M.L. 2013 Projects

MN Laws 2013, Chapter 52, Section 2 (beginning July 1, 2013)

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Subd. 06 Aquatic and Terrestrial Invasive Species

An Aquatic Invasive Species Research Center

Research Project

Subd. 06a $8,700,000 TF

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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.

Project Overview

Aquatic invasive species pose critical ecological and economic challenges for the entire state and beyond. They can cause irreparable harm to fisheries and aquatic habitat as well as damage to infrastructure. The problems posed by aquatic invasive species continue to grow as existing infestations expand and new exotic species arrive, most of which are poorly understood. New ideas and approaches are needed to develop real solutions. In 2012 the Minnesota Legislature provided the University of Minnesota with $3,800,000 ($2,000,000 from the Environment and Natural Resources Trust Fund; $1,800,000 from the Clean Water Fund) to launch a new, first-of-its-kind research center specifically focused on developing and implementing solutions to control aquatic invasive species. This appropriation provides this new center with additional initial operating funds for conducting research aimed at slowing the spread, reducing, controlling, and/or eradicating aquatic invasive species including Asian carp, zebra mussels, Eurasian watermilfoil, and more. Proven tools and techniques developed at the center are intended to be implemented statewide.

OVERALL PROJECT OUTCOME AND RESULTS

This project successfully established the Minnesota Aquatic Invasive Species Research Center (MAISRC) at the University of Minnesota, a vibrant and durable research program that develops research-based solutions to Minnesota's aquatic invasive species (AIS) problems. MAISRC has quickly become a global leader in the field and a go-to resource for managers, the public and researchers. In total, 32 subprojects were supported from this project – significantly advancing our scientific understanding and ability to manage AIS. New tools have been developed and knowledge gaps filled on many of Minnesota's most

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

PROJECT RESULTS USE AND DISSEMINATION

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

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

Sub-Projects M.L. 2013, 06a:

- *02: Metagenomic approaches to develop biological control strategies for aquatic invasive species* - $299,363
- *03: Attracting carp so their presence can be accurately assessed* - $682,269
- *04: Common carp management using biocontrol and toxins* - $384,231

- *07: Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents* - $445,210
- *08: Aquatic Invasive Species Research Center Sub-Project 8: Risk assessment, control, and restoration research on aquatic invasive plant species* - $822,000
- *09: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol.* - $427,950
- *10: Citizen Science and Professional Training Programs to Support AIS Response* - $566,550
- *12: Characterizing spiny water flea impacts using sediment records* - $207,766
- *14: Cost-effective monitoring of lakes newly infested with zebra mussels* - $266,500
- *16: Sustaining walleye populations: assessing impacts of AIS* - $198,700
- *17: Building scientific and management capacity to respond to invasive Phragmites in Minnesota* - $246,800
- *18: Eurasian and hybrid watermilfoil genotype distribution in Minnesota* - $221,375
- *19: Decision-making tool for optimal management of AIS* - $172,465
- *21: Early Detection of Zebra Mussels Using Multibeam Sonar* - $96,549
- *26: Updating an invasive and naitve fish passage model for locks and dams* - $90,827

Project Completed: 06/30/2019 **OVERALL FINAL REPORT**

Sub-Project 02: Metagenomic approaches to develop biological control strategies for aquatic invasive species - Phase II: Development of Potential Microbiological Control Agents for Aquatic Invasive Species - $299,363 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and zebra mussels (ZMs) pose a serious threat to the health and function of aquatic ecosystems. Traditional approaches for AIS management, including use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could be exploited to develop microbe-mediated AIS management strategies. As the first step towards the identification of potential biocontrol strategies, microbial communities associated with 'healthy' AIS were compared with that of 'diseased' AIS or to native species. Since no natural diseased mussels were available, we opted to develop an experimental model system, which allowed for the application of different intensities of stress – heat (17, 25, 33℃) 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.

PROJECT RESULTS USE AND DISSEMINATION

Our research findings were disseminated via oral and poster presentations at the following (international/ national/ local) conferences: 61st International Association for Great Lakes Research conference (Toronto, Canada), UNC Water Microbiology Conference 2019 (Chapel Hill, NC), 20th International Conference on Aquatic Invasive Species (Fort Lauderdale, FL), 5th Upper Midwest Invasive Species Conference (Rochester, MN), 119th General Meeting of the American Society for Microbiology (San Francisco, CA), and the AIS Research Management Showcase in 2017 & 2018 (St. Paul, MN). Two papers were published in the journals 'FEMS Microbiology Ecology' and 'Science of the Total Environment' during this project period. One manuscript is currently undergoing peer-review and two additional manuscripts are under preparation. All sequencing data generated in this project will be publicly available (via submission to NCBI Genbank) and all publications will list accession numbers to link to short read archive of all samples. Thus far, all sequence data mentioned in current publications is directly linked to a publicly available web site for download.

Subproject 02 Completed: 06/30/2019 **FINAL REPORT**

Sub-Project 03: Attracting carp so their presence can be accurately assesed - $682,969 TF

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OVERALL PROJECT OUTCOME AND RESULTS

This project developed several tools that can manage and control all species of invasive carp species in Minnesota. First, we developed ways using both food and sex pheromones to attract and measure the presence and density of carp using the environmental DNA (eDNA) they release to the water. This technique is superior to traditional netting because it can be performed in any habitat or water of any depth, including at low densities that are otherwise unmeasurable. eDNA can also determine carp gender. Second, we developed a deterrent system comprised of sound, light and air curtain that is 97% effective in the laboratory and could safely and effectively prevent invasive carp from swimming upstream through navigation locks in Mississippi River. If this deterrent system were to be paired with attractant-based eDNA surveillance methods in specific lock-and-dams whose gate was also adjusted to stop carp, it is extremely likely that enough carp could be prevented from passing through these lock-

and-dams that the remainder could be removed by targeted commercial fishing. Field tests of the deterrent system are now underway.

PROJECT RESULTS USE AND DISSEMINATION

The first invasive carp deterrent system in the world is now in place in southern Minnesota using the sensory cues we identified. The USGS is now exploring the pheromone and food attractants we developed in the Great Lakes, and the sound/light stimuli we developed are being used at Barkley Dam in Kentucky by the UAFWS with whom we have partnered with. Sorensen and colleagues have at 5 peer-reviewed scientific publications in high quality journals and several technical reports. A PhD and a MS thesis are being produced. A dozen talks were given as part of this project.

Subproject 03 Completed: 06/30/2019 **FINAL ABSTRACT**

Sub-Project 04: Common carp management using biocontrol and toxins: Phase II - $384,231 TF

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OVERALL PROJECT OUTCOME AND RESULTS

This project aimed to test new management tools for the common carp, Minnesota's most abundant invasive fish. We used a whole lake experiment to test if bluegill sunfish can reduce production of carp fry in shallow lakes (Activity 1). We also used a series of lab, pond and lake experiments to test if corn-based food pellets that contain a toxin can be used to selectively target carp without harming native fish (Activities 2, 3, 4). Activity 1 (bluegill experiment in 6 small lakes) showed that bluegills can suppress the production of carp fry in shallow lakes by 8-fold. Thus, maintaining healthy bluegill populations in lakes would serve as an important biocontrol strategy for carp in Minnesota.

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

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

PROJECT RESULTS USE AND DISSEMINATION

Information collected in these experiments were disseminated and will continue to be disseminated in a variety of ways. Presentations were given at MAISRC showcases, the Minnesota and National American Fisheries Society meetings, and will be given at the International Conference for Invasive Species.

Two manuscripts have been published:

- Poole, J. R., Sauey, B. W., Amberg, J. J., & Bajer, P. G. (2018). Assessing the efficacy of corn-based bait containing antimycin-a to control common carp populations using laboratory and pond experiments. Biological Invasions, 20(7), 1809-1820.
- Poole, J. R., & Bajer, P. G. (2019). A small native predator reduces reproductive success of a large invasive fish as revealed by whole-lake experiments. PloS one, 14(4), e0214009.

One manuscript has been submitted for publication:

- Hundt, P. J., Amberg, J. J., Sauey, B. W., & Bajer, P. G. 2019. Toward a new Common Carp (Cyprinus carpio) management tool: Laboratory and mesocosm experiments testing a species-specific corn-based bait containing a toxin. Submitted to Management of Biological Invasions

Subproject 04 Completed: 06/30/2017 **FINAL REPORT**

Assessing the efficacy of corn-based bait: Paper

Sub-Project 07: Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents - $445,210 TF

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OVERALL PROJECT OUTCOME AND RESULTS

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.

PROJECT RESULTS USE AND DISSEMINATION

We had learned during Phase 1 of this project (MAISRC Sub Project 7.1) that communication, outreach and transparency were very important for this type of project. To that end, the project team has spent considerable time engaging with managers, scientists, and the public in multiple formats. This has included formal in person meetings, local-national-international scientific conferences, peer-review publication, networking with internationally-renowned experts, video production, and print, radio and TV media. A summary of this is listed below:

Formal in-person meetings: Great Lakes Fish Health Committee, MN DNR Koi Herpes Virus Working Group.

Scientific conferences: American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop, MAISRC showcase (x3), International Conference on Aquatic Invasive Species, Minnesota Veterinary Diagnostic Laboratory, Aquatic Invaders Summit III, Freshwater Mollusk Conservation Society, International Symposium on Aquatic Animal Health. NOTE: Most of these conferences were supported by non-LCCMR funding.

Peer-review publication: Padhi, S. K., I. E. Tolo, M. McEachran, A. Primus, S. K. Mor, N. B. D. Phelps. In press. Koi herpesvirus and carp edema virus: Infections and coinfections during mortality events of wild common carp in the United States. Journal of Fish Disease. Several other publications are in progress.

Networking with experts: Dr. Ken McColl, Dr. Tom Waltzek, Dr. Mikolaj Ademek, and others.

Video production: Video 1 (viewed 822 times as of 8/8/19), Video 2 (viewed 96 times as of 8/8/19).

Media: New York Times, KSTP 5, KARE 11, Star Tribune, Minnesota Daily, MN DNR Press release, MAISRC newsletters.

Subproject 07 Completed: 06/30/2019 **FINAL REPORT**

Sub-Project 08: Aquatic Invasive Species Research Center Sub-Project 8: Risk assessment, control, and restoration research on aquatic invasive plant species - $822,000 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Aquatic invasive plants can lower native plant diversity, reduce habitat quality for fish and other animals, and interfere with recreation. To protect Minnesota's water resources, steps need to be taken to prevent new invasions, control existing populations, and support recovery of native biodiversity. These efforts require sound, science-based guidance. To provide such support, we conducted research to predict invasion risk, assess ecological impacts, evaluate control efficacy, and investigate factors limiting post-control recovery of native aquatic plants. This work was applied to three target species at different stages of invasion: (1) Nitellopsis obtusa (starry stonewort), first found in Minnesota in 2015 and now known in 14 lakes; (2) Myriophyllum spicatum (Eurasian watermilfoil), found in 1987 and established in >300 lakes; and (3) Potamogeton crispus (curly-leaf pondweed), here for >100 years and in >750 lakes. For starry stonewort, we developed models to predict risk of further spread and prioritize search locations for statewide volunteer search efforts, experiments to determine how long starry stonewort remains can survive out of water (i.e., remain transportable by boaters), and field and lab-based control experiments to guide management. For Eurasian watermilfoil and curly-leaf pondweed, we investigated relationships with native plant biodiversity, finding that they displace native species, an effect compounded by lower water clarity, and contribute to "biotic homogenization"—loss of ecological distinctiveness. We are investigating how to better control these invasive species and foster recovery of native vegetation by synthesizing thousands of aquatic plant surveys and management records collected in Minnesota and by conducting in-lake removal and restoration experiments. This work will continue under a follow-up project (MAISRC Subproject 8.2: Impacts of invader removal on native vegetation recovery). Our findings help Minnesotans by highlighting practices needed to protect lake ecosystems and refining approaches for preventing invasions, reducing populations of established AIS, and restoring native species.

PROJECT RESULTS USE AND DISSEMINATION

Information from this project has been disseminated through 10 peer-reviewed journal articles, 30 invited talks, 20 contributed presentations, 45 media stories, and resources published on the MAISRC website. Fully published articles (7 of the 10) are included as attachments. Project findings are being used to guide AIS spread prevention and management efforts involving the Minnesota Department of Natural Resources, lake associations, and other stakeholders. This project has also contributed significantly to MAISRC Subproject 10 ("Citizen Science and Professional Training Programs to Support AIS Response").

Subproject 08 Completed: 06/30/2019 **FINAL REPORT**

Lake and Reservoir Management Article: Response of the invasive alga starry stonewort *(Nitellopsis obtusa)*..
Journal of Aquatic Botany Article: Biology, ecology, and management of starry stonwort *(Nittellopsis obtuse;* Characeae)..
Journal of Ecology Article: Environmental filtering and competitive exclusion..
Realized niche shift associated with the Eurasian charophyte *Nitellopsis obtusa* becoming invasive in North America
Forecasting distributions of an aquatic invasive species *(Nitellopsis obtusa) under future climate scenarios*

Sub-Project 09: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol - $380,318 TF

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OVERALL SUBPROJECT OUTCOME AND RESULTS

Since arriving in Duluth Harbor in 1989, zebra mussels have infested more than 150 inland lakes and 17 rivers and streams in MN, with rising ecologic and economic costs. Efforts to block new invasions must be focused strategically on major sources of spread. To help achieve this, we used direct, forensic-like analyses to genetically identify waters from which mussels were carried to infest MN lakes. Using our new genome sequences and methods, we genetically classified mussels from more than 70 water bodies, with more than 6,000 DNA markers per mussel (compared to 9 markers/mussel in Subproject 9.1) – providing significantly increased clarity in the analysis. We found that lakes in the Detroit Lakes, Brainerd and Alexandria regions form large, unique genetic clusters found nowhere else. Additionally, mussels from the Mississippi and St. Croix Rivers, Lake Superior, and Lake Minnetonka (4 highly-likely source waters) are distinguishable from the clustered invasions with 6,000 genomic markers, but with our previous analysis of 9 markers, they were not. More research is needed across a larger, more regional landscape to determine the original sources of zebra mussels into MN, 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 MN and worldwide.

SUBPROJECT RESULTS USE AND DISSEMINATION

The results from this project were regularly communicated in presentations to public and professional audiences. McCartney delivered a total of 14 public presentations on research activities and outcomes at non-scientific meetings and events, and authored or co-authored a total of nine presentations on results of this work at professional conferences, meetings, and invited seminars, including talks at the University of MN Duluth, University of Montana Flathead Lake Biological Station, Montana Fish Wildlife and Parks, and the University of Iowa. As intended in the dissemination plan, outreach was accomplished at local, state and national levels with public talks in Douglas, Hubbard, Itasca, Meeker, Otter Tail, and Stearns Counties in MN, two in Wisconsin, two in Montana and one in Iowa. Media attention on this project was high and resulted in three print news items, including two front-page feature articles in the Minneapolis Star Tribune. A highlight was two podcasts by Montana Public Radio in which both the population genomics of spread and the genome sequencing projects were covered in

detail. Our research was regularly communicated in newsletter articles posted on the MAISRC website. Information about the zebra mussel genome project in the form of a white paper, written originally for a professional audience of scientists and managers in multiple disciplines (Activity 3), but accessible to members of the public with some background in AIS1. Two publications are in process (titles below)—one in revision2 and the other to be submitted soon. Two other manuscripts are in preparation, one on invasion genomics (Activity 1), and the other reporting on sequencing and analysis of the zebra mussel genome (Activities 2 and 3). All Next Generation Sequence data from Activities 1 and 2 will be publicly available in the MAISRC Data Repository at the University of MN or the National Center for Biotechnology Information database.

- McCartney, M.A., Mallez, S., Gohl, D. and K. Beckman (2018) The zebra mussel genome project: developing a new resource for invasion biology and biocontrol research. A white paper available from the author.
- McCartney, M.A., Mallez, S., Gohl, D. and K. Beckman (in revision) Genome projects in invasion and conservation genetics research programs. *Conservation Genetics*
- Mallez, S. and McCartney, M.A. (in prep) Moving zebra mussels into the 'omics' era: SNPs from NGS-based genotyping outperform microsatellites in discerning invasion sources. *Ecology and Evolution*

Subproject Completed: 12/31/2018 **FINAL REPORT**

The zebra mussel genome project

Sub-Project 10: Citizen Science and Professional Training Programs to Support AIS Response - $566,550 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Early detection of invasive species is critical. However, there are few professionals addressing aquatic invasive species (AIS) in MN 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 MNns 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 MN 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 MN and beyond. MNns 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 MNns 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.

PROJECT RESULTS USE AND DISSEMINATION

Information from our project has been disseminated through 2 publications (attached), 16 invited talks, 11 contributed presentations, 5 webinars, 69 media stories, and online resources. This project has also contributed significantly to MAISRC Subproject 8 ("Risk assessment, control, and restoration research on aquatic invasive plant species").

Subproject 10 Completed: 06/30/2019 **FINAL REPORT**

Journal of Extension: Flipping the Classroom to Train Citizen Scientists in Invasive Species Detection and Response

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Sub-Project 12: Characterizing spiny water flea impacts using sediment records - $212,266 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Although aquatic invasive species threaten MN'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 MN lakes. The results re-cast our understanding of the timeline of spiny water flea invasion in MN 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 MN, may be inadequate to detect spiny water flea when it is low or transient in abundance.

PROJECT RESULTS USE AND DISSEMINATION

We have disseminated our project results at a variety of conferences and meetings as summarized below:

- MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 12, 2016)
- MAISRC Research & Management Showcase (St. Paul, MN) – four laboratory presentations (September 12, 2016)
- Coe College Wilderness Field Station (Ely, MN) – platform presentation (July 22, 2017)
- MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 13, 2017)
- MAISRC All Members meeting (St. Paul, MN) – platform presentation (November 28, 2017)
- MAISRC Science-In-Seconds competition (St. Paul, MN) – platform presentation (May 30, 2018)
- MAISRC Research & Management Showcase (St. Paul, MN) – poster presentation (September 12, 2018)
- Upper Midwest Invasive Species Conference (Rochester, MN) – poster presentation (October 15-18, 2018)
- Association for the Sciences of Limnology and Oceanography Conference (San Juan, Puerto Rico) – poster presentation (Feb 23 – Mar 2, 2019)
- Rainy-Lake of the Woods Watershed Forum Conference (International Falls, MN) – poster presentation (March 13-14, 2019)
- MN Department of Natural Resources meeting (St. Paul, MN) – skype presentation (May 14, 2019)

We have included images of two poster presentations that were displayed at science conferences.

Subproject 12 Completed: 06/30/2019 **FINAL REPORT**

Sub-Project 14: Cost-effective monitoring of lakes newly infested with zebra mussels - $266,500 TF

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OVERALL PROJECT OUTCOME AND RESULTS

The current lack of standardized methods for surveying zebra mussels during their earliest stages of lake colonization limits our ability to track changes in density over time or to evaluate effectiveness of treatment programs (e.g., as required by DNR permits). We evaluated 5 different survey designs for estimating zebra mussel density (2 designs in 2017 and 3 designs in 2018), employing methods that utilize counts by two divers to estimate the probability of detecting mussels in the surveyed area. We also compared survey designs in terms of their density estimates, associated measures of uncertainty, and sampling efficiencies (time required to complete a survey), using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data. In 2017 in Lake Burgan, we estimated that a diver could detect between 5% and 41% of the mussels present in the surveyed area, depending on the specific diver and on whether the lake bottom was vegetated, with vegetation having the larger effect on detection. Accounting for low detectability of zebra mussels led to an estimate of density over three times higher than the observed density. Thus, for every zebra mussel detected by our divers, approximately two were missed. Using the data collected in 2018 and further simulation and analytical work, we found that double-observer survey designs that allow for imperfect detection are optimal when surveying lakes at low density, whereas quadrat counts that assume perfect detection are optimal at higher densities. We developed a training video, data collection worksheets, and an analysis tutorial so that others may implement our proposed survey designs in newly infested lakes. These tools benefit MNn's by providing better ways to monitor lakes infested with zebra mussels and to evaluate the effects of treatment options on zebra mussel density.

PROJECT RESULTS USE AND DISSEMINATION

We have developed several resources to facilitate uptake of our survey methods, including a website describing the project (https://zebramusselsurveys.netlify.com/), an instructional video demonstrating the survey methods (https://www.youtube.com/watch?v=E3ui8SVeBC0&feature=youtube), data sheets and google forms for data entry (https://zebramusselsurveys.netlify.com/forms), and an analysis vignette or tutorial using open-source software to analyze data collected from our survey designs (https://zebramusselsurveys.netlify.com/tutorial).

We have submitted a paper to Freshwater Science describing the survey methods we used in our first field season, along with estimates of density in Lake Burgan in 2017; we received a favorable review, and it has been forwarded to the editor for final consideration. We are currently working on an additional manuscript comparing the different survey methods in terms of their sampling efficiency (time required to complete a survey) and the resulting density estimates and associated measures of uncertainty using data collected in 3 lakes of varying density and using a simulation study and analytical framework informed by our data.

We have presented our research results via oral and poster presentations at professional conferences (Upper Midwest Invasive Species Conference, Hawaii Conservation Conference), MAISRC Research & Management Showcase events (oral presentations and a "hands on" demonstration of our survey designs), and a MAISRC outreach event sponsored by the Pelican River Watershed District. In the fall of 2019, we plan to offer a MAISRC-sponsored webinar to discuss our work, allowing us to reach a broad audience of scientists and managers interested in zebra mussel monitoring and control efforts.

Subproject 14 Completed: 06/30/2019 **FINAL REPORT**

Estimating densities of zebra mussels *(Dreissena polymorpha)* in early invasions using distance sampling

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Sub-Project 16: Sustaining walleye populations: assessing impacts of AIS - $198,700 TF

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OVERALL PROJECT OUTCOME AND RESULTS

MN 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 MN'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.

PROJECT RESULTS USE AND DISSEMINATION

- A manuscript documenting the results of our historical growth analysis has been submitted to the peer-reviewed journal Biological Invasions (submitted draft attached).
- We have delivered several presentations at scientific conferences, meetings with managers, and to the public:
- Our work has been covered in the popular press and University media:

- DNR Launches high-tech study of food webs in MN's largest walleye lakes. Tony Kennedy, Star Tribune. 19 August 2017 - http://www.startribune.com/dnr-launches-high-tech-study-of-food-webs-in-MN-s-largest-walleye-lakes/441088893/
- MN scientists dive deep to learn why walleye are stressed. Dan Gunderson, MN Public Radio. 18 July 2017 - https://www.mprnews.org/story/2017/07/18/scientists-digging-deeper-to-understand-factors-affecting-walleye
- Are lake invaders affecting walleye? June Breneman, NRRI news. 27 July 2017 - https://www.nrri.umn.edu/natural-resources-research-institute/news/ais-walleye

- We worked with MAISRC communications staff to develop a project fact sheet (Attached), which we distributed to interested citizens and to DNR offices.
- We have maintained an active social media presence (on Twitter) describing our ongoing research. The MNDNR and NRRI public information staff are in contact with the MAISRC communications coordinator to facilitate posting of information to social media posts of all three organizations.
- We worked with MAISRC staff to develop a video describing our work, viewable here: https://www.maisrc.umn.edu/news/walleye-video

Subproject 16 Completed: 06/30/2019 **FINAL REPORT**

Aquatic Invasive Species Fact Sheet

Sub-Project 17: Building scientific and management capacity to respond to invasive Phragmites in MN - $283, TF

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OVERALL PROJECT OUTCOME AND RESULTS

MnPhrag is an early detection and response effort targeting invasive Phragmites australis (common reed) (www.mnphrag.org), with the goal of supporting landscape-scale, strategic management throughout MN. 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 MN, 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 MN; 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 MNns 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 MN.

PROJECT RESULTS USE AND DISSEMINATION

Information from this project has been disseminated through 19 invited talks, 6 contributed presentations, 1 webinar, 1 radio interview, and reports and resources published on our website (www.mnphrag.org). Our Phragmites Identification Guide and the report "An assessment to support strategic, coordinated response to invasive Phragmites australis in MN" are included as attachments. Project findings are being used by the MN Noxious Weed Advisory Committee, the MN Department of Natural Resources, the MN Department of Agriculture, and the MN Pollution Control Agency to assess risk of Phragmites invasion in MN and review relevant regulations, permitting, and policy.

Subproject 17 Completed: 06/30/2019 **FINAL REPORT**

An assessment to support strategic, coordinated response to invasive *Phragmites australis* in MN
A Guide to Identifying Native and Non-native Phragmites australis

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Sub-Project 18: Eurasian and hybrid watermilfoil genotype distribution in MN - $221,375 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Eurasian watermilfoil (Myriophyllum spicatum) is one of the most problematic invasive aquatic plants in MN. 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 MN 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 MN 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.

PROJECT RESULTS USE AND DISSEMINATION

We disseminated our results with presentations at the MAISRC Research & Management Showcase, several regional meetings and the national Aquatic Plant Management Society. We met with DNR Specialists, lake managers, consultants and other stakeholders twice to present results and to seek input on further work. In conjunction with MAISRC staff, we developed a Google Map indicating the locations we sampled and found Eurasian, hybrid and northern watermilfoil (https://www.maisrc.umn.edu/hybrid-distribution). This map will be updated as we get new information. We also generated a preliminary report in March 2019 and a final report detailing the background, methods, results and conclusions for distribution to managers and stakeholders and posting on the MAISRC website. The DNR and managers are starting to take this information into account when planning control activities.

Subproject 18 Completed: 06/30/2019 **FINAL REPORT**

Eurasian and hybrid watermilfoil genotype distribution in MN

Sub-Project 19: Decision-making tool for optimal management of AIS - $299,363 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Understanding the patterns of historic AIS invasion can provide the framework for forecasting future invasions. To that end, we used a big data approach to combine hydrologic connectivity and boat movement to create a multiplex metacommunity model for both zebra mussel and Eurasian watermilfoil. We found that the hydrological corridors are important pathways of spread, even more so that previous research has suggested. While overland dispersal of AIS via boater movement is still a significant factor, additional management strategies should be developed to include intervention of hydrological pathways.

Using connectivity networks of boater movement, we developed county-based AIS management optimization models that prioritize inspection locations that will intercept the highest number of 'risky boats' (e.g. moving from infested to uninfested lakes). We piloted the models in Crow Wing, Ramsey, and Stearns Counties and had a very productive collaboration with county managers and citizen advisory boards during the development and evaluation for each. Ultimately, the application of this approach was well received and helped inform allocation of their inspection hours at the county level (for example: https://www.crowwing.us/1004/Aquatic-Invasive-Species-AIS).

Dissemination and usability of the models was a priority of this project. We created online tools to 1) visualize the spread risk for zebra mussels and Eurasian watermilfoil based on model predictions made in Activity 1, and 2) visualize and modify the decision optimization model at the county level based on management thresholds or funding availability. These tools and more detailed descriptions of the project has been disseminated through in-person stakeholder meetings and presentations to diverse audiences, including managers, researchers and the public.

PROJECT RESULTS USE AND DISSEMINATION

Efforts were made throughout the project to engage end-users, share findings and make deliverables broadly available. We used a combination of formal and informal dissemination strategies for this project given the direct application to AIS managers and broad interest among other stakeholders. We held in-person meetings with County representatives and citizen advisor boards from Crow Wing, Ramsey and Stearns Counties to present results and update our models according to their input. These meetings were highly valuable to the project team and the outcomes of the project. In addition, we provided scientific and/or outreach presentations at the International Conference on Aquatic Invasive Species, the Aquatic Invaders Summit, the Cass County Watercraft Inspectors annual training, the annual AIS Roundtable, and MAISRC's Research and Management Showcase. Several publications are currently in late-stage drafts and will be submitted for peer-review in the coming months.

Subproject 19 Completed: 06/30/2019 **FINAL REPORT**

Sub-Project 21: Early detection of zebra mussels using multibeam sonar - $96,549 TF

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OVERALL PROJECT OUTCOME AND RESULTS

Zebra mussels pose a serious threat to MN 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).

PROJECT RESULTS USE AND DISSEMINATION

Research results from Phase I will be disseminated through a peer-reviewed publication (in preparation) and will inform Phase II field testing starting July 2019 (MAISRC Subproject 21.2: Field validation of mulitbeam sonar zebra mussel detection). During this one-year project, we participated in MAISRC Fellows meetings and presented our project to the public at the annual MAISRC Research & Management Showcase. The MN Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

Subproject 21 Completed: 06/30/2019 **FINAL REPORT**

Sub-Project 26: Updating an invasive and native fish passage model for locks and dams - $90,827 TF

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OVERALL PROJECT OUTCOME AND RESULTS

The main purpose of the project was to develop an updated version of the Computational Fluid Dynamics Agent-Based (CFD-AB) fish passage model (Zielinski, et al., 2018) using the field/experimental data of fish passage through Lock and Dam #2. This updated CFD-AB model can better help stop invasive carps while allowing native fish to pass through Mississippi River locks and dams.

The subproject has been fulfilled for all the goals that were declared:

1. The computational code CFD-AB directed to enhance the simulation of swimming fish trying to pass through the navigation dams was updated/developed. The analysis of different fish passage index (FPI) showed that the values of FPI for the modified algorithm for a model channel (Gilmanov, et al., 2019, Water, under review) were greater than the FPI of the original algorithm

at about 16%. At this moment, no essential differences in fish passage index FPI for the original and modified model at LD2 and LD8 have been found. This effect can be explained by the special gate adjustments, which generate a rather high fluid flow prevented fish to pass through the dams. In other words, in case of blocking invasive species, the modified algorithm does not change the final results of FPI at LD2 and LD8. But the modified algorithm could play a positive role to help native fish to pass through the navigation dams in the case of changing gate adjustments leading to decrease flow velocity.

2. The modified algorithms now account for more realistic fish behavior, including placement of “attraction points”, such as resting zones characterized by low recirculating fluid flow. These parameters have been informed by the literature and unpublished field data collected on other projects.

3. Based on investigations of (Larson, et al., 2017, Kokotovich et al, 2017) it was reported that the “Invasive Front" is currently positioned in southern Iowa between Pool 14 and Pool 16. Therefore, the strategy of blocking bigheaded carp at Lock and Dams of MN 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 MN.

PROJECT RESULTS USE AND DISSEMINATION

The results of the “MAISRC Subproject 26: Updating an invasive and native fish passage model for locks and dams” were/will be presented at the following events:

- MAISRC Research & Management Showcase (2018) with a poster presentation "A computational model provides a way to stop invasive carp at two key MN Lock and Dams." Discussions and conversation with different groups of people were very informative and helpful.
- 2018 Upper Midwest Invasive Species Conference that was held with a joint conference of North American Invasive Species Management Association on October 15-18, 2018 - Mayo Civic Center - Rochester, MN and made an oral presentation "Computational model of fish swimming through Mississippi River locks and dams demonstrates ways to stop carp."
- The paper (Gilmanov, et al., 2019, under review) with the description of development/modification of CFD-AB model was submitted to the “Water” (an Open Access Journal from MDPI).
- MAISRC Research & Management Showcase (2019) with a poster “Mississippi River Dams: blocking invasive fish, helping natives”.
- Additional paper "Spillway gate settings in Mississippi River navigation lock and dams can be used to help native fish upstream passage" is in process and will be submitted for review in October-November 2019.

- The computer code of fish swimming through the navigation dam LD2 will be prepared and put in the publicly accessible Data Repository and the University of MN (DRUM) system.

Subproject 26 completed: 06/30/2019 **FINAL REPORT**

Project Completed: 6/30/2019 **OVERALL FINAL REPORT**