek for helping collect and process data. Thank you to our reviewers J. Delaney, M. Anderson, J. Sauer, M. Gaikowski, and two anonymous journal reviewers for improving this manuscript.

Author contributions All authors have made significant contributions to this work. DML designed research, analyzed data, and wrote the manuscript; DD designed research, collected data, analyzed data; MA designed research, analyzed data, wrote the manuscript; BK designed research, collected data, analyzed data; MF designed research, analyzed data; ES designed research, edited manuscript; BT designed research, wrote the manuscript.

Funding Funding was provided by the Minnesota Environment and Natural Resources Trust Fund, the Minnesota Department of Natural Resources, U.S. Geological Survey, and Augsburg University.

Data availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Code availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Material availability Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The peer-reviewed, analysis-ready dataset is available for public download using this citation: Larson, D.M., DeJong, D., Anteau, M.J., Fitzpatrick, M., Keith, B., Schilling, E.G., and Thoele, B., 2021, Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019: U.S. Geological Survey data release, <https://doi.org/10.5066/P9INBET3>.

Declarations

Conflict of interest The author declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Afton A, Hier R (1991) Diets of lesser scaup breeding in Manitoba. *J Field Ornithol* 62:325–334
- Afton A, Hier R, Paulus S (1991) Lesser scaup diets during migration and winter in the Mississippi flyway. *Can J Zool* 69:328–333
- Anteau MJ (2012) Do interactions of land use and climate affect productivity of waterbirds and prairie-pothole wetlands? *Wetlands* 32:1–9. <https://doi.org/10.1007/s13157-011-0206-3>
- Anteau M, Afton A (2006) Diet shifts of lesser scaup are consistent with the Spring Condition Hypothesis. *Can J Zool* 84:779–186
- Anteau MJ, Afton AD (2008a) Amphipod densities and indices of wetland quality across the upper-Midwest, USA. *Wetlands* 28:184–196. <https://doi.org/10.1672/07-53.1>
- Anteau MJ, Afton AD (2008b) Diets of Lesser Scaup during Spring Migration throughout the Upper-Midwest are Consistent with the Spring Condition Hypothesis. *Waterbirds* 31:97–106. [https://doi.org/10.1675/1524-4695\(2008\)31\[97:dolsds\]2.0.co;2](https://doi.org/10.1675/1524-4695(2008)31[97:dolsds]2.0.co;2)

- Anteau M, Afton A (2009) Wetland use and feeding by lesser scaup during spring migration across the upper Midwest, USA. *Wetl* 29:704–712
- Anteau MJ, Afton AD (2011) Lipid catabolism of invertebrate predator indicates widespread wetland ecosystem degradation. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0016029>
- Anteau MJ, Afton AD, Anteau ACE, Moser EB (2011) Fish and land use influence *Gammarus lacustris* and *Hyalella azteca* (Amphipoda) densities in large wetlands across the upper Midwest. *Hydrobiologia* 664:69–80. <https://doi.org/10.1007/s10750-010-0583-2>
- Austin JE, Afton AD, Anderson MG et al (2000) Declining scaup populations: issues, hypotheses, and research needs. *Wildl Soc Bull* 28:254–263
- Bal P, Tulloch AIT, Addison PFE et al (2018) Selecting indicator species for biodiversity management. *Front Ecol Environ* 16:589–598. <https://doi.org/10.1002/fee.1972>
- Bansal S, Lishawa SC, Newman S et al (2019) Typha (Cattail) invasion in North American Wetlands: biology, regional problems, impacts, ecosystem services, and management. *Wetlands* 39:645–684. <https://doi.org/10.1007/s13157-019-01174-7>
- Benoy GA, Nudds TD, Dunlop E (2002) Patterns of habitat and invertebrate diet overlap between tiger salamanders and ducks in prairie potholes. *Hydrobiologia* 481:47–59. <https://doi.org/10.1023/A:1021260920095>
- Brisson J, Rodriguez M, Martin CA, Proulx R (2020) Plant diversity effect on water quality in wetlands: a meta-analysis based on experimental systems. *Ecol Appl*. <https://doi.org/10.1002/eap.2074>
- Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. *Aquat Bot* 26:341–370. [https://doi.org/10.1016/0304-3770\(86\)90031-8](https://doi.org/10.1016/0304-3770(86)90031-8)
- Christensen JR, Crumpton WG (2010) Wetland invertebrate community responses to varying emergent litter in a prairie pothole emergent marsh. *Wetlands* 30:1031–1043. <https://doi.org/10.1007/s13157-010-0109-8>
- Engelhardt KAM, Ritchie ME (2002) The effect of aquatic plant species richness on wetland ecosystem processes. *Ecology* 83:2911–2924
- Euliss NH, Smith LM, Wilcox DA, Browne BA (2008) Linking ecosystem processes with wetland management goals: charting a course for a sustainable future. *Wetlands* 28:553–562
- Fast PLF, Clark RG, Brook RW, Hines JE (2004) Patterns of wetland use by brood-rearing lesser scaup in Northern Boreal forest of Canada. *Waterbirds* 27:177–182. [https://doi.org/10.1675/1524-4695\(2004\)027\[0177:POWUBB\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2004)027[0177:POWUBB]2.0.CO;2)
- Galatowitsch SM, van der Valk AG (1996a) Characteristics of recently restored wetlands in the prairie pothole region. *Wetlands* 16:75–83. <https://doi.org/10.1007/BF03160647>
- Galatowitsch SM, van der Valk AG (1996b) The vegetation of restored and Natural Prairie Wetlands. *Ecol Appl* 6:102–112. <https://doi.org/10.2307/2269557>
- Glazier DS (2014) Amphipoda. In: Reference Module in Earth Systems and Environmental Sciences. Elsevier
- Hanson JM (1990) Macroinvertebrate size-distributions of two contrasting freshwater macrophyte communities. *Freshw Biol* 24:481–491
- Hanson MA, Butler MG (1994) Responses to food web manipulation in a shallow waterfowl lake. *Hydrobiologia* 279–280:457–466. <https://doi.org/10.1007/BF00027877>
- Janke AK (2016) A Physiological Assessment of Wetland Habitats for Spring-migrating Ducks in the Agricultural Landscapes of the Southern Prairie Pothole Region. South Dakota State University
- Janke AK, Anteau MJ, Stafford JD (2019) Prairie wetlands confer consistent migrant refueling conditions across a gradient of agricultural land use intensities. *Biol Conserv* 229:99–112. <https://doi.org/10.1016/j.biocon.2018.11.021>
- Kahara SN, Chipps SR (2009) Predicting lesser scaup wetland use during spring migration in eastern South Dakota. *Gt Plains Res* 19:157–167
- Kalinkat G, Cabral JS, Darwall W et al (2017) Flagship umbrella species needed for the conservation of overlooked aquatic biodiversity. *Conserv Biol* 31:481–485. <https://doi.org/10.1111/cobi.12813>
- Larson DM, Anteau M, Isaacson C et al (2018) Restoring Wetland Invertebrates to Revive Wetland Habitats. Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g
- Larson DM, Cordts SD, Hansel-Welch N (2020a) Shallow lake management enhanced habitat and attracted waterbirds during fall migration. *Hydrobiologia* 846:3364–3379. <https://doi.org/10.1007/s10750-020-04342-7>
- Larson DM, Riens J, Myerchin S et al (2020b) Sediment excavation as a wetland restoration technique had early effects on the developing vegetation community. *Wetl Ecol Manag* 28:1–18. <https://doi.org/10.1007/s11273-019-09690-3>

- Larson DM, DeJong D, Anteau MJ, Fitzpatrick M, Keith B, Schilling EG, Thoele B (2021) Macrophyte and amphipod surveys in prairie wetlands of Minnesota in year 2019. U.S. Geological Survey data release. <https://doi.org/10.5066/P9INBET3>.
- Lindenmayer DB, Fischer J, Felton A et al (2007) The complementarity of single-species and ecosystem-oriented research in conservation research. *Oikos* 116:1220–1226. <https://doi.org/10.1111/j.2007.0030-1299.15683.x>
- Margules C, Pressey R (2000) A framework for systematic conservation planning. *Nature* 405:243–253
- McCauley LA, Anteau MJ, Van Der Burg MP, Wiltermuth MT (2015) Land use and wetland drainage affect water levels and dynamics of remaining wetlands. *Ecosphere* 6:1–22. <https://doi.org/10.1890/ES14-00494.1>
- McLean KI, Mushet DM, Sweetman JN et al (2019) Invertebrate communities of Prairie-Pothole wetlands in the age of the aquatic Homogenocene. *Hydrobiologia* 847:3773–3793. <https://doi.org/10.1007/s10750-019-04154-4>
- Merritt R, Cummins K, Resh V, Batzer D (2008) An introduction to the aquatic insects of North America, 4th edn. Kenall/Hunt Publishing Company, Dubuque
- Murkin H, Murkin E, Ball J (1997) Avian habitat selection and prairie wetland dynamics: a 10-year experiment. *Ecol Appl* 7:1144–1159
- Newman RM (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. *J North Am Benthol Soc* 10:89–114
- Oksanen J, Blanchet FG, Friendly M, et al (2019) Package “vegan” Community Ecology Package
- Olenick RJ, Gee JH (1981) Tiger salamanders (*Ambystoma tigrinum*) and stocked rainbow trout (*Salmo gairdneri*): potential competitors for food in Manitoa prairie pothole lakes. *Can Field-Naturalist* 95:129–132
- Ripley B, Venables B, Bates DM et al (2020) Package “MASS”
- Runge CA, Withey JC, Naugle DE et al (2019) Single species conservation as an umbrella for management of landscape threats. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0209619>
- Schilling EG, Loftin CS, Huryn AD (2009) Macroinvertebrates as indicators of fish absence in naturally fishless lakes. *Freshw Biol* 54:181–202. <https://doi.org/10.1111/j.1365-2427.2008.02096.x>
- Simberloff D (1998) Flagships, Umbrellas, and Keystones: Is single-species management passe in the landscape era? *Biol Conserv* 83:247–257
- Skawinski PM (2018) Aquatic Plants of the Upper Midwest: A photographic field guide to our underwater forests, 3rd edn.
- Strand K, Chipps S, Kahara S et al (2008) Patterns of prey use by lesser scaup *Aythya affinis* (Aves) and diet overlap with fishes during spring migration. *Hydrobiologia* 598:389–398
- Thomaz SM, Cunha ER (2010) The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages’ composition and biodiversity. *Acta Limnol Bras* 22:218–236. <https://doi.org/10.4322/actalb.02202011>
- Wasserstein RL, Schirm AL, Lazar NA (2019) Moving to a World Beyond “ $p < 0.05$.” *Am Stat* 73:1–19. <https://doi.org/10.1080/00031305.2019.1583913>
- Wiltermuth M (2014) Influences of climate variability and landscape modifications on water dynamics, community structure, and amphipod populations in large prairie wetlands. North Dakota State University
- Wiltermuth MT, Anteau MJ (2016) Is consolidation drainage an indirect mechanism for increased abundance of cattail in northern prairie wetlands? *Wetl Ecol Manag* 24:533–544. <https://doi.org/10.1007/s11273-016-9485-z>

Authors and Affiliations

Danelle M. Larson¹  · **Demey DeJong**² · **Michael J. Anteau**³  · **Megan J. Fitzpatrick**⁴ · **Breanna Keith**⁵ · **Emily G. Schilling**² · **Barry Thoele**⁶

Demey DeJong
demey.everett@gmail.com

Michael J. Anteau
manteau@usgs.gov

Megan J. Fitzpatrick
megan.fitzpatrick@state.mn.us

Breanna Keith
breanna.keith@my.smsu.edu

Emily G. Schilling
schillin@augsbury.edu

Barry Thoele
liveaqua@msn.com

¹ U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, USA

² Augsburg University, 2211 Riverside Avenue, Minneapolis, MN 55454, USA

³ U.S. Geological Survey, Northern Prairie Wildlife Research Center, 8711 37th Street SE, Jamestown, ND 58401, USA

⁴ Wetland Wildlife Population and Research Group, Minnesota DNR, 102 23rd St. NE, Bemidji, MN 56601, USA

⁵ Bemidji State University, 1500 Birchmont Dr. NE, Bemidji, MN 56601, USA

⁶ Lincoln Bait, LCC, 48301 County 21, Staples, MN, USA