M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract

For the Period Ending June 30, 2019

SUBPROJECT TITLE: Aquatic Invasive Species Research Center SUBPROJECT MANAGER: Nicholas Phelps AFFILIATION: University of Minnesota MAILING ADDRESS: 135 Skok Hall, 2003 Upper Buford Circle CITY/STATE/ZIP: Saint Paul, MN 55108 PHONE: 612-624-7450 E-MAIL: phelp083@umn.edu WEBSITE: www.maisrc.umn.edu FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF) LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

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

Sound bite of Project Outcomes and Results

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

Overall Project Outcome and Results

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

MAISRC currently has a social media following of just under 2,300 and an e-newsletter list with just under 3,500 recipients. Social media posts about research findings, events, AIS Detector workshops, and general invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects. In addition, MAISRC has recorded consistent growth in the number of unique visitors and total website views since the website launch in February 2016. This increase shows that MAISRC is

growing in name recognition and being seen as an important resource for different stakeholders around the state. Over the course of the last six years, MAISRC has been in approximately 350 news stories in roughly 117 different outlets. The most common outlets have been the *Star Tribune*, Minnesota Public Radio, and KSTP-TV. Other notable outlets include *The New York Times, The Washington Post*, and Minnesota Bound. Nine videos were created highlighting MAISRC subproject research. Six AIS Research and Management Showcases were held with 700+ unique attendees. The AIS Detectors program was formally launched in March 2017 and we now have 299 certified Detectors around the state.

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



Date of Status Update Report: November 11, 2019 FINAL REPORT Date of Work Plan Approval: June 25, 2013 Project Completion Date: June 30, 2019

Project Title: Aquatic Invasive Species Research Center

Project Manager: Nicholas Phelps

Affiliation: University of Minnesota

Address: 135 Skok Hall, 2003 Upper Buford Circle

City: St Paul State: MN Zipcode: 55108

Telephone Number: (612) 624-7450

Email Address: phelp083@umn.edu

Web Address: http://www.maisrc.umn.edu/

Location:

Counties Impacted: Statewide

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

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

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

Appropriation Language:

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

I. PROJECT TITLE: Aquatic Invasive Species Research Center

II. PROJECT SUMMARY:

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

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

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

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

plan. This first work plan has been written following advice provided by the DNR and LCCMR staff using knowledge available as of June 2013.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of August 30, 2013:

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

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

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

Amendment Request as of August 30, 2013:

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

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

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

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

Subproject 10: We slightly increased the salary based on updated information on this type of position. We also delayed the start and reduced it to a 75% position because of inadequate funds. We are seeking non ENRTF matching funds to make this a full time position. The job title of this position has also changed because conversations with the Extension service (Dr. M. Schmitt) have revealed that we cannot ask for formal status within extension service for this position (they lack space and funding, and have their own hiring procedures) so

we have dropped the 'extension' designation. Nevertheless, there is a good possibility that this individual may work with extension educators (which we will pursue) and language to that effect is now in the subproject description as well as the fact this individual will assist with communications. Together with the corrections mentioned above, this results in the budget for this project changing from \$395,416 to \$390,196; reserve from \$319,711 to \$283,694.

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

Project Status as of February 10, 2014

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

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

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

These subprojects are:

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.

Project Manager: Ray Newman Phase 1 Budget: \$214,995 Estimated Start Date: June 2014 This work will be guided by Professor Ray Newman over the next two and a half years and will have a phase 1 budget of \$214,995.

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

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

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

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

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

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure.

Project Manager: Susan Galatowitsch Phase 1 Budget: \$913,893

Estimated Start Date: April/May 2014

An administrative and communications assistant has been added, and a technician has been converted to a lab manager for the Engineering and Fisheries Laboratory, which was recently designated for the Minnesota Aquatic Invasive Species Research Center's use as a central holding and research facility. Additional funds were also included in supplies, capital equipment, and repairs in anticipation of MAISRC's increased responsibility for upkeep of this facility.

SUBPROJECT 2: Delaying the spread of AIS: Monitoring the abundance and distribution of AIS using new molecular tools and metagenomics to delay their spread.

Project Manager: Michael Sadowsky

Phase 1 Budget: \$365,756.00

Estimated Start Date: December 2014

University of Minnesota Professor and Director of the Biotechnology Institute, Mike Sadowsky, will now alone guide this subproject rather than the Center hiring a new research assistant professor to do so. This will allow the MAISRC to collaborate with a renowned expert in the field of metagenomics and also to get this research started sooner than previously planned.

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

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

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

5

Phase 1 Budget: TBD

Estimated Start Date: July 2015

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

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

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

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

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

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

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

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

Project Status as of August 31, 2014

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

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

Project Status as of February 28, 2015

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure Project Manager: Sue Galatowitsch

Phase I Budget: \$913,893

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

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

The Center's first research and management showcase, during which all Center faculty, staff, and students shared updates, information, and findings affecting AIS Management in Minnesota, was held in November,

2014, and was attended by over 200 people. Staff and faculty continue to give talks and serve in advisory and other roles outside the University, contributing to sound planning and coordination around Minnesota's collective AIS efforts.

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

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

SUBPROJECT 2: Delaying the spread of AIS: Monitoring the abundance and distribution of AIS using new molecular tools and metagenomics to delay their spread.

Project Manager: Michael Sadowsky

Phase 1 Budget: \$365,756.00

Estimated Start Date: July 2015

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

SUBPROJECT 3: Reducing and controlling AIS: Developing effective tools to attract and locate aggregations of invasive carp.

Project Manager: Peter Sorensen

Phase 1 Budget: TBD

Estimated Start Date: July 2015

This sub project proposal has been received and is currently undergoing peer review. This sub-project was envisioned to build upon and continue research being conducted as part of the ENRTF 2012 work plan, once those prior phases were complete. Work on subproject 3 will therefore begin July 2015 or as soon as work is completed and ENRTF 2012 funds for activities 3, 4, 5 and 6 are spent down.

SUBPROJECT 4: Reducing and controlling AIS: Developing effective bio-control techniques to control common and/or Asian carp.

Project Manager: Przemek Bajer

Phase 1 Budget: TBD

Estimated Start Date: July 2015

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

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.

Project Manager: Ray Newman

Phase 1 Budget: \$214,995

Start Date: July 2014

This subproject was approved on July 31, 2014 and is currently underway. Finding a postdoctoral associate has been harder than anticipated. A candidate has just accepted the position and started work on March 9, 2015.

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

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

Project Manager: Paul Venturelli

Phase 1 Budget: TBD

Estimated Start Date: July 2015

The project proposal has been received and is currently undergoing peer review, with an aim to start research July 2015. The proposal aims to address key knowledge gaps by providing, through modeling, an initial estimate of the threat caused by the parasite *Heterosporis* to populations of common game species, such as yellow perch, in Minnesota lake systems.

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

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.

Project Manager: TBD Phase 1 Budget: TBD

Estimated Start Date: Fall 2015

As previously reported, Dr. Galatowitsch was able to leverage this position from a term-limited position to a more competitive and permanent tenure- track position within the Department of Fisheries, Wildlife, and Conservation Biology. Per University procedures, a search committee was created, the position was posted, and candidates were interviewed. An offer was made recently; we hope the position will be filled this spring and the new hire will begin in August 2015.

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

SUBPROJECT 10: Implementing Findings: An educator-outreach position.

Project Manager: Susan Galatowitsch

Phase 1 Budget: TBD

Start Date: February 2015

Danielle Quist started work February 26, 2015 as the new Extension Educator for the Center. Ms. Quist is meeting with key partners and stakeholders while she works with Extension and MAISRC to develop a detailed program plan. This program plan will be focused on outreach and programming related to AIS control, which is consistent with the programming gaps identified by DNR, Minnesota Sea Grant, MAISRC, and Extension in preliminary outreach coordination meetings. Dr. Galatowitsch will continue to serve as project manager of this Subproject, with Ms. Quist as the key implementing staff.

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

Phase 1 Budget: \$110,185 Start Date: April 2014

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

Project Status as of September 24, 2015

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

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

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

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

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

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

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

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

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

Start Date: July 2014

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

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

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.

Project Manager: Nick Phelps

Phase 1 Budget: \$335,225

Start Date: May 2014

Significant progress has been made to perform diagnostic tests on the previously collected common carp with hundreds of carp testing negative for a variety of potential pathogens and with one still unknown virus identified. Two novel viruses have been identified from common carp and grass carp mortality events with one of them being the first report associated with fish mortality in the United States. Efforts are underway with new partners to collect silver carp this summer/fall. An update on this project was invited to be presented at the Great Lakes Fisheries Commission – Great Lakes Fish Health Committee meeting held in July 2015.

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

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

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

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

Amendment request as of September 24, 2015:

see amendment request below to add and fund this Phase 2 project.

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

and \$220,000 of the reserve budget line in Subproject 10 have been allocated within that subproject. The subprojects are as follows:

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species. Project Manager: Michael Sadowsky.

Phase I budget: \$303,217

LCCMR approval and start date: June 20, 2015 (2 years)

This sub- project was specified in original 2013 work plan, however it has been modified from *detection* of various AIS to *control* of water milfoil, zebra mussels. The budget has also shifted downward based on need. The subproject title, description, budget and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed

Project Manager: Peter Sorensen

Phase 2 Budget: \$500,000

LCCMR approval and start date: July 9, 2015 (2.5 years)

This sub-project was specified in the original 2013 workplan and has been revised to reflect findings from (and in some cases is intended to extend) work funded through 2012 ENRTF Activities 3,4,5,6, and 8. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 4: Common carp management using biocontrol and toxins

Project Manager: Przemek Bajer

Phase 1 budget: \$413,247

LCCMR approval and start date: July 7, 2015 (2 years)

This sub-project was specified in the original 2013 work plan, however it has been modified to include carpspecific toxins, a priority identified in MAISRC's 2015 research needs assessment. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control Project Manager: Paul Venturelli

Phase 1 budget: \$127,650

LCCMR approval and start date: June 15, 2015 (2 years)

The original 2013 work plan scoped this sub-project to model common carp populations; the project has been redirected to investigate the risk of an invasive pathogen identified as a priority in MAISRC's 2015 research needs assessment. The subproject title, description, and outcomes have been revised below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: to reflect these changes.

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.

Project Manager: Dan Larkin

Phase 1 Budget: \$130,000 (initial)

LCCMR approval and start date: August 31, 2015 (~4 years)

An initial workplan has been created by MAISRC and Dr. Larkin to cover program development, peer review, and workplan development and review. An updated workplan is expected to be submitted to LCCMR by March 15, 2016 at which time the subproject title, description, and outcomes below in IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES: will be revised to reflect changes.

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

LCCMR approval and start date: October 1, 2015 (~4 years)

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

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis

Project Manager: David Andow

Phase 2 Budget: \$123,128

LCCMR approval and start date: September 23, 2015

This project will conduct a risk assessment with a variety of experts and stakeholders by evaluating which adverse effects identified in Phase I are most salient and by determining the likelihood of impact and consequence in various watersheds. Through risk communication, the results and implications of these findings will be shared with a broader set of stakeholders, researchers, managers, and decision makers from relevant state and federal agencies. Areas of disagreement, remaining uncertainties, and additional research needs will be identified. By fostering conversation among researchers, managers, stakeholders, and decision makers, this project will promote needed dialogue and communication to support decision making in the face of complexity and uncertainty.

Amendment approved: October 14, 2015

Amendment request: October 29, 2015:

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

Amendment approved: October 30, 2015

Project Status as of February 29, 2016

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

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

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

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

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

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

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

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species. Project Manager: Michael Sadowsky.

Phase I budget: \$303,217

LCCMR approval and start date: June 20, 2015 (2 years)

A postdoctoral associate was hired to start August 31 and an undergrad has been assisting him. Sampling for Eurasian watermilfoil was conducted at three different sites in Cedar Lake and DNA extracts were submitted for sequencing. A milfoil decay experiment was also performed. Zebra and quagga mussels were collected from six lakes, were dissected, and DNA samples submitted for sequencing. Analysis for all will be conducted this spring.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed

Project Manager: Peter Sorensen.

Phase 2 Budget: \$500,000

LCCMR approval and start date: July 9, 2015 (2.5 years)

Experiments were conducted in late summer of 2015 to test food and pheromones as attractants to drive common carp aggregation. While data is still being analyzed, it is clear that food was able to drive aggregations, especially at night. Novel techniques for both eDNA and pheromone levels were able to measure the aggregations with more sensitivity. Plans for this coming summer will be formulated once we have analyzed all the data.

SUBPROJECT 4: Common carp management using biocontrol and toxins

Project Manager: Przemek Bajer.

Phase 1 budget: \$413,247

LCCMR approval and start date: July 7, 2015 (2 years)

Outcome goals have been achieved—experimental lakes have been selected for whole lake biocontrol experiments; monitoring is continuing over the winter; and next steps for stocking will be identified in the Spring. Winter aeration data were compiled and paired with DNR fish assessments, however the resulting

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

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

The Newman lab has received and collated curlyleaf pondweed datasets for 57 lakes from state and county agencies, watershed districts, and consultants. Several discussions regarding analytical approaches have taken place. Eight of 14 lakes surveyed for weevils/herbivores were resurveyed in August and September. Lower than average weevil densities were found in 5 of the 8 resurveyed lakes; only three showed an increase in weevil density. Enclosures were surveyed for weevils and plants and diets were collected from sunfish at six lakes.

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

Project Manager: Paul Venturelli

Phase 1 budget: \$127,650

LCCMR approval and start date: June 15, 2015 (2 years)

Model development is well under way. We have collected a quarter to a third of necessary parameter values, and beginning to code the subroutines that simulate disease and energy dynamics. In collaboration with the MN DNR, we collected 1,221 yellow perch and other fishes from three lakes in September. Preliminary results from the lab suggest that ~8% of fish are infected and that most of these fish were yellow perch. Winter gill netting is now under way so that we can determine if the frequency and intensity of heterosporosis infection is seasonal or temperature-dependent. To determine if infected fish are more or less susceptible to angling, we have also distributed to log books to resorts on all three lakes. Finally, we have obtained ~1100 yellow perch for laboratory experiments, which will begin once the new research facility is operational.

SUB-PROJECT 7. Developing eradication tools: Developing eradication tools for invasive carp species Phase 1: Understanding the virome of carp species in the Upper Midwest.

Project Manager: Nick Phelps

Phase 1 Budget: \$335,225

Start Date: May 2014

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

SUBPROJECT 8: Implementing findings: An applied ecologist position and program.

Project Manager: Dan Larkin

Phase 1 Budget: \$130,000 (initial)

LCCMR approval and start date: August 31, 2015 (~4 years)

This project is currently undergoing peer review. Additionally, an ecological niche model has been developed to determine the threat of starry stonewort spread in Minnesota. The model indicated that this species is persisting in novel habitats – meaning that it is occurring in areas here that are climatically distinct from its native range, and that conditions in portions of the upper Midwest and other regions in the U.S. are ideal for its growth and

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

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

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response Project Manager: Sue Galatowitch Phase 1 Budget: \$-566,550 LCCMR approval and start date: October 1, 2015 (~4 years)

A template for the online portion of the AIS Detectors course has been designed and is organized in six modules with specific learning outcomes. The course will initially focus on ten AIS species, which were chosen in consultation with the MAISRC technical committee. An educator for the AIS Trackers program has been hired and is expected to begin early April. We decided not to pursue additional funding for this program from the Initiative Foundation, so have had to rebudget this Subproject to make it whole. An amendment to this effect follows.

SUBPROJECT 11-1: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Project Manager: David Andow

Revised Phase 1 Budget: \$93,343

Start Date: April 2014

This phase of the project, which identified potential adverse effects from Asian carp to inform a subsequent risk assessment and characterized the tensions and conflicts that are hampering Asian carp management, completed in November. Two reports were released. An amendment was approved in November by LCCMR to move the remaining balance to Phase 2, which continues the work with a full risk assessment of Asian carp impacts and a risk communication session.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis

Project Manager: David Andow

Phase 2 Budget: \$139,970

LCCMR approval and start date: September 23, 2015

The risk assessment meeting, which will convene Asian carp experts from around the country, has been scheduled for March 8 and 9, 2016. An online survey to help guide the assessment meeting has been designed. Remaining funds from 11-1 (Phase 1) were transferred here, resulting in the budget to change from 123,128 to 139,970.

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

Amendment request as of February 29, 2016

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response Project Manager: Sue Galatowitch Phase 1 Budget: \$566,550 LCCMR approval and start date: October 1, 2015 (~4 years) MAISRC decided to withdraw its application to the Initiative Foundation for approximately 75% of the costs of Activities 1-2 due to difficulty meeting the prescribed match requirement for that program, which would have resulted in a need for us to secure additional private funds within a timeframe that was unfeasible. Additionally, it has been determined that additional staff assistance is needed for Activities 3-5. Therefore, we request that \$147,075 funds are transferred from the reserve to fully fund this Subproject 10. This would result in the overall reserve changing from \$4,416,986 to \$4,269,911. Subproject 10 budget would change from \$419,475 to \$566,550.

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

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

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

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

Amendment Approved March 3, 2016

Amendment request as of May 5, 2016

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

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

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

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

SUBPROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology Project Manager: Donn Branstrator (UMD) Phase 1 Budget: \$207,766 Estimated Start Date: August 2016 (~2.5 years) This project has completed peer review and is in process of revision and workplan development for approval by MAISRC and LCCMR. The work will be guided by Professor Donn Branstrator from University of Minnesota Duluth over the next two and a half years. We seek an amendment to move \$207,766 from the Subproject 1 Reserve budget into Subproject 12.

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

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

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

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

In summary, \$207,7<u>6</u>6 will be moved from the Subproject #1 Reserve to Subproject #12 reserve. \$215,000 will be moved from Suproject #1 Reserve to Subproject #13 Reserve. Therefore Subproject #1 reserve will decrease \$422,7<u>6</u>6 total, from \$4,269,911 to \$3,847,1<u>4</u>5.

Amendment Approved by LCCMR 5-11-2016

Project Status as of August 31, 2016

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

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

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

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

Planning was conducted and arrangements were made for the 2016 Showcase, which will be held September 12 on the St. Paul campus. Dissemination of research progress continues through talks, papers, newsletter, website

and other social media formats. An amendment is being sought to take Phase 2 of Subproject 1 out of reserve and into Subproject 1 budget to sustain this subproject to the end of the project period (June 30, 2019).

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species. Project Manager: Michael Sadowsky.

Phase I budget: \$303,217

LCCMR approval and start date: June 20, 2015 (2 years)

Sequence analysis has been completed for samples collected last year, and a broader sampling regime for both Eurasian watermilfoil (EWM) and zebra mussels (ZM) has been implemented this year. All samples were processed for nutrient and microbiological features and DNA extracts sent to UMGC for bacterial and fungal sequencing. A new tech was also hired.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed

Project Manager: Peter Sorensen.

Phase 2 Budget: \$500,000

LCCMR approval and start date: July 9, 2015 (2.5 years)

Water samples collected for eDNA and pheromone evaluation were analyzed and a baiting scheme perfected. Experiments from last summer showed that a third of the population of mature common carp could be attracted and then measured with eDNA and pheromones with a level of sensitivity, precision and accuracy previously unseen. Pheromone-releasing Judas carp were also attractive. A third study successfully measured common carp mating pheromones in waters near mating carp. Finally, a pilot study using food to attract Bigheaded carp was completed in Illinois with the University of South Illinois as collaborators. Whether this behavior enhanced our ability to measure them using eDNA or pheromones (as shown with carp) is presently being evaluated.

SUBPROJECT 4: Common carp management using biocontrol and toxins

Project Manager: Przemek Bajer.

Phase 1 budget: \$413,247

LCCMR approval and start date: July 7, 2015 (2 years)

Research continues to advance and outcome goals have been achieved. Experiments are underway for activity 1a: carp and bluegills have been stocked in ponds, egg and larval densities assessed, and water quality assessments taken to document productivity and zooplankton abundance. Activity 1b has been adapted to allow analysis of a higher quality dataset provided by DNR to determine which lakes are capable of supporting bluegill populations to control common carp. Corn-based bait containing antimycin has been formulated for Activity 2, and has been shown to be lethal to common carp through preliminary gavage studies, however leaching is occurring and rates higher than expected. The bait is currently being re-formulated.

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.

Project Manager: Ray Newman

Phase 1 Budget: \$214,995

Start Date: July 2014

Compilation of the curlyleaf pondweed data sets and ancillary data was completed and analyses conducted and a talk was given on the analysis and results at the Aquatic Plant Management Society meeting in Grand Rapids, MI in July. Enclosures (at Cedar and Peltier Lakes) have been installed, stocked with fish and pre- and mid-experiment samples have been collected. Fish diets were obtained and are now being collected from other lakes as well. Herbivore surveys have been conducted in 14 lakes and additional lakes are being selected for surveys in August. The milfoil weevil portion of the project will conclude December, while a possible extension will be requested in January to conduct additional curlyleaf pondweed analysis.

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

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

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

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

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

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

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

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

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response Project Manager: Sue Galatowitsch Phase 1 Budget: \$-566,550 LCCMR approval and start date: October 1, 2015 (~4 years)

The two part curriculum for the AIS Detectors program has been developed and will be pilot-tested in the September and early October. Part 1 is an online course and Part 2 is an all-day classroom session that will be

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

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

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods: Risk analysis

Project Manager: David Andow Phase 2 Budget: \$139,970

Start Date: September, 2015 (~1.2 years)

The two day risk assessment workshop was held with twenty-three experts on bigheaded carps and Minnesota's waterways. The risk assessment focused on the impacts to game fish, non-game fish, species diversity/ecosystem resilience, and recreation (from the silver carp jumping hazard). Four watersheds were chosen to be studied and participants characterized the likelihood that bigheaded carps would establish in each watershed, the resulting abundance of bigheaded carp in each watershed, and the severity of each potential adverse effect in each watershed. The risk assessment report is being written by project researchers and a subset of the workshop participants.

SUB-PROJECT 12: Characterizing long-term spiny water flea ecosystem impacts using paleolimnology Project Manager: Donn Branstrator Budget: \$207,766 This project was approved by LCCMR June 20, 2016 with a start date of August 1, 2016 (~3 years). A number

was transposed in the IV budget below and on the overall budget in the last update and has now been corrected.

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

Amendment Request as of August 31, 2016 and received October 4, 2016

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

The amendment would result in the following:

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

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

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

Amendment approved 10-10-16

Project Status as of February 28, 2017

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

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

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

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

The AIS Detectors program piloted to a small cohort of DNR staff and citizens in Fall of 2016 and substantial effort by MAISRC communications staff has continued in order to prepare for seven statewide sessions to be

held in 2017. Part of this effort includes creating a professional, scientifically vetted AIS (and look-alikes) identification book that will set a new standard in the state for this kind of publication.

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

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

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

SUBPROJECT 2: Metagenomic approaches to develop biological control strategies for aquatic invasive species. Project Manager: Michael Sadowsky.

Phase I budget: \$303,217

LCCMR approval and start date: June 20, 2015 (2 years)

The project has made significant progress since the last project update and is on schedule for completion in July. Field sampling in 2016 including collecting EWM, native macrophytes, zebra mussels, sediment, and water sampled from 25 lakes. Samples were processed, DNA extracted, and high-throughput DNA sequencing of bacteria and fungi was performed. Sequencing results showed a distinct clustering of microbes by each sample type with the greatest number of operational taxonomic units (OTUs) observed in sediment samples, and the lowest in EWM and ZM samples. Several OTUs were identified that were present in higher relative abundance in EWM and ZMs. Additionally, it was determined that EWM harbored elevated levels of fecal indicator bacteria, such as *E.coli* and *Enterococcus*.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed

Project Manager: Peter Sorensen.

Phase 2 Budget: \$500,000

LCCMR approval and start date: July 9, 2015 (2.5 years)

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

SUBPROJECT 4: Common carp management using biocontrol and toxins

Project Manager: Przemek Bajer.

Phase 1 budget: \$413,247

LCCMR approval and start date: July 7, 2015 (2 years)

The 2016 field season ended and data are currently being analyzed. Outcome goals have been achieved, or exceeded. Activity 1a has concluded and mark-recapture estimates were made for young-of-year (YOY) carp in

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

SUB-PROJECT 5: Reducing and controlling AIS: Developing and evaluating new techniques to selectively control invasive plants. Phase I: Manipulating sunfish to enhance milfoil weevils and factors influencing selective herbicide control of curlyleaf pondweed.

Project Manager: Ray Newman

Start Date: July 2014 (2.5 years)

Field work was completed in fall and all data were entered and analyzed. Eighteen lakes were assessed for milfoil weevil densities, which ranged from none found to 0.27/stem, lower than for most lakes in 2015. Densities were lower in 2016 compared to 2015, and 2015 generally had lower densities than in previous years. Sunfish stomach contents were analyzed. Benthic and macrophyte associated invertebrates were common in the diets but only one milfoil weevil was found. Enclosure experiments were completed in August. Despite methodological improvements and an earlier start in June we were unable to get definitive results from the enclosure experiments. Curlyleaf analysis was continued and the data sets were organized and systematized to allow an analysis of the effects of curlyleaf and curlyleaf control on the associated native plant communities. The final report and abstract for this project will be submitted by 2/28/17. The remaining funds are being moved to the overall project reserve. Please see amendment request below.

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

Project Manager: Paul Venturelli

Phase 1 budget: \$127,650

LCCMR approval and start date: June 15, 2015 (2 years)

We are on pace with model development, but not lab experiments. We have a working aggregate model to predict perch dynamics in a system with varying degrees of disease prevalence and virulence (Activity 1). We are now parameterizing this model so that it can generate predictions and perform a sensitivity analysis (Activity 3). We have finished microscope analysis on field samples from the fall and winter, resulting in a 6% and 1% prevalence of heterosporosis in Leech Lake, respectively. We are still processing samples from the spring and summer. We are behind on lab experiment due to delays in facility construction and difficulties in finding and culturing *Heterosporis*. We were able to run a small experiment and only one fish tested positive for the disease. Given our remaining timeline and the challenges associated with infecting perch in the lab, we are cancelling experiments to determine heterosporosis transmission rates via direct contact among fathead minnows. We have initiated work on Activity 3, and have started planning and structuring the model to best implement the sensitivity analysis.

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

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

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

Phase 1 Budget: \$822,000

LCCMR approval and start date: August 31, 2015 (~4 years)

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

SUBPROJECT 9: Implementing Findings: Applying new methods to control zebra mussels.

Project Manager: Michael McCartney

This project has undergone continuation review and its workplan has been approved by MAISRC and LCCMR. Please see amendment request below.

SUBPROJECT 10: Citizen Science and Professional Training Programs to Support AIS Response

Project Manager: Dan Larkin

Phase 1 Budget: \$566,550

Start Date: November 2015 (3.5 years)

Progress was made in several key areas of Detectors and Trackers. The full web-based Detectors course was pilot-tested this past fall and the participants provided feedback that is being used to revise the curriculum. Groundwork has been laid for full implementation of the AIS Detectors program in spring of 2017. Advanced training opportunities are being developed. Development of the Trackers program is in progress, with a detailed plan for program roll-out. In addition, progress has been made in refining the scope of the Trackers database and we have met with a vendor and agreed on a timeline for development of the data management system.

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

Final report submitted September 2015.

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

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

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

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

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

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

Amendment request as of February 28, 2017:

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

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

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

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

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

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

SUBPROJECT 9: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol. Project Manager: Michael McCartney

Phase 2 Budget: \$427,950 Start date: February 2017 (2.33 years)

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

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

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

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

Project Status as of August 31, 2017

SUBPROJECT 1: Coordinating, synergizing and promoting expertise: Establishing an administrative structure Project Manager: Nicholas Phelps Phase I Budget: \$1,805,859 Start Date: February 2015 (5.3 years) The Center is continuing to make significant advances in terms of implementing its strategic plan and ensuring high quality and high priority research and outreach is being conducted to solve AIS problems in Minnesota.

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

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

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

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

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

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

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

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

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

We continue efforts to get the MAISRC Containment Lab ("MCL") online and have worked to overcome obstacles related to proper functioning of the water decontamination system. We are also continuing to work

with the college and the Agricultural Experiment Station to create user policies, reservation and pricing systems, and maintenance procedures for its operation.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Final report submitted September 2015.

SUBPROJECT 11-2: Reducing and controlling AIS Phase 2: Risk assessment Project Manager: David Andow Phase 2 Budget: \$139,970 Start Date: September 2015 (~1.75 years) This project is complete. A final report is being finalized. We are requesting an amendment to transfer the remaining balance to the MAISRC reserve. Please see below.

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

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

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

Amendment request as of August 31, 2017

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

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

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

SUBPROJECT 2- Phase 2: Development of potential microbiological control agents for AIS Project Manager: Mike Sadowsky Phase 2 Budget: \$303,217 This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR 5/22/17. We wish to move \$303,217 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 3: Attracting carp so their presence can be accurately assessed; Determining if and how a soundbubble system can be combined with light in the laboratory to deter carp while examining potential impacts to native fishes.

Project Manager: Sorensen

Budget: \$682,969

Dr. Sorensen created a new activity (approved by LCCMR 4/18/17), funded through rebudgeting existing activities plus transferring \$182,968 from the overall reserve. We wish to move \$182,968 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below. The title on the budget spreadsheet has been updated.

SUBPROJECT 4- Phase 1: Developing realistic management solutions for common carp: testing the potential for biocontrol and assessing the possibility for developing carp-specific toxins

Project Manager: Przemek Bajer

Phase 1 budget: \$384,231

Phase 1 is now completed and we request that the remaining balance of \$29,016 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by August 31.

SUBPROJECT 4- Phase 2: Developing realistic management solutions for common carp: testing the potential for biocontrol and assessing the possibility for developing carp-specific toxins

Project Manager: Przemek Bajer

Phase 2 budget: \$406,000

This new subproject has undergone continuation review and its workplan and budget approved by MAISRC and LCCMR on 6/27/17. We wish to move \$406,000 from the overall reserve to fund this project. The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

SUBPROJECT 6: Determining Heterosporis Threats to Inform Prevention, Management, and Control Project Manager: Paul Venturelli

Phase 1 budget: \$111,889

Phase 1 has now completed and we request that the remaining balance of \$15,761 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by September 30.

SUBPROJECT 11-2: Reducing and controlling AIS: Risk analysis to identify AIS control priorities and methods Project Manager: David Andow

Phase 2 Budget: \$126,677

Phase 2 has now completed and we request that the remaining balance of \$13,294 be moved back into the MAISRC reserve to be reallocated to other priorities. The final report and abstract will be submitted to LCCMR by September 30.

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

SUBPROJECT 14: Cost- effective monitoring of lakes newly infested with zebra mussels Project Manager: John Fieberg Budget: \$266,500 LCCMR approval date: 6/27/17 The project description, outcomes, and budget have been added to section IV. PROJECT ACTIVITIES (SUB-PROJECTS), AND OUTCOMES, below.

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

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

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

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

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

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

Project Status as of February 28, 2018

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

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

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

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

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

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

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

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

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

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

We have also spent considerable effort with communicating the outcomes of our research. This is discussed in more detail in the dissemination section. We also presented at the International Conference on Aquatic Invasive Species on MAISRC's process for research prioritization, which is quickly becoming a model for other research organizations.
Also as part of our efforts to support our researchers, we have continued to provide LCCMR reporting and budgeting functions to ensure accurate and timely reflection of our efforts.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

This project has completed. Final report submitted September 2015.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Project status updates as of June 30, 2019:

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

3	Attracting carp so their presence can be accurately assessed	Peter Sorensen	Budget: \$682,969 Final:	July 9, 2015	June 30, 2019	Complete
4.1	Phase I: Common carp management using biocontrol and toxins	Przemyslaw Bajer	S003,713 Budget: \$413,247 Final: \$384,231	July 7, 2015	July 31, 2017	Complete
4.2	Phase II: Common carp management using biocontrol and toxins	Przemyslaw Bajer	Budget: \$406,000 Final: \$348,913	June 27, 2017	June 30, 2019	Complete
5	Developing and evaluating new techniques to selectively control invasive plants	Raymond Newman	Budget: \$214,996 Final: \$194,415	July 31, 2014	December 31, 2016	Complete
6	Determining Heterosporosis Threats to Inform Prevention, Management, and Control	Paul Venturelli	Budget: \$127,650 Final: \$111,889	July 31, 2016	August 31, 2017	Complete
7.1	Developing eradication tools for invasive carp species – Phase I: Understanding the virome of carp species in the Upper Midwest	Nicholas Phelps	Budget: \$335,224 Final: \$206,754	April 24, 2014	June 30, 2016	Complete
7.2	Developing eradication tools for invasive species – Phase II: Virus Discovery and evaluation for use as potential biocontrol agents	Nicholas Phelps	Budget: \$445,210 Final: \$422,667	February 1, 2017	June 30, 2019	Complete
8	Risk assessment, control, and restoration research on aquatic invasive plant species	Daniel Larkin	Budget: \$822,000 Final: \$820,251	August 13, 2015	June 30, 2019	Complete
9	Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol	Michael McCartney	Budget: \$427,950 Final: \$380,318	February 22, 2017	December 31, 2018	Complete

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

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

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

*Pending Approval; In Progress; Complete; Not Completed

Amendment Request August 31, 2018:

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

Amendment Request 1

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

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

Amendment Request 2

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

Amendment Request 3

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

Amendment Request 4

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

Amendment Request 5

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

Amendment Request 6

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

Amendment Request 7

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

Amendment Request 8

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

Amendment Request 9

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

Amendment Request 10

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

Amendment Request 11

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

Amendment Approved: [09/19/2018]

Amendment Request February 28, 2019:

Amendment 1

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

Amendment 2

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

Amendment 3

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

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

As we begin to wrap up work on M.L. 2013, we have identified sufficient budget savings among our projects to purchase a new electrofishing boat on M.L. 2013, in partnership with the FWCB Department. Purchasing a new boat will allow MAISRC to support multiple current projects through the coming field season and completion of

their research in June 2019. This will also provide much needed capacity as we launch new projects on M.L. 2017 and M.L. 2019 this summer, with several expected to need an electrofishing boat.

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

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

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

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

Amendment 4

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

Amendment 5

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

Amendment 6

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

Amendments Approved: 03/18/2019

Amendment Request May 28, 2019

As we wind down ML 2013 funding for the establishment of MAISRC, we request the following budget amendments:

Amendment 1

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

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

Amendment 2

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

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

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

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

Amendments Approved by LCCMR: 06/12/2019

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

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

Project Manager: Nicholas Phelps

Description: The promise of the center lies in its ability to promote synergies, share facilities, and disseminate information. These activities require scientific and administrative leadership that can organize meetings of center participants in the form of an advisory group as well as a technical group and faculty, while running peer-review, sponsoring symposia, raising funds, and both creating and disseminating reports to the legislature. Sub-Project 1 consolidates the framework for this leadership. As it becomes fully operational (an outcome of this work plan), the Center will be called 'The Minnesota Aquatic Invasive Species Research Center '(MAISRC) and it will be based in the College of Food, Agricultural and Natural Resource Sciences (CFANS) at the University of Minnesota. The MAISRC's Director is Dr. Susan Galatowitsch and she will devote approximately 30% of her time to administering the Center and providing overall leadership and direction. Dr. Galatowitsch will be assisted by

a fulltime Associate Director (1.0 FTE for 5 years) who will be fully funded by this activity after startup funding ends in 2014. The Associate Director will continue to work with the Director to run an advisory board (Center Advisory Board [CAB], that includes the DNR (see below), establish and coordinate a technical board (MTC), organize peer-reviews, organize working groups, compile and produce reports and budgets, track spending, produce media releases, and organize peer reviews. Working with the Director and Extension specialist, the Associate Director will also organize regular meetings of Center faculty and staff and a symposium on campus each year, and keep a website up to date. An annual report for the Center will also be produced and biannual reports to the LCCMR.

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

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

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

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

Initially there will be 11 Center sub-projects, each of which is described in this work plan. All will be peerreviewed within the first year of initiation when new staff will be asked to develop roughly two year sub-project proposals with budgets based on the outline provided herein. Staff will administer their own budgets and subproject work plans which will be shared (for approval) with the LCCMR staff after being approved by the Center Administrative Review Board. Subsequent sub-projects and sub-budgets will then be reviewed at least at three-year intervals depending on what the Director deems appropriate. It is expected that sub-projects will generally follow the outline of outcomes proposed in this work plan; however, changes may be proposed in activity scope, direction (specified outcome), FTE allocation, and budget. New sub-projects or activities may be created or old ones terminated by the Director according to process laid out in the Center's MOU with the department and college. Changes will be managed and implemented as described above.

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

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

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

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

Sub-Project Status as of February 10, 2014

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

Sub-Project Status as of August 31, 2014

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

Sub-Project Status as of February 28, 2015

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

The workplan is continuing to be implemented as originally laid out by the founder of the Center, with attention to expediting the initiation of sub-projects that had been delayed in the first year and a half of the Center's

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

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

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

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

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

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

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

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

Sub-Project Status as of September 24, 2015

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

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

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

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

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

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

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

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

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

Sub-Project Status as of October 29, 2015

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

Sub-Project Status as of February 29, 2016

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

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

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

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

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

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

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

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

Sub-Project Status as of August 31, 2016

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

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

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

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

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

The second biennial Research Needs Assessment process has begun. The interagency MAISRC Technical Committee (MTC) serves as the core of the Research Needs Assessment Team. Re-appointments and new twoyear appointments were made to this eleven-member committee, which then held its first meeting to review priority species and make modifications based on the present science and status of threats. The final draft list of species will be routed to the Center Faculty Group and Center Advisory Board for input before being finalized. Additional members were also selected to join with the MTC and serve on the 2016 Research Needs Assessment Team. This team represents researchers, AIS managers, and stakeholders from around the state. An added emphasis this year is on cross species issues, which will be informed by social scientists, a DNR conservation officer, and others. A request for input on research priorities from the general public as well as from the DNR's AIS Advisory Committee will be made. The results will be fed into the Research Needs Assessment process culminating in a list of research priorities that will be used for an RFP by the end of the calendar year. The MAISRC director and technicians have continued to be closely involved with the project designers and engineers in the commissioning process of the newly renovated research lab facilities. Our staff were able to identify several malfunctioning systems and equipment which has since prevented us from being able to fully occupy the space. Our continued time, attention, and expertise has been needed. We are now actively working with CFANS, UMN Capital Planning, and the design and construction firms to develop remedies. In the meantime, accommodations have been made to get as much research as safely possible running in the lab. Other research has been relocated or is on hold. The new storage facility is complete and we have facilitated the occupancy of this space for our faculty and their gear.

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

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

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

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

Sub-Project Status as of February 28, 2017

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

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

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

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

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

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

For example:

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

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

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

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

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

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

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

Sub-Project Status as of August 31, 2017

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sub-Project Status as of February 28, 2018

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

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

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

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

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

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

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

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

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

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

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

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

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

Sub-Project Status as of August 2, 2018

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

We are currently supporting 14 subprojects from M.L. 2013 – summaries of the progress of these subprojects are included below. Subproject 13 – Eco-epidemiological Model to Assess Aquatic Invasive Species Management – has completed and a final report has been submitted to LCCMR. Additionally, we are in the process of

approving seven new research projects that will be funded on M.L. 2013 until funding concludes on June 30, 2019. New subprojects are listed in the update table above and are included in the project summaries below.

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

https://www.maisrc.umn.edu/sites/maisrc.umn.edu/files/maisrc_five_year_report.pdf

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

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

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

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

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

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

before being finalized by MAISRC staff for our 2018 Request for Research Proposals, which we plan to announce in November 2018.

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

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

Sub-Project Status as of February 28, 2019

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

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

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

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

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

- $\circ \quad \text{The AIS Detectors program} \\$
- o Starry stonewort research
- Spiny waterflea research
- \circ $\;$ The impact of zebra mussels and spiny waterflea on walleye
- Using pathogens to control invasive carp
- Novel methods for controlling common carp

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

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

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

https://www.maisrc.umn.edu/news/showcase-presentations-1

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

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

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

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

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

Final Report Summary:

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

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

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

Project Manager: Michael Sadowsky

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

Summary Budget Information for Sub-Project 2:

ENRTF Budget**:	\$299,364
Amount Spent:	\$299,364
Balance:	\$0

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

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

Sub-Project Status as of February 10, 2014

No progress to report as project is not anticipated to start until December 2014

Sub-Project Status as of August 31, 2014

The proposal process for this subproject has begun with an estimated start in early 2015.

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

This subproject was approved to begin June 20, 2015.

Sub-Project Status as of February 29, 2016

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

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

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

Sub-Project Status as of August 31, 2017

This project has completed and a final report will be submitted by 9/30/17. We are seeking an amendment to return the remaining balance of \$3854 to the MAISRC reserve so that it may be redistributed to other priorities.

Final Report Summary:

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

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

Project Manager: Michael Sadowsky

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

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

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

Summary Budget Information for Sub-Project 2:	ENRTF Budget ^{**} :	\$303,217
	Amount Spent:	\$286,610
	Balance:	\$16,607

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

Sub-Project Status as of February 28, 2018

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

Sub-Project Status as of August 31, 2018

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

Sub-Project Status as of February 28, 2019

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

Final Report Summary:

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

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

Project Manager: Peter Sorensen

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

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

ENRTF Budget ^{**} :	\$682,969
Amount Spent:	\$663,719
Balance:	\$19,251
	ENRTF Budget ^{**} : Amount Spent: Balance:

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

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

Sub-Project Status as of February 10, 2014

No progress to report as initial work is being funded with 2012 ENRTF funds through June 2015

Sub-Project Status as of August 31, 2014

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

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

This subproject was approved to begin July 9, 2015.

Sub-Project Status as of February 29, 2016

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

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

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

Sub-Project Status as of August 31, 2017

Research is proceeding well and is on schedule. Three specific approaches to use sex pheromones as attractants have now been identified while two approaches have been identified for using feeding stimuli. Experiments on

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

Sub-Project Status as of February 28, 2018

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

Sub-Project Status as of August 31, 2018

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

Sub-Project Status as of February 28, 2019

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

Final Report Summary:

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

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

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

Project Manager: Przemek Bajer

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

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

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

Summary Budget Information for Sub-project 4-1:

ENRTF Budget ^{**} :	\$384,231
Amount Spent:	\$384,231
Balance:	\$0

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

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

Sub-Project Status as of February 10, 2014

No progress to report as project is not anticipated to start until approximately March 2015

Sub-Project Status as of August 31, 2014

The proposal process for this subproject has begun with estimated start Spring 2015.

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

This subproject was approved to begin July 7, 2015

Sub-Project Status as of February 29, 2016

Research continues to progress and outcome goals have been achieved. Experimental lakes were selected for experiment 1a. Monitoring will continue over the winter and planning will be done for stocking and monitoring these lakes in the spring. For activity 1b, winter aeration data from aeration permit surveys were compiled. Surveys were paired with DNR fish assessments. The number of fish assessments that paired with aeration surveys proved to be too few in number to analyze. A higher-resolution case-study approach is now being pursued. For activity 2, experimental design has been finalized for activity and experimental ponds have been selected at the USGS facility. Activity 2 experiments will begin in the spring. A detailed account of each activity follows.
Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

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

Final Report Summary:

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

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

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

Project Manager: Przemek Bajer

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

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

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

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

Summary Budget Information for Sub-project 4-2:

ENRTF Budget ^{**} :	\$406,000
Amount Spent:	\$348,913
Balance:	\$57,087

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

Sub-Project Status as of February 28, 2018

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

Sub-Project Status as of August 31, 2018

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

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

Sub-Project Status as of February 28, 2019

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

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

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

Final Report Summary:

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

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

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

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

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

Project Manager: Ray Newman

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

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

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

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

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

Sub-Project Status as of February 10, 2014

A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of August 31, 2014

A workplan and budget for this subproject were approved July 31, 2014 and initial work is now underway.

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

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

Sub-Project Status as of February 29, 2016

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

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

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

Sub-Project Status as of August 31, 2016

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

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

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

Sub-Project Status as of February 28, 2017

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

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

Final Report Summary:

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

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

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

Project Manager: Paul Venturelli

Description:

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

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

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

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

We will develop a population model of yellow perch and couple this model with a disease model that describes *H. sutherlandae* dynamics as well as a generic population model that describes the dynamics of other fish hosts. We will base model parameters on current knowledge, and fill any gaps using related species, professional

opinion, simple lab experiments, and field observations. We will use the model to estimate the threat that heterosporosis poses to yellow perch harvest in Minnesota, and prioritize future empirical research for improving model predictions. The overall project (Phases 1 and 2) will generate advice related to heterosporosis spread prevention, monitoring, control, and management; and establish a framework for approaching other invasive species that are relevant to Minnesota.

EINKIF Dudget	ŞIII,009
Amount Spent:	\$111,889
Balance:	\$0
	ENRIF Budget**: Amount Spent: Balance:

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

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

Sub-Project Status as of February 10, 2014

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

Sub-Project Status as of August 31, 2014

The proposal process for this subproject has begun with estimated project start summer 2015.

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

This subproject was approved to begin June 15, 2015

Sub-Project Status as of February 29, 2016

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

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

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

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

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

Sub-Project Status as of August 31, 2017

This project has completed. A final subproject report will be submitted by 9/30/17.

Final Report Summary:

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

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

Project Manager: Nick Phelps

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

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

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

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

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

Sub-Project Status as of February 10, 2014

A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of August 31, 2014

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

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

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

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

Sub-Project Status as of February 29, 2016

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

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

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

Sub-Project Status as of August 31, 2016

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

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

Final Report Summary:

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

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

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

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

Summary Budget Information for Sub-Project 7-2:

ENRTF Budget**:	\$445,210
Amount Spent:	\$422,667
Balance:	\$22,543

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

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

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

Sub-Project Status as of February 28, 2017

This subproject was approved in February 2017. An updated project description, budget, and outcomes are provided above.

Sub-Project Status as of August 31, 2017

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

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

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

Sub-Project Status as of February 28, 2018

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

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

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

Sub-Project Status as of August 31, 2018

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

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

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

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

Sub-Project Status as of February 28, 2019

We have continued to make good progress in the previous six months of the project period. We are nearly complete with collection of fish kills and healthy common carp – we plan to work with commercial fishermen in the coming months to collect common carp from the final two lakes. We have spent considerable time this

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

Final Report Summary:

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

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

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

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

Project Manager: Dan Larkin

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

Despite strong interest and investment in preventing new invasions, controlling existing infestations, and supporting the recovery of impacted waterbodies, there are still key gaps in scientific knowledge needed to support effective management. To help address these gaps, this subproject will involve applied research on four

high-priority aquatic plant species that are invasive or potentially invasive in Minnesota lakes. These species are at different stages of invasion in Minnesota. Because of this, management priorities and associated research needs differ, from evaluating risk of future invasion and spread, to improving the toolkit available for control, to identifying strategies for aiding recovery of lakes affected by AIS:

(1) (Discontinued)

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

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

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

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

Summary Budget Information for Sub-Project 8:	ENRTF Budget**:	\$822,000
	Amount Spent:	\$820,251
	Balance:	\$1,749

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

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

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

Sub-Project Status as of February 10, 2014

No progress to report at this time as the project is not anticipated to start until approximately January 2015

Sub-Project Status as of August 31, 2014

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

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

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

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

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

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

Sub-Project Status as of February 29, 2016

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

Sub-Project Status as of May 2, 2016

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

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

Sub-Project Status as of August 31, 2016

This Sub-project was just approved in May with an understanding that its next status update would be provided January 31, 2017

Sub-Project Status as of February 28, 2017

This funding has enabled an active research program addressing applied issues in aquatic invasive plant management in Minnesota lakes. Research on starry stonewort has addressed spread risk using ecological niche modeling and ongoing work to predict vulnerability of individual Minnesota lakes to starry stonewort invasion based on environmental characteristics. Culturing of starry stonewort is being refined to enable laboratory experiments addressing starry stonewort climate and desiccation tolerance and chemical control. Field sampling and experimental germination of starry stonewort bulbils from areas treated with algaecides and/or mechanical harvesting revealed high capacity for reinvasion of treated areas. In-lake outcomes of starry stonewort management efforts are being monitored in collaboration with DNR and other external partners. Research on Eurasian watermilfoil and curly-leaf pondweed has shown that shallow lakes with higher native plant diversity are more vulnerable to invasion, and that these invasive plants are associated with rapid biotic homogenization of vegetation in these lakes (loss of plant community distinctiveness). We are compiling monitoring data from past treatments of Eurasian watermilfoil and curly-leaf pondweed in Minnesota lakes to investigate how management decisions and environmental conditions influence effectiveness of control and capacity for recovery of native plant communities. The curly-leaf pondweed component incorporates and builds upon previously ENRTF-funded work by Dr. Ray Newman (Subproject 9). Finally, our research is being integrated with joint MAISRC-Extension efforts to develop the Trackers citizen science program (Subproject 10). Research related to this project has been presented in peer-reviewed publications (one complete, two in revision, several in preparation), research and outreach talks (13 total, 12 invited), and media coverage (7 total, including print, television, and radio).

Sub-Project Status as of August 31, 2017

We have advanced progress of our research on several fronts. The completion dates for some outcomes have been amended and three small budget adjustments have been made. These updates are described below.

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

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

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

Sub-Project Status as of February 28, 2018

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

Since the last workplan update, we have disseminated our findings through (1) peer-reviewed publications (one paper has been accepted since the last update and two manuscripts are currently in revision and one is in

review); six invited talks to agency staff, other researchers, and the public; two contributed talks at national scientific meetings; and 12 print, television, and radio stories.

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

Sub-Project Status as of August 31, 2018

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

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

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

Sub-Project Status as of February 28, 2019

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

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

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

Final Report Summary:

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

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

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

Project Manager: Michael McCartney Description:

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

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

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

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

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

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

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

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

Sub-Project Status as of February 10, 2014

Project is currently being peer reviewed and will be funded with Clean Water Funds through June 2016.

Sub-Project Status as of August 31, 2014

Subproject has been approved and is underway with funding from the Clean Water Funds through June 2016.

Sub-Project Status as of February 28, 2015

The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of September 24, 2015

The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of February 29, 2016

The preliminary phases of this sub project continue to advance with funding from the Clean Water Fund.

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

This subproject was approved in February 2017. An updated project description, budget and outcomes are provided above.

Sub-Project Status as of August 31, 2017

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

of 75 new MN sites listed in the research addendum, Appendix 2; 8 water bodies either had too few mussels to collect, or had access issues that we will solve). For Great Lakes samples, it was necessary to develop a test to quickly and reliably distinguish between zebra and quagga mussels, because Great Lakes collections contain both species, and most are dominated by quagga mussels. Our SNP test is refined for zebra mussels, and to avoid the expense of submitting samples of the wrong species, we tested a quick molecular assay (modified from the literature) validated it on sequenced DNA from zebra and quagga mussels, and now use it routinely on these collections.

We processed all samples that were genotyped in Phase I (with microsatellites), as well as the newly extracted Great Lakes samples, and submitted them for genomic SNP analysis [using the University of Minnesota Genomic Center's (UMGC) assay refined for zebra mussels that was completed in Phase I (December 2016)]. We performed initial analyses of SNP data (examining effects of filtering parameters, filtering SNP data, scoring SNP markers, initial clustering analysis...) and found that with conservative parameters 3320 SNPs could be scored for each of 439 mussels, with no missing data); 10 times or more can be scored with less filtering. This important step shows that the SNP analysis generates a very large number of scorable markers (approximately the number expected), and shows the route we can take to increase the number of markers to study relationships between important source water bodies (e.g. Lake Minnetonka, St. Croix and Mississippi Rivers, Great Lakes).

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

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

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

Sub-Project Status as of February 28, 2018

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

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

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

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

Sub-Project Status as of August 31, 2018

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

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

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

Sub-Project Status as of February 28, 2019

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

Final Report Summary:

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

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

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

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

Project Manager: Dan Larkin

Description:

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

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

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

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

Both programs will recruit and train professionals (i.e., AIS managers and service providers) and citizen scientists (lake association leaders, county AIS task forces members, Master Naturalists and other motivated citizens). Successful completion of these programs will be recognized by certification. To maintain their status as a certified AIS Detector or AIS Tracker, volunteers must perform a minimum level of service and maintain and increase their expertise through continuing education opportunities offered by the programs. Annual service

will include activities that are self-initiated as well as those that are organized by the programs, such as surveys of high risk lakes for new AIS occurrences or providing outreach related to reporting AIS.

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

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

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

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

Sub-Project Status as of February 10, 2014

No progress to report as project is not anticipated to start until approximately March, 2015

Sub-Project Status as of August 31, 2014

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

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

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

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

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

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

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

Sub-Project Status as of February 29, 2016

An online template for the online portion of the AIS Detectors course has been designed and created in Moodle, the University of Minnesota's course delivery platform. The course information for the online portion of the course was organized into six modules and for each module, the specific learning outcomes were developed.

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

Sub-Project Status as of August 31, 2016

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

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

Sub-Project Status as of February 28, 2017

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

Sub-Project Status as of August 31, 2017

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

Sub-Project Status as of February 28, 2018

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

On August 5, 2017, 200 volunteers and over 20 local host coordinators throughout the state participated in Starry Trek, our first AIS Detectors advanced training opportunity. Our volunteers discovered what was, at the time, the tenth known population of starry stonewort in Minnesota (Grand Lake, Stearns Co.). Early detection of

this small, likely recent infestation through Starry Trek enabled a rapid response plan to be developed by the Grand Lake Association, MnDNR, and MAISRC and implemented by MnDNR, whose AIS Specialists dove and hand-removed all visible plants.

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

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

Sub-Project Status as of August 31, 2018

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

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

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

Sub-Project Status as of February 28, 2019

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

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

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

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

Final Report Summary:

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

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

Project Manager: Dave Andow

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

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

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

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

Sub-Project Status as of February 10, 2014

A project proposal has been written, peer reviewed, and recommended for funding by the Scientific Director. After Center Administrative Review committee approval is granted, a subproject work plan and budget will be submitted to LCCMR.

Sub-Project Status as of August 31, 2014

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

Sub-Project Status as of February 28, 2015

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

Sub-Project Status as of September 24, 2015

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

Final Report Summary:

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

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

Project Manager: Dave Andow

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

To help address this complicated situation, the project will conduct a risk assessment to prioritize issues and areas for Asian carp management and to reduce the uncertainty about how Asian carp will impact Minnesota's waterways. The risk assessment will assume that silver and bighead carp will arrive in all Minnesota waterways and will focus on determining which potential adverse effects are most likely and consequential in the different watersheds of Minnesota. It will first use a qualitative approach to determine which adverse effects are most salient and then work towards quantifying their likelihood and consequence of the most salient effects. The risk assessment will follow a deliberative process involving a variety of relevant experts and expert stakeholders, and will be designed in coordination with the MNDNR. Important areas of disagreement, remaining uncertainties, and additional research needs will be characterized so as to foster a productive discussion about them. This project will also include a risk communication component to share findings and foster a conversation about the findings' implications for management. The risk communication process will involve organizing a meeting where those involved with the risk assessment will discuss its results and implications with the relevant and interested

parties who were not involved with the risk assessment, including a broader set of stakeholders, researchers, managers, and decision makers from relevant state and federal agencies.

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

ENRTF Budget:	\$126,677*
Amount Spent:	\$126,677
Balance:	\$0
	ENRTF Budget: Amount Spent: Balance:

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

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

Sub-Project Status as of February 29, 2016

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

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

Since the last project update, the report for the Minnesota Bigheaded Carps Risk Assessment has been drafted and reviewed by risk assessment workshop participants. In working on the risk assessment report, project

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

Sub-Project Status as of August 31, 2017

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

Final Report Summary:

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

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

Project Manager: Donn Branstrator

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

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

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

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

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

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

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

Sub-Project Status as of May 2, 2016

This project is currently undergoing revision and workplan development following peer review. The Subproject workplan and budget will be submitted to LCCMR following approval by MAISRC, at which point the above budget, description, and outcomes will be revised as needed.

Sub-Project Status as of August 31, 2016

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

Sub-Project Status as of February 28, 2017

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

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

Sub-Project Status as of August 31, 2017

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

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

Sub-Project Status as of February 28, 2018

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

Sub-Project Status as of August 31, 2018

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

Sub-Project Status as of February 28, 2019

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

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

Final Report Summary:

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

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

Project Manager: Nicholas Phelps

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

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

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

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

Outcome	Completion Date
Activity 1	
1.Validated next generation ecological niche model for Zebra mussel	July 2016
2. Validated next generation ecological niche model for Eurasian watermilfoil	Nov 2016
3. Validated next generation ecological niche model for Heterosporis	Feb 2017
Activity 2	
1.Validated network model of lakes and rivers	Nov 2016
2. Validated network model of boater movement	Mar 2017
Activity 3	
1. First workshop: Categorization of management strategies	Nov 2016
2. Final cumulative risk model for the three AIS selected	June 2017
3. Second workshop: Evaluation of final cumulative risk model	Sept 2017
Activity 4	
1.Scientific and public presentations (n=6; i.e. MAISRC Showcase, research	March 2018
meetings, etc)	
2. Publication of peer-reviewed manuscripts (n=6)	March 2018

Sub-Project Status as of May 2, 2016

This project is currently undergoing revision and workplan development following peer review. The Subproject workplan and budget will be submitted to LCCMR following approval by MAISRC, at which point the above budget, description, and outcomes will be revised as needed.

Sub-Project Status as of August 31, 2016

This Sub-project was just approved in September with an understanding that its next status update would be provided January 31, 2017

Sub-Project Status as of February 28, 2017

The ecological niche model for Heterosporosis was developed to achieve outcome 1 from Activity 1. Thus, we were able to identify the geographic areas in Minnesota with suitable conditions for the establishment or presence of this fish disease and produce risk maps for use by managers and researchers. These findings will be submitted for peer-review in late January to the open access journal *Frontiers in Veterinary Science* (Working title: "Novel methods in disease biogeography: A case study with Heterosporosis").

The early results of this project were presented at the 2016 MAISRC showcase, to more than 200 participants (https://goo.gl/atJ1Zm). The audience was interested in the project's outputs and requested future presentations showing how the suitability and network models will identify lakes where preventive measures should be implemented and prioritized. A second manuscript is currently under review in the scientific journal *Reviews in Fisheries Science and Aquaculture,* with a broad overview of MAISRC studies, including this project, ("Aquatic invasive species in the Great Lakes region: An overview.").

Data for the zebra mussels risk maps were collected and cleaned and models are under development. Data for the network models is currently being organized and cleaned by Dr. Huijie Qiao, the visiting researcher involved with the project. This status provides us confidence to achieve the results according to our schedule.

Sub-Project Status as of August 31, 2017

The project attempts to forecast invadable areas for an invasive pathogen, a plant, and an animal, assessing risk of invasion and establishment in Minnesota. The ecological niche model for the pathogen Heterosporosis has been completed and was published. Thus, results are currently available to the international scientific community and the managers in Minnesota (Escobar, L. E., Qiao, H., Lee, C., & Phelps, N. B. D. (2017). Novel methods in disease biogeography: A case study with Heterosporosis. Frontiers in Veterinary Sciences doi:10.3389.fvets.2017.00105). The second manuscript of the project ("Aquatic invasive species in the Great Lakes region: An overview.") has received the first round of reviews. We expect to publish this manuscript as a guide for students and citizens about the state of aquatic invasive species in Minnesota, including the gaps in the knowledge and the ongoing research at the Minnesota Aquatic Invasive Species Research Center at the University of Minnesota (MAISRC). The ecological niche model for zebra mussel was completed and predictions to Minnesota were done at a fine spatial resolution. We are now working on the forecasts for the invasive plant starry stonewort.

A second part of this project includes the exploration of pathways for the spread of invasive species to suitable lakes in which species can establish populations. For this component, a visiting scholar, Dr. Huijie Qiao, worked at MAISRC from December 2016 to June 2017. During his collaboration, Dr. Qiao developed a first of its kind database with spatial distances between lakes and the connection of lakes via streams/rivers. These databases are essential to the development of network models and will likely have value in many other water resource issues.

A workshop was hosted in August to present the current status of this project to key stakeholder groups. This will result in the development of management scenarios that, when hypothetically implemented in the models in the coming months, could affect the risk of AIS establishment.

Sub-Project Status as of February 28, 2018

The project is progressing nicely and has made significant progress. For Activity 1, we have spent considerable effort cleaning the massive boater survey database provided by the MN DNR. We developed a data cleaning algorithm that improved inclusion of available data from 21.1% to 99%, a significant increase and fills in a much more complete assessment of boater movement. This now includes 1,690,613 total boater movements among 2,588 unique lakes during the 2014-2017 survey years. We have also created a network of water connectivity in the state – also the most detailed dataset of its kind at a statewide scale. These networks, along with geographic proximity, are now being integrated to evaluate the risk of AIS introduction based on historical invasion patterns.

For Activity 2, we hosted a workshop with AIS stakeholders to develop and evaluate hypothetical (but realistic) management scenarios that could be integrated into our risk models. The group ultimately came to consensus on likely effectiveness of 12 management options that ranged from not effective (but easy to implement) to very effective (but difficult to implement). These will be used to modify our risk models and be presented back to the same group for reaction in May of 2018.

Results of this project have been presented at the International Conference on Aquatic Invasive Species, as well as regional and local meetings to a wide range of AIS stakeholders. We have also published two manuscripts highlighting the results of this project.

Final Report Summary:

Aquatic invasive species (AIS) are spreading at an alarming rate in Minnesota, putting the urgent need for prevention at odds with limited budgets and capacity. To inform decision making, we have developed a series of integrated models that provide the cumulative risk of introduction and establishment of zebra mussels and starry stonewort in all Minnesota lakes. We first answered the question of 'can the species get there?' using network models to describe lake connections. The watercraft network was built with 1.6M MN DNR watercraft inspections from 2014-2017, with gaps and biases accounted for with a variety of statistical approaches. The water connectivity network was created at a finer resolution and larger geographic area than currently available using multiple sources of GIS data and satellite imagery. Next, we answered the question of 'will the species survive?' using advanced methods of ecological niche modeling. With current species distribution of the invaded and native ranges, paired with local environmental data, we projected suitability at the lake level. These three massive data sources fed into the development of an integrated model that quantified the risk of AIS invasion for each waterbody from 2018-2025. Not surprisingly the results suggest the number of infested waterbodies will increase in the years to come. However, with the integration of hypothetical management scenarios developed and incorporated during two project workshops, we demonstrated the value of this approach to assess management effectiveness by determining the number of new infestations averted. While the model is not perfect (no models are), the results are robust and provide useful information from which to make decisions. When considered across a watershed, county or state, the ability to rank waterbodies based on actual, not perceived, risk is a game changer for the prioritization of intervention strategies.

SUB-PROJECT 14: Cost-effective monitoring of lakes newly infested with zebra mussels

Project Manager: John Fieberg

Description: Our objective is to develop recommendations for underwater survey methods and methods for estimating population abundance and distribution of zebra mussels, accounting for imperfect detection, which can be used to monitor newly infested lakes.

Advice regarding appropriate survey methods is desperately needed by Minnesota Department of Natural Resources' (MNDNR) staff, citizen groups, MN Counties, watershed districts, and lake managers confronted with new infestations of zebra mussels. The earliest stages of lake colonization are difficult to monitor because abundance is low, mussels are sparsely distributed, and they are hard to locate and count. In 2015, the MN DNR initiated a Pilot Project Program to evaluate effectiveness of pesticide treatments, focusing on water bodies where zebra mussels have been determined to be "limited in size and localized" using "an established monitoring protocol" (http://www.dnr.state.mn.us/invasives/aquaticanimals/zebramussel/pilot_project.html). This program issues treatment permits and provides protocols for survey and monitoring of zebra mussel larvae, juvenile recruitment, adult densities and pesticide mortality to evaluate outcomes following treatment efforts (http://files.dnr.state.mn.us/natural_resources/invasives/aquaticanimals/zebramussel/zebra_mussel_monitoring g_2015-09-10.pdf). Lakes in the program must be surveyed for 3 successive years post-treatment, but the Pilot Project Program currently lacks guidelines for allocating survey effort (e.g., through a valid statistical sampling design), which makes extrapolation to unsampled areas and comparisons over time problematic. Additionally, no guidelines exist to account for imperfect detection (i.e., mussels present but not observed) when sampling.

Sampling designs for zebra mussels must be feasible to implement by SCUBA divers and result in data that allow for efficient estimation of abundance and spatial distribution patterns while also accounting for imperfect detection. Methods must also be standardized to allow comparisons across lakes. We will take advantage of

recent methodological advances for collecting and modeling spatial data using line-transect surveys. Linetransect sampling designs are appealing for several reasons: 1) divers can quickly survey large contiguous areas; 2) methods for estimating and correcting for imperfect detection are well developed; and 3) recent advances in spatial modeling can be used to estimate the distribution of mussels throughout the lake.

We will survey lakes in 2017 and 2018 using a variety of line-transect sampling designs. In addition, we will conduct an extensive simulation study to evaluate the efficiency of alternative survey designs and to provide recommendations regarding appropriate sampling effort. We plan to select lakes that were first listed and confirmed infested in years 2015 and 2016 from a publicly available database maintained by the MN DNR Invasive Species Program (http://www.dnr.state.mn.us/invasives/ais/infested.html: updated 12/29/16). We will draw untreated reference lakes from 2015 and 2016 to bracket a range of initial densities, and will select lakes that have been treated with pesticides from MN DNR's Pilot Project Program. We will estimate abundance and distribution patterns by fitting density surface models to the resulting data. These density estimates will also allow us to develop realistic simulation scenarios for comparing alternative sampling designs and to evaluate how sampling effort affects our ability to detect changes in abundance and distribution over time and therefore the efficacy of pesticide treatments.

This work will result in the following outcomes:

- 1. Recommended, cost-effective monitoring programs for estimating distribution and abundance of mussels that can be implemented in recently infested lakes, allowing for targeted control efforts.
- 2. Estimates of population distribution and abundance patterns in 10 newly infested lakes.
- 3. Comparisons of mussel abundance and distribution in lakes that are and are not treated with pesticides as part of MNDNR's Pilot Project Program.

Summary Budget Information for Sub-Project 14:	ENRTF Budget*:	\$266,500
	Amount Spent:	\$225,553
	Balance:	\$40.947

Outcome	Completion Date
Activity 1	
1. Survey up to 10 lakes in 2017	November 1, 2017
2. Survey 5 lakes in 2018, test feasibility of adaptive line-transect design	November 1, 2018
Activity 2	
1. Report preliminary estimates of distribution and abundance patterns from	January 31, 2018
lake surveys conducted in 2017	
2. Report final estimates of distribution and abundance patterns from lake	June 20, 2019
surveys conducted in 2017 and 2018	
Activity 3	
1. Senior capstone project, simulation study to compare alternative sampling	June 1, 2018
designs	
2. Develop recommendations for monitoring newly infested lakes	June 30, 2019

Sub-Project Status as of February 28, 2018

We visited a total of eleven lakes reported to have low/moderate-density zebra mussel populations and conducted SCUBA surveys in six of them. Five lakes were excluded because zebra mussel populations were too high or, in one case, there was an active algae bloom that prevented the survey from occurring. Zebra mussels are a cryptic species so we knew they would be difficult to detect, even with SCUBA surveys. We surveyed lakes using two different methods that can, if certain model assumptions are met, provide estimates of the number of mussels encountered but not observed within the surveyed area. We had two dive teams survey the same areas in Lake Burgen in Douglas County. This "double-observer" dive allowed us to evaluate important assumptions of

our approach and to quantify differences in detection ability of the divers. Our estimates of the probability of detecting a mussel within 1 meter of a diver was between 3% and 30% depending on who the observer was and the environmental conditions (e.g., water clarity) near the mussel. When averaged across surveyed areas and environmental conditions, we estimated divers detected 16% (diver 1) and 28% (diver 2) of the mussels present in the surveyed area. Thus, we may expect low detection probabilities even with experienced divers. Our data also suggest that divers are likely to miss zebra mussels that are on (or very near) the transect line. This result challenges a critical assumption of conventional survey designs, namely that observers are able to detect all objects on the transect line. We can get around this assumption by conducting surveys with multiple dive teams, but the additional personnel will increase survey costs and may reduce the total amount of area that can be surveyed. Our initial results suggest that to estimate zebra mussel densities accurately, we need to implement double-observer surveys.

An amendment was approved by LCCMR on 02/06/2018 to reduce the number of lakes to be surveyed in 2018 from 10 to 5. By concentrating on fewer lakes, we can save time and money allocated to travel and devote it to increased survey efforts on the 5 lakes we choose. This proposed change in sampling effort will ensure we are able to collect sufficient data to evaluate the assumptions of our survey methods and will also better facilitate comparisons among survey methods (e.g., single and double observer dives). In particular, we would be able to resurvey lakes multiple times, using different survey methods, and compare results. The disadvantage of this shift in survey effort is that we would estimate zebra mussel density and distribution patterns in a smaller number lakes.

Sub-Project Status as of August 31, 2018

We have developed initial plans for sampling lakes this summer (2018). To increase time spent in lakes with appropriately low densities, we have decided to sample lakes in 3 "phases". The first 2 phases will be used to quickly assess relative abundance and spatial distribution of mussels in a set of candidate lakes without attempting to estimate detection probabilities or correct for imperfect detection. The third phase will be used to more rigorously compare alternative survey methods useful for estimating abundance (i.e., correcting, as necessary, for mussels not observed in the surveyed area) in a small number of low density lakes.

In June, we visited 18 lakes and sampled 15 of these using 20-minute timed surveys (phase 1). Based on these initial surveys, we chose 6 lakes for phase 2 sampling, in which we surveyed 15 transects spread throughout the lake. In July and August, we plan to compare 3 different survey methods in a subset of these 6 lakes (phase 3 sampling).

We have analyzed the data from last year's surveys and recently completed a first rough draft of a manuscript describing these methods and results. Lastly, students from Carleton College completed a simulation study to explore the efficiency of different survey designs using simulations. Their results support the use of distance sampling for estimating density of zebra mussels in lakes, but point to the need for increased sampling effort to reduce uncertainty associated with density estimates.

Sub-Project Status as of February 28, 2019

We completed our field surveys associated with Activity 1. In particular, we implemented 3 different survey techniques (double-observer surveys with and without distance sampling, quadrat counts) in three lakes capturing a range of zebra mussel densities: Lake Florida in Kandiyohi County, Lake Burgan in Douglas County, and Little Birch Lake in Todd County.

We developed two approaches for analyzing the data from our first field season in Lake Burgan, a straightforward approach that can be implemented with existing open-source software and a more refined approach that can be used to explore the effect of covariates (e.g., plant presence, substrate) on detection probabilities and zebra mussel density. Both methods produced density estimates that were 3 times larger than the observed densities (uncorrected for detection). These results demonstrate the importance of estimating and

adjusting for detection probabilities <1 rather than relying on observed counts when comparing densities over time or space.

We compared estimates of detection probabilities and zebra mussel density from data collecting during our second field season using 3 different survey methods (double-observer with and without distance sampling, quadrat counts). We found that estimates of detection probabilities were fairly similar in all three sampled lakes (Lake Burgan, Lake Florida, and Little Birch Lake), and the different survey methods all gave similar estimates of density. The estimated detection probability using double-observer surveys without distance sampling was 0.94, suggesting we may be able to achieve near perfect detection, provided we use 2 observers and survey a smaller width transect. However, we detected a pattern of slightly lower density estimates when using this approach (compared to double observer surveys with distance sampling may be most efficient at low densities and quadrat or double-observer surveys (without distance data) may be more efficient when densities are high. This spring, we will further evaluate relative efficiencies of these methods using simulated data across a range of zebra mussel densities.

Final Report Summary:

The current lack of standardized methods for surveying zebra mussels during their earliest stages of lake colonization limits our ability to track changes in density over time or to evaluate effectiveness of treatment programs (e.g., as required by DNR permits). We evaluated 5 different survey designs for estimating zebra mussel density (2 designs in 2017 and 3 designs in 2018), employing methods that utilize counts by two divers to estimate the probability of detecting mussels in the surveyed area. We also compared survey designs in terms of their density estimates, associated measures of uncertainty, and sampling efficiencies (time required to complete a survey), using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data. In 2017 in Lake Burgan, we estimated that a diver could detect between 5% and 41% of the mussels present in the surveyed area, depending on the specific diver and on whether the lake bottom was vegetated, with vegetation having the larger effect on detection. Accounting for low detectability of zebra mussels led to an estimate of density over three times higher than the observed density. Thus, for every zebra mussel detected by our divers, approximately two were missed. Using the data collected in 2018 and further simulation and analytical work, we found that double-observer survey designs that allow for imperfect detection are optimal when surveying lakes at low density, whereas quadrat counts that assume perfect detection are optimal at higher densities. We developed a training video, data collection worksheets, and an analysis tutorial so that others may implement our proposed survey designs in newly infested lakes. These tools benefit Minnesotan's by providing better ways to monitor lakes infested with zebra mussels and to evaluate the effects of treatment options on zebra mussel density.

SUB-PROJECT 15: Determining Highest Risk Vectors of Spiny WaterFlea Spread

Project Manager: Valerie Brady

Description: Spiny water flea is a predatory species of zooplankton that represents a serious threat to the ecology and recreational value of Minnesota waters. As of 2015, spiny water flea (SWF) was reported in 36 lakes in Minnesota, including some of the largest basins (Superior, Kabetogama, Lake of the Woods, Mille Lacs, Rainy, Vermilion) that now unfortunately serve as potential source populations to uninfested waters. A major potential risk for the health of Minnesota lakes is that spiny water flea is a carnivore that feeds aggressively on native herbivorous zooplankton, a food resource that is shared as prey by many species of young fish including walleye, northern pike, and yellow perch. This potential competitive interaction with young fish could slow the growth and health of many native fish species in Minnesota. A second potential risk for the health of Minnesota lakes is that spiny eas grazers on algae, the microscopic plants that form the base of aquatic food webs. Higher concentrations of algae are directly related to lower water clarity. Thus, through removal of herbivorous zooplankton, spiny water flea threatens to reduce the health of fish through

competition and to reduce water clarity through eliminating native grazers. These impacts could bring changes to Minnesota lakes that have serious implications for recreation and wildlife. Estimates are that >40% of northern Minnesota lakes provide suitable habitat for spiny water flea, indicating that management programs that foster best practices for containment are critical.

Human recreational activity is believed to be the primary vector of spread; however, little is known about the specific pathways by which dispersal occurs. Current best management practices direct recreationalists to clean, drain, and dry their equipment before moving it to another water body (this is the core message of the "Stop Aquatic Hitchhikers!" [SAH!] campaign). While this message should be effective if followed stringently, it is broad and fails to draw attention to what may be high risk equipment where decontamination effort could be focused or whose usage could be minimized or avoided altogether. Hence, while we have an opportunity to prevent further spread of spiny water flea in Minnesota, clear evidence-based educational messages and policies are urgently needed. A key aspect of spiny water flea behavior is that it migrates closer to a lake's surface at twilight to feed. This behavior increases its potential contact with surface-based equipment (e.g., boat live wells, bait buckets) that could boost the likelihood of a transport event. To increase the effectiveness of the SAH! campaign against the spread of spiny water flea, we need answers to two critical questions: 1) What forms of recreational equipment pose the highest-risk pathway for spiny water flea over midday equipment usage?

Goal: The goals of this project are 1) to measure and rank recreational (mostly fishing) gear in its ability to spread the adult free-swimming spiny water flea using Lake Mille Lacs as the test lake; and 2) to widely disseminate the results, our recommendations, and gear-cleaning tips both in the Mille Lacs area and throughout the state to anglers, the tourism industry, AIS managers, agency staff and legislators, and lake associations.

How: The goal will be accomplished by deploying commonly-used forms of recreational equipment including anchor ropes, angling lines, bait buckets, downrigger cables, and live wells and then cleaning them and comparing the "load" (total number) of spiny water flea relative to the flea's natural abundances in surrounding Mille Lacs lake water. We will use NRRI's boats to test the different types of gear in Lake Mille Lacs. We will set out three different types of anchor rope and have three fishing poles each rigged with a different type of fishing line, with a hookless weight on the end. One boat will also be set up for downrigging gear to determine the numbers of spiny water flea that accumulate on the steel cable and the monofilament line. One of the boats will also have a bait bucket in the water and be running water into a live well.

At the same time as the fishing gear are in the water potentially encountering and being fouled by spiny water flea, we will determine the fleas' abundance in the water using zooplankton nets. Spiny water flea will be cleaned from all gear being tested, and will be collected out of the plankton nets to determine ambient flea densities. Collected spiny water flea will be preserved and returned to the laboratory for microscopic analysis.

Field work will be done from July to September 2018 in Lake Mille Lacs. Lake Mille Lacs has supported spiny water flea since 2009 and is a major sport-fishing and recreational destination in the Midwest, elevating its potential threat as a source population for new infestations in other lakes. For statistical rigor, we plan to collect 30 samples per type of gear during daylight and again during twilight (evening). We anticipate collecting approximately 1000 samples total from the recreational gear and the sampling nets. Analyzing spiny water flea numbers on each gear type versus the spiny water flea densities in the lake at the same time will allow us to create a ranking of the threat that each type of gear poses for spiny water flea spread to other water bodies. We will use this information to create specific outreach messages for the public, including reminder stickers with gear cleaning tips. We will provide this information to lake associations, lake managers, anglers, and recreationalists.

Our long-term goal is to provide science-based information that will improve the effectiveness of current best management practices used in Minnesota to minimize pathways for AIS introduction. **Our long-term outcome** is to help slow the spread of spiny water flea to uninfested lakes.

Summary Budget Information	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a	M.L. 2017, Chp. 96, Sec. 2, Subd. 06a
for Sub-Project 15:		

Subproject Budget:	\$92,932	Subproject Budget:	\$26,581
Amount Spent:	\$92,756	Amount Spent:	\$7,456
Balance:	\$176	Balance:	\$19,125

Outcome	Completion Date
Activity 1	
1. Test anchor ropes, angling lines, bait buckets, downrigger cables, and live wells in Lake Mille Lacs for entanglement with spiny water flea on 6 different daylight and evening trips, as well as collect water column samples of spiny water flea.	Fall 2018
2. Microscopically examine samples in the lab and count the number of spiny water flea on each gear type.	Dec. 2018
3. Determine spiny water flea transfer risk from each gear type using appropriate statistics.	April 2019
4. Write detailed report of results and conclusions; provide report to agency AIS personnel.	June 2019
5. Write peer-reviewed manuscript for submission to a scientific journal to inform other AIS researchers of findings.	June 2019
Activity 2	
1. In collaboration with MAISRC, U Extension staff, and Wildlife Forever, create up to 10,000 waterproof, UV-protected stickers with plain-English outreach messages for anglers and boaters on gear cleaning. For example: "Clean, Drain, Dry, and don't forget your anchor rope!" Stickers will be placed at bait shops, gas stations near boat launches, and where fishing licenses are sold.	March 2020
2. In collaboration with MAISRC, the Aquatic Nuisance Species taskforce, and Sea Grant outreach staff, we will create radio and TV PSA-type ads highlighting what anglers should do; purchase spring/summer ad time for the Mille Lacs area.	April 2020
3. Presentations to AIS managers, agency staff, lake associations, tourism industry (esp. Dock Boys and Girls), policy makers, and fishing groups. Also, social media outreach messages targeted to connect with anglers and boaters.	May 2020
4. Outreach article for Minnesota Sportsman (or similar) magazine.	June 2020
5. Service for MAISRC, including participation in the 2018 and 2019 Showcase Events and participation on 1-2 committees.	June 2019

Sub-Project Status as of February 28, 2018

This project (by design and approval of MAISRC) has not yet started. We will begin planning for our first field season within the next couple of months with fieldwork to start in July. However, our companion project to do similar work in Island Lake Reservoir, near Duluth, funded by St. Louis County, had a full and successful sampling season last year. In the process of working on the St. Louis County-funded project we have been able to test and

refine our sampling methods to ensure that they will work. These changes are detailed below under Activity 1. None of these changes affects the budget.

Sub-Project Status as of August 31, 2018

This project (by design and approval of MAISRC) is just getting started. We have planned for our fieldwork and will start sampling by July 23 on Lake Mille Lacs using methods we tested and refined during our companion project on Island Lake funded by St. Louis County.

Per an approved amendment request, we are postponing our outreach activities (Activity 2) to ensure that we are able to craft an outreach message that is supported by project data being collected in the 2018 summer season. To avoid confusion and increase the effectiveness of our outreach campaign, we need to carefully word and test our message about preventing the spread of spiny water flea. An unclear or inconsistent message about AIS prevention could actually decrease the likelihood that anglers will be motivated to carefully clean and dry their gear. Delaying our campaign and testing our message increases the effectiveness and likelihood of compliance.

Under this new timeline, we will target the 2020 fishing season (beginning with walleye opener) and will purchase TV and radio ad time in the late winter/early spring of 2020. We will be coordinating our outreach on this project with the outreach on our companion St. Louis County funded spiny water flea project. Combining the outreach efforts on these companion projects will allow us to generate more outreach for the same amount of money since we will not have to pay designers twice for similar products. All efficiency savings will go into purchase of more outreach materials, particularly TV and radio ads. St. Louis County has agreed to provide a no-cost extension to Activity 2.

Sub-Project Status as of February 28, 2019

This past summer we completed all fieldwork associated with this project by conducting 7 sampling events on Lake Mille Lacs for spiny water flea entanglement on fishing gear, as described in Activity 1. This sampling resulted in collection of 718 samples. Samples collected included zooplankton tows, spiny water flea on fishing gear (downrigger, surface lines, bait bucket, and live well), and spiny water flea on anchor ropes. In the lab, we have counted and aged spiny water flea in 195 of the 718 samples collected. The remaining samples will be processed in February and March.

MAISRC staff hired a videographer and drone operator to come with us on one sampling trip. The resulting video has been used for a number of presentations to great reviews. PIs Brady and Branstrator participated in the 2018 MAISRC showcase event. In addition, graduate student Nicole DeWeese gave a presentation on this project at the Upper Midwest Aquatic Invasive Species Conference in Rochester, MN, in October 2018.

An amendment request is included in this report to transfer \$3,127 of surplus funds from Subproject 15, back into MAISRC reserves.

Sub-Project Status as of August 31, 2019

This past winter we completed processing of the samples collected during the 7 sampling events on Mille Lacs during the summer of 2018. Processing involved counting and aging all spiny water flea in samples. In total we processed 360 zooplankton tows; 36 braided nylon, 36 twisted nylon, and 36 polypropylene anchor ropes; 35 bait bucket samples; 21 livewell samples; 36 downrigger steel cable samples; 35 downrigger monofilament lines; and 36 braided, 36 monofilament, and 35 fluorocarbon fishing lines.

All data from samples was entered and QC'd. We ran data analyses and summaries for each gear type and have presented the findings at a number of meetings and conferences. We are currently crafting and testing our outreach message for distribution in the spring of 2020. We have tested potential messages with different user groups to determine which short phrase will best convey our message most effectively.

Activity 1 and Activity 2, Part I were funded on M.L. 2013, which ended on June 30, 2019. Activity 2, Part II will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 16: Sustaining walleye populations: assessing impacts of AIS

Project Manager: Gretchen Hansen

Description: Minnesota's walleye fisheries are vulnerable to ecosystem changes following the introduction of invasive species such as zebra mussels and spiny water fleas. For example, zebra mussels reduce zooplankton, limiting the amount of food available for fish in the open water zone of lakes. At the same time, the high filtering capacity of zebra mussels creates an "energy shunt" that moves food and energy from the water column into the bottom of the lake and nearshore areas, changing the structure of the food web by providing extra resources for fish that feed primarily in nearshore areas. Spiny water fleas are large predatory zooplankton that also reduce the abundance of other, smaller zooplankton. They themselves are inedible to some fish species and life stages due to their long protective tail spine. The zooplankton declines associated with both of these invaders are likely to affect predatory fish such as walleye, because both young walleye and many of their prey species rely on zooplankton as a food source. However, the impacts of zebra mussels and spiny water fleas on sport fish populations are not well understood.

The impacts of zebra mussels and spiny water fleas on fish likely depend upon the ability of fish to switch to alternative food sources if and when invaders cause zooplankton to become scarce. This ability to switch food sources likely depends on lake characteristics including size, depth, productivity, and fish community composition. Determining how these invasive species affect walleye, and identifying characteristics of walleye populations that can withstand these invasions with minimal effect, will allow managers to set realistic goals for future walleye production and harvest. Managers will also be able to assess the impacts of current and future invasions, and separate these effects from other potential causes of walleye population changes.

In this collaborative effort among the Minnesota Department of Natural Resources (MNDNR), the Natural Resources Research Institute, University of Minnesota-Duluth (NRRI), and Voyageurs National Park (VNP), we will quantify the impacts of zebra mussels and spiny water fleas individually and together on walleye and their food webs in Minnesota's large lakes. Minnesota's nine largest walleye lakes (all greater than 15,000 acres) are at different stages of invasion by zebra mussels (Cass, Winnibigoshish, Leech), spiny water fleas (Kabetogama, Lake of the Woods, Rainy, Vermilion), both (Mille Lacs), or neither (Red). Notably, we have an unprecedented opportunity to track the effects of each invader on walleye populations throughout all stages of invasion by tracking impacts early in the invasion. Zebra mussel veligers (larvae) were first discovered in Leech Lake in 2016 and no adult zebra mussels have yet been found. Similarly, spiny water fleas were discovered in Lake Vermilion in 2015 but have not reached high abundances and currently only occur in one of the lake's two major basins. Each of the nine study lakes will be sampled once in either 2017 or 2018.

We will use two approaches to evaluate the impacts of zebra mussels and spiny water fleas on walleye and food webs in Minnesota's large lakes. First, we will determine which habitats and food resources support walleye and other fish species in each lake by examining stable isotopes in their bodies. Naturally occurring stable isotopes show what a fish has been eating in the past few weeks to months. This analysis will allow us to determine the amount of food resources various fish species and ages (young or adult) are eating from different habitats (nearshore or open water), and at what trophic level they are feeding (their position in the food web). The

results of this analysis in each lake will tell us to what degree walleye and their prey rely on zooplankton in the open water as a food source to sustain their populations. This will allow us to assess how likely it is that walleye could switch to other food sources if zooplankton abundances are greatly reduced by zebra mussels or spiny water fleas.

We will also assess the effects of reduced zooplankton abundance due to zebra mussels and/or spiny water flea invasion on the growth rates of walleye and yellow perch in their first year of life. These young fish rely on small zooplankton prey in their early life stages, but they also can eat invertebrates (for example, insects, snails, small mussels) that are less likely to be reduced by zebra mussels or spiny water fleas. We will assess whether young fish may be less affected by the negative impacts of zebra mussels and spiny water fleas if they can successfully switch to other prey even as zooplankton food resources decline. Growth rates will be compared both among lakes with and without zebra mussels and/or spiny water fleas, and within lakes pre- and post-invasion using historical data collected by the Minnesota DNR.

The MNDNR will serve as lead and project manager, ensuring that timely and accurate reporting on the project is completed. MNDNR is also responsible for coordinating and carrying portions of Activities 1 - 3 as described in each section below with a focus on describing whole lake food webs. Funds requested here will support benthic invertebrate sampling for all 9 lakes; fish sampling from all 9 lakes in coordination with existing MNDNR sampling programs; stable isotope analysis for each trophic level; and organizing historical data for pre- invasion comparison. The MNDNR budget includes fieldwork conducted under contract by VNP on Rainy and Kabetogama lakes as well as other project activities. The MNDNR's funds will be provided through a subaward with MAISRC. MNDNR co-PI salaries, as well as additional sampling work already planned through the MNDNR's Large Lakes Program, are provided in-kind.

NRRI will be responsible for portions of Activities 1-3 as described below with a focus on fish diet sampling in 6 lakes; and age-0 fish sampling in 2 of the 9 lakes. NRRI will receive \$81,116, which will be awarded internally through a subproject child account similar to other MAISRC projects.

This project will provide a greater understanding of the impacts of zebra mussels and spiny water fleas on food webs and fish in Minnesota lakes, and will facilitate better walleye management in the face of these invasions. Quantifying how these invaders disrupt food webs supporting walleye will allow managers to project realistic levels of walleye production. Additionally, understanding the most important prey supporting walleye will allow us to assess the vulnerability of each population to the impacts of invasion. This project will provide a critical supplement to the existing MNDNR Large Lakes program by incorporating the community and ecosystem-level data required for understanding the lake-wide impacts of AIS.

Summary Budget Information for Sub-Project 16:	Sub-Project Budget:	\$198,700
	DNR Portion: \$88,139	
	NRRI Portion: \$29,445	
	UMN Portion: \$81,116	
	Amount Spent:	\$197,568
	Balance:	\$1,132

Outcome	Completion Date
Activity 1	
1. Collect benthic macroinvertebrates from nearshore and deepwater lake bottom	10/2018
areas to quantify baseline isotopic positions to determine which fish feed on these	
invertebrates. To be done in Mille Lacs, Red, and Leech lakes in 2017 and in Cass,	
Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018. Co-	
Lead: MNDNR and NRRI	

2 Collect muscle tissue from fich sampled during fall gillnetting (nart of MNDNR large	10/2018
2. Conect muscle tissue from his sampled during fair grintetting (part of wirdow large	10/2018
lakes core sampling) of while Lacs, Red, and Leech lakes in 2017 and in cass,	
Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018. Fish	
targeted from this sampling include walleye, yellow perch, northern pike, cisco	
(where present), black basses, and other Centrarchids such as bluegill, black crappie,	
and rock bass (where present). Lead: MNDNR	
3. Collect age-0 walleye, age-0 yellow perch, and littoral prey fish in summer for	10/2018
isotopic analysis for food web assessment via seining in Leech and Red lakes in 2017	
and Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in 2018.	
Lead: MNDNR (including subcontract to NPS)	
4. Collect age-0 walleye, age-0 yellow perch, and littoral prey fish in summer for	10/2018
isotopic analysis via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI	-,
5. Process fish and invertebrate samples from Mille Lacs. Red. and Leech lakes in 2017	12/2018
and in Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in	,
2018 to prepare samples for stable isotope analysis. Processing includes dissecting	
zuisele tissue from small fish and combining invertebrate taxa across sites and	
tavanemia grauna as appropriate to angura sufficient hismass is available for stable	
iastene enclusis Load MNDND	
isotope analysis. Lead: MinDINR	
6. Process Zooplankton samples from Mille Lacs, Red, and Leech lakes in 2017 and	
from Cass, Kabetogama, Lake of the Woods, Rainy, Vermilion, and Winnibigoshish in	
2018 (part of MNDNR large lakes core sampling) to prepare for stable isotope	
analysis. Processing includes separating major taxonomic groups (spiny water flea,	
large native predatory zooplankton, large herbivores, small herbivores, etc.) Lead:	
MNDNR	
7. Quantify stable isotope composition of Carbon and Nitrogen of each trophic group	2/2019
collected by MNDNR in all study lakes using an external lab. This will provide the data	
for the food web analysis. Lead: MNDNR via subcontract to an external stable isotope	
laboratory.	
8 Determine how much food/energy is coming from nearshore versus open water	6/2019
habitats contributing to walleye production in each study lake, and how this varies	
with invasion status. Lead: MNDNR	
Activity 2	
1. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via	10/2018
seining in Leech and Red lakes in 2017 and Kabetogema, Lake of the Woods, Leech,	-
Rainy, Red. Vermilion, and Winnibigoshish in 2018. Lead: MNDNR (including	
subcontract to NPS)	
2 Collect age-0 walleve and age-0 vellow perch length and weight data in summer via	10/2018
2. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI	10/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille 	10/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs. Leech. and Red Lakes in 2017 to target additional littoral prev species sampling 	10/2018 10/2017
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI 	10/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data 	10/2018 10/2017 2/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including 	10/2018 10/2017 2/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) 	10/2018 10/2017 2/2018
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following 	10/2018 10/2017 2/2018 3/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR 	10/2018 10/2017 2/2018 3/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR 	10/2018 10/2017 2/2018 3/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) 	10/2018 10/2017 2/2018 3/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Activity 3 	10/2018 10/2017 2/2018 3/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Dissemination of findings – at least two presentations at stakeholder meetings, and including subcontract to a state and for meetings. 	10/2018 10/2017 2/2018 3/2019 6/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: 	10/2018 10/2017 2/2018 3/2019 6/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR 	10/2018 10/2017 2/2018 3/2019 6/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR Dissemination of findings – at least one presentation at stakeholder meetings, 	10/2018 10/2017 2/2018 3/2019 6/2019 6/2019
 Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: 	10/2018 10/2017 2/2018 3/2019 6/2019 6/2019
 2. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI 3. Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI 4. Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) 5. Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Activity 3 1. Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR 2. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI 	10/2018 10/2017 2/2018 3/2019 6/2019 6/2019
 2. Collect age-0 walleye and age-0 yellow perch length and weight data in summer via seining in Mille Lacs in 2017 and Cass in 2018. Lead: NRRI 3. Collect age-0 walleye and age-0 yellow perch diets in summer via seining in Mille Lacs, Leech, and Red Lakes in 2017 to target additional littoral prey species sampling in 2018 in support of Activity 1 littoral food web work. Lead: NRRI 4. Gather and organize historical MNDNR age-0 walleye and yellow perch growth data from each study lake for pre- and post-invasion comparison. Lead: MNDNR (including subcontract to NPS) 5. Analyze data to estimate changes in walleye and yellow perch growth following invasion of spiny water fleas and/or zebra mussels. Co-lead: NRRI and MNDNR (including subcontract to NPS) Activity 3 1. Dissemination of findings – at least two presentations at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: MNDNR 2. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI 3. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI 3. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI 3. Dissemination of findings – at least one presentation at stakeholder meetings, policy and planning meetings, conferences, and/or research showcase events Lead: NRRI 	10/2018 10/2017 2/2018 3/2019 6/2019 6/2019 6/2019

Sub-Project Status as of February 28, 2018

We successfully collected fish, benthic macroinvertebrates, and zooplankton from the three lakes targeted for 2017 (Mille Lacs, Red, and Leech lakes). Multiple species were obtained from multiple sites in each lake, which will allow us to characterize the food webs of these lakes with a high degree of accuracy. A total of 1,481 tissues samples were collected and are ready for stable isotope analysis (Activity 1).

We collated hundreds of thousands of historical fish records for historical data analysis of growth rates of age-0 walleye and yellow perch. We also collected additional age-0 fish from Mille Lacs. These data will be used to assess whether any changes have occurred in the growth rates of young fish corresponding to invasion by zebra mussels or spiny water flea. Diets of age-0 walleye and yellow perch were also collected and analyzed to ensure that our sampling of the food web included important diet items.

Finally, we have delivered three presentations describing our work in progress to MNDNR staff, stakeholders, and at the MAISRC showcase. Our project has been featured in the popular press and the University media.

An amendment was approved by LCCMR on 02/06/2018 to amend the sampling design based on the results of the first field season. In the original proposal, we planned to sample three of our nine study lakes in each of two study years. The remaining six study lakes were to be sampled only once. Under this proposed amendment, we would sample each of our nine study lakes one time. This proposed change will allow us to more fully characterize the food web of each lake to better understand ongoing and future impacts of zebra mussels and spiny water fleas. Additionally, the amendment changes the lab with which we will contract for our stable isotope analysis. We are pursuing permission to send our samples to the Cornell University Stable Isotope lab, which can analyze samples in our desired timeline and at lower cost. The amendment requires that funds are shifted between budget categories, though the overall budget remains the same.

Sub-Project Status as of August 31, 2018

We sent our fish, invertebrate, and zooplankton samples from Leech, Mille Lacs, and Red Lakes to the Cornell stable isotope laboratory for analysis. We have received a subset of results from these samples and begun developing a workflow for analysis to facilitate analysis of the complete dataset when it becomes available.

We analyzed age-0 walleye and yellow perch data from each of the 9 lakes. Preliminary results suggest changes in growth associated with zebra mussel invasion, but these results are heavily influenced by data from a single lake. We will collect additional data from Cass Lake that will provide further data to test the hypothesis that zebra mussel invasion negatively affects the growth rates of young of year fish.

Finally, we have delivered 3 presentations since January describing our work in progress to MNDNR staff and interested stakeholders.

Sub-Project Status as of February 28, 2019

We have collected fish, invertebrate, and zooplankton samples from all 9 lakes and sent them to the Cornell stable isotope laboratory for analysis. We have received most of our stable isotope composition results from these samples and have initiated preliminary analysis of the large lake food webs and how energy sources supporting walleye differ among lakes.

We analyzed age-0 walleye and yellow perch data from each of the 9 lakes through 2018. Our results demonstrate slower growth of walleye in their first year of life in lakes invaded by zebra mussels and spiny water flea. Yellow perch growth rates were somewhat slower in lakes invaded by zebra mussels, but these differences were not statistically significant. We detected no changes in yellow perch growth associated with spiny water flea invasion. We are writing a manuscript reporting these results to be submitted before the completion of this project in June 2019.

Finally, we have delivered 3 presentations since July describing our work in progress at the MAISRC showcase, a professional scientific conference, and one lake association meeting.

Final Report Summary:

Minnesota lakes experience ecosystem-level changes following the introduction of aquatic invasive species (AIS), specifically zebra mussels and spiny water fleas. However, the effects of these AIS on fish are poorly understood and vary among lakes. We evaluated the impacts of zebra mussels and spiny water fleas on walleye and yellow perch in Minnesota's nine largest walleye lakes. We compared age-0 walleye and yellow perch growth over 35 years, including pre- and post-invasion. Age-0 walleye were >10% smaller at the end of summer following invasion by either AIS. Age-0 yellow perch growth decreased following zebra mussel invasion, although this effect was not statistically significant. Smaller length at the end of the growing season was associated with decreased survival to later life stages for walleye in 7 of the 9 study lakes.

We used stable isotope analyses to understand which habitats and food resources support walleye and other fish and to assess their position in the food web in each lake. We documented a high degree of variability in the resources supporting all life stages of walleye. In general, juvenile walleye relied on offshore prey resources in invaded lakes. Combined with reduced growth rates, these results suggest that as zooplankton food resources decline following invasion, young walleye are not sufficiently accessing alternative prey resources to maintain pre-invasion growth rates. Variability in walleye diets among lakes may reflect differences in lake productivity or morphology, not necessarily the presence of AIS.

Our results demonstrate that zebra mussels and spiny water flea influence the growth rates of age-0 walleye and that a wide range of food resources and habitats support walleye in these lakes. Declines in growth rates of young walleye are an early signal of potential negative effects on walleye. This information can guide managers on the most effective and sustainable walleye harvest and stocking strategies in invaded lakes.

SUB-PROJECT 17: Building scientific and management capacity to respond to invasive Phragmites (common reed) in Minnesota

Project Manager: Daniel Larkin

Description: European strains of common reed (*Phragmites australis*), a highly invasive wetland grass, have been introduced to multiple locations in Minnesota and appear to be spreading. Invasive populations of *Phragmites* can have strong negative impacts on biological diversity, wildlife, habitat quality, and recreation. Thus far, there have been no systematic attempts in Minnesota to map and monitor spread of invasive *Phragmites* and develop coordinated control efforts. **The aims of this project are to: 1**) **Map the current distribution of invasive** *Phragmites* **in Minnesota, 2**) **Determine its capacity for further spread in Minnesota, and 3**) **Formulate and disseminate model management protocols for this species.** The products of this work will support a comprehensive statewide response to this aquatic invasive species (AIS).

Like many AIS, *Phragmites* does not quickly spread immediately after introduction. The initial barrier to rapid spread is overcome when *Phragmites* can produce viable seed—in addition to its ability to spread vegetatively. This occurs when there is enough genetically diverse *Phragmites* on the landscape to support sexual reproduction. In Minnesota, seed production may also be limited by climate because of our relatively short growing season. Once viable seeds start spreading by wind and water, eradication is no longer feasible and control is much more difficult and expensive. Compared to other Midwestern states, we have relatively little invasive *Phragmites*, but this is changing. The window of opportunity to limit invasion in Minnesota is now. For this reason, it is crucial to map the current distribution of invasive *Phragmites* in Minnesota, assess its potential for further spread, and promote coordinated control and spread prevention efforts.

The distribution of invasive, European *Phragmites* in Minnesota is unknown because it is not easy for non-experts to distinguish it from native *Phragmites*. *Phragmites* is a "cryptic" invader in the U.S. because there are both native and non-native lineages here. Native *Phragmites* is an important component of wetlands that

can be displaced by invasive *Phragmites* and harmed by indiscriminate control efforts that do not distinguish invasive from native forms. Resource managers need support in distinguishing and targeting the invasive.

An efficient statewide response to *Phragmites* requires effective management techniques for different invasion scenarios found in Minnesota. For example, treating a large infestation in a high-quality wetland presents different challenges than a new infestation along a roadside. We will develop management protocols that identify and communicate optimal responses to different scenarios. These protocols will consider different factors, such as: How large is the population? Is it producing seed? Is the invaded site connected to other water bodies? Is the population a threat to resources of special concern such as wild rice waters?

The proposed project will generate critical data on statewide distribution and reproduction of invasive *Phragmites*. We will collaborate with external partners to use findings to respond to *Phragmites* invasion. We will also leverage a separately funded workshop for managing *Phragmites* in Minnesota. This workshop will engage resource managers from state, federal, and other agencies and will inform the proposed project by helping us identify invasion scenarios in the state and key areas of uncertainty. Project partners will also help us focus capacity-building efforts on solutions that are feasible within the context of their agencies' broader missions. Management protocols will be developed for different *Phragmites* invasion scenarios and disseminated to partner agencies and other stakeholders through in-person meetings, webinars, and online resources. While this project is focused on invasive *Phragmites*, this approach to research-management collaboration will serve as a model that could be applied to other invasive species issues. In particular, use of a collaborative network ("crowdsourcing") for sampling statewide distribution and development of custom response protocols for different invasion scenarios will be applicable to other invasive species.

Revised ENRTF Budget:	\$283,568
Amount Spent:	\$269,773
Balance:	\$13,795

Outcome	Completion Date
Activity 1	
1. Adapt GLEDN (EDDMapS) portal and develop submission system	August 15, 2017
2. Morphological identification and genetic fingerprinting	December 15, 2018
3. QA/QC crowdsourcing/identification approach	May 1, 2018
4. Publish/update distribution map for non-native Phragmites	November 15, 2018
Activity 2	
1. Microsatellite results to quantify genetic diversity of subset of statewide	December 15, 2018
populations	
2. Collection of seed heads from subset of populations	February 15, 2018
3. Evaluation of seed viability from subset of populations	June 15, 2018
Activity 3	
1. Project website	May 1, 2018
2. Project webinars	April 15, 2019
3. Decision making resources and meetings	June 30, 2019
4. Assessing potential for landscape-scale Phragmites control	June 30, 2019

Sub-Project Status as of February 28, 2018

Our invasive *Phragmites* early detection and response effort ("MNPhrag") engaged 155 volunteer observers to assist us in searching for populations of invasive *Phragmites* throughout Minnesota (Activity 1). This crowdsourcing approach, combined with our project staff's own search efforts throughout the state, resulted in more than 290 populations of *Phragmites australis* (both non-native and native) being documented in fall 2017. Plant samples and/or reports were submitted by 50 observers and project staff. Morphological and genetic analyses were then used to confirm the identification of the samples as either native or non-native. Of the submitted reports, 188 have already been confirmed or are suspected to be invasive *Phragmites*. Our project has identified populations of invasive *Phragmites* in 28 different counties to date. More than 100 of the

occurrences of invasive *Phragmites* are closely geographically associated with rural wastewater treatment plants permitted to use non-native *Phragmites* in their dewatering basins. In general, most populations of non-native *Phragmites* occurred in roadside and/or wetland habitats.

In the next phase of the project (Activity 2), we will assess seed viability of invasive *Phragmites* populations from 9 regions throughout the state that differ in growing season length and other climatic factors that may influence potential for development of viable seed. Seed heads from 48 populations were collected in December 2017 and January 2018 for this assessment by project staff.

We have also initiated work related to Activity 3 (building response capacity). A graduate student research assistant has been hired for the spring 2018 semester to work on a literature review/synthesis of management strategies in preparation for a structured decision making workshop scheduled for April 9-11, 2018.

Sub-Project Status as of August 31, 2018

We continued to accept reports and vouchers of invasive *Phragmites* throughout the winter and have sent out an update with a request that our volunteers continue to report new populations of invasive *Phragmites* in summer and fall 2018 (Activity 1). The MNPhrag mailing list continues to grow as more agency staff and citizen scientists learn about the effort to document invasive *Phragmites*. In addition, we were invited to speak to agency staff at both DNR and USFWS regional meetings and presented at the State of Water conference, which targeted lake association members and lake managers. Through our project, we have documented more than 200 populations of invasive *Phragmites* in 33 counties.

We performed initial testing of seed viability in relation to climatic factors (Activity 2). Most of the 33 populations tested produced viable seed. There was a significant effect of latitude, with populations further south having greater reproductive potential in terms of both seed numbers and seed viability. We will perform additional seed viability assessment in fall 2018 to increase the robustness of these results.

We created resources for *Phragmites* control efforts (Activity 3) through development of management recommendations, convening of a structured decision-making workshop with agency staff, and launch of a new website providing information on invasive *Phragmites* identification, impacts, and control.

MASIRC and LCCMR also approved an amendment request and budget adjustment to add an additional task (Outcome 4) to Activity 3 and hire an additional person to complete the work.

Sub-Project Status as of February 28, 2019

The MNPhrag program continued to accept reports and vouchers of invasive *Phragmites* throughout the 2018 growing season and has encouraged its volunteers to continue to document and report new populations of invasive *Phragmites* through the end of the project (Activity 1). Additional reporters have been engaged and added to the MNPhrag mailing list during the last reporting period. We had several opportunities to speak to citizens, agency staff, and researchers at workshops, meetings, and conferences. To date, we have documented and verified nearly 400 populations in 38 counties.

Seed head samples have been collected from the subset of invasive *Phragmites* populations that were sampled in January 2018 in order to repeat the seed viability assessment conducted last winter (Activity 2). We are processing seed heads at this time. This additional seed viability assessment will increase the robustness of our results, showing whether the patterns we observed previously are consistent year to year, i.e., under a different annual climate. In addition, leaf tissue was collected in August 2018 from the same populations to assess their genetic structure and diversity, which has important implications for sexual reproduction potential. These results are forthcoming.

We are currently drafting an assessment of capacity and needs for a strategic response to invasive *Phragmites* and have held meetings with several partners critical to collaborating on and supporting such a response effort (Activity 3). Our assessment highlights 12 distinct "*Phragmites* regions" of the state—based on current distribution of invasive *Phragmites*, stakeholder capacity, and potential for coordinated regional partnerships. It describes for each region its *Phragmites* invasion context; suggests opportunities for coordination between local, regional, and state entities; funding sources; control approaches and cost estimation; and training needs. We expect to have a complete draft by late winter and will be hosting a webinar and engaging partners in the coming months to solicit feedback. We have also met with partners at MNDNR and MPCA to share project findings and begin discussing response approaches, and are providing information to the Noxious Weed Advisory Committee to fill gaps in knowledge about invasive *Phragmites* that were identified as key areas of uncertainty during the last review of *Phragmites*' noxious weed classification, which is expected to be revisited during the next reporting period.

Final Report Summary:

MnPhrag is an early detection and response effort targeting invasive Phragmites australis (common reed) (www.mnphrag.org), with the goal of supporting landscape-scale, strategic management throughout Minnesota. We mapped the distribution of invasive Phragmites, investigated its spread potential, and developed strategies for coordinated response in collaboration with agency staff and other resource managers. We engaged professionals and citizen scientists in reporting suspected populations; conducted intensive search efforts in under-sampled regions; and revisited unverified reports from a web-based invasive species reporting system. Over 70 active observers helped us identify 435 invasive Phragmites populations statewide, and we showed that non-experts can reliably distinguish invasive from native *Phragmites* using an identification guide we developed (www.maisrc.umn.edu/identifying-phragmites). The value of this "crowdsourcing" approach to surveillance is reflected in most invasive stands we identified being small populations (90% are <0.25 acres), for which effective control is much more feasible. Invasive Phragmites is producing viable seed in Minnesota, which increases spread risk; however, the extent of seed production varies across populations, and there is still time to prevent further spread through sound, sustained control efforts. We are working closely with diverse stakeholders to support coordinated response efforts. Our work has also brought state agencies together to address crosscutting issues related to invasive *Phragmites'* regulatory status, including its use in some wastewater treatment facilities in "reed beds" for removing water from biosolids. We recently published an action plan outlining how Phragmites spread could be stopped and reversed in Minnesota; this assessment includes management recommendations, cost estimates, and region-specific response guidance (www.maisrc.umn.edu/ reversing-spread). Our findings reveal a window of opportunity to slow and reverse spread of invasive Phragmites, which would benefit Minnesotans by protecting vital natural resources. This approach to statewide surveillance, and framework for a coordinated, landscape-scale response, are strategies that could be applied to other invasive species issues in Minnesota.

SUB-PROJECT 18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota

Project Manager: Ray Newman

Description: Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most troublesome aquatic weeds in North America. In addition to suppressing native plant communities, inhibiting recreation and use and suppressing property values, hundreds of millions are spent annually on its control, with over \$2 million per year in Minnesota. Recently concern has arisen for hybrid watermilfoil, which may respond differently to management or be more invasive than pure Eurasian. This study will determine the distribution and extent of the hybrid milfoil problem in Minnesota to define the scope of the problem and develop specific hypotheses that can be tested with future studies to improve management.

In Minnesota, Eurasian watermilfoil was first found in Lake Minnetonka in 1987 and White Bear Lake in 1988. It now occurs across the state in more than 300 waterbodies in 35 counties. Permits are issued for larger

scale control of Eurasian watermilfoil on 80 to 100 lakes per year in Minnesota, and most control efforts are with auxinic herbicides: 2,4-D and triclopyr.

Eurasian watermilfoil hybridizes with the native northern watermilfoil (*M. sibiricum*). Hybrids are difficult to distinguish from Eurasian watermilfoil, and as a result, populations identified as "Eurasian watermilfoil" may be composed of "pure" Eurasian watermilfoil, hybrids, or both. Although managers and aquatic botanists increasingly recognize Eurasian and hybrid watermilfoil as distinct taxa, they are not frequently distinguished when it comes to operational management strategies, control tactics, or evaluations of management actions. As a result, there is still uncertainty regarding whether, and to what extent, hybrid watermilfoils may exhibit unique ecologies and/or pose distinct challenges for management (e.g., will they exhibit faster growth and/or herbicide tolerance?).

However, there is increasing concern that hybrid watermilfoil might be more invasive than Eurasian watermilfoil. A laboratory study found that hybrid watermilfoils in Michigan had faster vegetative growth rates and increased tolerance to 2,4-D, on average, compared to Eurasian watermilfoil. Similarly, a field study found that efficacy of the auxinic herbicides 2,4-D and triclopyr were much greater on pure Eurasian compared to hybrid watermilfoil in Houghton Lake, MI (93% versus 44% reduction, respectively). Overall, the number of quantitative comparisons of Eurasian watermilfoil and hybrids is low, and more comparisons are needed to determine whether generalities exist in terms of differences between Eurasian watermilfoil and hybrids.

Recent molecular genetic studies demonstrate that genetic diversity is much higher in watermilfoils than previously recognized. Although clonal reproduction is common, sexual reproduction is also common, as indicated by genetic diversity, including evidence for sexual reproduction by hybrid watermilfoils. Genetic variation is generally higher for hybrid and northern watermilfoil compared to Eurasian watermilfoil. It is therefore possible that differences among Eurasian watermilfoil and hybrids depend on the specific genotypes being compared.

Several studies have identified clear tolerance by some hybrid genotypes to some herbicides, including fluridone and the auxin mimics 2,4-D and triclopyr, whereas studies on other genotypes have not found any evidence for tolerance. Because the properties of populations likely vary as a function of their genetic composition, an important first step in being able to predict the growth and control response of populations is to delineate and quantify genetic variation within and among populations. These observations regarding hybrid watermilfoil illustrate the need for a structured effort to document the occurrence and distribution of hybrid milfoil in Minnesota.

Although hybrid watermilfoil has been documented in Minnesota since the early 2000s and additional occurrences have since been reported, a comprehensive assessment of the distribution and genetic diversity of hybrid watermilfoil in Minnesota has not been conducted. We have identified 12 lakes with verified hybrid watermilfoil (out of 330 + waterbodies with verified Eurasian, which includes hybrids). All of these lakes are in the Twin Cities Metro Region (Anoka, Dakota, Hennepin, Ramsey and Washington counties), but few lakes outside the Metro Region have been genetically analyzed. Furthermore, analysis for specific genotypes has only been conducted on Christmas Lake and several bays in Lake Minnetonka and these analyses showed considerable diversity. Hybrid watermilfoil had 34 distinct hybrid genotypes compared to nine Eurasian genotypes and 24 northern watermilfoil genotypes. One hybrid genotype appeared to be more prevalent after bay-wide herbicidal control. There was also evidence that northern watermilfoil was restricted to shallower sites and Eurasian and hybrid were found in deeper water. The distribution and occurrence of hybrid milfoil is unknown around the state and even less is known about distribution of milfoil genotypes.

To address this gap, we will assess the distribution and occurrence of hybrid watermilfoil in Minnesota and determine relations to factors that may affect its ecology and management. Specifically, our project has the following objectives:

Objective 1: Describe the frequency of occurrence and the geographic distribution of hybrid watermilfoil in Minnesota in order to determine the extent of this AIS problem and evaluate factors that are relevant to its biology and management. Specifically, test whether it is a) geographically widespread versus restricted to the Metro Region, b) more likely to occur in lakes with native northern watermilfoil, or c) more likely to occur in lakes with a longer invasion history.

Objective 2: Delineate and quantify genetic variation in hybrids in order to determine the role different genotypes and genetic diversity might play in its distribution and management. Specifically, A) assess whether specific genotypes are associated with a) geography and distribution extent, b) invasion history, or c) management history. B) Determine whether genetic diversity or the occurrence of specific genotypes is related to a) local environment and aquatic plant communities or b) management history or actions.

To address these objectives, we will conduct a statewide survey of lakes infested with Eurasian watermilfoil to determine the occurrence and distribution of hybrid milfoil across the state. We will use molecular genetic techniques to identify hybrids and genotypes of hybrid, Eurasian and northern watermilfoil. Finally, we will conduct more detailed study on a small subset of lakes to determine the relationship of local scale factors such as depth and plant community with hybrid genotypes, and the influence of management actions to hybrid milfoil genetic diversity.

With the results of this study, we will be able to determine if hybrid watermilfoil is a widespread or limited problem, if there are few or many genotypes that are of potential concern, and if specific approaches will be needed to manage hybrid watermilfoil. We will be able to identify specific genotypes or populations in need of further study and develop specific hypothesis for future studies to test to improve management and effectively deal with hybrid milfoil in control programs.

Summary Budget Information for Sub-Project 18:	ENRTF Budget*:	\$221,375
	Amount Spent:	\$220,412
	Balance:	\$963

Outcome	Completion Date
Activity 1	
1. Select and sample 50-60 lakes across the state for milfoil, process and	August 2018
preserve samples and send material to Thum for genetic analysis.	
2. Extract DNA and identify plant taxa with internal transcribed spacer DNA	December 2018
sequence (ITS).	
3. Analyze distribution of hybrid and co-occurring milfoils across state.	March 2019
4. Develop a manuscript describing the distribution of hybrid milfoil and	June 2019
addressing the relationship of hybrid and Eurasian milfoil with geographic	
location, time since invasion, depth, and co-occurrence with northern milfoil.	
Activity 2	
1. Decide whether to use microsatellites and AFLPs versus SNPs to genotype	January 2018
plants.	
2. Analyze 25-100 DNA samples from each lake for identification of genotypes.	January 2019
3. Analyze distribution of genotypes and genetic diversity across lakes in	March 2019
relation to geography, invasion history and management	
4. Develop a manuscript describing the distribution of genotypes and genetic	June 2019
diversity.	
Activity 3	
1. Select and sample 10 lakes for intensive study	September 2018
2. Analyze DNA samples for identification of genotypes.	January 2019
3. Analyze intensive study lakes for relationships of genotypes and genetic	April 2019
diversity to depth, plant community and management actions.	
4. Develop a manuscript that addresses local scale factors associated with	June 2019
genotype occurrence or the response of hybrid genotypes to management	
actions.	
Activity 4	

1. Disseminate preliminary results at MAISRC showcase 2017, 2018 and	December 2018
coordinate with MAISRC Extension Specialist Dan Larkin and communicator to	
address hybrids and milfoil genetics on MAISRC website.	
2. Host meeting with stakeholders to present results and discuss management	April 2019
strategies	
3. Submit one or more manuscripts to peer-reviewed scientific journal(s)	June 2019

Sub-Project Status as of February 28, 2018

The project got started in summer 2017 and we were able to collect milfoil samples from 33 lakes. Due to the somewhat later than anticipated start date, we mostly sampled lakes in the Twin Cities Metro region, but we sampled a good coverage of lake types and age of infestation. At most lakes, we sampled 100 points for milfoil (Eurasian, northern or hybrid); we found no milfoil at one lake (previously known to be infested) but got a good distribution of samples at most of the lakes. Samples of all taxa were processed and have been shipped to the Thum lab for genetic analysis. Thum has started DNA extractions and completed ITS identifications on a subset of lakes. These results indicate that our visual determinations of milfoil taxon (hybrid, Eurasian or northern) are not always correct and corroborate the need for genetic analysis. Thum will complete the taxonomic identifications this winter and the genotyping by April. At that time, we will host a meeting with the DNR and cooperators to determine lakes to sample in summer 2018, including lakes for intensive analysis.

An amendment was approved by LCCMR on 02/06/2018 to re-budget resources to poster printing and publication charges. We did not budget for poster printing and publication charges but these are important to our outreach and scientific publication efforts. We will allocate \$200 out of the current Services – Office and General Operations that was for mailing and shipping. We currently have spent less on shipping than anticipated. If we later need more resources for shipping or publications we will request a re-budget from another budget category.

Sub-Project Status as of August 31, 2018

Genetic identifications of plants with ITS has been completed for up to 20 plants for each lake sampled in 2017. Eurasian watermilfoil was found 19 lakes, hybrid in 18 lakes, northern in 10 lakes and all three taxa in just one lake. A comparison of our visual identifications with the genetic IDs indicated that overall our visual IDs were correct 80% of the time but most of the miss-matches were hybrid misidentified as Eurasian or vice versa. Although we can often visually detect hybrids, genetic analysis is needed for certain identification. Genotypic characterization with microsatellites has also been completed for the samples identified with ITS. Northern watermilfoil was most diverse with different genotypes in each lake and generally several different genotypes within a lake. Only three genotypes of Eurasian watermilfoil were found; one that was widespread and two others that occurred each in a different lake. Hybrid watermilfoil showed intermediate diversity; most lakes with hybrid had only one genotype of hybrid but several bays in Lake Minnetonka had 5 to 7 different genotypes. One hybrid genotype was found in 6 lakes in the northeast metro; most other lakes had unique hybrid genotypes. The Thum lab will process additional samples from lakes that had more than one taxa or different genotypes this summer.

We selected and sampled 5 treatment and 5 control lakes with point intercept surveys (generally 150 or more points) to characterize the genetic composition and plant community structure. Treatment lakes were subjected to a range of herbicide treatments including fluridone, 2,4-d, and ProcellCOR. We will resample these lakes in August to assess changes in relation to management or changes over time.

We have presented our results at several local and national meetings addressing lake users, managers and scientists and have been interacting with the DNR, consultants and applicators in lake selection. We will start surveying additional lakes for the presence of hybrids in July to further characterize the distribution of taxa and genotypes in the state.

Sub-Project Status as of February 28, 2019

Genetic identifications of plants has been completed for up to 20 plants from 31 lakes sampled in 2018. Across both years we sampled 62 lakes and found Eurasian in a total of 43 lakes, hybrid watermilfoil in 27 lakes, and northern in 23 lakes; all three taxa were found in four lakes. Overall most lakes tend to either contain just EWM (29%) or just hybrid (21%). This indicates that a lake does not necessarily have to have Eurasian or northern in order to have hybrid present.

Amongst the three taxa, EWM was the least diverse. Overall we have identified 8 Eurasian genotypes, 76 northern genotypes, and 57 hybrid genotypes in Minnesota. For EWM most of the lakes sampled in 2018 (21 lakes) contained the same genotype that was the dominant genotype within the lakes sampled in 2017. A total of 37 lakes overall contained this same genotype. There was no within-lake diversity for EWM, and overall we have found seven EWM genotypes that were different from the common widespread genotype. Hybrid watermilfoil showed intermediate diversity in comparison to EWM and NWM. Ten lakes had multiple hybrid genotypes, with there being particularly high diversity in one lake and in three bays of Lake Minnetonka. A few lakes shared common genotypes, which indicates some clonal spread of hybrids in Minnesota. There are numerous hybrid genotypes that could become problematic, but there are relatively few hybrid genotypes that have been more widely distributed. Northern watermilfoil was the most diverse, with most lakes having multiple different genotypes within lakes and no genotypes shared between lakes.

Ten lakes were intensively sampled based on recommendations by the DNR, consultants and applicators. Five treatment lakes and five reference or control lakes were surveyed in 2018 to characterize the plant community and milfoil genotypes to assess the response to herbicide treatment and characterize the native plant community. The lakes with a lake-wide fluridone application both had significant decreases in milfoil abundance following treatment, with almost complete elimination of milfoil (<2% frequency remaining). The lakes with 2,4-d and ProcellaCor had more focused treatments and less overall control. One lake treated with ProcellaCor needed a second treatment in the fall to further target the milfoil population. It is unknown if the poor response to it was due to application issues or the presence of tolerant genotypes.

We presented our results at several local and national meetings addressing lake users, managers and scientists and have been interacting with the DNR, consultants and applicators in lake selection. We had a productive meeting with applicators, consultants and DNR staff to discuss results and strategies to address key management questions during the MAISRCshowcase and users are keenly interested in our results.

Final Report Summary:

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most problematic invasive aquatic plants in Minnesota. It can hybridize with the native northern watermilfoil (*M. sibiricum*) and reproduce sexually. Previous studies show that some genotypes of hybrid are resistant to specific herbicides and some may be more invasive. We determined the distribution of hybrid, Eurasian, and northern watermilfoil in Minnesota and assessed factors related to this distribution. We also assessed genetic variation (diversity) and distribution of specific genotypes and began an assessment of the response of watermilfoil and genotypes to management with herbicides. We sampled 64 lakes across the state stratified by county, size, and duration of infestation and collected milfoil from random points. The DNA from the milfoil samples was analyzed to determine taxon (Eurasian, northern or hybrid) and specific genotypes.

We found Eurasian in 43 lakes, hybrid in 28 lakes, and northern in 23 lakes. Hybrid was much more common in the metro, whereas Eurasian was broadly distributed. Northern watermilfoil was the most diverse with 84 genotypes, none shared across lakes. In contrast, we found one widespread genotype of Eurasian and six others found in indivdual lakes. Hybrid was intermediate in diversity with 53 genotypes; most lakes had only 1 unique genotype but 40% had multiple hybrid genotypes. Several genotypes were found in multiple lakes indicating clonal spread. The high diversity of hybrid watermilfoil indicates there is much potential for selection of

problematic genotypes that are resistant to herbicides or that are competitively superior. There are numerous hybrid genotypes that could become problematic, but few have been widely distributed. We have not yet identified any clearly problematic genotypes in Minnesota but lakes with unexplained treatment failures, and populations with high diversity should be assessed. We will implement a strategy to identify and test problematic genotypes in Phase II of this project – MAISRC Subproject 18.2: Genetics to improve hybrid and Eurasian watermilfoil management.

SUB-PROJECT 19: Decision-making tool for optimal management of AIS

Project Manager: Nicholas Phelps

Description: Effective management of aquatic invasive species (AIS) in complex and dynamic systems, considering variable needs, values, and constraints, has proven difficult. AIS managers at the local and state levels urgently need science-based tools to inform planning and decision-making. For example, mathematical and optimization models using robust and updated information can be used for developing effective intervention strategies, predicting impacts, testing what-if scenarios, increasing stakeholder buy in, and designing cost-effective surveillance programs to mitigate and prevent AIS spread. We have been moving in this direction with previous and ongoing research led by the Project Manager and collaborators to describe environmental suitability and pathways of spread for high priority AIS. We have reached a point where the previously developed risk maps could be incorporated into dynamic system models to visualize risk and evaluate optimization approaches for management.

The aim of this proposal is to build upon and refine previous research to develop and deploy a decision-making tool for optimal management intervention on a county and statewide scale to minimize the spread of high priority AIS.

Based on the dynamics of AIS and the systems in which they live and move, we will develop models to forecast the invasion of zebra mussels and Eurasian watermilfoil in Minnesota at the lake level. These models will be subjected to strict verification and cross-validation to ensure confidence in model predictions. The risk scores for each waterbody will then be used to inform AIS management optimization models at the county level. Optimization models are a useful approach to identify a set of actions that make the best use of available resources while achieving a desired outcome. Therefore, in addition to the risk scores, values and management objectives such as types of lakes to prioritize for prevention (e.g. All lakes equally? Large/popular lakes?) will be incorporated to recommend the allocation of available funds and strategic locations for prevention and control activities to reduce the risk of new AIS introductions within each county. Similarly, cumulative risk models will be developed to help inform statewide allocation of the County AIS Prevention Aid, compared to the current approach of total boat ramps and parking spots. Local and state AIS managers will be engaged throughout the project to ensure consistency with management goals and realities. Ultimately, the models will be visualized through a user-friendly and interactive application for online or mobile viewing to empower AIS management stakeholders.

Summary Budget Information for Sub-Project 19:	ENRTF Budget*:	\$172,465
	Amount Spent:	\$80,469
	Balance:	\$91,996

Outcome	Completion Date
Activity 1	
1. Development and validation of multiplex network metacommunity (MnM) model	May 2018
2. <i>Result dissemination: MAISRC communications, scientific presentation, peer-reviewed publication</i>	August 2018

Activity 2	
1. Development of county-based AIS management optimization models	September 2018
2. Development of risk-based statewide funding allocation model	September 2018
3. Deploy models at AIS manager workshops	October 2018
4. Result dissemination: MAISRC communications, scientific presentation,	January 2018
peer-reviewed publication	
Activity 3	
1. Development of visualization tool for AIS management	April 2019
2. Deployment of visualization tool to AIS managers	June 2019
3. Result dissemination: MAISRC communications, peer-reviewed publication	June 2019

Sub-Project Status as of February 28, 2018

Project is progressing as expected, despite a small delay in data availability. The first step in developing AIS risk estimates for each lake in Minnesota is complete, with the creation of a hydromorphological network models. As hypothesized, the model suggests that while water connectivity is important (explains ~35% of distribution for ZM and EWM), other factors are clearly influencing the spread of AIS. In the coming months, we will be adding other variables, such as environmental suitability and boat movement, to increase complexity and predictability of the models. In addition, theoretical optimization model has been created to conceptually evaluate AIS management tradeoffs, considering prevention (focus on uninfested lakes), containment (focus on infested lakes), or a mix of the two. We have found with early conversations that the DNR's strategy has been largely focused on containment, while most local groups have largely focused on prevention. We will continue to explore various scenarios with two counties (likely Ramsey and Crow Wing) in the coming months.

An amendment was approved by LCCMR on 1/31/2018 to reduce one service contract identified in the budget and add another service contract. Under the new workplan, funding will be split \$15,000 to TheBlackTechGuy for app development and \$10,000 to SMART Solutions for Questions and Decisions model website and webservice in connection to the dynamically updated predictions of the multiplex network metacommunity model. This update does not change the scope of the project, timeline or overall budget.

Sub-Project Status as of August 31, 2018

This has been a productive phase of the project, with additional data made available with the completion of MAISRC Subproject #13. Significant progress has been made with the multiplex metacommunity model development. With the application of the model, we verified the importance of the Hydrologic Network (HN) to be higher for Zebra Mussel (ZM) than Eurasian Watermilfoil (EW); the latter seems more affected by local environmental variability and characterized by a more confined dispersal. ZM and EW fluctuate more proportionally to systemic runoff and local rainfall, respectively. Thus, runoff as an output from lakes informs a more dynamic risk determinant of species invasion vs. local lake features. Certainly, it is clear that it is not sufficient to consider only the environment as a determinant of a higher or lower chance of species invasion downstream or upstream an invasive population. Furthermore, these results emphasize once again the importance to consider physical basin boundaries rather than political lines for effective management. This paradigmatic shift creates some tension with the management of AIS because a basin can belong to different counties and decisions are typically taken at the county scale. These models are being incorporated into a new application that can be used to visualize risk of AIS.

We have also begun to evaluate 'optimal management scenarios' based on the data available for lake connectivity and suitability. We evaluated Ramsey and Washington counties to inform the location of a limited number of watercraft inspection sites to intercept the largest number of 'at-risk' boats. The mathematically optimal results have been counter-intuitive to some, demonstrating this as a valuable exercise for managers. We will continue to develop these models for other counties and a statewide approach in the months to come.

Sub-Project Status as of February 28, 2019

The January 31, 2019 status update for this subproject has been delayed due to the federal government shutdown from December 2018 – January 2019. A status report is currently being drafted and will be included in the next update.

Final Report Summary:

Understanding the patterns of historic AIS invasion can provide the framework for forecasting future invasions. To that end, we used a big data approach to combine hydrologic connectivity and boat movement to create a multiplex metacommunity model for both zebra mussel and Eurasian watermilfoil. We found that the hydrological corridors are important pathways of spread, even more so that previous research has suggested. While overland dispersal of AIS via boater movement is still a significant factor, additional management strategies should be developed to include intervention of hydrological pathways.

Using connectivity networks of boater movement, we developed county-based AIS management optimization models that prioritize inspection locations that will intercept the highest number of 'risky boats' (e.g. moving from infested to uninfested lakes). We piloted the models in Crow Wing, Ramsey, and Stearns Counties and had a very productive collaboration with county managers and citizen advisory boards during the development and evaluation for each. Ultimately, the application of this approach was well received and helped inform allocation of their inspection hours at the county level (for example: https://www.crowwing.us/1004/Aquatic-Invasive-Species-AIS).

Dissemination and usability of the models was a priority of this project. We created online tools to 1) visualize the spread risk for zebra mussels and Eurasian watermilfoil based on model predictions made in Activity 1, and 2) visualize and modify the decision optimization model at the county level based on management thresholds or funding availability. These tools and more detailed descriptions of the project has been disseminated through inperson stakeholder meetings and presentations to diverse audiences, including managers, researchers and the public.

SUB-PROJECT 20: A Novel Technology for eDNA Collection and Concentration

Project Manager: Abdennour Abbas

Description: In a very recent informal survey of Minnesota Department of Natural Resources (DNR) managers and researchers, it became evident that a major need for aquatic surveys is not developing new detection methods but improving the sampling tools. A number of promising techniques are available today including environmental DNA (eDNA) amplification using PCR and LAMP assays or metagenomics sequencing. However, the major problem is that the results obtained from eDNA techniques do not always correlate with traditional netting data (e.g., some species are missed, or abundance relationships are weak) in part due to sample size and quality. Current attempts to use eDNA for detecting species typically require numerous samples from each site, especially when detecting rare species such as a newly invading aquatic invasive species (AIS). Improving detection probability or precision of abundance estimates by increasing the number of samples leads to high costs using current sampling methods. To convert these techniques into reliable species detection tools and enhanced quantitative tools (offering a good correlation between eDNA copies and species abundance) new efficient and cost-effective sampling methods need to be developed.

Environmental DNA (eDNA) is the genetic material (genomic DNA) obtained directly from environmental samples such as soil and water. The collection of eDNA is an emerging cost-effective alternative or complement to traditional sampling (mostly nets and electrofishing for fish, visual surveys or net tows for inverts). When combined with DNA sequencing technology or quantitative PCR (qPCR), eDNA could represent a cost-effective and reliable tool for biodiversity monitoring, including species detection and abundance. However, current eDNA sampling methods may result in significant false positives or negatives that prevent wide-spread adoption for management purposes. To avoid failure to detect a species across an entire site of interest (e.g., lakewide,

stream reach), several to tens of individual water samples are typically collected. The need for a large number of samples is greatest when targeting rare species, such as a newly invading AIS where limited concentrations of DNA may be present in the water. Our improved sampler aims to reduce these per sample costs directly but could also provide savings elsewhere, including reduced staff time per site and ability to sample more locations in a single trip.

This proposal aims at developing a novel aquatic eDNA collection and concentration technology for more efficient, reliable and cost-effective screening for not only invasive aquatic organisms and pathogens but also native and endangered species. The technology would significantly enable and empower aquatic ecosystem survey and management programs in Minnesota.

Specific aims: The proposed eDNA aquatic sampling technology will be developed and tested in three major steps:

- 1. Develop an eDNA nanofilter that specifically and rapidly captures nucleic acids (DNA, RNA) from water
- 2. Develop a housing system for the nanofilter to allow field deployment and continuous sampling of large water volumes or large areas
- 3. Verify increased eDNA sampling efficiency of the new device in field settings (proof-of-concept)

M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2013, Chp. 52, Sec. 2, Subd. 06a M.L. 2017, Chp. 96, Sec. 2, Subd.		2, Subd. 06a
Summary Budget Information for Subproject 20:	Subproject Budget: Amount Spent:	\$94,599 \$90,263	Subproject Budget: Amount Spent:	\$96,264 \$39,876
	Balance:	\$4,336	Balance:	\$56 , 388

Outcome	Completion Date
Activity 1	
1. Development of eDNA nanofilter using a polymeric membrane modified with	March 2019
nanotechnology	
2. Development of a housing system for the eDNA nanofilter	July 2019
3. Evaluation of the performance of the eDNA nanofilter	November 2019
Activity 2	
1. Collection of eDNA from selected locations	April 30, 2020
2. Sample analysis: quantitative PCR of collected samples	April 30, 2020
3. Dissemination of research findings to AIS managers, policy makers, and planners,	June 30, 2020
including at the annual Showcase event; coordination with MAISRC and Extension on media	
efforts and communications; and participation on 1-2 committees	

Subproject Status as of January 31, 2019:

The project is currently progressing as expected and no amendment is needed. A full-time researcher (category 4), Mr. Akli Zarouri and one undergrad student were hired to work on the project. Mr. Akli started his position on December 20, 2018. Both hires received on week-long research and safety training.

Currently, we are working on Phase 1 of Activity 1, related to the development of an efficient eDNA filter. This phase will be completed in March. Details of the technical progress of the development of an eDNA filter is provided below in the Activity 1 summary below. Activity 2 will be initiated early April 2019 as planned.

Subproject Status as of July 31, 2019:

The project is progressing as expected. We have successfully developed a new eDNA filter that captures > 90 % of DNA (our objective was 50%) within 10 seconds. The filter is a cellulose membrane functionalized with a polysiloxane polymer and put in contact with eDNA solution with concentration ranging from 10 ng/L to 1000

ng/L. The loading capacity of the new filter is up to 5 mg/g, meaning that 1 g of filter can capture up to 5 mg of DNA. This is a record-breaking capacity that enables the filtration of large volumes of water with one filter, knowing that surface water contains usually 10 ng/L of eDNA.

We are currently working on Phase 3 of Activity 1 that involves the development of a housing system for the eDNA filter to enable field use. This is expected to be completed as planned in November 2019.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 21: Early detection of zebra mussels using multibeam sonar

Project Manager: Jessica Kozarek

Description: Zebra mussels (*Dreissena polymorpha*) pose a serious threat to water supply and power plant infrastructure, and to Minnesota lake and river ecosystems, including native mussel species (Baker and Hornbach 1997). Current methods for detection and quantification of zebra mussel colonies rely on time consuming and expensive diving surveys, video imaging, or sampling of veligers (larvae) in the water column. Survey sampling design would be made much more efficient given spatially extensive information on the on the presence/absence of zebra mussel beds. Such remote sensing technology would also be useful for early detection and warning in rivers, lakes and reservoirs through routine monitoring, or to follow changes in zebra mussel density (boom or bust cycles).

This study will test the utility of swath mapping systems such as multibeam sonar for detecting and quantifying the abundance of invasive mussels at a very large scale. Multibeam sonar can map tens to hundreds of square kilometers of river or lake bed in a single day from a moving vessel. Ostensibly an instrument for bathymetric mapping, each sounding from a multibeam sonar also records the echo from the bed surface, which can be analyzed to provide information about the roughness and composition of the ensonified bed. This echo can be used to reliably distinguish among various substrates (Brown et al., 2011). Acoustics are also increasingly being used to map and monitor shellfish (e.g. Sanchez-Carnero et al. 2014) and submerged vegetation (e.g. Buscombe et al., 2017). There is a strong likelihood that mussels have a distinct acoustic response (echo) compared to their surrounding substrate. If so, this acoustic signature can be readily used to detect and map zebra mussel beds at cm to m resolution in any navigable waterway of sufficient water depth.

This study will define the methodology needed to detect, distinguish and quantify mussels from a moving vessel by studying backscattering of sound by mussels and common mussel-supporting substrates. Mussels are soft-bodied invertebrates with hard shells. The acoustics of backscattering by mussels might depend on many physiological and morphological factors such as size, shape, shell thickness/roughness/composition, and the composition of soft tissues. In concert, these factors manifest as differences in scattering due to differences in roughness and hardness. It should therefore be possible to discriminate between different species of mussel (zebra mussels vs. native species) using acoustics alone, or acoustics in combination with measurable environmental variables that govern the spatial distributions of mussels. In lakes and rivers, this methodology will enable the scanning of large areas for the early detection of zebra mussel colonies. In river systems, it could be applied to detect longitudinal changes in zebra mussel populations downstream from a source population to evaluate the role of downstream drift in zebra mussel spread.

The first phase of this study, laboratory experiments, is designed as a proof-of-concept to utilize multibeam sonar to distinguish amongst substrate, native and zebra mussels in a controlled setting. We will study the acoustic backscattering properties of zebra mussels (*Dreissena polymorpha*) and native mussels, Threeridge (*Amblema plicata*), under controlled laboratory settings. Experiments in self-contained tanks at the St. Anthony Falls Laboratory will be used to determine the acoustic parameters that will maximize the discrimination between mussels and substrates. Following this study, a second research phase is planned to validate and further develop methodology in the field. Field measurements will allow the incorporation of a larger range of variables (mussel density, mixed substrates, water depth, etc.), once methodology has been tested in carefully controlled laboratory conditions.

Summary Budget Information for Subproject 21:	Subproject Budget:	\$96,549
	Amount Spent:	\$96,175
	Balance:	\$374

Outcome	Completion Date
1. Acoustic parameters to detect zebra mussels	June 2019
2. Acoustic parameters to detect native mussels	June 2019
3. Effect of substrate on detection	June 2019

Subproject Status as of January 31, 2019:

We successfully completed the planned lab experiments over 4 weeks in September 2018. Using the data, we have developed machine-learning-based substrate classifiers hypothetical situations of abiotic (bare) and biotic (mussel-supporting) substrates. The input into each model is measured backscattering strength of the bed over prescribed combinations of several acoustic frequencies and pulse lengths. The model output is the likelihood of each substrate class. Each model is trained only on distributions of uncalibrated acoustic backscatter measured in the lab over ten unique substrates, namely: 1) sand, 2) mix sand-gravel (MSG); 3) gravel; 4) sand-supported *A. plicata*; 5) MSG-supported *A. plicata*; 6) gravel-supported *A. plicata*; 7) sand-supported *D. polymorpha* (low density); 8) sand-supported *D. polymorpha* (high density). Phase I, experiments to examine the feasibility of using multibeam sonar to detect zebra mussels, is considered complete when the following objectives have been met: * indicates objective has already been met

- 1. Conduct lab experiments (summer 2018)*
- Develop an empirical substrate classifier based on measured uncalibrated backscatter (fall/winter 2018)*
- 3. Develop an analytical substrate classifier based on measured calibrated backscatter (spring 2019)
- 4. Develop a prototype field protocol for zebra mussel detection (spring/early summer 2019)
- 5. Write and disseminate findings (spring/early summer 2019)

Final Report Summary:

Zebra mussels pose a serious threat to Minnesota lake and river ecosystems. However, monitoring zebra mussel populations is challenging because current methods for detecting and counting zebra mussel colonies rely on time consuming and expensive diving surveys, video imaging, or sampling of veligers (larvae), which limits the areas surveyed. Remote sensing techniques have been shown to quickly and efficiently gather spatially extensive information. Using this technology to detect zebra mussels would likely be much more efficient and more effective than traditional methods and could be used for early detection and warning in rivers, lakes and reservoirs and to track changes in zebra mussel density.

This project was the first phase of research designed to test the utility of a swath mapping system, multibeam sonar, for detecting the presence and abundance of invasive mussels. Laboratory experiments were conducted to test the feasibility of using multibeam sonar to distinguish zebra mussel containing substrates. Acoustic backscatter data were collected in a two meter deep tank over sand, gravel, and mixed substrate containing high and low densities of zebra mussels and with native mussels using combinations of different sonar settings (frequencies and pulse lengths). Machine-learning was used to differentiate the acoustic backscattering signatures in a data-driven substrate classifier approach. Using these methods, we were able to classify substrate by size and mussel density. Classification errors decreased with more sonar settings. For minimum errors of less than 20%, 8 sonar settings are required, and for minimum errors of 10% or less for all substrates, 12 sonar settings. Each sonar setting corresponds to a separate boat survey of an area with a multibeam sonar in the field. Therefore, the next phase of this research is to further develop and test multibeam sonar monitoring approaches in the field (MAISRC Subproject 21.2: Field validation of mulitbeam sonar zebra mussel detection).

SUB-PROJECT 22: Copper-based control: zebra mussel settlement and non-target impacts

Project Manager: James Luoma

Description: Development of population level management techniques that have potential to reduce the environmental and economic impacts of zebra mussels while also protecting and preserving native species and habitats are critically needed. Targeting treatments to kill zebra mussel larvae and prevent their settlement also has potential use for zebra mussel containment or eradication in small, hydrologically isolated inland water bodies. Potential users include the MN DNR, local governmental units, and water infrastructure owners/users.

This project builds upon previous work (McCartney 2016) which identified the susceptibility of larval zebra mussels to much lower doses of copper compared to adult zebra mussels. This project will involve a 10-day, lowdose (60-ppb) copper treatment of an entire enclosed bay in Lake Minnetonka. St. Albans Bay (treated bay) and Robinson's Bay (control bay) will be sampled before and after application to determine treatment-related impacts on zebra mussel veliger abundance and settlement success. Treatment-related impacts to adult zebra mussels, algal, zooplankton, benthic invertebrates, and fish communities will be assessed. The three main objectives in this project are: 1) evaluate the efficacy of low-dose copper treatments to control populations of zebra mussel veliger larvae, 2) evaluate the use of low-dose copper treatments to suppress zebra mussel larval settlement, and 3) evaluate the effects of low-dose copper treatments on native aquatic animals and algal biomass.

	Balance:	\$4,430	Balance:	\$42,003
	Amount Spent:	\$62,436	Amount Spent:	\$106,457
	(USGS Portion: \$12,428)		(USGS Portion: \$121,790)	
Information for Subproject 22:	(UMN Portion: \$54,438)		(UMN Portion: \$26,670)	
DRAFT Summary Budget	Subproject Budget:	\$66,866	Subproject Budget:	\$148,460
	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	

Outcome	Completion Date
Activity 1	
1. Refine methods to assess zebra mussel settlement	December 2018
2. Complete acquisition contract for EarthTec QZ	May 2019
3. Develop project protocol and obtain necessary permits for application and test	May 2019
cages	
Activity 2	

1. Conduct pretreatment collection of veliger/zooplankton tows, benthic invertebrate	July 2019
samples, water chemistry samples, secchi disk readings, and chlorophyll samples.	
2. Placement of buoys, nontarget fish and unionid mussels, adult zebra mussels, and	July 2019
zebra mussel plate samplers in control and treated bays.	
3. Entire bay applications of EarthTec QZ over 10 days, consisting of 5 independent	August 2019
applications.	
Activity 3	
1. Conduct post-treatment collection of veliger/zooplankton tows, benthic	August 2019
invertebrate samples, water chemistry samples, secchi disk readings, and chlorophyll	
samples.	
2. Conduct survival assessments of adult zebra mussels, unionid mussels and fish	August 2019
3. Complete assessments of settlement success on plate samplers	December 2019
4. Complete data entry, proofing, and summarization	January 2020
5. Prepare study report and peer-reviewed manuscript	June 2020

Subproject Status as of January 31, 2019:

Since work plan approval in November 2018, the project teams at the USGS and MAISRC have been working on administrative set-up for project budgets and subawards and refining methodology for the 2019 field season. Project activities detailed in the workplan and spending have not yet begun.

Subproject Status as of July 31, 2019:

The project teams at the USGS and MAISRC have completed action items under Activity 1 and have initiated action in Activity 2 to include buoy and settlement sampler placement and all preparations leading up to application of the EarthTec QZ. Pretreatment sampling is scheduled to begin on July 18, 2019 and test animals will be placed within the treated and control bays by July 21, 2019. Treatment applications are scheduled to begin on July 22, 2019 and be completed on July 30, 2019.

Additional non-sponsored funding was secured by MAISRC to enhance the data collection for the study. The additional labor provided by a graduate student will allow for more robust water sampling to allow for water copper concentration profiling and test animals will be analyzed for tissue residues after the exposure is completed. More information is provided in section VI.B.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 23: Public Values of Aquatic Invasive Species Management

Project Manager: Amit Pradhananga

Description: Emerging evidence shows that Aquatic Invasive Species (AIS) management can be used to restore ecosystem services. For example, management of the invasive common carp (*Cyprinus carpio*) can lead to increases in water clarity and declines in nutrient concentrations in a more cost-effective manner than other management practices (Vilizzi et al. 2015; Bartodziej et al., 2017). Yet, management of AIS is often not considered an option when planning ecosystem restoration. Even if the direct costs of AIS management are known, lack of information about the potential benefits of AIS management makes informed decision making

difficult. With an accurate assessment of the costs and benefits of AIS management strategies, as well as information on public perception, resource managers will be better prepared for the efficient investment of management resources. The overall goal of this project is to quantify and analyze the ecological and economic value of AIS damages and AIS management as they relate to ecosystem services (e.g., fishing, swimming, biodiversity, navigability). The specific objectives of this project are to:

- Assess the use and non-use values assigned to ecosystem services impacted by AIS. Use values are those values generated from using a resource, such as recreation values. Non-use values are those values generated even when a resource is not directly used-- the value a person has for a resource they never visit and never will visit. An example would be existence value—valuing a resource just for existing, or bequest value—valuing a resource for the benefit of future generations.
- 2. Investigate the costs and effectiveness of carp management as a strategy for water clarity restoration
- 3. Develop a flexible ecological and economic optimization modeling framework to inform AIS management decisions

We will employ a multi-pronged approach with five activities: estimating public benefits of AIS management (Activities 1 and 2), analyzing costs of carp management (Activity 3), and the development of a broad AIS analysis framework (Activity 4) which we will use to estimate efficient carp management (Activity 5). The main goal of Activities 1 (mail survey of residents and lakeshore owners) and 2 (onsite survey of recreationists) is to produce data which can be used to estimate the lost public value attributed to AIS. The on-site surveys will target recreationists to generate use values related to boating, fishing, swimming, and general hiking/wildlife viewing/enjoyment of nature. The third activity, a cost analysis, will focus on common carp, an established AIS with long management history. This activity will generate cost and effectiveness information for various methods of carp management, potentially including removal, prevention, and barriers. Activities 4 and 5 include the development of a programming framework both to analyze the data generated in activities one, two, and three, and to provide guidance for AIS management in other regions of the state.

This project will provide multiple benefits to stakeholders and natural resources throughout Minnesota, as well as other areas with AIS concerns. This project will provide both natural resource managers and water quality regulators with information that will help to prioritize AIS and water quality management projects, permitting them to make more effective use of limited conservation dollars. This project will quantify the dollar value of the public benefits of AIS management, as well as the costs of managing a specific AIS (i.e., common carp) for water quality outcomes. Expected outcomes of this project include a decision support tool that will help resource managers assess the costs and benefits of AIS management. Specific outcomes of the study include a comprehensive AIS valuation data compilation for use by other researchers, and an eco-economic programming model to predict the economic and ecological repercussions of using AIS prevention and control initiatives.

	Balance:	\$696	Balance:	\$59,589
for Subproject 23:	Amount Spent:	\$131,149	Amount Spent:	\$50,656
Summary Budget Information	Sub-Project Budget:	\$131,845	Sub-Project Budget:	\$110,245
	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	

Outcome	Completion Date
Activity 1	
1. Develop survey questionnaire for residents and lakeshore owners	January 31, 2019
2. Administer survey to 2,000 MN residents and lakeshore owners	July 31, 2019
Activity 2	
1. Develop the survey questionnaire for recreationists (e.g. boaters, anglers), sampling	April 30, 2019
plan, and sampling schedule	

2. Administer onsite surveys to recreationists at boat docks	September 30, 2019
Activity 3	
1. Compile list of management cases and supporting lake and watershed data in MN	January 31, 2019
2. Conduct preliminary cost-benefit analysis and identify data gaps	July 31, 2019
3. Finalize the database by scouring out-of-state data and conducting global literature	January 31, 2020
review	
4. Finalize cost-benefit analysis, submit manuscript, present the results to stakeholders	July 31, 2020
(e.g. Minnesota Association of Watershed Districts (MAWD))	

Subproject Status as of January 31, 2019:

We have made substantial progress in Activity 1 (general resident survey), Activity 2 (onsite survey of recreationists), and Activity 3 (cost-benefit of carp management). Because this is the first phase of this study, we conducted literature review to identify survey topics and questions (for Activities 1 and 2) from past research. We are currently developing the questionnaire that will be administered with Minnesota residents and lakeshore owners. We have also collected secondary data on lakes and AIS establishment from multiple sources (e.g., DNR, USGS). We developed, piloted, and revised a carp management questionnaire that will be used for data collection in Activity 3.

Subproject Status as of July 31, 2019:

We have made progress in Activity 1 (general resident survey), Activity 2 (onsite survey of recreationists), and Activity 3 (cost-benefit of carp management). For Activity 1, we developed a draft survey that will be administered with 2,000 residents across Minnesota. The survey is currently being reviewed by experts in survey design. For Activity 2, we developed the survey questionnaire, sampling plan, and sampling schedule. We have also hired and trained field surveyors. The survey is being administered at 6 lakes across Minnesota. For Activity 3, we developed and administered a questionnaire with watershed districts and other carp management agencies to collect information about cost estimates (for each management action) and water quality (clarity and Phosphorus) before and after AIS management.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 24: Genetic method for control of invasive fish species

Project Manager: Michael Smanski

Description: Invasive fish species present an estimated \$5.4 billion burden on our domestic economy, and much of that extends to the lakes and rivers of Minnesota. For example, the foraging habits of the invasive common carp, *Cyprinus carpio*, diminishes water quality, reduces vegetative cover and waterfowl numbers, and reduce the ability of lakes to absorb nutrients that enter water systems through agricultural runoff. Current control methods have not been able to stem the tide of invasive carp and other fish species, so improved strategies are needed. The overall goal of this project is to demonstrate a novel approach for controlling aquatic invasive species using invasive carp species as proof-of-concept. Success of this project would lead to its implementation in other aquatic invasive species (AIS), including Asian carp and zebra mussels.

We have three activities in this subproject. Activity 1 aims to develop state-of-the-art carp transgenesis capabilities at the MAISRC Containment Lab. Obtaining freshly laid eggs and fertilizing them with freshly collected sperm is a prerequisite for generating the young carp embryos needed for carp transgenesis. In Minnesota, wild carp only spawn during late spring/early summer, creating a very short window of opportunity for performing genetic engineering experiments. A serious effort towards developing new biocontrol methods in carp requires year-round access to young carp embryos, and we will achieve this be maintaining several independent tanks of captive carp that have been slowly 'trained' to be on different annual cycles.

Activity 2 aims to transition our new genetic biocontrol strategy into carp. We have done proof-of-concept experiments in simple laboratory organisms to demonstrate the feasibility of our approach. In this aim, we begin engineering these genetic components in carp. The complete engineering effort will require more time than is funded in this current subproject, but we have listed milestones that will demonstrate substantial progress towards our engineering goals.

Activity 3 accomplishes two tasks. First, we use computer modeling to predict the efficacy of our approach when combined with existing strategies for carp management. Second, we engage the public to develop a better understanding of their attitudes and opinions on using genetically engineered organisms as one part of an integrated pest management plan.

	M.L. 2013, Chp. 52, Sec. 2, Subd. 06a		M.L. 2017, Chp. 96, Sec. 2, Subd. 06a	
Summary Budget Information for Subproject 24:	Subproject Budget: Amount Spent:	\$110,112 \$109,000	Subproject Budget: Amount Spent:	\$140,004 \$36,693
	Balance:	\$1,112	Balance:	\$103,311

Outcome	Completion Date
Activity 1	
1. Begin husbandry of 4 separate carp populations synced to unique annual cycles	July 2018
2. Demonstrate the ability to harvest and fertilize carp eggs/sperm from laboratory carp	December 2019
during Summer, Fall, and Winter (seasons when wild carp are not actively spawning)	
3. Generate transgenic carp expressing the genes needed to engineer our biocontrol	June 2020
system	
Activity 2	
1. Assess genetic diversity in wild populations of common carp	June 2019
2. Generate and validate point mutations in promoters of GATA5, SSH1, and ERN, which	June 2020
are three genes in carp that we need to modify for our genetic biocontrol approach.	
3. Transfer sex-ratio biasing construct to the C. carpio chromosome	June 2020
4. Introduce genetic components into carp that will drive the incompatibility between	June 2020
wild carp and engineered fish. These components will not be toxins but will cause	
natural carp genes to be turned on at the wrong time during development and lead to	
inviable offspring.	
Activity 3	
1. Complete optimal IPM plan based on agent-based simulation models	July 2019
2. OUTREACH: Survey state-wide Watershed District Managers about GMO technologies	September 2018
3. OUTREACH: Oral presentation at MAISRC open houses	September 2018/19
4. OUTREACH: Public survey via MAISRC Detectors volunteers and 2019 MN State Fair	September 2019

Subproject Status as of January 31, 2019:

We have made significant progress towards developing a first-of-its-kind biocontrol approach to combat invasive carp using Sterile Male Accelerated Release Technology (SMART) carp. Since we received notice of the LCCMR-MAISRC award in August 2018, we have created protocols for creating and rearing transgenic carp at the MAISCR Containment Facility. We have built genetic constructs encoding components of our technology and prototyped them in model laboratory fish. Lastly, we have designed and conducted a survey concerning the public perceptions surrounding genetic biocontrol of invasive carp. He learned that the public is more likely to embrace genetic biocontrol compared to alternative options, although there are major knowledge gaps concerning the potential risks and benefits of this technology.

Subproject Status as of July 31, 2019:

We have made significant progress towards developing a first-of-its-kind biocontrol approach to combat invasive carp using Sterile Male Accelerated Release Technology (SMART) carp. Since our last status update, we have successfully spawned carp during 'off-cycle' calendar periods. We have tested several genetic constructs in the model laboratory fish, *Danio rerio*. We have not yet found a genetic design that is suitable for introduction to carp. Lastly, we have organized a second iteration of our public engagement survey that will be administered at the 2019 Minnesota State Fair.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 25: What's in Your Bucket? Quantifying AIS Introduction Risk

Project Manager: Nicholas Phelps

Description: The use of baitfish for recreation angling results in billions of farm-raised and wild-caught fish (and accompanying hitchhikers) being moved long distances overland and intentionally introduced into new environments. As a result, baitfish movement has been considered a high-risk activity for the spread of aquatic invasive species (AIS), with potentially major economic, ecological, and societal consequences. Consequently, state legislatures and management agencies across the country, including Minnesota, are considering dramatic overhauls of their baitfish regulations. This has put supporting a multimillion-dollar bait industry at odds with conserving a multibillion-dollar recreational fishery. The lack of a structured framework to evaluate risk in the face of differing perceptions and great uncertainty (ie. minimal data) for many aquatic hazards is limiting our collective ability to understand and mitigate the risk that baitfish movement could spread potentially devastating AIS.

While the baitfish trade has the potential to move all varieties of AIS, perhaps most vexing are invasive pathogens that can move as passengers undetected at high prevalence, have little or no management options, and can cause long lasting population-level impacts on important fish species. In Minnesota alone, numerous novel baitfish viruses have been discovered in recent years, highlighting the limited information we have regarding the health status of baitfish. There is a clear need for a rigorous risk analysis, but the lack of an informed framework to do so has limited our ability to quantify the risk and make risk-based decisions. The goal of this study is to assess the risk of introduction of important fish pathogens through the recreational use of baitfish. We will synthesize existing knowledge to identify priority hazards for the baitfish trade, develop a risk analysis framework, and characterize the volume, patterns, and complexity of baitfish use by anglers in Minnesota, to develop a tool for estimating risk of AIS introduction via the baitfish pathway. The tool will be
tested with three pathogens of concern to estimate the number of likely introductions to wild fish populations - a useful metric when considering trade-offs for risk management.

This work builds upon, and will be informed by, an ongoing baitfish risk assessment led by the MN DNR, previous baitfish hazard assessments, and previous and ongoing research by members of the project team. By quantifying the actual, not just perceived risks, we will help to facilitate discussions among agency, industry, and public stakeholders, inform risk-based management decisions, and ultimately lead to better outcomes that support the state's bait and fishing industries while protecting natural resources. This project aligns with MAISRC High Priority Research Needs (Research Priority A.8), builds upon existing MAISRC research, forms a new collaborative team, and will fill critical knowledge gaps identified by managers and industry alike.

	M.L. 2013, Chp. 52, Sec.	2, Subd. 06a	M.L. 2017, Chp. 96, Sec.	2, Subd. 06a
Summary Budget Information for Subproject 25:	Subproject Budget: Amount Spent:	\$111,642 \$101,540	Subproject Budget: Amount Spent:	\$88,142 \$25,556
	Balance:	\$10,102	Balance:	\$62,586

Outcome	Completion Date
Activity 1	
1. Identification of 2-4 priority pathogen hazards for further research (Activity 4)	November 2018
and to create an overall Hazard Report.	
2. Finalization of the hazard prioritization matrix	January 2019
Activity 2	
1. Create process model for the baitfish supply chain and points of risk that will	December 2018
feed in to the design of angler survey (Activity 3).	
2. Development of initial introduction risk assessment framework to assess the	March 2019
risk of baitfish as a pathway for pathogen entry into MN waters.	
Activity 3	
1. Finalization of survey design and initial contact for mailed survey	March 2019
Survey coding and data analysis of survey responses	November 2019
3. Final boat launch surveys administered and evaluated	December 2019
4. Technical report on angler bait-related behaviors and peer reviewed	March 2020
manuscript	
Activity 4	
1. Updated risk assessment framework to inform decision making on AIS in the	June 2020
baitfish trade	
2. Peer-reviewed manuscript and policy brief	June 2020

Subproject Status as of January 31, 2019:

We have made substantial progress on the project, including the completion of Activity 1 and laying the groundwork for Activities 2 and 3. We completed our hazard prioritization matrix, which selected viral hemorrhagic septicemia virus (VHSV), *Ovipleistophora ovariae*, and the Asian tapeworm from among 30+ pathogens initially considered. Selection criteria included the pathogen's ability to evade detection, the impact of its establishment, and its current distribution in the state. We also outlined a conceptual model designating the steps in the bait pathway that will be evaluated for their contribution to overall risk by our quantitative model in Activity 2. Finally, we began development of angler survey questions, the answers to which will provide quantitative data to inform the risk model.

Subproject Status as of July 31, 2019:

We have made substantial progress, particularly for Activity 3. After finalizing a design for the mailed paper survey in consultation with our project advisory team and our survey design collaborators at UMN Liberal Arts Technology and Innovation Services (LATIS), we completed the mailing procedures for the written survey protocol. We mailed invite letters, paper questionnaire surveys, and reminder postcards to 4,000 anglers across the state between May and June 2019. To date we have received approximately 600 completed mail surveys and expect more to come (see amendment request). We have also distributed 1,000 postcard surveys to trained MAISRC AIS Detector volunteers who are in the process of administering them at boat launches and other accesses around the state during the summer of 2019. We have been recording data from the surveys as they arrive as well as monitoring the online portal by which some survey participants responded. Once the data from these two methods have been recorded we can begin analysis and parameterization of the risk assessment model. Finally, we are drafting a manuscript explaining the process and importance of our risk ranking exercise in Activity 1, which we expect to submit September 1, 2019.

Year 1 funding for this project on M.L. 2013 ended on June 30, 2019 and Year 2 activities will continue on M.L. 2017 funding.

Subproject Status as of January 31, 2020:

Status update on subproject activities through 01/31/2020 are recorded on M.L. 2017 report.

Final Report Summary:

Final report summary is recorded on M.L. 2017 report.

SUB-PROJECT 26: Updating an invasive and native fish passage model for locks and dams

Project Manager: Anvar Gilmanov

Description: Bighead and silver carps (together known as Bigheaded carps (*Hypophthalmichthys spp.*) and sometimes "Asian carp") were introduced to the Arkansas in the 1970's and are now threatening to enter Minnesota waters of the Mississippi River from Iowa where they presently exist as self-sustaining populations. This would become a significant problem for Minnesota aquatic ecosystems which are already burdened with high populations of invasive Common carp (*Cyprinus carpio*), which were introduced over a century ago. To preserve the Mississippi and St. Croix Rivers ecosystems, it is crucial to stop this invasion. One way to accomplish this is to use existing Mississippi River lock and dams (LDs), through which all fish must pass to go upstream. Existing data and numeric models suggest that carp passage through the spillway gates of these LDs systems is already hindered by the high velocities the gates create. Of course, it would highly desirable to avoid hindering native fish passage, and if possible even improve it, while stopping invasive carp passage through gates. Because of the complexity of LDs, and the high costs of conducing field work, a numeric model is the best way to achieve these goals in the immediate future. It is important that this model be as accurate as possible.

This project aims to create an updated version of Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model using new field data that can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams. These new field data presently being generated by an ongoing Sorensen laboratory field study of fish behavior and passage at Lock and Dam 2 (LD2) will be analyzed. Parameters on fish behavior will then be updated in the CFD-AB fish passage model already developed by [Zielinski et al., 2018] to improve it. We will then use this updated CFD-AB model to predict fish passage for invasive carp (silver carp, common carp) and two native fishes (channel catfish, lake sturgeon) at two model lock and dams (LD2, LD8). If the updated model predicts better than the old one, we will then determine new optimum spillway gate positions to stop carp for these sites and will share these new data with the US Army Corps of Engineers (USACE) and the MN DNR.

It is crucial to protect the freshwater ecosystems of Minnesota by stopping the invasion of bigheaded carp from Asia and promoting native fish passage through Mississippi River locks and dams. We have the opportunity to do this by altering operating procedures for spillway gate openings at existing lock and dam structures. The CFD-AB has already been developed to do this and is being implemented at LD8 but new field data on fish movement suggest that there are some divergences from the model. These finding contrast with the CFD-AB model and suggest that improvement of this computational model must be developed. This project will do that. Application of our updated proposed model to LD8 could prevent invasive species such as silver and bighead carp from colonizing Minnesota.

Summary Budget Information for Subproject 26:	Subproject Budget:	\$90,827
	Amount Spent:	\$88,296
	Balance:	\$2,531

Outcome	Completion Date
Activity 1	
1. Developed and validated updated version of CFD-AB model based on LD2	November 2018
experimental data.	
2. Provide numerical simulations of invasive and native fish passage through LD2 based	December 2018
on the updated version of CFD-AB model.	
Activity 2	
1. Provide numerical simulations of invasive and native fish passage through LD8.	April 2019
2. Prepare 1 papers for submission to an engineering/biological journal.	May 2019
3. Organize meeting with all interested agencies: MN Department of Natural Resources,	May 2019
US Fish and Wildlife Service, US Army Corps of Engineers to report our progress and take	
into account any critical remarks.	
4. Give recommendations to USACE to improve gate regulation at LD8 to block invasive	May 2019
fish passage and to help native fish.	
5. Final Report to MN Department of Natural Resources	June 2019

Subproject Status as of January 31, 2019:

The following progress has been made so far. For Activity 1, the code development and validation of Computational Fluid Dynamics – Agent Based (CFD-AB) model has been done:

(a) The current CFD-AB code used the nodes of the fluid grid to locate fish position. We have changed the algorithm so that the new approach would allow the fish to be at any spatial location (vs only at fluid grid nodes). The accuracy of fish swimming calculation in the modified version of the CFD-AB model has increased, which was demonstrated on a test problem of fish swimming in a channel.

(b) Numerous simulations with common carps, which were trying to pass through LD2, have been performed. In contrast with our previous simulations (Gilmanov et al., 2017, 2018), a new approach with actual initial fish distribution as described by the experimental data from Lock and Dam 2 (Finger J., Riesgraf A, and Sorensen P., 2019, unpublished) has been prepared. These simulations provided excellent comparisons between the percentage of passing common carp of computational results and the experimental field data.

(c) A recent modification of the CFD-AB model which considers fish swimming up and downstream the Mississippi River has been finished. Presently, work on debugging of the code is performed. In order to validate the modification of the CFD-AB model, we have proposed an idea of "Attractive Zones" (resting, migration, feeding zones, etc.). We get the positions of resting zones from the field data of (Finger J., Riesgraf A, and Sorensen P., 2019, unpublished).

Final Report Summary:

The main purpose of the project was to develop an updated version of the Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model (Zielinski, et al., 2018) using the field/experimental data of fish passage through Lock and Dam #2. This updated CFD-AB model can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams.

The subproject has been fulfilled for all the goals that were declared:

- 1. The computational code CFD-AB directed to enhance the simulation of swimming fish trying to pass through the navigation dams was updated/developed. The analysis of different fish passage index (FPI) showed that the values of FPI for the modified algorithm for a model channel (Gilmanov, et al., 2019, Water, under review) were greater than the FPI of the original algorithm at about 16%. At this moment, no essential differences in fish passage index FPI for the original and modified model at LD2 and LD8 have been found. This effect can be explained by the special gate adjustments, which generate a rather high fluid flow prevented fish to pass through the dams. In other words, in case of blocking invasive species, the modified algorithm does not change the final results of FPI at LD2 and LD8. But the modified algorithm could play a positive role to help native fish to pass through the navigation dams in the case of changing gate adjustments leading to decrease flow velocity.
- 2. The modified algorithms now account for more realistic fish behavior, including placement of "attraction points", such as resting zones characterized by low recirculating fluid flow. These parameters have been informed by the literature and unpublished field data collected on other projects.
- 3. Based on investigations of (Larson, et al., 2017, Kokotovich et al, 2017) it was reported that the "Invasive Front" is currently positioned in southern Iowa between Pool 14 and Pool 16. Therefore, the strategy of blocking bigheaded carp at Lock and Dams of Minnesota should be reconsidered. It is well documented that the navigational dams have significantly altered the movement, spawning, feeding and other activities of native fish (Wilcox et al. 2004). Hence, managers should consider alternative strategies whereby navigation dams are adjusted to *help* native fish pass, instead of *blocking* invasive fish. This strategy could help with ecosystem restoration efforts and potentially improve natural resistance to invasion by bigheaded carps. To evaluate this strategy, simulations of walleye passing through LD2 have been executed. It has been shown that by changing gate adjustments, FPI=4% is for the original algorithm and FPI=12% for the modified algorithm. We have to note, that for current gate adjustments from USACE the FPI=0% for original and modified CFD-AB models. By utilizing active monitoring data of bigheaded carp managers could *instantly* change gate adjustments at LD2-LD8 by using our CFD-AB approach if the invasion front threatens Minnesota.

V. DISSEMINATION:

Description: Findings will be disseminated by annual public workshops organized by the Center, the Center's web site, collaborative meetings with our advisory boards, peer-reviewed publications and student theses.

Status as of February 28, 2015

Updates and research findings continue to be published in a (roughly) bi-monthly e-newsletter and through the MAISRC website, Facebook, and Twitter.

MAISRC organized and hosted the "2014 Minnesota Aquatic Invasive Species Research and Management Showcase" on November 19, 2014. This public workshop was attended by over 220 people from around the state and included 13 talks and demonstrations given by 23 MAISRC-affiliated researchers, an Extension educator and DNR scientist. Participants saw demonstrations of methods used to advance the science of AIS detection and control, gained some basic skills for working on AIS issues in their communities, and learned about some of the current research on invasive carps, zebra mussels, aquatic invasive plants, and harmful fish diseases. An anonymous participant survey showed 98% of respondents found the information presented at the Showcase relevant or extremely relevant to their work on AIS; 92% said they learned new skills and information that will help their efforts to prevent and control AIS; and 90% reported they plan to take at least 3 actions as a result of something they learned at the Showcase. A press release was disseminated about the Showcase event.

The Center initiated its first systematic research needs assessment to determine state priorities for the next "wave" of research projects and disseminated information about the process and ways to provide input. The process included consideration of 33 different species of fish, plants, invertebrates, and harmful microbes and involved input from UMN scientists, agency biologists, statewide AIS managers, and the public. In addition to emails, the newsletter, and Facebook and website postings, a press release was disseminated to solicit input from the public. The process in still underway; results will be likely be shared with the public later in 2015.

Three candidates were interviewed for the Extension Specialist position during the month of March with each candidate providing research seminar and outreach seminar. The DNR, the public, and professional stakeholders were invited to attend these seminars in person or by Webex, to provide evaluations, and to meet one-on-one with the candidates as well. These opportunities were advertised by email, Facebook and on the MAISRC websites.

Status as of September 24, 2015

MAISRC organized and hosted the "2015 Minnesota Aquatic Invasive Species Research and Management Showcase" on September 16, 2015. This public workshop was attended by 175 people from around the state and included 16 talks and demonstrations given by MAISRC-affiliated researchers, an Extension educator and two DNR staff. Participants received updates on current research and saw demonstrations of methods used to advance the science of AIS detection and control, including through on-campus talks, lunch with researchers, and field trips to nearby lakes and research sites.

Updates and research findings continue to be published in a (roughly) bi-monthly e-newsletter and through the MAISRC website, Facebook, and Twitter.

Status as of February 29, 2016

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

Status as of August 31, 2016

Efforts to educate, inform, and share findings are continuing via the website, Facebook, Twitter, media efforts, and our annual Showcase event. Research Center faculty and staff also continue to give talks and meet with stakeholders.

Planning began for the 2016 Showcase and involved recruiting a committee, finding a date, securing facilities, sending out a save- the- date, and beginning to rough out a program. The event will be held on the St. Paul Campus on September 12.

After a significant effort designing, editing, and creating new content, the newly revamped website is live. It is continually updated with descriptions of research projects underway, progress and results, MAISRC events, researcher information, and opportunities for input by our stakeholders. Our average monthly views have grown from approximately 400 to over 1,000.

Newsletters continue to be written every other month, which includes seeking input from researchers, drafting stories, getting them reviewed by scientists, taking photographs, and formatting materials for dissemination. We now have over 1,700 subscribers with an even mix of agency personnel, non-governmental and lakeshore association members, private industry, and higher ed. We have a consistently high open rate (30-40% versus industry average of 18%). We also leverage Facebook and Twitter to get our messages out and have consistently high reach and engagement there as well.

MAISRC has also planned a special session at the upcoming Upper Midwest Invasive Species Conference taking place in October.

Status as of February 28, 2017

Efforts to broadcast research progress continue through talks, attendance at statewide AIS Advisory Committee meetings, papers, newsletters, website and other social media formats. We continue to reach larger audiences and receive high engagement from our followers.

We held our 2016 Showcase in September, with attracted 171 non-MAISRC attendees and provided 16 presentations spread out among 21 speakers, including 5 grad students and 4 postdocs, and faculty and non-Twin Cities campus- based researchers. <u>Copies of most of these presentations can be found on our website</u>. Tours of the lab were also provided. 90% of attendees rated the event as excellent or very good.

MAISRC core staff also attended conferences to stay abreast of current work and research needs around the state and also gave a presentation on MAISRC's RNA process, which has gained attention as an efficient, inclusive solutions-oriented model. We have also submitted an abstract to present at the 20th International Conference on Aquatic Invasive Species in October, 2017.

Status as of August 31, 2017

Efforts to broadcast research progress continue through talks, attendance at statewide AIS Advisory Committee meetings, papers, newsletters, website and other social media formats. We continue to reach larger audiences and receive high engagement from our followers.

Since our last update, we have had 56 news stories published about MAISRC, the work we are conducting, and the results our work is generative. We have also had 13% growth in followers on Facebook, 15% growth in followers on Twitter, 20% growth in newsletter subscribers, and have had 10,170 unique visitors to our website. We consider these to be positive indicators of more people being engaged in the issue of AIS, becoming informed on the science, and at some level supporting the investment in research to help solve our state's AIS problems.

We are currently planning for our 2017 Management and Research Showcase, scheduled for September 13. Approximately half of the people registered this far have never attended a Showcase before—another indication of our expanding reach. 18 talks are scheduled by 31 MAISRC researchers plus lab tours with demonstrations, including by Whooshh Innovations, a collaborator in an ENRTF- funded carp project. New this year will be a poster session during the end of day reception. We were accepted to present at the 20th International Conference on Aquatic Invasive Species in October 2017.

Status as of February 28, 2018

MAISRC is continuing its efforts to educate, inform, and share our research findings. Key outreach and communications activities include:

MAISRC currently has a social media following of 1,500 and an e-newsletter list with 2,700 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories on our research projects.

Since the last workplan update, MAISRC has been featured in the news approximately thirty times, with stories on Asian carp, zebra mussels, and pathogens, as well as a podcast from Montana Public Radio that focused specifically on zebra mussels and featured many MAISRC researchers and stakeholders. We recently worked with Minnesota Public Radio for a story about our invasive plants research which will be appearing soon.

To mark MAISRC's fifth anniversary in late December 2017, staff put together a comprehensive five-year report that includes key findings and accomplishments, big wins, and plans for the future for each of MAISRC's twelve species of research. It also includes an overview of our outreach programs and our strategic plan process. It was mailed to numerous MAISRC stakeholders and pushed heavily through e-newsletter and social media. It has now been viewed online over 21,000 times.

Since the last workplan update, over 10,500 unique visitors have visited the website a total of 15,520 times; viewing 30,940 pages. These statistics are routinely increasing and we view this as a sign that MAISRC is growing in name recognition and being seen as an important resource.

We held the 2017 AIS Research and Management Showcase on September 13 and hosted just under 200 attendees, not including anyone affiliated with MAISRC. Three legislators attended. Planning is now beginning for the 2018 Showcase, to be held on Sept. 12.

Many MAISRC researchers are giving talks around the state, including the Aquatic Invaders Summit, the New Brighton Sportsmen's Club, the State of Water Conference, the Itasca Area Business Water Summit, the Pelican Lakes Association of Crow Wing County annual meeting, the Cass County watercraft inspection conference, and more.

Status as of August 2, 2018

MAISRC currently has a social media following of over 1,700 and an e-newsletter list with just under 3,000 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

Since the last workplan update, MAISRC has been featured in 48 stories in the press. Stories have included our AIS Detectors program, invasive carp research, invasive plants research, a full feature on Minnesota Bound, and an op-ed from members of University administration.

Staff continued to push out the five-year report that was created in early 2018. We followed it up with an interactive online map that shows all points of MAISRC research and outreach activities. It can be seen online at www.maisrc.umn.edu/maisrc-map.

Since the last workplan update, over 18,000 unique visitors have visited the website a total of 24,000 times; viewing 41,500 pages. This is a significant increase over the last reporting period. We feel that our consistent growth in these communications areas is a sign that MAISRC is growing in name recognition and being seen as an important resource around the state, nation and world.

Planning is underway for the 2018 AIS Research and Management Showcase, which is scheduled for September 12, 2018. Registration is moving quickly and we expect to have 200+ attendees.

This spring and summer, many MAISRC researchers gave talks around the state, including the Pelican Lakes Association of Crow Wing County, the AIS Roundtable (organized by the Whitefish Area Property Owners Association and attended by members of 17 lake associations), an all-day event with MAISRC speakers in Detroit Lakes, and more. Several researchers are slotted to speak at the Upper Midwest Invasive Species Conference in October.

Lastly, MAISRC is partnering with a videographer this summer to create a series of videos about our research. The videos will cover:

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

These videos will help us keep legislators, managers, and interested members of the public informed by explaining our research in a new and different way.

Status as of February 28, 2019

MAISRC currently has a social media following of just under 2,000 and an e-newsletter list with just under 3,250 recipients. Social media posts about research findings, events, AIS Detector workshops, and invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

Since the last workplan update, MAISRC has been featured in 35 stories in the press. Stories have included research updates on starry stonewort, zebra mussels, common carp, spiny waterflea, as well as the Showcase and the AIS Detectors program.

Staff created a <u>2018 Annual Report</u> in late 2018. An electronic version was sent to all newsletter subscribers and shared on social media, and a print version was sent to donors and other interested stakeholders.

In late summer, MAISRC released its first-ever white paper, <u>Treatment options for the eradication of limited-</u> <u>scale zebra mussel infestations at various water temperatures</u>. This white paper was shared at the Showcase and distributed through our newsletter, website, and social media.

Since the last workplan update, 22,500 unique visitors have visited the website a total of 31,000 times; viewing 56,000 pages. This is an increase of 25%, 23%, and 35%, respectively, over the last reporting period. This consistent growth shows that MAISRC is growing in name recognition and being seen as an important resource for different stakeholders around the state.

The 2018 AIS Research and Management Showcase had over 200 attendees (who were not affiliated with MAISRC). Roughly half of these attendees had never attended the event before.

In summer 2018, MAISRC created a series of videos about our research which were very well-received. The videos covered: the AIS Detectors program, starry stonewort research, spiny waterflea research, the impacts of AIS on walleye, using pathogens to control invasive carp, and novel methods for controlling common carp. In total, the videos were viewed 36,000 times.

MAISRC staff will coordinate in-person talks from the MAISRC Director and other MAISRC researchers around the state this spring and summer, and will share these event announcements through the newsletter and social media.

Final Report Summary:

Social media and e-newsletter

MAISRC currently has a social media following of just under 2,300 and an e-newsletter list with just under 3,500 recipients. Social media posts about research findings, events, AIS Detector workshops, and general invasive species news are posted daily. An e-newsletter goes out every other month and includes more in-depth stories about our research projects.

MAISRC's Facebook, Twitter, and e-newsletter accounts were all created after the start of this workplan in July 2013.



Newsletter list growth, 2014 – 2018:

Growth in followers and average engagement on Facebook:



Growth in followers and average engagement on Twitter:



Media relations

Since the last workplan update, MAISRC has been featured in 62 stories in the press. Stories have included research updates on zebra mussels, the annual Starry Trek event, invasive carp, starry stonewort, and more.

Over the course of the last six years, MAISRC has been in approximately 350 news stories in roughly 117 different outlets. The most common outlets have been the *Star Tribune*, Minnesota Public Radio, and KSTP-TV. Other notable outlets include *The New York Times, The Washington Post*, and Minnesota Bound.



News stories featuring MAISRC research:

MAISRC website

Since the last workplan update, 26,584 unique visitors visited the MAISRC website a total of 35,660 times; viewing 62,645 pages. This is an increase of 18%, 15%, and 12%, respectively. This consistent growth shows that MAISRC is growing in name recognition and being seen as an important resource for different stakeholders around the state.

Average number of unique users and pageviews per month: Pageview information unknown prior to launch of new MAISRC website in February 2016.



MAISRC Showcase

The 2019 AIS Research and Management Showcase will be held on Sept. 18, and registration is already at its highest of any year. Over 200 attendees (who are not affiliated with MAISRC) will attend; roughly half of whom have never attended the event before. In total, roughly 700 different people have attended the AIS Research and Management Showcase since 2014.

Videos

In summer 2019, we created three videos about our research which will be released soon. The videos covered the Whooshh fish transport system (project led by Przemek Bajer), evaluating public values of AIS management (project led by Amit Pradhananga) and the genetic biocontrol of invasive fish (project led by Mike Smanski). A MAISRC project on the control of zebra mussels (project led by Jim Luoma) was also chosen by University Relations to be highlighted in upcoming *Driven* campaign. A video will be released and widely promoted in October 2019.

Statewide talks

MAISRC staff also coordinated in-person talks rom the MAISRC Director and other MAISRC researchers around the state this spring and summer, including the Stillwater Rotary Club, the Bay Lake Improvement Association, the Clamshell-Bertha Lake Association, the Pelican Lakes Association of Crow Wing County, and the Whitefish Area Property Owners Association.

Summary of notable MAISRC communications and outreach activities

Summer 2013 – summer 2019

Events and trainings

- Have held six AIS Research and Management Showcases with roughly 700 different attendees
- Held a lab ribbon-cutting ceremony in March 2016
- Hosted new University President Gabel for a lab tour and research demonstration in September 2019

- Have held three Starry Trek events, through which volunteers have found new infestations of starry stonewort, Eurasian watermilfoil, and Chinese mystery snails
- Formally launched the AIS Detectors program in March 2017; have now certified 299 Detectors around the state

Videos

- Created nine videos, highlighting MAISRC subproject research:
 - o <u>AIS Detectors</u>
 - <u>Starry stonewort research</u>
 - o Spiny waterflea research
 - Impacts of AIS on walleye
 - o <u>Using pathogens to control invasive carp</u>
 - o <u>Novel methods for controlling common carp</u>
 - o Valuing AIS management
 - Genetic control of invasive carp
 - Using the Whooshh fish transport system (not released yet)
- <u>Featured in U of M Driven campaign in summer 2018</u>
- Featured in U of M Driven campaign in fall 2019

Reports and other materials

- <u>Treatment options for the eradication of limited-scale zebra mussel infestations at various water</u> <u>temperatures</u>
- An assessment to support strategic, coordinated response to invasive *Phragmites australis* in Minnesota
- 2018 Research Report
- Five years of AIS Research | 2012 2017
- Interactive map: MAISRC work around the state
- <u>Aquatic Invasive Species ID Guide</u>

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget: See budget attachments.

Explanation of Use of Classified Staff: *n.a.*

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

SUBPROJECT 1: MAISRC portion of a new electrofishing boat purchased in partnership with the Fisheries, Wildlife, and Conservation Biology (FWCB) Department at the University of Minnesota (\$65,000). The new electrofishing boat will be available for use by any MAISRC funded or MAISRC partnership project. MAISRC use of the boat will be in proportion to the percent investment by MAISRC/LCCMR in its purchase. MAISRC staff will also provide oversight of the management of the boat, to ensure that it is being used proportionally for the purpose of advancing AIS research in Minnesota. This oversight will continue throughout the useful life of the boat. If for some reason the use of the boat changes, MAISRC will pay back the Environment and Natural Resources Trust Fund an amount equal to the proportional residual value (approved by the director of the LCCMR), or the proportional cash value received if it is not sold.

For capital expenditures made by MAISRC subprojects, see the subproject final reports.

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:

Subproject 1: 0 FTE Subprojects 1-26: 2.58 FTE

B. Other Funds (related projects that can synergize this one):

	\$ Amount	\$ Amount	
Source of Funds	Proposed	Spent	Use of Other Funds
Non-state			
National Science Foundation	\$234,000	\$232,520	Radio-tags for Judas fish
USGS	\$129,646	\$124,343	Preliminary work with Asian carp
Riley Purgatory Bluff	\$2,728,771	\$2,728,771	Preliminary work on Judas carp
Watershed District			
State			
ENRTF –M.L. 2012, chp 264, art4. Sec 3- Aquatic Invasive species (AIS) Cooperative research center	\$2,000,000	\$2,000,000	Startup funds for Center (eDNA work, facility repair, Judas carp study, administrative costs)
Clean Water Legacy Funds	\$1,800,000	\$1,794,028	Startup for Center (Zebra mussel position, facility repair, administrative costs)
TOTAL OTHER FUNDS:	\$6,892,417	\$6,879,662	

VII. PROJECT STRATEGY:

A. Project Partners:

DNR (a full partner and co-lead on CAB with whom the University will have a memoradum of understanding), USGS (LaCrosse WI; and Columbia, MI; former with a memorandum of understanding), Riley Purgatory Bluff Watershed District (Chanhassen, MN), Ramsey Washington Metro Watershed District (Maplewood, MN), Minnehaha Watershed District (Minnetonka, MN)

B. Project Impact and Long-term Strategy: This project will establish a new national center of excellence for AIS in Minnesota that will develop and disseminate new information and useful techniques for their control to public agencies and the private sector.

C. Spending History:

Funding Source	M.L. 2005	M.L. 2007	M.L. 2008	M.L. 2009	M.L. 2010
	or	or	or	or	or
	FY 2006-07	FY 2008	FY 2009	FY 2010	FY 2011
ENRTF – M.L. 2008 Chp 367,		550,000			
Sec 2, Subd. 04b -					
Accelerating plans for					
integrated control of common					
carp					
ENRTF –M.,L. 2005, First	550,000				
Special Session, Chp.1, Art					
2, Sec 11, Subd. 05g –					
Integrated and pheromonal					
control of the common carp					

VIII. ACQUISITION/RESTORATION LIST: n.a.

IX. MAP(S): Entire state of Minnesota

X. RESEARCH ADDENDUM: *not applicable (peer review of all activities will be completed by the Center)*

XI. REPORTING REQUIREMENTS: Periodic work plan status update reports will be submitted not later than February 28 and August 31 each from February 10, 2014 through February 28, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019 as requested by the LCCMR.

Environment and Natural Resources Trust Fund									
M.L. 2013 Sub-Project Budget of M.L. 2013-06a: Aquatic Inva	sive Species I	Research Cent	er						
Project Title: Aquatic Invasive Species Research Center Subproject 1:	Coordinating, S	ynergizing, and I	Promoting Expe	rtise: establishin	g an Administrati	ve Structure			
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a Project Manager: Nicholas Phelps									*
Organization: University of Minnesota – Minnesota Aquatic Invasive S	pecies Research	n Center							
Subproject Budget: \$1,805,859								13	VIRONMENT
Subproject Phase 1 Length and Completion Date: 3 years, June 30,	2016								DIST FUND
Project Length and Completion Date: 6 Years, June 30, 2019								1	
Date of Report: November 11, 2019									
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND	Activity 1: Coc Promoting Exp Administrative	ordinating, Syne pertise: establis e Structure (Pha	rgizing, and hing an se 1)	Activity 2: Res	erves				
	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL	TOTAL BALANCE
Personnel (Wages and Benefits) - Total	\$1,199,487	\$1,194,619	\$4,868	\$0	\$0	\$0	\$1,199,487	\$1,194,619	\$4,868
Associate Director Professional & Admin: \$83,000 Salarv	φ1,100, 4 07	<i>w</i> 1,10 4 ,019	φ-7,000	ψυ	ψŪ	ψŪ	¥1,100, 1 07	¥1,10 1 ,013	ψ-,000
(66.4%Salary, 33.6% benefits, 1 FTE)									
Scientific Director Professional & Admin: \$79,000 (66.4%Salary,									
33.6% benefits, 0.5 FTE)									
Name- Post Doctoral Fellow: \$Salary; (79.25% Salary, 20.75% benefits) 1.0 FTE									
Undergraduate Student: \$6000 (93% salary, 7% benefits) 0.25 FTE Admin and Communications Assistant: \$28,000 (63.2% salary, 36.8% benefits) 0.75 FTE									
Field Technician (Civil Service): \$42,000; (63.2% salary, 36.8%									
Lab Manager (Civil Service): \$49,000; (63.2% salary, 36.8% benefits)									
1.0 FTE									
Professional/Technical Services and Contracts - Total	\$35,675	\$32,263	\$3,412	\$0	\$0	\$0	\$35,675	\$32,263	\$3,412
Services- office & gen oper. (printing/duplication, mailing, printer repairs, audio visual associated with seminars & conferences, conf. calls, surveys, insurance for pontoon, etc.)	\$16,221	\$14,241	\$1,980			\$0	\$16,221	\$14,241	\$1,980
Services- lab & medical (data storage, sequencing, biochemistry, microscopy, well permits, discharge licences and fees, preventative	\$49	\$49	\$0			\$0	\$49	\$49	\$0
Professional Services & contracts- (fees or honoraria for guest lecturer	\$165	\$165	\$0			\$0	\$165	\$165	\$0
Repairs- lab & field (vehicle, EFL holding facility, or other shared	\$19,240	\$17,808	\$1,432			\$0	\$19,240	\$17,808	\$1,432
Rentals- space and facilities for conferences and events (e.g. annual	\$0	\$0	\$0			\$0	\$0	\$0	\$0
Showcase)	¢ 40, 047	* 00.407	<u> </u>	* 0	* 0	* 0	* 40.047	* 00.407	<u> </u>
Supplies- office & gen oper. (paper, toner, folders, brochures,	\$48,317 \$22,108	\$39,497 \$19,025	\$8,820 \$3,083	\$0	\$0	\$0 \$0	\$48,317 \$22,108	\$39,497 \$19,025	\$8,820 \$3,083
Supplies- lab & field (piping, glue, hardware and plumbing for facilities,	\$5,005	\$4,853	\$152			\$0	\$5,005	\$4,853	\$152
Equipment- non capital lab & field (primarily equipment for central holding facilities if needed for repair or replacement, pumps for	\$21,204	\$15,619	\$5,585			\$0	\$21,204	\$15,619	\$5,585
washing down boats, storage containers, etc)									
Capital Expenditures Over \$5,000 - Total	\$65,000	\$65,000	\$0	\$0	\$0	\$0	\$65,000	\$65,000	\$0
Cap expenditures over \$5,000: MAISRC portion of new electrofishing	\$65,000	\$65,000	\$0			\$0	\$65,000	\$65,000	\$0
boat, purchased in partnership with UMN Dept of Fisheries, Wildlife,									
and Conservation Biology	\$22.660	¢10.406	¢ / 172	0.9	¢0	¢0	¢22 660	¢10.406	¢ / 172
Travel - MN (mileage, meetings, conferences, guest speakers, out of town experts for research needs assessment, travel etc. Field tech	\$15,669	\$11,890	\$3,779	φ υ	φυ	\$0 \$0	\$15,669	\$11,890	\$3,779
mileage will be paid from specific subprojects)									
I ravel - Domestic (mileage, conferences, mtgs for Center	\$8,000	\$7,605	\$395			\$0	\$8,000	\$7,605	\$395
	¢E00	¢550	¢22	¢^	¢0	¢∩	¢593	¢550	¢20
Telecommunications (voicemail service for MAISRC researchers and staff)	\$582 \$582	\$550	\$32	ბ ე		\$0 \$0	\$582 \$582	\$550	\$32
Budget Reserve Pending Progress and Peer Review - Total	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
Funds for future phases to be allocated to specific budget categories at a future date pending sub-project progress	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
COLUMN TOTAL	\$1,372,730	\$1,351,424	\$21,306	\$0	\$0	\$0	\$1,372,730	\$1,351,424	\$21,306

Environment and Natural Resources Trust Fund

M.L. 2013 Project Budget - Overall Budget of Aquatic Invasive Species Research Center Project Title: Aquatic Invasive Species Research Center Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a Project Manager: Nicholas Phelps Organization: University of Minnesota M.L. 2014 ENRTF Appropriation: \$8,700,000 Project Length and Completion Date: 6 Years, June 30, 2019 Date of Report: November 11, 2019

Date of Report: November 11, 2019																																																			
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	SUBPROJECT 1 promoting experi administrative st	- Coordinating, sy tise: Establishing ructure	nergizing and an	SUBPROJECT 2 - develop biological invasive species	Metagenomic approaches control strategies for aqu	to SUBPROJE latic Develop Bio Invasive Sp Potential Mi	ECT 2.2 - Metagenon ological Control Stra pecies, Phase II: Dev licrobiological Contr	mic Approaches t rategies for Aquat velopment of rol Agents for AIS	o SUBPROJECT 3 - Attracting carpo so assessed; Determ system can be cor deter carp while e	Reducing and cont o their presence ca nining if and how a s mbined with light in examining potential	ntrolling AIS: an be accurately sound-bubble n the laboratory to I impacts to native	SUBPROJECT 4 - using biocontrol a	Common carp man nd toxins	agement SUBPR using b	ROJECT 4.2 - Co biocontrol and to	ommon carp managen oxins, Phase II	nent SUBPROJE Developing selectively c	CT 5 - Reducing and and evaluating new to control invasive plants	controlling AIS: techniques to ts	SUBPROJECT 6 - De Threats to Inform Pre Control	etermining Heteros evention, Managen	porosis SUBPRC nent, and for invas Understa Upper M	JECT 7 - Developi ve carp species. nding the virome dwest.	ing eradication to Phase I: of carp species in	ols SUBPROJECT 7 for invasive spe the and evaluation f agents	7.2 - Developing er cies Phase II: Viru for use as potentia	eradication tools Si rus Discovery re tial biocontrol sp	UBPROJECT 8 - I estoration researd pecies	Risk assessment, cont ch on aquatic invasive	rol, and SUBPR plant mussel and and strateg	ROJECT 9 - Population I spread pathways, ge alysis to select target gies for genetic biocor	on genomics of zebra enome sequencing et genes and ontrol.	SUBPROJECT 10 - Professional Trainir Response	Citizen Science ar ng Programs to Su	nd SUE upport AIS Risl met	BPROJECT 11 - Reduk k analysis to identify thods, Phase 1: Prob	icing and controllin AIS control prioriti lem Formulation	ing AIS: SUBPROJEC ties and AIS: Risk and priorities and	T 11.2 - Reducing and lysis to identify AIS co methods, Phase 2: R	controlling SU ontrol flea sk Analysis	BPROJECT 12 - Ch impacts using sec	naracterizing spiny wate	r SUBPROJECT Assess Aquati	13 - Eco-epidemio c Invasive Species	ogical Model to SUF Management lak	IBPROJECT 14 - Cos ces newly infested wi	t-effective monitor th zebra mussels	ring of SUBPROJ	ECT 15 - Determining F Spiny WaterFlea Spre	Highest Risk S ead F	SUBPROJECT 1 Populations: As:
BUDGET ITEM	Subproject 1 Budget	Amount Spent	Subproject 1 Balance	Subproject 2 Budget	Subproje	ect 2 Subproject	t 2.2 t Amount Spen	Subproject 2.	2 Subproject 3 Budget	Amount Spent	Subproject 3 Balance	Subproject 4	Amount Spent	ubproject 4 Subpro	oject 4.2 Idget Amou	Subproje	ct 4.2 Subprojec	5 Amount Spent	Subproject 5 Balance	Subproject 6 Budget Am	ount Spent B	project 6 Subpro	ject 7	Subproje Spent Balanc	ct 7 Subproject 7.2 e Budget	Amount Spent	Subproject 7.2 Balance	Subproject 8 Budget A	Amount Spent Bala	oject 8 Subpr	roject 9 udget Amount Sr	Subproject 9 Balance	Subproject 10 Budget Ar	Sul	ubproject 10 Su Balance	Ibproject 11 Budget Amour	Subproj	ject 11 Subproject 1 nce Budget	I.2 Sent	ubproject 11.2 Su Balance	Ibproject 12 Budget Amo	Subprojec	.t 12 Subproject 1	Amount Spent	Subproject 13 S Balance	ubproject 14 Budget Amou	Subprount Spent Ba	roject 14 Subproje	ect 15	Subproject 15	Subproject 16 Budget
Personnel (Wages and Benefits) - Overall Total	\$1,199,487	7 \$1,194,619	\$4,868	\$226,717	\$226,717	\$0 \$198	8,070 \$198,07	070	\$0 \$518,950	\$518,950	\$0	\$286,024	\$286,024	\$0	\$257,740	\$221,115 \$3	36,625 \$160	771 \$160,771	1 \$0	\$98,836	\$98,836	\$0	570,855 \$7	570,855	\$0 \$238,650	\$227,552	2 \$11,098	\$761,604	\$761,604	\$0	\$297,752 \$297	7,752 \$C	0 \$394,038	\$393,172	\$866	\$89,274	\$89,274	\$0 \$104,9	23 \$104,923	\$0	\$159,732	\$159,271	\$461 \$163,3 ^r	1 \$163,351	\$0	\$206,384	\$184,302	\$22,082 \$ ^f	3,085 \$83,085	,5 \$0	\$16,275
Professional/Technical Services and Contracts - Overall Tota	\$35,675	5 \$32,263	\$3,412	\$45,251	\$45,251	\$0 \$54	4,500 \$42,33	333 \$12,10	68 \$57,289	\$46,701	\$10,588	\$59,261	\$59,261	\$0	\$73,460	\$62,074 \$	1,386 \$4	769 \$4,769	9 \$0	\$0	\$0	\$0	60,403 \$6	60,403	\$0 \$114,413	\$110,607	7 \$3,806	\$14,946	\$13,358	\$1,587	\$73,334 \$73	3,334 \$0	0 \$48,491	\$47,969	\$522	\$1,400	\$1,400	\$0 \$7,5	43 \$7,543	\$0	\$45,250	\$45,248	\$2 \$17,8	1 \$17,871	\$0	\$38,563	\$19,697	\$18,866	\$0 \$C	<u>0</u> \$0	\$44,245
Equipment/Tools/Supplies - Overall Total	\$48,317	\$39,497	\$8,820	\$23,000	\$23,000	\$0 \$37	7,500 \$37,00	000 \$50	00 \$90,102	\$82,709	\$7,393	\$13,079	\$13,079	\$0	\$50,000	\$49,182	\$818 \$18	265 \$18,265	5 \$0	\$5,645	\$5,645	\$0	\$47,123 \$4	47,123	\$0 \$70,429	\$67,300	0 \$3,129	\$25,307	\$25,223	\$84	\$5,609 \$5	\$5,609 \$0	0 \$61,085	\$58,349	\$2,736	\$1,304	\$1,304	\$0 \$1,7	57 \$1,757	\$0	\$2,338	\$2,252	\$86 \$4,17	4 \$4,124	\$0	\$6,617	\$6,617	\$0 <u></u>	,1,577 \$1,401	,1 \$176	\$5,370
Capital Expenditures Over \$5,000 - Overall Total	\$65,000	\$65,000	\$0	\$0	\$0	\$0	\$0 \$	\$0	\$0 \$0	\$0	\$0	\$5,925	\$5,925	\$0	\$0	\$0	\$0	\$0 \$0) \$0	\$0	\$0	\$0	\$23,776 \$2	23,776	\$0 \$7,718	\$ \$7,718	8 \$0	\$0	\$0	\$0	\$0	\$0 \$C	0 \$0	\$0	\$0	\$0	\$0	\$0	\$0 \$0	\$0	\$0	\$0	\$0 \$6,8*	7 \$6,817	\$0	\$0	\$0	\$0	\$0 \$0	,0 \$0	\$0
Travel - Overall Total	\$23,669	9 \$19,496	\$4,173	\$4,396	\$4,396	\$0 \$13	3,147 \$9,20	208 \$3,93	39 \$16,628	\$15,359	9 \$1,270	\$19,943	\$19,943	\$0	\$24,800	\$16,542	\$8,258 \$10	610 \$10,610) \$0	\$7,408	\$7,408	\$0	\$4,597	\$4,597	\$0 \$14,000	\$9,490	0 \$4,510	\$20,143	\$20,066	\$77	\$3,623 \$3	\$3,623 \$0	0 \$21,260	\$20,846	\$415	\$1,365	\$1,365	\$0 \$12,4	54 \$12,454	\$0	\$4,946	\$4,937	\$9 \$3,08	6 \$3,086	\$0	\$14,936	\$14,936	\$0 5	¢8,270 \$8,270	0 \$0	\$22,249
Other - Overall Total	\$582	2 \$550	\$32	\$0	\$0	\$0	\$0 \$	\$0	\$0 \$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0 \$0) \$0	\$0	\$0	\$0	\$0	\$0	\$0 \$0	\$0	0 \$0	\$0	\$0	\$0	\$0	\$0 \$C	0 \$515	\$515	\$0	\$0	\$0	\$0	\$0 \$0	\$0	\$0	\$0	\$0	0 \$0	\$0	\$0	\$0	\$0	\$0 \$C	,0 \$0	\$0
Budget Reserve Pending Progress and Peer Review - Overal Total	\$0	\$0	\$0																																																1
	\$1,372,730	Total spent adjusted to	\$21,306 Balance adjusted to	\$299,364	\$299,364	\$0 \$303	3,217 \$286,61 Total spent adjusted	510 \$16,60	97 \$682,969	\$663,719	Balance adjusted to	\$384,232	\$384,232	\$0	\$406,000	\$348,913 \$	57,087 \$194	415 \$194,415	5 \$0	\$111,889	\$111,889	\$0 \$	206,754 \$20	06,754	\$0 \$445,210	\$422,667	7 \$22,543	\$822,000	\$820,251 Balance a	\$1,749	\$380,318 \$380	0,318 \$0	0 \$525,389	\$520,850	\$4,539	\$93,343	\$93,343	\$0 \$126,6	77 \$126,677	\$0	\$212,266	\$211,708	\$558 \$195,24	9 \$195,249	\$0	\$266,500 Total spe	\$225,553 ent adjusted to Balance	\$40,947 \$5	/2,932 \$92,756	6 \$176	\$88,139
		discrepancy	discrepancy				discrepancy	ng			discrepancy																		account to discre	pancy			aco	discrepancy												disc	srepancy discre	repancy			

Page 161 of 162

Environment and Natural Resources Trust Fund

M.L. 2013 Project Budget - Overall Budget of Aquatic Invasive Species Research Center Project Title: Aquatic Invasive Species Research Center Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a Project Manager: Nicholas Phelps Organization: University of Minnesota M.L. 2014 ENRTF Appropriation: \$8,700,000 Project Length and Completion Date: 6 Years, June 30, 2019 Date of Report: November 11, 2019

Date of Report: November 11, 2019																																															
	6 (DNR)- Susta	ning Walleye	SUBPROJECT	16 (UMN)- Sust	taining Walleye	SUBPROJE	ECT 16 (NNRI)- S	Sustaining Walle	eye SUBP	PROJECT 17- Bu	ilding scientific a	and SL	BPROJECT 18-	- Eurasian and h	hybrid	SUBPROJEC	19 - Decision-I	making tool for	SUBPRO	DJECT 20 - A N	ovel Technolog	gy for eDNA S	UBPROJECT 21	- Early detect	ion of zebra	SUBPROJEC	T 22 (UMN) - Co	pper-based cont	ntrol: SUBP	PROJECT 22 (USG	S) - Copper-bas	sed SU	BPROJECT 23	- AIS Manageme	nt: An Eco-	SUBPROJECT 2	24 - Genetic m	ethod for control o	of SUBPROJECT	25 - What's In Y	/our Bucket?	SUBPROJECT	26 - Updating av	in invasive and			
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	sessing impacts	of AIS	Populations: As	ssessing impac	ts of AIS	Populations	s: Assessing imp	bacts of AIS	manag Phrag	agement capacity gmites in Minnes	ota	invasive wa	termilfoil genot	type distribution	n in Minnesota	optimal mana	gement of AIS		Collectio	on and Concent	ration (Year 1)	m	iussels using mu	ultibeam sonar		zebra mussel (Year 1)	settlement and	non-target impa	acts contro impact	ol: zebra mussel s cts (Year 1)	settlement and n	non-target ecc 1)		s of Ecosystem s	Services (Year	invasive fish sp	ecies (Year 1)		Quantifying Als	introduction Ri	sk (Year 1)	native fish pase	sage model for lo	ocks and dams			
BUDGET ITEM	Amount Snort	Subproject 16	Subproject 16	Amount Succ	Subproject	t 16 Subprojec	t 16	Subproj	ect 16 Subp	project 17	Sut	Ibproject 17 S	ubproject 18	Amount Sucut	Subproject 1	8 Subproject 1	9 Amount Suc	Subprojec	ct 19 Subpro	ject 20	Sub	oproject 20	Subproject 21	Amount Snow	Subproject 21	Subproject 2	22	Subproject	t 22 Subp	project 22	Subp	project 22 Su	ubproject 23	Amount Sport	Subproject 23	Subproject 24	Amount Sno	Subproject 24	4 Subproject 2	5 Amount Suc	Subproject 2	5 Subproject 2 [/]	ô Amount Sno	Subproject 2f		TOTAL	
Personnel (Wages and Benefits) - Overall Total	S16.27	5 S) \$28,645	5 \$28.6	ht Balance	\$0 \$7 ²	1.309 \$7	1.309	<u>ве в</u>	\$244.663	\$243.864	\$799	\$105.671	\$105.671	Balance	Бийдет 50 \$117.4	Amount Spe	911 \$7	се Бий 78,554	\$66,599	\$66.599	Salance \$0	\$37,743	<u>Amount Spen</u> \$37.65	5 Salance	Budget	\$0	<u>ent Баlance</u> \$0	<u>е в</u> \$0	\$12,428	<u>s12.428</u>	salance \$0	\$122,603	\$121,908	Balance \$695	5 \$90,588	Amount Spe 3 \$90.5	it Balance	<u>Бийдет</u>	42 \$78.5	92 \$2.0	50 \$86.8	24 \$84.3	309 \$2.5 ¹	5 \$6,597,6 [′]	98 \$6.436.	996 \$160,701
	÷·•,=·	-	<i> </i>	· · · · · · · · · · · · · · · · · · ·		**	,	.,	+ -	<i>+_</i> , <i></i>	<i> </i>		<i> </i>	<i> </i>		÷• • • • • • • • • • • • • • • • • • •	÷••,	····	-,	+,	+,	<i>+-</i>	<i>••••</i> ,••••	÷••,••	-		+-	<i>+-</i>	<i>v</i> ·	<i>•••••••••••••••••••••••••••••••••••••</i>	<i>•••••••••••••••••••••••••••••••••••••</i>	τ.	¢:,	<i> </i>	+	÷••,•••	+,-		, , , , , , , , , , , , , , , , , , ,		,	+++++++++++++++++++++++++++++++++++++++		··· +=,• ··	<i><i><i>vc</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>cci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>ccci</i>,<i>cccci</i>,<i>cccccci</i>,<i>ccccci</i>,<i>cccccci</i>,<i>ccccccccccccc</i></i></i>	÷,,	•••
Professional/Technical Services and Contracts - Overall To	otal \$44,24	5 \$() \$(0	\$0	\$0	\$0	\$0	\$0	\$15,120	\$15,120	\$0	\$94,704	\$94,395	\$30	09 \$47,0	00 \$39,	774 \$	57,226	\$6,805	\$6,805	\$0	\$48,771	\$48,56	6 \$20	ō	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0) \$6,538	8 \$6,5	538 \$	0 \$11,00	0 \$9,61	16 \$1,38	4 \$,0 (\$0 \$1	\$1,026,60	,2 \$955,1	⁴¹ \$71,461
Equipment/Tools/Supplies - Overall Total	\$5,37	D \$() \$(0	\$0	\$0 \$ [^]	1,130 \$	51,130	\$0	\$5,025	\$2,786	\$2,239	\$8,000	\$7,926	\$7	74 \$3,0	00 \$1,	784 \$	51,216	\$21,195	\$16,859	\$4,336	\$10,035	\$9,95	4 \$8	1 \$54,4	38 \$50,	,008 \$4	4,430	\$0	\$0	\$0	\$4,742	\$4,741	\$	\$11,020	5 \$10,6	608 \$41	2 \$19,00	0 \$12,97	71 \$6,02	.9 \$2,49	2 \$2,47	, <mark>77 \$1</mark> ′	\$658,62	.5 \$616,0	J <u>50</u> \$42,575
Capital Expenditures Over \$5,000 - Overall Total	\$	D \$() \$(0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	5	\$O	\$O	\$0	\$0	\$0	\$0	\$0	\$0	\$	0 \$	D	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0) \$C	D	\$0 \$.0 \$, <mark>0</mark>	\$0 \$,0 \$, <mark>0 </mark>	\$0 \$'	ג \$109,23	y <mark>6 \$109,2</mark>	236 \$0
Travel - Overall Total	\$21,29	5 \$954	4 \$800	0 \$7	739	\$62 \$8	3,677 \$	68,561	\$116	\$13,010	\$8,003	\$5,007	\$13,000	\$12,420	\$58	30 \$5,0	00	\$0 \$	65,000	\$0	\$0	\$0	\$0	\$	0 \$	D	\$0	\$0	\$0	\$0	\$0	\$0	\$4,500	\$4,500	\$(\$1,000	D \$3	\$69	4 \$1,00	1 <mark>0 \$3</mark> f	ð 1 \$6?	,9 \$1,51	1 \$1,5'	ر 10 \$	\$300,02	.8 \$264,3	327 \$35,704
Other - Overall Total	\$	D \$(D \$0	0	\$0	\$0	\$0	\$0	\$0	\$5,750	\$0	\$5,750	\$0	\$0		5O	5O	\$0	\$0	\$0	\$0	\$0	\$0	\$	0 \$	D	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$966	5 \$9	960 \$	6 \$	؟ (0,	\$0 ?	\$ 0,	<u>ې 0</u> ر	\$0 \$ ⁷	ر \$7,81	.3 \$2,0)25 \$5,788
Budget Reserve Pending Progress and Peer Review - Over Total	rall																																												\$,0	\$0 \$0
COLUMN TOTAL	\$87,18	5 \$954	\$29,44	5 \$29,3	383	\$62 \$8 ²	1,116 \$8	31,000	\$116	\$283,568	\$269,773	\$13,795	\$221,375	\$220,412	\$9	63 \$172,4	65 \$80,	469 \$9	91,996	\$94,599	\$90,263	\$4,336	\$96,549	\$96,17	5 \$37	4 \$54,4	-38 \$50,	,008 \$4	4,430	\$12,428	\$12,428	\$0	\$131,845	\$131,149	\$690	\$\$110,112	2 \$109,0	900 \$1,11	12 \$111,6/	42 \$101,5	40 \$10,1	J2 \$90,87	27 \$88,2	296 \$2,53	1 \$8,700,0r	J0 \$8,383, ⁻	770 \$316,230
					Balance adjuste	ed to																																							Total Budget adjuste	d Total Spent adjust	ed Total Balance
					discrepancy	y																																							rounding discrepan	<i>sy</i> rounding discrepa	incy rounding discrepancy



	τοται
96	\$160,701
41	\$71,461
50	\$42,575
36	\$0
27	\$35,704
25	\$5,788
\$0	\$0
70	\$316,230
d	Total Balance
	adjusted to account for
су	rounding discrepancy