

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

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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, 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 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

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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 Bieganeck, 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 Bieganeck 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)
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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 Bieganek. Bieganek 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 Bieganek, 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 amphibods. 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 scaup.

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, Eglund, 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, Eglund, 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 Eglund 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 Eglund 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 Bieganek (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. Bieganek 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. Bieganek 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 Bieganeck (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 Bieganeck (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 Wetroksy, 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 Bieganeck (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 Bieganeck 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 Bieganeck (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/ P9INB ET3](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
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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
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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:

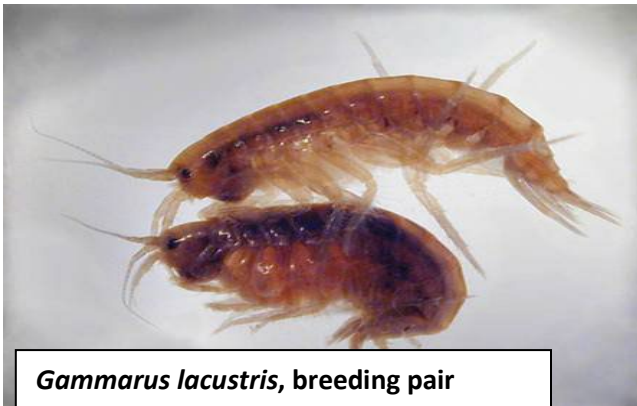
- **The project is for 4 years, will begin on July 1 2018, and end on June 30 2022.**
- **Periodic project status update reports will be submitted January 31 and June 30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2022.**

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 Dick Daniels

Photo by Glenn Bartolotti



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.



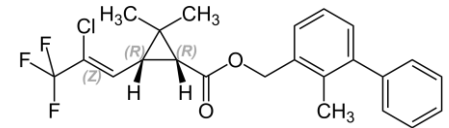
Aquatic plants (+)

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



