2012 Project Abstract
For the Period Ending June 30, 2018

PROJECT TITLE: Aquatic Invasive Species (AIS) Cooperative Research Center; Appropriation
PROJECT MANAGER: Peter Sorensen
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2012, Chp. 264, Art.4, Sec. 3

APPROPRIATION AMOUNT: $2,000,000
AMOUNT SPENT: $2,000,000
AMOUNT REMAINING: $0

This project established a new research center at the University of Minnesota dedicated to developing sustainable solutions to the problems posed by aquatic invasive species (AIS) and developed a solution for bigheaded carp from Asia ("invasive carps"), two of the primary issues faced by our region. The Minnesota Aquatic Invasive Species Research Center (MAISRC) still exists at the University although it now has a new leadership, administrative structure, and vision. As part of this project, associate and scientific directors for MAISRC were hired; they then initiated the process of hiring the state's only zebra mussel and aquatic plant experts, acquired funding for a new research laboratory, renovated an extant laboratory, and established a communications plan. A memorandum of understanding with the DNR was created as well as an administrative structure that included boards dedicated to self-governance, research, and strategic vision. In addition, research on invasive carp was conducted which identified a possible affordable and sustainable solution that does not cause collateral damage. This solution entails strategically adjusting gate openings in river locks and dams to prevent carp passage and adding sound systems to lock gates; it is now being implemented at Lock and Dam 8 with new ENRTF funding as well as a site in Kentucky by the U.S. Fish and Wildlife Service. This solution was enabled by new developments in molecular survey techniques ("eDNA") also instigated by this study, which showed that, contrary to public fears, few invasive carp had reached Minnesota. Finally, this study showed that an important fish disease (VHS) is not in Minnesota water and that invasive carps use novel foods and social signals (pheromones) that could be deployed in control were they to enter Minnesota. All this information is publically available and in the hands of the DNR awaiting full implementation.

Project Results Use and Dissemination
The first invasive carp deterrent system in the world is now in place in southern Minnesota and is now being upgraded. The only known state-directed AIS research center is also up and running. Information about this research center and its solutions are being disseminated via a website, an e-newsletter and a Facebook account, as well as via both radio and TV coverage. Sorensen and colleagues have at 11 peer-reviewed scientific publications in high quality journals and several technical reports while other MAISRC investigators have also published others. eDNA survey results conducted by MAISRC were used by the DNR and USFWS to make decisions about invasive carp survey techniques while information on feeding attractants is now being considered for use by the U.S. Geological Survey in fish toxin design. Over 3 dozen public talks were given as part of this project.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2012 Work Plan Main Document Final Report

Date of Status Update: August 15, 2018

Final Report

Date of Work Plan Approval: June, 2012

Project Completion Date: 6/30/2018

Project Title: Aquatic Invasive Species (AIS) Cooperative Research Center; Appropriation

Project Manager: Peter Sorensen

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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 $:	2,000,000
	Amount Spent $:	2,000,000
	Balance $:	0

Legal Citation: M.L. 2012, Chp. 264, Art.4, Sec. 3

Appropriation Language:
$2,000,000 is appropriated in fiscal year 2013 from the environment and natural resources trust fund to the Board of Regents of the University of Minnesota to develop and implement an Aquatic Invasive Species Cooperative Research Center, including equipment and facility development. As a condition of receiving this appropriation, the University of Minnesota is requested to collaborate with the commissioner of natural resources in developing solutions to control aquatic invasive species. Money appropriated in this section may not be spent on activities unless they are directly related to and necessary for the purposes of this section. Money appropriated in this section must not be spent on indirect costs or other institutional overhead charges that are not directly related to and necessary for the purposes of this section. This is a onetime appropriation and is available until June 30, 2018.

I. PROJECT TITLE: Establishing an AIS Cooperative Research Center and Evaluating Threats and Solutions for Asian carp

II. PROJECT SUMMARY: The legislature granted the University of Minnesota $2,000,000 from the LCCMR to start an Aquatic Invasive Species Cooperative Research Center to address and solve aquatic invasive species (AIS) problems in the state. The University will use this initial funding to establish the administrative structure for this center, establish and renovate its facilities, start studies of Asian carp biology designed to control this species, and develop work plans for the LCCMR to ensure continuing funding for the center. This three-year project is designed to stand alone while establishing a solid foundation for a second phase of operating funding being requested from the ENRTF for 2013-2019, and coordinating with ongoing zebra mussel work at the University which will be supported by the Clean Water Fund.

III. PROJECT STATUS UPDATES:

Project Status as of *June 30, 2013*

The Minnesota Aquatic Invasive Species Research Center (MAISRC) started conducting studies on AIS control in December 2012 when it also hired an associate director to work with the scientific director. A memorandum of understanding with the Minnesota Department of Natural Resources (MN DNR) has been signed to establish MAISRC's structure and function, and an advisory board meeting is being scheduled for July. A website and Facebook page have been created. An initial evaluation of eDNA as a technique to measure the presence of invasive Asian carp was completed with the assistance of the USGS. It demonstrated that at least in its present form (that endorsed by the US Army Corps of Engineers), this technique is prone to false negatives and positives as well as being slow and expensive. Accordingly, the MN DNR was informed that the eDNA technique is presently unsuited for use in routine fisheries management. No evidence of Asian carp in Minnesota waters was described during the course of this work which was jointly announced in a press release with the MN DNR. A postdoctoral associate was hired to start developing new and better eDNA techniques for measuring the abundance of common carp and this work is showing promise with the development of two new markers and a metagenomics approach. A dozen lakes have also been surveyed for the viral hemorrhagic septicemia virus (VHS; an invasive fish virus), and these results have shown no indication that it is present in Minnesota. Research facilities have been repaired in Hodson Hall and plans are now underway to renovate a central holding facility for AIS while using Hodson as temporary holding facility. A search for a zebra mussel biologist is also now underway. Several grant proposals have been submitted.

Amendment Request (June 21, 2013):

Activity #1: The scientific director has discovered that his time is needed in the summer to manage the center and budget so he has added three weeks of salary and benefits. Additionally, a quarter time technician whose time was previously split among three activities (4,5, and 6) is now being fully accounted for under Activity #1 in order to simplify record- keeping. The position has also increased from quarter time to full time. Some savings were recognized by a delayed hiring of administrative staff. The net result is an Activity #1 budget increase of $33,760 in the personnel budget category.

Activity #3: The unexpectedly poor performance of eDNA analysis has lead us to move funds from eDNA analysis by the USGS to supplies, staff and travel costs for eDNA collection and archiving by MAISRC. The net result is a decrease in Activity #3 budget by $25,065.

Activity #4: We have identified a reduced need for a P.I.'s summer salary and also realized some staff and supplies cost savings due to a late start. We have also added an undergraduate student assistant. Net result is a decrease in Activity #4 budget by $71,638.

Activity #5: The fish technician time is being consolidated and moved to Activity #1 as explained above to simplify record keeping, we have added an undergrad student assistant, and we have added some PI time for summer of 2013, which had previously been inadvertently left out. The net result is a budget decrease for Activity #5 of $5,935 in the personnel budget category.

Activity #6: The fish technician time is being consolidated and moved to Activity #1 as explained above to simplify record keeping, we have increased supplies, and we have added some PI time for summer of 2013, which had previously been inadvertently left out. The net result is a decrease in the Activity #6 budget by $14,790.

Activity #8: This activity was created to address a critical and unanticipated need in carp deterrent system development and assessment. This new project will examine air bubble deterrents for Asian carp. The new activity will have a budget of $83,668.

Summary of budget amendments:

Activity	**Budget Decrease**	**Budget Increase**
Activity #1		$33,760
Activity #3	$25,065	
Activity #4	$71,638	
Activity #5	$5,935	
Activity #6	$14,790	
Activity #8		$83,668
Total	$117,428	$117,428

In reformatting this amendment request and Attachment A as requested by LCCMR, it became apparent that some budget items needed to change. Consequently, the numbers shown above and some amount spent, remaining balances, and outcome budget numbers have changed slightly from those shown on the amendment request that was conditionally approved on 6/28/13.

Amendment approved: August 6, 2013

Project Status as of *April 1, 2014*

The Minnesota Aquatic Invasive Species Research Center (MAISRC) research is well underway with progress and discoveries being made in several of its scientific activities.

Collection of eDNA samples in previously sampled areas of the Mississippi River continued for archival for future analysis by the MAISRC once eDNA technology has been optimized. In the meantime a subsample is being analyzed to assist the DNR. With no cost extension in funding from MAISRC, the USGS used our 2012 archived eDNA samples to develop and test new qPCR markers and techniques for both Silver and Bigheaded carp that have reduced the incidence of false negatives and might eventually be published and used as part of a coordinated and multifaceted systematic sampling scheme for Bigheaded carps in Minnesota with the US Fish&Wildlife Service which we are now developing.

Using common carp as a model, we have developed a valid and reliable qPCR assay marker and used it to determine optimal eDNA sampling and extraction procedures and available eDNA extraction kits. Future work includes further investigation into degradation dynamics and the relationship between species abundance and eDNA. Metagenomic sequencing has also identified a number of bacteria specific to bigheaded and common carps that have potential to be developed into a testable bacterial

source-tracking markers that could be used to determine the presence of these fishes. We want to supplement funding in this topic via future amendment as it shows great promise.

MAISRC experiments have now conclusively demonstrated that juvenile silver carp shoal (aggregate) in the laboratory, meaning that the Judas fish concept has potential to be used to locate and control this species. Sterilization techniques have been developed for common carp and show promise for future application to Bigheaded carps. Other experiments planned for the summer of 2014 will determine whether and how sexual receptivity and pheromone release might enhance the probability of shoaling.

Laboratory experiments have also shown that food attractants have the potential to be used for control of Bigheaded carps: experiments using juvenile bighead and silver carp have demonstrated that ingestion behavior (buccal pumping) in both species is largely mediated by food-related chemicals and can be stimulated with highly filtered food extracts. Upcoming work will examine the chemosensory system that is responsible for this behavior and the chemical identity of the cues.

3,340 fish from 33 lakes and rivers have now been tested for VHSV. All results have been negative and results were posted on the Minnesota Veterinary Diagnostic Laboratory's fish health website and on the MDNR's Lake Finder website. This activity will conclude after about 15 additional bodies of water are tested this Spring.

We have tested the effects of sound alone on common carp and bigheaded carps and were able to demonstrate that carp are strongly repelled by sound in a highly directional manner that is associated with local acoustic particle motion. Two experimental setups have been constructed and fish pit tagged to test how bubble curtains might now be used at low cost to deflect bigheaded carp movement in controlled manners for possible application in small streams. Initial experiments are expected to be complete by July.

Administrative progress has been made, including finalizing an MOU between the Center, the Department of Fisheries Wildlife, and Conservation Biology (the Center's administrative home) and the College of Food, Agriculture and Natural Resources Sciences that establishes policies and procedures for, among other things, ENRTF subproject approval and hiring of non-tenured Center faculty. Budget reporting forms and procedures with the LCCMR have been streamlined. A new zebra mussel faculty member has been hired, the Center Advisory Board has met twice, and several committees have been established. A number of educational and outreach events have been hosted, including a joint effort with the Freshwater Society featuring Asian carp expert Duane Chapman. Future efforts will focus on providing technical and scientific assistance for the Scientific Director. A part-time communications position has been advertised to help the Administrative Director.

Two existing aquatic invasive species research projects headed by Sorensen have been voted in as Next Generation Projects by Center Faculty and funding for two additional projects (LCCMR and Minnehaha Creek Watershed District) is being pursued. The College reimbursed MAISRC for work already completed on Hodson Hall renovations and MAISRC's ENRTF funds have been redirected toward drilling a new well (which is due to be operational soon) and for future security upgrades to the holding and research facility. Extensive planning for more complete holding and research facility renovations has taken place and additional (bonding) dollars is being sought by the University from the 2014 Legislature. If awarded, construction (including aforementioned security upgrades) will begin in Fall 2014.

Amendment Request (April 1, 2014): We request the following amendments to accommodate the new budget format, to correct some errors discovered while transitioning to the new budget format, and to account for expenses incurred and planned for the next 6 months:

Activity 1

- To change personnel from $393,444 to $401,693 to account for a previous error. This allows our total budget to correctly add up to $2,000,000.
- To redistribute $6,700 funds from personnel to services, repairs, and non-capital equipment.
- To increase services from $0 to $1,730 to cover printing duplication, mailing expenses etc (for example to receive shipments of new Asian carp)
- To increase repairs from $0 to $5,045 to cover blower, well pump, and other repairs
- To reduce office supplies from $8,000 to $3,187 and redistribute to the proper UMN budget categories of lab supplies ($332) and non-capital equipment ($6,028).
- To decrease funds in travel from $8,000 to $6,378 and use them to cover supplies and equipment needs instead.

Activity 4
- To change personnel from $146,396 to $152,398 to account for a previous error. This results in our total budget accurately adding up to $2,000,000.
- To redistribute $4,436 from supplies to the proper UMN budget category of non-capital equipment

Activity 5
- To add $5000 in services to Activity 5 from Activity 6 for carp sterilization and other lab services. This results in an Activity budget change.

Activity 6
- To change personnel from $94,928 to $94,849 to account for a previous error. This results in our total budget accurately adding up to $2,000,000.
- To add a professional and admin position until that staff person became a grad student
- To move $5000 in services from Activity 6 to Activity 5

Activity 7
- To change personnel from $4,071 to $4,047 to account for a previous error. This results in a slight change to the Activity budget and results in the total budget accurately adding up to $2,000,000.
- To move $20,000 from supplies to cover lab and field services instead based on the needs for the next several months.

Activity 8
- To redistribute $1,883 lab & field supplies to the proper UMN budget category of non-capital equipment,

Amendment Approved: April 23, 2014

Amendment Request (May 22, 2014):

In response to the recent report of late-stage Bigheaded carp embryos being found in Mississippi River Pool 9, the MAISRC requests approval to use 2012 funds to immediately purchase and install underwater transducers at Lock & Dam #8 before the spawning season and before July 1, when a 2014 ENRTF appropriation is anticipated to become available to continue this work and conduct associated research. The purchase and installation work would be completed under Activity 8, which aims to use sound deterrents to control the movement of bighead and silver carp in Minnesota's rivers. The anticipated budget for this expedited purchase and installation at Lock & Dam #8 is $75,000.

Specific funds for this expedited work would come from the transfer of $45,224 in personnel costs from Activity #1 to Activity #8 and re-allocation of $24,476 within the Activity #8 budget.

$15,000 of the revised Activity #8 budget will be used to meet the bubble deterrent outcomes originally identified under Activity #8 by June 30, 2014.

Specific budget re-allocation under Activity #8 includes:

- Decrease personnel from $65,668 to $51,706 because these funds are no longer needed
- Addition of $18,000 in professional services to pay scuba divers and an electrician to install and power the transducers. These contracts will be granted to contractors acceptable to Army Corps of Engineers and in an accordance with University contracting procedures
- Increase in supplies from $11,117 to $14,469 to pay for cable, sub-surface attachment supplies, and amplifier housing for the transducers
- Increase in non- capital equipment from $1,883 to $5,883 to pay for an equipment rack, MP3 player and signal splitter, wireless alarm system, and sensors for the transducer work plus 4 small transducers for the bubble deterrent work.
- Increase of $35,810 in capital equipment to pay for 5 underwater transducers at $8,200 each
- Decrease in Minnesota travel from $5,000 to $523 because it's no longer needed
- Addition of $1,600 domestic travel (some shifted from Minnesota travel) for Doctors Sorensen and Zielinski to attend the 2014 International Conference on Engineering & Ecohydrology for Fish Passage conference in Madison, Wisconsin as part of their completion of the bubble deterrent work.
- Addition of $900 utilities to pay for electricity to power the transducers at Lock & Dam #8, an expense the Army Corps of Engineers is not permitted to pay.

Net change is increase in Activity #8 budget from $83,668 to $128,891 and decrease in Activity #1 from $424,393 to $379,170.

Please note, if MAISRC learns prior to installation that there is evidence of successful Bigheaded carp reproduction upstream of Lock & Dam #8, this plan may shift to install transducers at Lock & Dam #5 rather than at #8.

MAISRC also requests an amendment in Activity #4 to shift $340 from the supplies budget line to the non-capital equipment budget line to accommodate the purchase of an additional piece of non-capital equipment (a pipette). This amendment results in an:

- Increase in non- capital equipment budget from $4,436 to $4,776 and
- Decrease in supplies budget from $73,300 to $72,960.

Amendment Approved May 28, 2014

Project Status as of *June 30, 2014*

Research at the MAISRC continues to progress with objectives being met, new discoveries being made, and an administrative structure in place to support it. The Center has undergone a change in leadership with Dr. Susan Galatowitsch becoming the Center Director and the *de facto* project manager for Activity #1. New positions as proposed with ENRTF 2013 funds are being pursued, Dr. McCartney's zebra mussel project has been reviewed through the peer review process with his summer field season launched, and new project proposals are being scheduled for this Fall.

The Center Faculty Group and Center Advisory Board are engaging in discussions about strategic planning and future Center priorities. 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.

Activity #2.The new well at the Engineering and Fisheries Lab is now fully functional and funds have been secured for more significant renovations that are expected to occur sometime this winter/spring.

For Activity #3, Mississippi River water samples collected from Lock and Dam #1 in 2012 have been analyzed and do not to contain measurable amounts of Silver carp eDNA. A collaborative plan is being developed with the USFWS and MN DNR to accommodate and enhance University eDNA research whereby the USFWS would take responsibility for eDNA sampling and analysis downstream of Lock and Dam #5 while collecting samples for the University for research and archival purposes above that site. The University would guide the MN DNR through data analysis and interpretation.

For Activity #4, experiments on eDNA technology continue using the common carp as a model. In the process of validating markers using this well established model, it has been discovered that at least for this species, eDNA does not persist across large expanses of natural waters, calling to attention a new, critical need to sample waters in an extremely strategic manner (i.e. only in locations where carp are expected to be).

For Activity #5, masculinization of carps using steroid implants has been characterized and appears to be a viable technology for use with Judas fish and invasive carp detection but also one that requires several months and thus advance planning. While follow-up studies are ongoing with Silver carp, feminization is also now being examined as an alternative.

For Activity #6, experiments with feeding stimulants have now shown for both Bighead and Silver carp species that novel – and potentially powerful- chemicals found in algal food can drive food sampling (buccal pumping). It appears that potency would be enhanced in the presence of particles.

For Activity #7,1,212 fish from 15 bodies of water were tested for VHSV this spring. Testing for six locations is complete, with all results negative. Testing from the remaining nine locations is still in progress as of June 30 and will be updated prior to the final report for this activity being submitted. While Minnesota remains free of VHS and the funding for this project concludes, the threats of introduction remain a concern for the State's fish health managers.

For Activity #8, tests of enhanced bubble curtains in the laboratory have shown that this simple and inexpensive technology can, on its own, deflect nearly 90% of both Bighead and Silver carp from entering experimental channels in the laboratory. Other work shows that sound can deter all invasive carps in controlled, directional manners and might be used to enhance bubble curtains. Plans to install a sonic deflection shield for invasive carps at Lock and Dam #8 near the Iowa border are complete (transducers have been purchased along with other supplies) and installation simply awaits the flood waters to recede and a real estate plan that will allow the University to pay contracted electricians and divers to install the transducers. This is work in progess and we expect will be complete within a few weeks, in time to block summer migrations.

Amendment request as of June 30, 2014:

Activity #8: Transducers have been purchased and the divers and electrician are on standby to complete their work once a real estate agreement with the Army Corps of Engineers is in place and waters recede. Actual costs for much of this work have been confirmed and new needs have been identified for Activity #8. The following amendment is therefore requested:

- Increase in capital equipment from $35,810 to $38,781 because the 5 underwater transducers were slightly more expensive than anticipated.
- Decrease in supplies from $14,469 to $8,646, as the cable, sub-surface attachment supplies, and amplifier housing for the transducers were less expensive than anticipated.
- Decrease in non- capital equipment from $5,883 to $4,402, as the equipment rack, MP3 player and signal splitter, wireless alarm system, sensors, and small transducers (the latter for the bubble deterrent work) were less expensive than anticipated.
- Decrease in professional services from $18,000 to $14,487 because the scuba divers and electrician work to install and power the transducers will cost less than what we had anticipated.

If additional work is required than is budgeted for, we will propose to pay these costs with ENRTF 2014 funds.

- Elimination of the $900 utilities to pay for electricity to power the transducers at Lock & Dam #8. We have learned that our first bill for power from the Army Corps of Engineers will likely come in July, 2015. These costs will be paid from the ENRTF 2014 appropriation.
- Increase in Minnesota travel from $523 to $1070 because an extra trip to Lock & Dam #8 was needed and more are anticipated. If additional travel is required for the transducers to be fully installed, then we will propose to pay these costs with ENRTF 2014 funds.
- Increase in travel for a non-employee from $0 to $297 to cover the hotel stay of a visiting expert on barriers to look at the experimental design and consider future work with the Center.
- Increase in personnel from $51,706 to $59,608 to pay for continued part-time technician help with experiments related to the Lock & Dam project.

Additional shipping is required to support work on Asian carps (for example to ship new experimental fish to the lab). Therefore, the following amendment is also requested:

- Activity # 4: Shift $1,500 from travel to services to pay for shipping needs, resulting in travel budget changing from $21,000 to $19,500 and services from $0 to $1,500.

- Activity # 5: Shift $1,500 from travel to services to pay for shipping needs, resulting in travel budget changing from $16,000 to $14,500 and services from $0 to $1,500.

- Activity # 6: Shift $1,500 from travel to services to pay for shipping needs, resulting in travel budget changing from $16,000 to $14,500 and services from $0 to $1,500.

Amendment approved July 31, 2014

Project Status as of *August 8, 2014:* Activity 7 has concluded and the activity is now being closed out. Please refer to Section IV for the final summary report.

Amendment request as of August 8, 2014: We would like to request a minor budget shift of $120 from the personnel budget line to the services budget line. This is due to a small savings in personal costs being realized and then spent on services instead.

Amendment approved August 18, 2014:

Project Status as of *December 31, 2014*

For an update on the overall MAISRC status, including highlights of the Center's first two years, please see the attached 1-page summary dated December 22, 2014.

Research at the MAISRC continues to make headway: work on invasive carp biology and their eDNA is making excellent progress and research on carp barriers and disease testing have successfully completed. The operational components of the Research Center funded with this appropriation are now winding down or transitioning to the ENRTF 2013 appropriation.

Briefly summarizing:

Activity #1 ("Establishing administrative structure…) Candidates for 2 new positions as proposed with ENRTF 2013 funds are being interviewed, 4 additional projects to be funded with the ENRTF 2013 appropriation are now in the proposal stage, and a research needs assessment is underway to help determine the Center's next research priorities. The Center Advisory Board has initiated a strategic planning process and 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. With the amendment request sought below, work under this Activity will be complete and all funds will have been spent down according to the plan; this effort will now be funded with the ENRTF 2013 appropriation to the Center.

Activity #2 ("Establishing dedicated holding facilities) is still in progress but nearing the construction phase; the schematic design plan for the new facility has been completed, the detailed design phase began this month, and construction is planned for May, 2015 to December, 2015.

Activity #3 ("Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp…") is still in progress although an amendment is now requested because routine sampling activity is in place now financed by the US Fish & Wildlife Service and there is a need to conduct new experiments to understand the fate of carp eDNA in river water, and interpret the USFWS data. No new Asian carp eDNA has been detected in the Mississippi River.

Activity #4 ("Determining the ability of two approaches to measure eDNA … of invasive common carp in Minnesota Lakes") is making excellent progress and has discovered that while eDNA is an excellent indicator of the presence of carp, it degrades quickly in lake water so sampling design must be considered in eDNA monitoring strategies; in the meantime, there is a need to finish microbial source tracking to see whether this technique might supplement eDNA. An amendment to do this work is requested.

Activity #5 ('Testing whether carp can be located using 'Judas fish'…) is on track but a re-budgeting is requested as some costs differed from original projections.

Activity #6 ("Developing food attractants…") is making excellent progress but re-budgeting is now requested to accommodate an increasingly clear need to identify the chemicals involved for carp control and monitoring.

Activity #7 ("Screening for VHS") is complete and no VHS has been found.

Activity #8 ("Developing an enhanced bubble curtain") has found that bubble curtains can be strongly repellent and is complete. The requested amendments and re-budgeting in Activities 3, 4, 5 and 6 fit with activities being planned for a new project (proposed for funding with the Center's ENRTF 2013 appropriation) that aims to determine if attractants can be used to enhance the power of eDNA in rivers so it can guide lock and dam modification, and effect Asian carp control.

Details of progress on all activities and re-budgeting are found below and in the Activity Status sections.

Amendment request as of *December 31, 2014:*

Activity #1 ("Establishing an administrative structure…"): We request that the remaining balances in Activity #1—repairs ($1,048) and travel ($345)—be shifted to personnel so that these funds can be immediately spent down, this account can be closed, and core Center operational expenses be transitioned to the ENRTF 2013 appropriation.

	Activity 1 Proposed Amendments		
BUDGET ITEM (please see budget spreadsheet for more detailed information)	From:	To:	Change:
Personnel-	$356,470	$357,863	$ 1,393
Repairs-	$5,045	$3,997	$ (1,048)
Travel - MN	$6,378	$6,033	$ (345)
Column TOTAL	$367,893	$367,893	$ -

Activity #3 ("Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp…"): The USFWS has now taken over sampling for eDNA and our results have raised the possibility that eDNA is likely short-lived in natural waters. We therefore request to determine this possibility and propose to use funds no longer needed for eDNA sampling to instead conduct a key experiment on carp eDNA fate in river water using the Outdoor Experimental Stream Lab at St. Anthony Falls Laboratory. We request that the following budget changes be made to accommodate additional staff time, services, repairs, and travel to a conference* (likely out of state) to present results of research while acquiring new information on eDNA methods for Asian carp eDNA —all of which is needed to accomplish the new outcomes proposed (please see the IV Activity #3 Status Update section of the work plan for more detailed information):

	Activity 3 Proposed Amendments		
BUDGET ITEM (please see budget spreadsheet for more detailed information)	From:	To:	Change:
Personnel-	$9,968	$66,576	$ 50,813
Services- lab & medical	$0	$1,000	$ 1,000
Professional Services & contracts-	$187,967	$150,000	$ (37,967)
Repairs-	$0	$2,000	$ 2,000
Supplies- lab & field	$32,000	$18,383	$ (8,617)
Travel - MN	$15,000	$3,976	$ (11,024)
Travel – Domestic (for conference attendance by to present results and gain information on new techniques and technologies).	$0	$3,000	$ 3,000
Column TOTAL	$489,870	$489,870	$ -

Activity #4 ("Determining the ability of two approaches to measure eDNA … of invasive common carp in Minnesota Lakes"): Progress understanding eDNA has been rapid but this has delayed our ability to address microbial markers. An additional year is needed to accomplish the outcomes of this activity, specifically to complete a promising project on microbial source tracking (metagenomics) as an alternative tool to monitor carp. This project was one of the original outcomes and its completion appears more promising but complex than originally expected. The following budget amendments are being requested to pay for this extended staff time and travel to a conference* (likely out of state) to both present results of research and acquire new techniques on eDNA measurement (all other eDNA research is occurring out of state). This amendment request also includes purchase of capital equipment (a FastPrep and/or plate reader with estimated cost of $10,000).

	Activity 4 Proposed Amendments		
BUDGET ITEM (please see budget spreadsheet for more detailed information)	From	To	Change:
Personnel (Wages and Benefits) – Total	$152,399	$199,183	$ 46,784
Services- office & gen oper.	$1,500	$4	$ (1,496)
Services- lab & medical	$16,000	$12,398	$ (3,602)
Repairs- lab & field	$10,000	$1,964	$ (8,036)
Supplies- office & gen oper		$100	$ 100
Supplies- lab & field	$72,960	$60,920	$ (12,040)

Cap expenditures over $5,000: (FastPrep and/or plate reader, est. $10,000)	$60,000	$48,670	$ (11,330)
Travel - MN	$19,500	$6,570	$ (12,930)
Travel - Domestic (for conference attendance to present research results and gain information on new techniques and technologies).	$0	$2,550	$ 2,550
Column TOTAL	$332,359	$332,359	$ -

Activity #5 ('Testing whether carp can be located using 'Judas fish'…): Work is in progress and all outcomes are being met; however, we have identified a need for additional assistant time, repairs, slightly more supplies, and travel to out of state conference* to present final results of research and receive feedback for publication as this project comes to an end, so are requesting a budget amendment as follows:

	Activity 5 Proposed Amendments		
BUDGET ITEM (please see budget spreadsheet for more detailed information)	From	To	Change:
Personnel (Wages and Benefits) - Total	$144,043	$147,931	$ 3,888
Services- office & gen oper.	$1,500	$1,000	$ (500)
Services- lab & medical	$5,000	$1,167	$ (3,833)
Repairs- lab & field	0	$1,000	$ 1,000
Supplies- office & gen oper	0	$500	$ 500
Supplies- lab & field	$24,000	$27,170	$ 3,170
Travel - MN	$14,500	$7,775	$ (6,725)
Travel - Domestic (for conference attendance to present research results and gain information on new techniques and technologies).	0	$2,500	$ 2,500
Column TOTAL	$189,043	$189,043	$ -

Activity #6 ("Developing food attractants…"): Progress understanding food attractants has been significant, faster, and less expensive than expected, leading us to the next step: chemical identification of the active components so they might be applied. Several agencies are keenly interested in this work for carp control. To accomplish this we will add additional outcomes to the project and request to move funds set aside for travel, supplies, and general services to graduate student support, lab & medical services, office supplies, and travel to out of state conference* to present results of research and acquire information on new techniques and technologies accordingly:

	Activity 6 Proposed Amendments		
BUDGET ITEM (please see budget spreadsheet for more detailed information)	From	To	Change
Personnel (Wages and Benefits) - Total	$94,849	$106,966	$ 11,717
Services- office & gen oper.	$1,500	$490	$ (1,010)
Services- lab & medical	$0	$6,000	$ 6,000
Supplies- office & gen oper	$0	$500	$ 500
Supplies- lab & field	$20,930	$13,226	$ (7,704)

Travel - MN	$14,500	$2,597	$ (11,903)
Travel - Domestic (for conference attendance to present research results and gain information on new techniques and technologies).	0	$2,000	$ 2,000
Column TOTAL	$131,779	$131,779	$ -

*****Please note that different investigators are working on each of the above mentioned activities. Therefore, the conference attendance requested is for each investigator to attend a conference specific to his/her research as described in the activity.

Amendment approved January 14, 2015:

Project Status as of *June 30, 2015*

Activity #1 ("Establishing administrative structure…) was closed with the approval of the prior amendment request and a final summary is provided below. Core Center operational expenses continue to be supported though Subproject #1 of the ENRTF 2013 appropriation.

Activity #2 ("Establishing dedicated holding facilities) continues to be in progress: demolition of the facilities began this month and construction is still planned to extend through December 2015.

Activity #3 ("Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp…"), which is investigating eDNA in river waters, continues to make excellent progress and key samples have been collected from the open river and an experimental facility.

Activity #4 ("Determining the ability of two approaches to measure eDNA … of invasive common carp in Minnesota Lakes") has succeeded in optimizing eDNA measurement techniques and field validation of one of them in in local lakes and is now nearly complete.

Activity#5 ('Testing whether carp can be located using 'Judas fish'…) has shown that Bigheaded carp are attracted to sex pheromones and that sterilization may be possible. A final report is expected in December.

Finally, activity #6 ("Developing food attractants…") has shown that olfaction drives feeding responses in Bigheaded carps and we are testing promising candidate compounds for possible use in field attraction.

Activities #7 and #8 were completed August 1, 2014 and December 31, 2014 respectively.

Project Status as of *December 31, 2015*

We continue to make good progress continues on our four active studies, one of which (Activity #5) ends with this report. Our understanding of how carp eDNA can be used as an indicator in natural waters is improving. Using the Outdoor Stream lab at the University of Minnesota SAFL lab, we discovered that carp DNA does not degrade rapidly (i.e. with 5 min) in stream water (Activity #3 "Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp…"). Our next step will now be to examine release rates by the carps in open stream water as we work towards developing monitoring protocols that are biologically meaningful. Meanwhile the USFWS has not reported the presence of any Asian carp DNA in the upper Mississippi River. Studies of common carp eDNA in lakes has revealed strong relationships between carp biomass and eDNA in lakes in the summer but not in winter, suggesting temperature may alter release rates and that eDNA sampling protocols have to be carefully designed (i.e. not randomized) to be useful (Activity #4

“Determining the ability of two approaches to measure eDNA … of invasive common carp in Minnesota Lakes”). Work on Judas fish also progresses and we believe we have identified an anesthetic treatment (euthanol) that could be used to euthanize pheromone-implanted Judas fish after they have been released (Activity #5--“Testing whether carp can be located using ‘Judas fish’”). Sterilization may be practical because of unique anatomical feature in the male Asian carp reproductive system. This project is now complete but we hope to continue the work. Testing of the Judas fish technique in lakes and/or ponds is continuing as part of our 2013 ENRTF Subproject 3 effort (“Attracting carp so their presence can be measured”). Studies on Asian carp feeding attractants suggest that fatty acids may have role (Activity #6 “Developing food attractants…”) A technician will continue this work because the graduate student who might have done this work has graduated. Renovations on our facilities are now almost complete (Activity #2 “Establishing dedicated holding facilities for AIS”) and this activity will close with this report. Activities #1, #7 and #8 were completed last year.

Amendment request December 24, 2015:
We seek an amendment in Activity #5 to transfer all non-personnel remaining balances to personnel and close the project out. Specifically, this would result in:

Operating services budget decreasing by $613 from $1,000 to $387
Lab & medical services decreasing by $515 from $1,167 to $652
Repairs decreasing by $1,000 from $1,000 to $0
Operating supplies decreasing by $500 from $500 to $0
Lab & field supplies decreasing by $3,399 from $27,170 to $23,771
Travel- MN decreasing by $5,996 from $7,775 to $1,779
Travel- domestic decreasing by $68 from $2,500 to $2,432

Personnel increasing by $12,090 from $147,931 to $160,022

More time and fewer non personnel costs were needed to bring the project to completion.

As a result of Activity #5 completing, all personnel breakdown in column A of the budget spreadsheet has been updated to the current status.

LCCMR approved language has also been added at the beginning of section VI.A below.

Amendment approved January 4, 2016:

Project Status as of *June 30, 2016*

Good progress is being made on all three remaining activities (#3, #4, #6). Three manuscripts were published in good peer-reviewed journals For Activity #3, DNA release into flowing river by carp is now being measured at the Saint Anthony Falls Laboratory under various conditions. A manuscript on eDNA release lake carp is also now being finalized for submission. For Activity #4, final analyses of carp metagenomics are being performed for a manuscript on this topic that was submitted and is now undergoing final revisions. For Activity #6, we have discovered that Spirulina is a major component of Asian carp food attractants and are now starting definitive physiological and behavior experiments to identify what chemical components are most important and could be used in control. Activities #3 and #4 will be successfully completed by December 2015 and Activity #6 in 2017, all on schedule.

Project Status as of December 5 , 2016
Good progress is being made on all three remaining activities (#3, #4, #6), one of which, Activity #4, is now complete. For Activity #3, work on how to use eDNA to assess the presence of bigheaded (Asian) carp is now examining how DNA released by carp disperses in flowing river water. We designed and conducted two initial experiments using the river water flume at the University river lab (SAFL) whose

initial results seem to suggest that release rates may be relatively low in flowing stream water and that eDNA release, not decay, is thus the primary determinant of eDNA distribution. his unexpected and important result will need to be confirmed in a new testing flume we are now building. A new half-time investigator, Dr. Ping Wang, is now leading this project because the primary investigator, Dr. Eichmiller, left for another more permanent position. This change in leadership has resulted in our needing 6 additional months to complete the work and that is requested as part of a proposed amendment. Activity #4 is now complete; it demonstrated that while both conventional eDNA and microbial decal NA (metagenomics) can be very useful to measure the presence of carp but that the former is the most useful, Four peer-reviewed publications by Dr. Eichmiller have resulted to date. For Activity #6, we have found that fatty acids in Spirulina appear to be an important new component in the food odor stimulants for bigheaded carp which nevertheless appear to be mixtures. This unexpected and important possibility will be tested next as part of a proposed 6 month extension (see below). A new activity on dam operations is also proposed for 2018 at the request of the LCCMR.

Amendment request as of December 5, 2016:
An amendment is being requested at the request of the LCCMR to accommodate new work on lock and dam modeling using surplus funds from Activities #3 and #6, most of whose objectives have been met and whose personnel are both now part-time. This new project (Activity #9) will model fish passage through Lock and Dam #4 in 2017-2018 and is designed to identify changes to gate operations that could stop carp movement upstream. It accompanies a related amendment to Sorensen's ENRTF2014 project to make changes to gate operations at Lock and Dam #5 in 2017 as well as a yet-to-be proposed amendment to ENRTF2013 for more work on sound deterrents : by pairing all of these projects, a coordinated, new 2-year project is created as requested by the LCCMR. It is described below along with a brief update for Activity #3 and Activity #6, and a final report for Activity #4. As part of the update, we are proposing a 6 month extension in outcomes for both Activity #3 and Activity #6, which will still occur within the original appropriation timeframe. For activities that have been completed or for which no additional funding is needed, we propose zeroing out remaining balances and moving those funds to create a new Activity #9 as described in detail below. The following re-budgeting is requested:

Activity #3 is under budget, largely complete, and its lead investigator is now half-time .We would like to both extend the activity outcomes by 6 months (but still within the original appropriation timeframe) and reduce budgets in the following lines and then move the cost savings to a new Activity #9:

- Decrease personnel from $66,576 to $45,174 because less personnel will be needed than anticipated. The $18,100 balance will support a week of Dr. Sorensen's summer salary and approximately 25% time for Dr. Ping Wang who will replace Dr, Eichmiller and will work until July 2017 to help complete the Activity
- Decrease repairs from $2,000 to $0.
- Decrease lab & field supplies from $18,383 to $8,670
- Decrease travel in Minnesota from $3,976 to $100
- Decrease Domestic travel from $3,000 to $2,273 to account for funds spent. No more domestic travel will be needed.
- Total reduced from Activity #3: $37,718

Activity #4 is complete. We were able to achieve all outcomes for less funding than anticipated. The work is now complete and we would like to move all unspent funds from here to the new Activity #9. This would result in the following budget reductions:

- Decrease personnel from $199,183 to $184,884
- Decrease office and gen oper services from $4 to $0
- Decrease lab and medical services from $12,398 to $10,205
- Decrease repairs from $1,964 to $1,675

- Decrease gen oper supplies from $100 to $0
- Decrease lab and field supplies from $60,920 to $53,772
- Decrease capital expenditures from $48,670 to $48,597
- Decrease travel from $9,120 to $5,957
- Total reduced from Activity #4: $27,269

Activity #6 is under budget, almost complete, and its personnel is also now part-time. We therefore would like to extend outcomes to 2017, reduce budgets in the following lines, and then move the cost savings to the new Activity #9:

- Decrease personnel from $106,966 to $87,378.
- Decrease office and gen oper services from $490 to $193 to account for funds spent; no more services will be needed
- Decrease lab and medical services from $6,000 to $860
- Decrease office and gen oper supplies from $500 to $0
- Decrease lab and field supplies from $13,226 to $11,940
- Decrease Minnesota travel from $2,597 to $1,162. The remaining $900 will be used for in-state sample collections and meetings.
- Decrease Domestic travel from $2,000 to $1,401 to account for funds spent. No more domestic travel will be needed.
- Total reduced from Activity #6: $28,845

We would like to use the $93,832 savings from above to create a new Activity #9, in which we will model fish passage through Lock and Dam #4 in 2017-2018 and suggest changes to gate operations. This accompanies a related amendment to Sorensen's ENRTF2014 project to make changes to gate operations at Lock and Dam #5 in 2017. By pairing these projects, a coordinated, new 2-year project (and position) is created to inform and implement needed redundancies to better protect Minnesota's waters from the advance of invasive carps. The budget changes would result in the following:

- Personnel would increase from $0 to $91,200 to support Dr. Anvar Gilmanov who will be working on ENRTF 2014 until June 2017 and then will work on this Activity #9 from July 2017 to July 2018.
- Travel in Minnesota would increase from $0 to $1,000 to cover one scientific workshop.
- Domestic Travel would increase from $0 to $1,631 to pay for Dr. Gilmanov to attend one scientific conference to present results of his research.
- Total increase in Activity #9: $93,832

Amendment approved by LCCMR 12/17/16

Project Status as of *June 30, 2017*
Activity 3 on eDNA has been completed. Recent tests of bigheaded carp in flowing waters have both confirmed the eDNA release is relatively slow and discovered that it is strongly stimulated by feeding activity. This possibility had previously not been considered in eDNA sampling schemes and will be very useful. Activity #6 on feeding stimulants is also now complete. We discovered that feeding stimulants are also attractive to bigheaded carps and the fatty acids are more important as attractants than stimulants as part of multiple-component food mixtures. Amino acids are a key of this mixture and synergize each other's activity. This information is now being used in the field tests conducted as a part of ENRTF2013 and has been provided to the USGS to assist in carp sampling and removal. A manuscript was published on carp feeding stimulants and another is now being prepared. Activity #9 on spillway gate modification at Lock and Dam #4 will start July 1as scheduled.

Project Status as of *December 30, 2017*
Activity 9 (our only ongoing activity) is proceeding on schedule. Initial numeric models of Lock and Dam #4 spillway function are complete and now being validated and perfected. We met with the US Army Corps to discuss implementation three times with another meeting planned.

Project Status of: June 30, 2018
Activity 9, our only ongoing activity has been successfully completed. Initial numeric models of Lock and Dam #4 spillway function are complete and have been validated and perfected, and can reduce carp passage by about 50%. We met with the US Army Corps of Engineers.

Amendment request as of: August 15, 2018
The project is now complete but a final amendment is being requested to the budget for Activity 9 to balance out a small surplus in both travel ($2441) that was less than expected and use it to cover salaries which were slightly higher than anticipated ($2441).

Final Report Summary for Project: August 15 2018
Subprojects 1-8 were previously completed and are reported on above; here, we report on Activity 9, the last subproject. This project, which sought to identify ways that spillways gates could be managed more effectively at Lock and Dam #4 to block invasive carp (without effecting scour, safety or safety), identified ways that carp passage could be reduced by about 50% from already very low numbers. These procedure were reported to the US Army Corps of Engineers and if used in conjunction with previously reported recommended changes at Lock and Dam #5 as well as #8 (ENRTF2014) would reduce invasive passage by about 90%.

Project Abstract:
This project established a new research center at the University of Minnesota dedicated to developing sustainable solutions to the problems posed by aquatic invasive species (AIS) and developed a solution for bigheaded carp from Asia ("invasive carps"), two of the primary issues faced by our region. The Minnesota Aquatic Invasive Species Research Center (MAISRC) still exists at the University although it now has a new leadership, administrative structure, and vision. As part of this project, associate and scientific directors for MAISRC were hired; they then initiated the process of hiring the state's only zebra mussel and aquatic plant experts, acquired funding for a new research laboratory, renovated an extant laboratory, and established a communications plan. A memorandum of understanding with the DNR was created as well as an administrative structure that included boards dedicated to self-governance, research, and strategic vision. In addition, research on invasive carp was conducted which identified a possible affordable and sustainable solution that does not cause collateral damage. This solution entails strategically adjusting gate openings in river locks and dams to prevent carp passage and adding sound systems to lock gates; it is now being implemented at Lock and Dam 8 with new ENRTF funding as well as a site in Kentucky by the U.S. Fish and Wildlife Service. This solution was enabled by new developments in molecular survey techniques ("eDNA") also instigated by this study, which showed that, contrary to public fears, few invasive carp had reached Minnesota. Finally, this study showed that an important fish disease (VHS) is not in Minnesota water and that invasive carps use novel foods and social signals (pheromones) that could be deployed in control were they to enter Minnesota. All this information is publically available and in the hands of the DNR awaiting full implementation.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Establishing the administrative structure of an AIS Cooperative Research Center at the University

Description: An AIS cooperative research center will be established with a Scientific Director and a fulltime Administrative Director (100% time). The Administrative Director will work with the Scientific

Director (P.I.) to organize and run yearly workshop(s), establish an advisory board (commission), establish (renovate) AIS holding facilities, offices and laboratories, coordinate a collaboration with the Minnesota Department of Natural Resources (DNR), produce media releases, coordinate progress reporting, and ensure that new faculty members are hired for the second phase of the project (funded by another LCCMR project proposed to start in 2013). This process will take two years after which it expected that operating costs will be subsumed by another, larger ENRTF work plan.

Summary Budget Information for Activity 1: **ENRTF Budget: $379,170**
Amount Spent: $379,170
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Hold public workshop #1, Advisory board established, Coordinate lab repairs, Develop work plans for the second phase of the Center	2013	$91,148
2. Public workshop #2, Advisory board meetings, Coordinate renovations, Recruit and hire new positions	2014	$130,028

Activity Status as of *June 30, 2013:*

The Minnesota Aquatic Invasive Species Research Center (MAISRC) officially started work in December, 2012. Peter Sorensen was hired as its Scientific Director (50% time) in September 2012 and Becca Nash was hired as its Associate Director (100% time) in December 2012. A memorandum of understanding has since been written and signed with the Minnesota Department of Natural Resources that describes most components of MAISRC's collaborative relationship, including the makeup, role, and operations of the Center Advisory Board, a Center technical Committee, and a DNR- MAISRC collaboration committee. We now have 10 advisory board members and the board's first meeting is being scheduled for July. The administrative structure of MAISRC has also been established. Considerable effort has been spent with the University trying to raise funds to create new laboratories for faculty and to identify space needs and solutions for these needs and while we have not raised new external funding, a plan for renovations is now in place to make us operational. Already completed under this activity include completing first phase of renovations to AIS holding facilities in Hodson Hall (St. Paul); work towards securing space and initiating phase two of renovations to the AIS facilities (Fisheries and Engineering Lab); initiating advertising for a zebra mussel scientist; completing media releases related to Activity #3; coordinating work among Center-affiliated researchers; grant writing (two submitted), budgeting, and reporting. It was determined that a work shop might be premature given the early nature of the research, however information sharing opportunities (e.g. American Fisheries Society annual meeting, Upper Midwest Regional AIS meetings) were pursued. Website and brochures were developed.

Activity Status as of *April 1, 2014*

Considerable effort has been expended to get the Center's administration in order. A memorandum of understanding (MOU) between Dr. Sorensen, the Department of Fisheries Wildlife, and Conservation Biology (the Center's administrative home) and the College of Food, Agriculture and Natural Resources Sciences was signed on 12/02/2013. The MOU describes how the AIS Research Center operates within the College and with other colleges , including how non-tenure track faculty positions are created and then reviewed, how committees function, the responsibilities of the Scientific and Administrative Directors, how Center research projects are proposed and reviewed, and how certain costs are shared. Procedures for reporting on ENRTF funding were changed and clarified since the last progress report, as has the format for budget reports.

The Center Advisory Board has been established and has met twice. Additional ENRTF funding for detering Bigheaded carps from migrating up the Mississippi, St. Croix, and Minnesota Rivers is being pursued. Lab and holding facilities and a failing well have continued to prove challenging, however a new well has been drilled and is approximately 4 weeks from being operational. Considerable time has been expended to plan for badly needed facilities upgrades that will be possible if bonding funds from the legislature are awarded in 2014.

The Center brought well known Asian carp researcher, Duane Chapman, in town for a successful event co-hosted with the Freshwater Foundation as well as for educational exchange with Center researchers. Dr. Bill Haller from the University of Florida's Center for Aquatic and Invasive Plants (and a Center Advisory Board Member) provided a seminar that was advertised to the public. A new zebra mussel researcher has been hired; the Center Faculty Group has started meeting; and a guest speaker program for informational exchange and coordination has been established. A position description for our next faculty hire in applied ecology is being developed by a faculty workgroup. Dr. Sorensen has identified a need for scientific support staff including a technician and Scientist (the later will require some re-budgeting). A part-time position is being advertised to help with communications and office work.

Four existing research projects have been voted into the Center as Next Generation Core Projects, including: 1) Developing and Implementing a Sustainable Program to Control Common Carp in the Riley Purgatory Bluff Creek Watershed District (Peter Sorensen and Przemek Bajer, faculty, Joey Lechelt, Graduate Student) 2) Adaptive Carp Management in the Ramsey Washington Metro Watershed District (Justine Koch, graduate student; Sorensen as faculty) 3) Collaborative Research; A Robotic Network for Locating and Removing Invasive Carp from Inland Lakes (Nate Banet, graduate student; Sorensen as faculty) and 4) Restoration and Maintenance of Native Vegetation in Lakes in the Riley Purgatory Bluff Creek Watershed District (Ray Newman, faculty, and John Jaka, graduate student). These projects are already underway and bring with them expertise and additional resources.

Additionally, the Scientific and Administrative Director have been involved in collaborations to help serve the needs of the state and to strengthen ties with AIS managers around the state (in particular, the MN DNR). Examples include serving on the technical review committee for the electrical barrier planned for Lock and Dam 2, the State's Asian Carp Action Plan update working group, the MDNR AIS Advisory Committee, and the Mississippi River Basin Panel's AIS task force.

Activity Status as of *June 30, 2014*

The Center has undergone a change in leadership and as of May, 2014, the new Center Director and project manager for Activity #1 is Dr. Susan Galatowitsch. Current AIS- related awards and activities are being reviewed together by Dr. Galatowitsch, Dr. Sorensen, and Dean Buhr to ensure a smooth transition.

Progress is being made on launching new science and getting positions filled: job descriptions for one of the faculty positions and the educator position proposed with FY 2013 funds have been fleshed out and are currently receiving input and approvals from various parties before being posted. Dr. McCartney's zebra mussel research proposal has been reviewed and revised through the Center's peer review process and his summer field session has been launched. The next research proposal is anticipated to be Dr. Sadowsky's metagenomics work on zebra mussels and Eurasian watermilfoil submitted later this Fall.

Bonding dollars (and University match) have been secured to pay for additional renovations at the Engineering and Fisheries Lab. Work by MAISRC staff and consultants on the renovation predesign has been completed. Final design work is anticipated to start soon with construction expected to begin this winter.

The Center Advisory Board is scheduled to meet in July to discuss strategic planning and other Center priorities. The Center Faculty Group is engaging in similar discussions.

Dr. Sorensen continues to serve on the MN DNR AIS Advisory Committee, Becca Nash continues to serve as Co-Chair of the working group to update the State's Invasive Carp Plan, and a symposium organized by Dr. Sorensen with talks by several MAISRC researchers and a talk by Dr. Galatowitsch are planned as part of the Upper Midwest Invasive Species Conference in Duluth in October.

Funds in Activity #1 (primarily budgeted in personnel) will be spent down as budgeted and then the ENRTF 2013 award will begin paying for these costs. Funds are anticipated to be spent down before the next workplan update.

Activity Status as of *December 31, 2014*

Progress with getting positions filled to conduct the Center's work has continued. A search is under way for an Extension educator to lead statewide science- based AIS education efforts, leveraging regional and local Extension capacity, and tying Center research with field implementation and activities. A search is also under way for an aquatic plants management and restoration faculty member to work on invasive plant control. Both of these positions, proposed with FY 2013 funds, have been posted and interviews will be taking place in January and February, respectively.

Significant strides have also been made in getting underway the Center's science funded through our ENRTF 2013 appropriation: Since the last update, Dr. Newman's research project (Subproject 5) has been launched and Dr. Sadowsky's (Subproject 2), Dr. Sorensen's (Subproject 3), Dr. Bajer's (Subproject 4), and Dr. Venturelli's (Subproject 6) project proposals have now all begun the project proposal and peer review process.

The Engineering and Fisheries Lab renovation schematic designs have been completed with considerable involvement by MAISRC staff and researchers. The detailed design phase was launched in December and construction is expected to start in May 2015 and last through December 2015. Alternate plans are being made for projects that will be displaced during this time.

The Center Advisory Board has created a strategic planning work group to help plan for the Center's future. The Center's first systematic research needs assessment process is also now underway to determine state priorities for the next "wave" of research projects. This process involved input from UMN scientists, agency biologists, statewide AIS managers, and the public. Over 300 research ideas were submitted, organized, and vetted. The Center Director, Center Faculty, and Center Advisory Board will help determine next steps.

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, who heard about opportunities, gained skills, and gathered information to advance AIS efforts in their communities.

Becca Nash continues to serve as Co-Chair of the working group to update the State's Invasive Carp Action Plan and will be the MAISRC representative to the Minnesota Invasive Species Advisory Council.

With approval of the amendments proposed, Activity #1 will be completely spent down and core Center work conducted under this activity will be supported through the ENRTF 2013 appropriation.

Final Activity Summary as of *June 30, 2015*

Significant progress has been made in creating and building capacity for AIS research for the State of Minnesota as a result of funding through the Environment and Natural Resources Trust Fund. Following is a final summary of the work made possible through funding of the Center's core operations via Activity 1. Center operations continue to be funded and reported on through Subproject 1 of the ENRTF 2013 appropriation.

Research projects initiated

- ✓ 10 ENRTF-funded research projects were proposed, peer reviewed, and launched.
- ✓ Post docs and graduate students were hired for those projects, labs set up where needed, and research begun.
- ✓ 4 additional subprojects have been peer reviewed and approved by MAISRC and are awaiting workplan approval by LCCMR.
- ✓ Two of the originally-scoped ENRTF projects remain: one included the hire of a new full time Extension educator and the other included the hire of a new, full time, tenure-track faculty member. Both hires are now complete. The former project is in proposal stage; the latter project will begin following the new faculty member's start date of August 31.
- ✓ Additional research projects have been adopted as part of MAISRC and additional projects have been developed and pursued to expand AIS research capacity
- ✓ MAISRC completed its first systematic research needs assessment process to determine state priorities for a next "wave" of research projects. Input was gathered from the MAISRC Technical Committee plus additional UMN scientists, agency biologists, statewide AIS managers, and the public. Over 300 research ideas were submitted, organized, and vetted. A list was finalized with help from the Center Faculty Group and the Center Advisory Board. The Center Advisory Board provided its official support for the priorities at its May 2015 meeting. MAISRC now intends to use existing funding to seek collaborations through a request for proposals to address these 2015 research priorities. The RFP release is anticipated for Fall 2015.

Staffing

- ✓ Hired Minnesota's first full time dedicated zebra mussel researcher
- ✓ Leadership at MAISRC was changed in May, 2014 to Dr. Susan Galatowitsch
- ✓ A new tenure track faculty position in aquatic plants management and restoration was created and filled. The new researcher will start Fall 2015 with a focus on invasive plant control
- ✓ Hired a new Extension Educator to lead statewide AIS education and science- based programming efforts, leveraging regional and local Extension capacity, and tying Center research with field implementation & activities. She started March 2015
- ✓ Staffing is currently at 8 part-time faculty, 2 full-time faculty, 6 post docs, 7 graduate students, 8 junior scientists/technicians, a part-time Director, a full-time Associate Director, a full-time Extension educator, and a part- time communications and administrative specialist.

Space and facilities

- ✓ Office space was secured within a USFS building on campus for MAISRC staff
- ✓ Renovations were conducted at Hodson Hall as planned and budgeted. This charge was later reimbursed by the College and funds were spent to drill a new well
- ✓ A new well was drilled to replace the old failing well that was jeopardizing years of research
- ✓ Approximately $7m in bonding was secured to fund renovation of the Engineering and Fisheries Laboratory, the main animal holding and research facility of the MAISRC
- ✓ Staff and researchers engaged in significant effort to secure needed research facilities, including participating in extensive research facility needs scoping meetings and feasibility studies, spec'ing lab function and major equipment as part of pre-design, schematic design, and detailed design processes, reviewing bids, and coordinating with UMN capital planning, government affairs, and project contractors
- ✓ Laboratories were moved to accommodate research during demolition and construction, which began June 2015

Communications & outreach

- ✓ The Center brought well known Asian carp researcher, Duane Chapman, in town for a successful event co-hosted with the Freshwater Foundation as well as for educational exchange with Center researchers
- ✓ MAISRC hosted guest talks by national invasive plant experts Bill Haller and Mike Nederland
- ✓ The "The 2014 Minnesota Aquatic Invasive Species Research and Management Showcase" was held November 19, 2014 and attended by over 220 people from around the state, who heard about opportunities, gained skills, and gathered information to advance AIS efforts in their communities.
- ✓ The 2015 Minnesota AIS Research and Management Showcase planning began
- ✓ Several conferences were attended with informational booths, posters, and brochures
- ✓ A website (www.maisrc.umn.edu) was created, expanded, and is undergoing additional development
- ✓ Staff and researchers participated in technical groups, panels, and working groups, including the state Invasive Carp Action Plan committee.
- ✓ MAISRC was mentioned in more than 60 newspaper articles and radio interviews
- ✓ Facebook and Twitter accounts were created and are actively managed
- ✓ E-newsletters are now published every two months.

Organizational structure

- ✓ An MOU was created with the DNR to establish a working relationship with the agency, including the makeup, role, and operations of the Center Advisory Board and a MAISRC cross-agency Technical Committee
- ✓ An advisory board was created, members were recruited, and meetings held approximately every four months to advise on broad policy issues and research direction
- ✓ Agreement was reached on an MOU between the MAISRC, the Department, and the college that describes how the MAISRC operates within the College, including how non-tenure track faculty positions are created and reviewed, how committees function, the responsibilities of the Scientific and Administrative Directors, how MAISRC research projects are proposed and reviewed, and how certain costs are shared.
- ✓ Members of the MAISRC Technical Committee were recruited and meetings held to evaluate species in need of additional research in the State of Minnesota
- ✓ A strategic planning process was initiated with the Center Advisory Board and the Center Faculty Group to ensure continued ability for MAISRC to conduct critical work on AIS in the future. In coordination with a consultant, several work sessions were held, input was sought from key stakeholders, and goals and strategies are currently being developed.
- ✓ Procedures for reporting on ENRTF funding were created and refined; reporting was conducted
- ✓ Center Faculty Group meetings were regularly held to receive input on Center policy, direction, and activities and to review proposed research

ACTIVITY 2: Establishing dedicated holding facilities for AIS

Description: The Hodson Hall Fish Holding Facility (St. Paul Campus, U of MN) will be repaired and the invasive species presently held in in the Fisheries and Aquaculture Facility (St. Paul Campus) moved into it. The Fisheries and Aquaculture laboratory will then be refurbished with a new well, circulating and filter systems so that it can hold AIS including Asian carp and zebra mussel for long-term studies. The latter facility will be maintained as a permanent, central facility for this work in the state.

Summary Budget Information for Activity 2:	**ENRTF Budget:**	**$565,000**
	Amount Spent:	**$565,000**
	Balance:	**$0**

Activity Completion Date:

Outcome	Completion Date	Budget
1. Hodson Hall fish holding facility repaired (half of total)	2013	$65,000
2. Fisheries and Aquaculture Laboratory renovated (contribution to total cost)	2014	$500,000

Activity Status as of *June 30, 2013*
The Hodson Hall Fish Holding Facility (St. Paul Campus, U of MN) was repaired and many of the invasive species presently held in the Fisheries and Aquaculture Facility (St. Paul Campus) moved into it. Work is underway now to drill a new well in the Fisheries and Aquaculture laboratory by September 30, 2013. Following the new well, other key components will be repaired as funding permits so that it can hold AIS including Asian carp and zebra mussel for long-term studies. The latter facility will be maintained as a permanent, central facility for this work in the state.

Activity Status as of *April 1, 2014*

It has been determined that the repair and upgrade needs for the Research Center's facilities greatly exceed the funds on hand and that a major renovation and additional funding is needed. Significant planning has taken place and a bonding request through the University is being pursued in the 2014 Legislature for major renovations to the AIS research and holding facility. The costs previously incurred for repairing Hodson Hall and for conducting a feasibility study for renovations were reimbursed by the College and will be considered part of the University's 1/3 required match if 2014 bonding dollars are secured. 2012 ENRTF funds are being used now to drill a new well (which is approximately 4 weeks from being operational), repair iron filters, and will be used for Phase I demolition and construction at the Engineering and Fisheries Lab (the "MAISRC Central Research and Holding Facility") which will include preliminary work on building security, piping, electrical, and water treatment. Additional renovations will be required to the Hodson Hall holding facility to house AIS while the Engineering and Fisheries Laboratory is eventually renovated.

Activity Status as of *June 30, 2014*

The new well has been drilled and is now operational, providing a steady supply of well water to the Engineering and Fisheries Laboratory. Funding for major renovations was secured in the 2014 legislative session. The preliminary work mentioned in the April 1 status report will be conducted at the same time as these more extensive renovations in order to reduce disruption to the research. The University is currently securing approvals to proceed with the final design work required before construction begins this winter/spring. At that time, all animals will be transferred to Hodson Hall and several research projects may have to be suspended for several months until construction is completed.

Activity Status as of *December 31, 2014*

The schematic designs for the entire renovation have been completed and the detailed design phase was launched December 12. Plans include safe, secure, and fully operational spaces for the growth, holding, and study of aquatic invasive fish, plants, invertebrates, and pathogens. Construction is planned to begin May 2015 and last through December 2015. Alternate plans are being made for animals and projects during this construction time.

Activity Status as of *June 30, 2015*

All equipment and animals in the Engineering and Fisheries Laboratory have been moved to alternate locations and demolition on the site has begun. Some research will be possible in Hodson Hall during construction; other work will be paused until the new facilities are available, which is still planned to be

after December 2015. The funds will be fully expended and this Activity closed within this same approximate timeframe.

Final Report Summary as of *December 31, 2015*
The Hodson Hall Fish Holding Facility (St. Paul Campus, U of MN) was repaired so that it could be used as a temporary holding space for invasive species while the larger laboratory and holding facility, The Engineering and Fisheries Laboratory ("EFL"), was renovated. Funds from this appropriation were used to replace a failing well at the EFL and then have contributed to this larger renovation that is also being funded with state bonding dollars appropriated in 2014. The newly renovated, state-of-the-art facility should be operational by February 1 and will make possible holding and study of a range of invasive species, including fish of all stages; invertebrates; pathogens; and aquatic plants. It will serve as the official lab and holding facility for the MAISRC and will be a resource for the state.

ACTIVITY 3: Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp in large Minnesota rivers

Description: In collaboration with the United States Geological Service (USGS LaCrosse, WI), DNR, and National Park Service (NPS), the University will reinitiate sampling of Minnesota waters for the presence of eDNA from both Asian and common carps. The DNR will work with NPS to collect all needed samples while the study design will be under the immediate guidance of the University and USGS. A technician will be hired at the USGS to do this work under the auspices of a reimbursable agreement. Both existing and improved DNA primers will be used in this two-year study to determine if previous results from the Mississippi and St Croix rivers can be duplicated and if they are credible (additional controls will be built in). Work will follow protocols outlined by the U.S. Army Corps of Engineers so as to be valid in a legal context. The second year of this study will seek to confirm the first and allow us to refine sampling designs so that specific recommendations can be made to the DNR for possible future work. This work will synergize and complement another study aimed at validating and developing these techniques with common carp (Activity #4). Results from both studies will assist future efforts of a research assistant professor focused on implementing new molecular tools to systematically measure AIS across the state, funding for which will be requested in a new ENRTF proposal to start in 2015.

Summary Budget Information for Activity 3:

ENRTF Budget: $207,217
Amount Spent: $207,217
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. eDNA sampling for Asian carps in MN developed/ completed	2012	
2. eDNA results for 2012 analyzed	2012	
3. Recommendations made to DNR about further eDNA work	2013	
4. eDNA samples collected and archived	2013	
5. eDNA samples from 2013 analyzed for two sites	2014	$150,000
6. eDNA recommendations made available to DNR	2014	
7. Recommendations made to DNR about further eDNA work	2014	
8. eDNA samples collected and archived	2014	
9. eDNA samples from 2014 analyzed for two sites	2015	$4,837
10. eDNA sampling for Asian carps in MN completed with USFWS	2015	
11. Initial analysis of eDNA stability in river water	2015	$45,000

12. Further study of eDNA release rate study by carp and stability in flowing river and well water	2016	
13. Complete study, final report of eDNA in flowing water	2017	7380

Activity Status as of *June 30, 2013*

Over five hundred water samples were collected from the Mississippi River (n = 50 per site) and other key control locations for analysis of eDNA from Asian carp. Sites included above and below St. Croix Falls Dam on the St. Croix River, and above and below the Coon Rapids Dam and below Lock and Dam 1 on the Mississippi River. In addition, control samples were tested from a positive control site at Lock and Dam 19 (Keokuk, Iowa), two negative control lakes, Square Lake and Lake Riley, and laboratory water controls. Genetic testing was then conducted by the U.S. Geological Survey laboratory in La Crosse, WI, using the U.S. Army Corps of Engineers' Quality Assurance Project Plan (QAPP) standardized protocols with the exception that all samples (rather than 5%) were sequenced. The DNA of silver carp was detected at the Lock and Dam 19 positive control site (where both species of Asian carp are known to exist in high abundance) but at no location in Minnesota waters, including multiple sites that had positive detections reported for 2011 samples. The DNA of bighead carp was not detected in any environmental sample, including Lock and Dam 19 where bighead carp are known to be abundant. A report on the results was provided to the Minnesota DNR: *Interim report: Detection of environmental DNA of Bigheaded Carps in samples collected from selected locations in the St. Croix River and in the Mississippi River*. Data were released to the press at a news conference. Our eDNA report documented the possibility of false negatives (i.e. failure of this technique to measure eDNA released by live fish when it is known to be present) using QAPP protocols, an inability to repeat 2011 findings, and other studies that have described the possibility of false positives (i.e. measurement of eDNA in the absence of live fish) from vectors of DNA such as bird-eating fish (we saw no indication of this in 2012). Together, these findings, along with the relatively complex and slow analysis time required for eDNA using current protocols, led us to suggest that this particular variant to the eDNA technique not be adopted by the MN DNR as a routine tool to guide Asian carp management. Instead, we recommended that research continue to improve this technique (which we think has great promise) while water samples are taken for archival and research purposes, and that DNR monitoring for Asian carp uses standard fisheries techniques.

In accord with this recommendation, we propose to amend this research activity to reduce the scale of sampling to include five sites. Most samples will then be archived although two will be analyzed. These data will not be released to the public and instead will be used to improve the eDNA technique and for tentative early warning to the DNR. Much of this new work will be conducted in-house but we will re-initiate a new (smaller) research contract (collaboration) with the USGS. In particular, if any suspect positives are determined, duplicate sets will be sent to USGS for confirmation and other archived samples will be analyzed while the DNR is alerted,

Activity Status as of *April 1 2014*

Working with the MN DNR we collected and extracted 50 water samples in duplicate from three sites in September 2013 for Bigheaded carp eDNA analysis (Lock&Dam 1, Lock&Dam 5 and St Croix Fall [all sites previously examined]). This effort will become part of scientific archival program for future analysis by the Center once eDNA technology has been optimized. One group of 50 samples is being analyzed for Silver carp eDNA in-house (the cPCR Bigheaded carp marker is not reliable, and the cost of having the USGS do this work exceeded our budget). Information from this analysis will be provided to the MN DNR. We continue to collaborate with the USGS In particular we have re-directed the funds that might have been spent on eDNA field studies with them to develop new and hopefully more useful qPCR markers and techniques for both Silver and Bigheaded carp, They have been tested using archived 2012 samples. The new qPCR marker for Bighead carp is able to measure the presence of that species in river water with a few false positives while Silver carp marker is more rapid and maintains a detection rate of about 60%. Sequencing will still be necessary as there are some PCR-false positives. It seems likely that these markers will eventually be incorporated in the US FWS monitoring program.

Having a specific understanding of these new markers will be extremely helpful to the science and possible management (MN DNR). A publication is anticipated from this work

In the meantime, we are leading and coordinating discussions with the US FWS, MN DNR and WI DNR to develop a coordinated, scientifically-valid, and largely federally funded Asian Carp monitoring program in the Upper Mississippi River that will include at least three sampling approaches in addition to eDNA and be connected with response items. Coordinating this larger program may become a major function of ours instead of the relatively simple eDNA program we envisaged two years ago..

Activity Status as of *June 30, 2014*

In September 2013, 50 Mississippi River water samples were collected from below Lock and Dam #1, Lock and Dam #5, and St. Croix Falls as planned. All samples were filtered, and stored at –20 °C. While our original intention had been to have the USGS analyze these samples for both Silver and Bighead carp eDNA (i.e. Bigheaded carp [*Hypophthalmichthys* spp.] from Asia or "Asian carp"), this proved to be overly expensive (approximately $50,000), complex (i.e. subject to federal reporting protocols and, as mentioned in the April report, their markers have been subject to difficulty), so we decided to develop eDNA analysis at the University. Developing the ability to independently confirm results at the University seemed efficient and wise. Given the evolving nature of eDNA techniques, it was decided early on that results from University analyses of these archival collections would be considered experimental and would not be broadly released to the public at this time. We have now completed analysis of Lock and Dam #1 samples for Silver carp eDNA. No amplification of Silver carp eDNA was observed for any of these samples, cooler blanks, extraction controls, or PCR negative controls. Work focused on this particular site and species because the marker for Silver carp is much better than that for Bighead carp and this site has a better sampling record and it is considered to be representative of the region. All Silver carp tank positive controls had a band at 190 bp, indicating successful amplification of the desired product. Ten DNA extracts were also randomly selected to assess potential inhibition. All but one DNA extract was successfully amplified by universal fish primers. This confirmed that inhibition was not the cause of the negative results for Silver carp eDNA, and that the cause was insufficient target DNA. We are fairly confident that no measurable Bigheaded carp was located in the Upper Mississippi River in the fall of 2013. This information was shared with the MN DNR.

Dr. Sorensen has recently been heavily involved in the discussions spearheaded by US Fish and Wildlife Service (USFWS) on future joint monitoring efforts for Silver and Bighead carp in the Upper Mississippi River. A plan is now being formalized. The MN DNR has also been involved in these discussions and have deferred to, and worked closely with, Dr. Sorensen to develop a reasonable science-based sampling plan for the state. As promoted by Dr. Sorensen based on his extensive experience with sea lamprey control in the Great Lakes, this plan will involve multiple techniques including: adult carp surveys, trap netting for juveniles, larval netting and eDNA sampling. A response component (modifying lock and dam function) has also been proposed. The USFWS should be conducting eDNA sampling and analysis below Lock and Dam #5 in the future and will likely collect samples above this location and at Lock and Dam #1 for the University to archive and, if necessary/appropriate, analyze as part of Activity #3 for the state. Archiving samples makes good sense because this discipline is rapidly changing and historical perspective and ability to re-analyze samples is likely to be of critical importance. The University will also be available to help the USFWS and MN DNR interpret data internally and if necessary re-analyze samples for the MN DNR. The activities described by Activity #3 are thus likely to change to include archival, reanalysis if appropriate, providing scientific guidance as part of larger coordinated USFWS plan, and conducting experiments to facilitate data interpretation. For Minnesota, we are primarily concerned with failure to date to detect Bigheaded carp when a small population is present (i.e. false negatives). In any case, when and as the USFWS plan and is finalized and it ramifications to Activity 3 clarified, an amendment to Activity #3 will be proposed that reflect these changes (likely the end of 2014 at the next update).

Activity Status as of *December 31, 2014*

The US Fish Wildlife Service (USFWS) has officially assumed responsibility for eDNA sampling (and analysis) as part of an Upper Mississippi River Asian carp plan developed by a large group of agencies including the University of Minnesota (Dr. Sorensen). As part of this agreement Dr. Sorensen continues to assist the MN DNR in interpreting eDNA, and his lab will receive samples collected in the St Croix River and Upper Mississippi for archival purposes and possible future analysis when the procedure has been perfected. In the meantime, ongoing experiments with common carp eDNA in lakes conducted as part of Activity #4 strongly suggest that eDNA is extremely short-lived in natural waters (in lakes most eDNA degrades in just one to three days and is not measurable as little as a few tens of meters from fish [see Activity #4 result below]) – meaning that there is likely a high risk of false negatives (failure to measure live carp when they were recently present) -- this risk must be defined to help us and the DNR evaluate ongoing USFWS eDNA analyses in the Mississippi River. To the best of our knowledge no such analysis to determine this risk is either underway or has been conducted. Accordingly, we propose (below) a new set of experiments using funds that are no longer needed for routine sampling/analysis (as the USFWS will do this) to answer this important question using remaining funds.

To answer the question of how rapidly carp eDNA degrades in rivers (whose dynamics differ greatly from the lakes we have measured to date), and thus how to interpret negative eDNA data from the USFWS, we propose to conduct a new set of experiments in the Outdoor Experimental Stream Lab at Saint Anthony Falls Laboratory (U of MN) to measure eDNA persistence and retention in river water and sediments. This experiment would start in the summer of 2015 and be completed by December 2016, thus extending the project and adding new outcomes as proposed above. The results of this experiment could also ultimately help guide new sampling protocols in rivers for Asian carp eDNA (e.g. it may be best to sample more frequently and at targeted locations if decay is rapid). We will use the Stream Lab, a one of kind facility, to directly examine the effects of flow and “time since spring flood” on our ability to measure released eDNA into its natural waters which come directly from the Mississippi River and can be precisely controlled. We select these variables as they are thought most likely to affect the residence time of eDNA in natural systems. We will conduct a total 15 experiments which will examine several combinations of flow rate and time since spring flood (a combination of temperature and biological activity seen in rivers). Experiments will last two days and be conducted in triplicate. On day 1 of each experiment, silver carp eDNA (and possibly common carp eDNA) will be released as a single pulse to mimic the pulse of eDNA released by a passing fish. After release, water samples will be taken along the length of the stream, and sediment cores will be taken to examine the penetration of eDNA into the stream bed. On day 2 (after flushing), eDNA will again be released over an extended 24 h period to examine eDNA dynamics in a scenario which mimics a fish maintaining its position within the river. Samples will be analyzed with both the conventional PCR markers used by USFWS for monitoring and other new qPCR markers in development by government labs. A report and publication will be produced.

Activity Status as of *June 30, 2015*

We have been coordinating with the US Fish and Wildlife Service (USFWS) who completed eDNA sampling in the Mississippi River in Minnesota in August 2014. Archival samples from Lock and Dam #1 and Pool 5a were collected and are now stored at The University of Minnesota (UofMN) for possible future analysis. Meanwhile, a second set of samples from Pools 5a, 6, 8, and 9 has been analyzed by USFWS. Of a total of 476 samples, only one sample was positive for Bigheaded carp in Pool 8, which corresponds to a less than 1% detection rate in this pool. A detailed report of these monitoring results can be found at: http://www.fws.gov/midwest/fisheries/eDNA/Results-umr.html. The next sampling for eDNA in Minnesota waters will be in fall 2015 with the MN DNR, USFWS, and UofMN. We will continue to coordinate and advise this effort. Notably the USFWS has changed protocols for extracting eDNA which lessens sample value as the technique they now use (centrifugation) is less efficient than

filtration techniques. Changes in protocol also diminish historical value. We have made our findings and manuscripts available to the USFWS as well as USGS.

We started new pilot studies of Bigheaded carp eDNA in river water. On May 29, 2015 common carp DNA was released in the Saint Anthony Falls Outdoor Stream Lab facility with an inert fluorescent dye tracer to examine the fate and transport of eDNA in river water and sediment. As part of this experiment, 105 water samples and 48 sediment samples were collected over the course of two days. All samples have been filtered, weighed, and are currently in freezer storage awaiting eDNA analysis. Preliminary results of the data for the fluorescent tracer revealed that the experimental release of eDNA went well, with good mixing of the tracer (dye + eDNA) within the stream. These data will be used to refine subsequent releases in the fall of 2015 to examine the effect of temperature on the fate and transport of Bigheaded carp eDNA in river water. As part of this project, we have also obtained primers and probes for 6 separate quantitative PCR (qPCR) assays used by USFWS for quantification of Bigheaded carp eDNA. As part of our preliminary analyses, we will also evaluate which assays to use for subsequent release experiments.

Activity Status as of *December 31, 2015*

Mississippi River water samples continue to be collected as part of the eDNA monitoring scheme for Bigheaded carp run by the USFWS. The most recent monitoring event was September 28, 2015. Samples were collected by the USFWS from Pools 5a (50 samples), 6 (50 samples), 8 (100 samples), and 9 (100 samples). No samples were found to be positive for either bighead or silver carp eDNA. Results are available from http://www.fws.gov/midwest/fisheries/eDNA/results/upper-miss/2015-10-29/2015-10-29.html. Archival samples will no longer be collected for the University due to methodological changes in the eDNA collection method by USFWS that render possible comparisons to be of low value; our joint sampling efforts have thus ceased.

We continue to focus our effort to examine the dynamics of Bigheaded carp eDNA in river water. We finished data analysis of a preliminary experiment to examine the fate of carp eDNA in river water using the Outdoor Stream Lab at Saint Anthony Falls Laboratory (SAFL). In this experiment, eDNA was released in conjunction with a conservative tracer to examine eDNA degradation and retention in both river water and sediment. We found that eDNA suspended in the water column did not degrade along the 50 meter length of the experimental stream (5.5 min). Further, after eDNA release had ceased, the eDNA concentration in the water column rapidly returned to pre-release levels suggesting sediment plays little role; 1.5 hours post-release, eDNA was detectable but less than 1% of peak release concentration. In sediment, eDNA went from below detectable levels to above detectable levels within 15 minutes of the release start. However, within 12 hours post-release, eDNA levels fell below detectable levels. The distribution of eDNA in the sediment immediately after release was extremely patchy. The standard deviation of replicate sediment samples was similar to or exceeded the average concentration of eDNA. We conclude from our experiments that eDNA fate in river water is largely determined by release rate and hydrologic factors (dilution), rather than decay. In other words, because water flow in Mississippi River is greater than in the experimental stream used in this study, we conclude that dissipation of eDNA signal in rivers is primarily due to dilution rather than degradation. We now plan to continue our work at SAFL in spring 2016 but not at the Outdoor Stream Lab which appears to be too small to test dilution or degradation effects. Rather, we will now focus on determining release rates by different species of carps and possible effects of temperature on it, and then seek to develop a model to explain fates of eDNA in rivers and how to determine sampling regimes. These upcoming experiments will likely use indoor flow-through flume channels, likely at SAFL. We will test some the new markers we have acquired.

Activity Status as of *June 30, 2016*

Mississippi River water samples continue to be collected as part of the eDNA monitoring scheme for Bigheaded carp run by the USFWS. There have been no recent collections. We continue to examine the production and fate of carp eDNA in river water. The current project examines the release of eDNA from carp in flowing waters. We are presently working with Saint Anthony Falls Lab staff and engineers to devise a containment apparatus for live fish within a 20 inch interior flow-through flume to run experiments to address this important question. Containment mechanisms include an upstream wire grate, two downstream wire grates, and a final double layer metal grate just prior to the flume outflow. The final outflow grate is constructed from steel flooring material placed securely over the outflow pipe. The gap size is ¾ inch square, ensuring effective containment of experimental fishes. We have hypothesized that most eDNA is released from the exterior mucus layer of carp. Therefore, it is likely that eDNA release from live fish is dependent on flow velocity and temperature. Preliminary experiments using dye tracer showed that the flume outflow is well-mixed at low to high velocity flows, allowing for effective and representative sampling of the flume outflow. Experiments with common carp under flow velocities ranging from 0.05 to 0.45 m/s showed effective containment of experimental fish. Experiments will be conducted with Bigheaded carps, pending DNR approval. Accurate measures of eDNA release, coupled with degradation estimates obtained under Activity 4, would aid in the interpretation of eDNA monitoring results. We will attempt to use the resulting data from this experiment to create a one-dimensional model of eDNA transport distance. We anticipate this project will be completed on schedule in December 2016.

Activity of *December 5, 2016*

We have been examining how DNA released by common carp dissipates in flowing river water. We designed and conducted two initial experiments using common carp in a flow-through flume at the University river lab (SAFL). Several dozen water samples were collected and analyzed for eDNA. Very low (about 10 copies per ml) quantities of DNA are being measured, seeming suggesting that release rate may be unexpectedly and relatively low in fast flowing stream and that eDNA release, not decay, is the primary determinant of eDNA distribution in rivers. We need a new apparatus that has slower flows to test this and are now building a new testing flume that uses lower volumes of temperature controlled well water and which should also allow us to test bigheaded carp. A new half-time investigator, Dr. Ping Wang, is now leading this project because the primary investigator, Dr. Eichmiller, left for another position that has the promise of being permanent. This change, and the surprisingly low eDNA release rates in flowing waters that now require new study to resolve, have lead ask for an additional 6 months to complete the work. The question is of fundamental importance to interpreting eDNA measurements presently being taken in the Mississippi River by the USFWS and results

Final Status Report as of *June 15, 2017*

Activity 3, which addressed implementing eDNA as a management tool for invasive (Asian) carp, has now been completed. Our last key experiment was to determine instantaneous eDNA release rates in slowly flowing water designed to mimic a river. In the most recent six-month period, we successfully estimated instantaneous eDNA release by bigheaded carp held into flowing water. These are the first such measurements described and are thus extremely useful to managers who previously could not ascribe significance to eDNA copy numbers in flowing river waters. Briefly, six bighead carp (30 g) were put into custom-built flow-through lab tanks (8’ x 2’ x 2’) with a slow laminar flow rate (13 L/min), so the water turnover time was less than 20 minutes. Water temperature was then adjusted to 21°C. After fish were acclimated and feeding, the experiment started. Three 1L water samples were collected at the outlet, 30 min before feeding, and then 30 min, 3.5 hr, 6.5 hr, 9.5 hr, 12.5 hr, 15.5 hr and 25.5 hr after feeding, stored at 4°C in fridge before filtering or filtered with the Glass Microfiber filters 934-AH™ (Whatman). DNA was then extracted from the filters by using FastDNA Spin Kit (MP Bio) and extensively purified through OneStep PCR Inhibitor Removal Kit (Zymo Research). The copy number of two molecular markers (BH-TM1 and BH-TM2) of mitochondria DNA released by bighead carp was next measured through duplex qPCR by using iTaq™ Universal Probes Supermix (Bio-Rad) and

StepOnePlus Real-Time PCR Systems (Applied Biosystems). This experiment was repeated once a week after the first run when the carp were replaced. The eDNA copy number in water samples were calculated based on the measured standard curve. It is shown in Figure 3.1: the eDNA basal release rate measured by both markers was in the range of 10^3-10^4 copies/100 mL water. However, this rate increased to over 10^5 copies/100 mL after 30 min of feeding. It appears that feeding stimulated the eDNA releasing – an important new finding that is relevant to management. A manuscript is now being prepared.

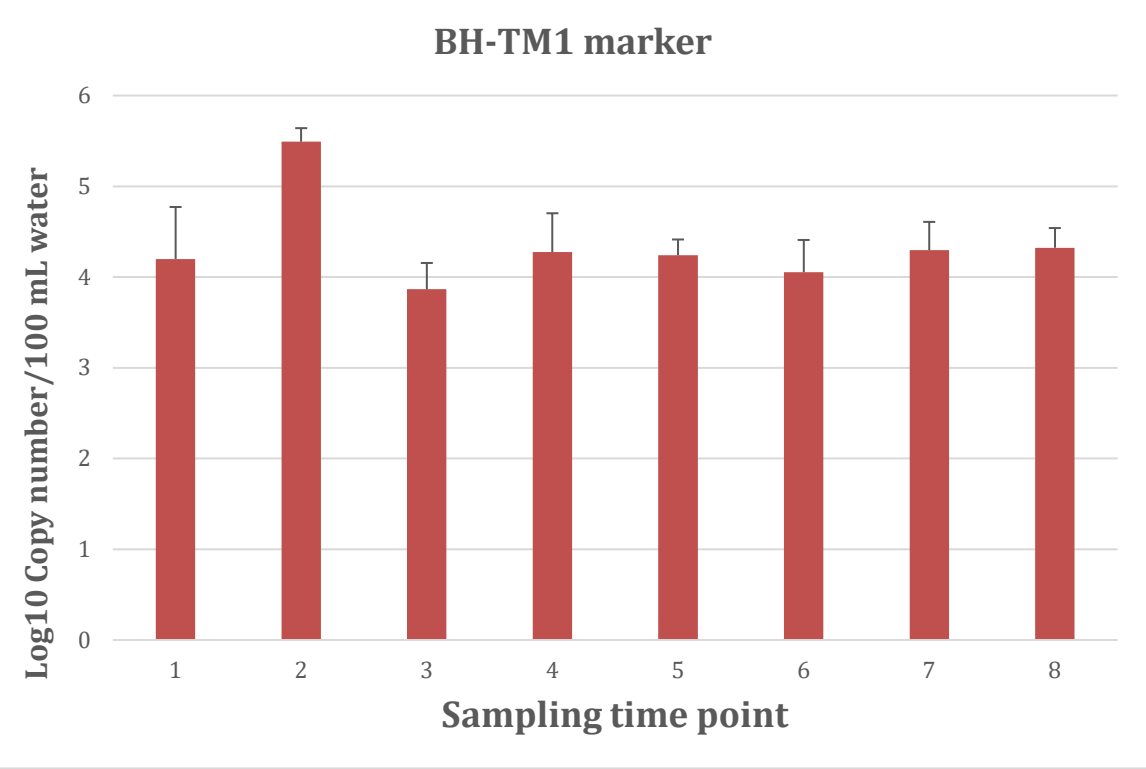


Figure 3-1. eDNA release of 6 bigheaded carp in flowing lab waters. Note peak in release at the time of feeding (2).

In summary, this activity has demonstrated that initial 2011 measurements of eDNA in the Mississippi River were almost certainly fallacious and attributable to poor markers as new measurements conducted after that by the Sorensen lab, and later the USFWS (with our assistance) have been unable to confirm these measures while our studies also developed new/ better techniques. The release and decay rates we have calculated for eDNA by bigheaded carp have added even greater resolution and significance to eDNA techniques and ongoing studies which can now be interpreted with much greater certainty. Our finding that eDNA release is closely correlated with feeding activity is especially germane future eDNA measurement will be much more relevant

ACTIVITY 4: Determining the ability of two approaches to measure environmental DNA (eDNA) to reliably quantify the abundance of invasive common carp in Minnesota lakes.

Description: The University will develop and test the ability of eDNA to estimate the abundance of an important AIS, the common carp, in Minnesota lakes. This study will complement the eDNA study of Asian carps (Activity 3) which focuses on presence /absence measures of a rare AIS. Here, we use the common carp as a model because it is extremely abundant, well understood, and the subject of

management efforts; i.e. this study will be sure to produce easily interpretable results. Dr. Michael Sadowski (Biotechnology Institute) will direct a postdoctoral fellow in this effort. The primary objective will be to determine if we can accurately and reliably measure the abundance of common carp in waters they are known to inhabit. We will develop and validate two techniques: quantitative PCR (qPCR) of carp mitochondria (conventional eDNA analysis) and qPCR of microbial rDNA from carp feces combined with sequencing ('metagenomics'). Metagenomics is promising because it could offer greater sensitivity and more information on species abundance and ecosystem health. Initial efforts will identify molecular markers for common carp by comparing mitochondrial sequences from the common carp and close relatives. Quantitative PCR assays will then be developed. In a second step, the effects of key environmental variables on the abundance of these markers will be examined in laboratory mesocosms. Lastly, we will apply the most promising technique(s) to study the relationship between the abundance of these markers and the abundance of common carp in local lakes. A publication is expected and results will be disseminated via workshops. Results from this study will guide future studies by a research assistant professor focused on perfecting molecular tools to systematically measure a wide variety of AIS across space and time in Minnesota waters (funding requested in a new ENRTF proposal that would start funding this enhanced activity in 2015).

Summary Budget Information for Activity 4:

ENRTF Budget: $309,866
Amount Spent: $309,866
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Identify molecular markers for common carp and its microfauna, and develop assays to measure both	2013	$36,849
2. Validation of the most useful markers/ approach to quantify the abundance of common carp in lake waters	2013	$109,147
3. Validation of optimized eDNA measurement techniques for the measurement of common carp in the lakes	2015	$109,147
2. Validation of optimized microbial trace markers for the measurement technique for carp in lakes	2016	
4. Final report and publication	2016	$117,795

Activity Status as of *June 30, 2013*

A postdoctoral associate (Jessica Eichmiller) was hired to do this work. In early 2013, equipment purchases were completed including that of a real-time quantitative PCR (qPCR) system and a –80°C freezer for samples storage. Seven candidate markers for common carp have been identified to date. We have eliminated five of these so far due to non-specific amplification of DNA from a library of 34 DNA extracts of native and non-native fish species that we have assembled. Our results show that markers within the cytochrome *b* gene have lower rates of non-specific amplification, and produce more stable and efficient qPCR assays. Therefore, all subsequent test markers will target this gene. Our preliminary lab studies have also shown that copies of cytochrome *b* are correlated with fish biomass, are abundant in feces, milt, and mucus, and have a linear short-term release rate. We plan to conduct a more detailed study of the release and degradation of cytochrome *b* in future lab experiments. We have also completed metagenomic sequencing of 14 fish fecal samples in order to identify bacterial (microfauna) markers specific for common carp. These samples included common carp, goldfish, silver carp, and bighead carp housed in laboratory facilities, as well as wild silver and bighead carp. We have identified two particularly abundant bacterial families in all of these species. We have also identified 22 strains that were specific to common carp, and 4 that were specific to silver carp. However, the microbial communities of lab and wild Asian carps were distinct; therefore, we are expanding the dataset with more replicate samples of wild-caught fish on order to validate these results

Activity Status as of *April 1 2014*

We completed development of a quantitative PCR (qPCR) assay to target a 149 bp region of the Common carp cytochrome *b* gene. The assay has been optimized, and it meets all established criteria for valid qPCR assays, including a low limit of detection, optimal amplification efficiency, and reproducible standard curves. The marker is specific for common carp, and the assay did not amplify DNA from 34 native and non-native fish species. Although the marker has proven to be extremely sensitive for common carp in the lab setting, initial field testing proved that detection is much less efficient in the natural environment. Therefore, a series of experiments were conducted to examine the role of sampling methodologies and the distribution of eDNA in a small lake while optimizing eDNA collection and extraction protocols. From the results of these experiments we have concluded that in order to improve detectability, eDNA must be collected via filtration with a small pore-size filter in water where fish are presumed to frequent. Comparison of commercial DNA extraction kits revealed a wide range of DNA yield and degree of inhibitor removal. The MoBio Power Soil kit performed well overall and will be used for future experiments. Two manuscripts are currently in preparation that will describe these findings. This work will involve future eDNA sampling schemes for Bigheaded ('Asian') carp in the Mississippi River (they add critical background information and point to unrealized weakness and strengths in the technique). A future experiment will examine the release and degradation dynamics of the common carp marker to further understand the relationship between fish abundance/presence and eDNA. For examination of fish microfauna, 102 samples of feces were collected from 5 fish species. including common and bigheaded carps. Metagenomic sequencing has been completed, and the data analysis is underway. From each sample, 150 000 bacteria were classified, and the number of species per sample ranged from 2000 to 7000. A number of bacteria were identified that were unique to a particular fish species. Over 30 000 and 50 000 such bacteria were identified for bigheaded and common carps, respectively. Future analysis will aim to develop testable bacterial source-tracking markers that could be used to determine the presence of these fishes. In sum, field validation of both eDNA and metagenomic approaches to measure invasive carps in natural waters is already underway and describing insightful results, with new, critical experiments planned for this summer. These results are extremely promising and a future amendment will request to re-direct funds to this activity so it can be extended.

Activity Status as of *June 30, 2014*

Work on final validation of the marker is progressing. Last year we successfully developed a molecular assay for common carp using a mitochondrial DNA marker. Although the primary literature suggested that validation of this technique in natural waters would be straightforward, preliminary sampling revealed that the ability to detect carp eDNA using standard methods was not optimal. This has significant implication for the federal (and now state) eDNA sampling program (see above). For example, a low frequency of detection was observed in lake waters with a well-documented, large population of common carp. Subsequently, we completed two studies to validate field sampling and laboratory processing methodology. In March 2014, we submitted a manuscript titled "The relationship between the distribution of common carp and their environmental DNA in a small lake" to a scientific journal and are awaiting the results of peer review. The first draft of a second manuscript entitled, "Influence of environmental DNA collection and extraction techniques on the ability to detect and quantify fish" has also been completed and is currently undergoing internal revisions. Further laboratory validation is now underway to understand the effect of environmental variables on this marker, and an experiment is in progress to determine the decay rate of the carp marker as well as the effect of temperature and nutrient status on decay. This summer, sampling will commence on metro area lakes using our optimized methods for eDNA collection and extraction to examine the biomass and eDNA relationship for common carp using the mitochondrial DNA marker: a critical step in field validation in local waters. We plan to repeat this sampling in winter to examine seasonal effects. The fish fecal metagenomics research has revealed numerous potential source-tracking markers for common carp. These sequences will be screened against a large dataset of fecal metagenomes from different animal sources to further remove sequences that are not specific to common carp. This work will be done in

collaboration with Michael Sadowsky. The resulting list of potential source-tracking markers will be validated in the laboratory setting. The budget for this project will need to be adjusted and reconciled at the next update to reflect the increased amount of time Dr. Sorensen is spending on this and other projects because of inherent needs and the fact he no longer is Center Director. A manuscript describing the preliminary findings of the metagenomic project is expected by Fall 2014.

Activity Status as of *December 31, 2014*

Final validation of our new common carp-specific mitochondrial DNA marker and optimization of field and laboratory techniques are nearly completed, and we are continuing to evaluate metagenomic data, which we now propose to fully address next year (see below). An initial field validation testing of this eDNA marker revealed that its distribution correlated strongly with fish distribution and this is now described in a publication in *PloS One*. We have also identified optimal techniques for capturing and measuring eDNA that include an optimal filtration technique (that differs from USFWS protocols) and an extraction kit which needs a proprietary lysis device (FastPrep-24) and possibly a plate reader which we would like / need to purchase instead of the autoclave initially budgeted (and which we no longer need). A manuscript which describes optimized eDNA collection and extraction techniques has been written and is presently under peer review. Because this document reveals likely major shortcomings in the U.S. Fish and Wildlife Service Quality Assurance Project Plan for eDNA Monitoring of Bighead and Silver Carps- and ways it might be improved- we believe it to be significant as does the USGS with whom we have communicated on this issue but which is not examining it. We have also completed a laboratory study of common carp eDNA decay, showing that decay is extremely rapid, even at low temperatures. With validated field sampling and laboratory protocols, we have completed one of two rounds of lake sampling to create a robust eDNA biomass relationship for common carp. We aim to complete this project by July 2015 (provided we are able to purchase the FastPrep Instrument).

In the meantime, while we have completed initial metagenomic analysis of fish fecal microbial communities and identified numerous bacteria that are species-specific (showing metagenomic analysis might be viable alternative technique to eDNA for measuring invasive carps), we will need more than 6 months to reach our goal of developing and testing microbial-source-tracking (MST) markers for common, silver, and bighead carps. Therefore, we propose to extend this project for a year using remaining funds. This research would consist of *in silico* screening of our candidate markers against a database of existing fecal metagenomics data from a diverse array of sources in collaboration with Dr. Michael Sadowsky. Once the suite of candidate markers is refined, they will be amplified, sequenced, and screened for specificity and sensitivity *in vitro*. Specificity and sensitivity testing will consist of testing the candidate markers against other fecal sources using quantitative PCR. Finally, the markers will be validated by screening lake or river water with different population levels of the target species. We hypothesize that metagenomic markers may be a powerful tool to augment traditional eDNA sampling. Due to the rapid degradation of eDNA, MST markers may be a more sensitive method to detect invasive fish if, as we hypothesize, these MST markers are more abundant than carp eDNA and persist longer in the environment. A revised budget is proposed to accommodate this activity.

Activity Status as of *June 30, 2015*

We published a laboratory validation of eDNA measurement techniques in *Molecular Ecology Resources* titled, “Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish” and are now completing a field validation of these optimized methods for measuring common carp biomass using eDNA. This analysis uses water samples collected from 13 local lakes (50 samples from each lake) with known biomass of common carp. It explores the two most promising eDNA capture and extraction techniques identified in the aforementioned publication. We first analyzed all samples using the method identified to be optimal for biomass quantification in the laboratory setting; however, this method had poor detection rates of common carp in the field. For example, one study lake had high biomass of carp, but carp eDNA was not detected at all. Consequently, we are testing the second method, which was optimal for detection (rather than

quantification) of lab carp. Currently, we have analyzed 4 out of 13 lakes (31%), and the results are promising. Detection rate in the field presently appears to be correlated with biomass, but we have too few sites analyzed at present to confirm this statistically. We have also submitted a manuscript, which is currently under peer-review, which measured the decay rates of common carp eDNA in natural freshwaters. This is the first manuscript to measure the decay rate of eDNA in natural freshwaters, and we found that eDNA decay was extremely rapid (which likely explains some of the results mentioned above). Potentially, winter sampling may thus be more conducive to biomass measurement of common carp, due to potentially lower decay rates. We expect completion of the analysis and final report for the validation of optimal measurement techniques for common carp in lakes by December 2015. Statistical analysis of metagenomic data of fish fecal microbial communities is now well underway.

Activity Status as of *December 31, 2015*

We have completed the analysis of common carp eDNA in the lake waters that we collected in the fall of 2014 and winter of 2015. We found that eDNA is strongly correlated with common carp biomass and density in the summer months but not the winter months. We are currently working in collaboration with John Fieberg, a computation biologist at the University of Minnesota, to develop a statistical model to describe the relationship of carp eDNA and density. The results will be used to create a guide for eDNA sampling in lakes, given a desired detection probability. We foresee that this guide will be broadly useful to the scientific community and managers, as no such relationship between eDNA and density in lakes has yet been found by other workers. The results of the summer study are currently being prepared for publication in a peer reviewed journal. Future analysis of the winter data will likely examine the relationship between winter samples in distribution of eDNA and aggregated radio tagged carp to examine the causes of the winter-summer discrepancy. Our statistical analysis of metagenomic data of fish fecal microbial communities has now been completed and we are in the process of synthesizing these data for publication. The manuscript on eDNA degradation rates has been revised to address reviewer comments and resubmitted.

Activity Status as of *June 30, 2016*

The project is wrapping up. We published a manuscript titled, “Effects of temperature and trophic state on degradation of environmental DNA in lake water,” in Environmental Science and Technology in January 2016. We finished writing and have submitted a manuscript reporting the results for the metagenomic analysis of fecal microbial communities of invasive carps. The manuscript is currently in revision. Characterization of the carp microbiome and the factors that affect its composition is an important step toward understanding the biology and interrelationships between these species and their environments. We compared the fecal microbiomes of common, silver, and bighead carps from wild and laboratory environments using Illumina sequencing of bacterial 16S ribosomal RNA (rRNA). Environment played a large role in shaping fecal microbial community composition, and the microbiomes of wild fishes were more distinct than those of laboratory-housed fishes. Although differences among wild fishes could be attributed to feeding preferences, diet did not strongly affect microbial community structure in laboratory-housed fishes. Comparison of wild and lab invasive carps revealed five shared OTUs that comprised approximately 40% of the core fecal microbiome. This work is an important advancement in the understanding of the factors that control the fish microbiome, and may be useful for management applications. Work is ongoing on another manuscript detailing the relationship of eDNA and carp biomass in lakes. Further analysis of winter data has shown that eDNA detections correlated with distribution of fish. For example, the percentage of detections were highest when the nearest neighbor distance between radio tagged carp was lower, indicating that highly clustered distributions of fish lead to a lower overall percentage of positive eDNA results and showing that sampling schemes need to be targeted and very strategic to be meaningful (but then can be very informative). No new data collection is needed. We anticipate this project will be completed on schedule in December 2016.

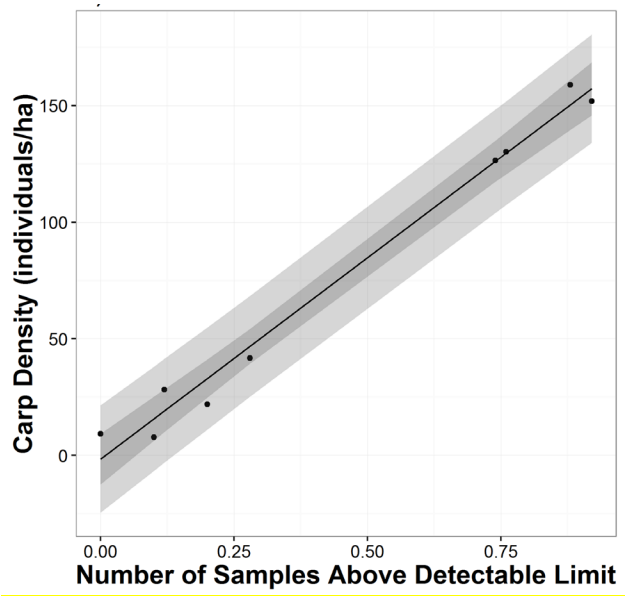
Final Status Report as of *December 5, 2016*

This project has been completed. We (Dr. Eichmiller) have published four peer-reviewed studies that address: (1) the development and validation of eDNA markers for common carp, (2) the optimization of field and lab protocols for processing eDNA samples, and (3) the factors that control degradation of eDNA in lake water and 4) the genetic and evolutionary factors that determine the fecal microbiome of invasive carps. Together, these studies demonstrate that eDNA is useful and valid technique to measure carp densities while the microbiome is complicated by environmental factors.

A final, as yet unpublished study of this project used optimized methods for developing a model to predict carp density from eDNA. The project was done in collaboration with John Fieberg, an Assistant Professor in the Department of Fisheries and Wildlife at University of Minnesota. This study has been completed, the results analyzed, and the final manuscript is in now preparation by Eichmiller (who is now at new position) for submission to *Canadian Journal of Fisheries and Aquatic Sciences*. This manuscript is the culmination of our work in this activity to measure fish populations in small MN lakes using eDNA. Our results show that the density of common carp in MN lakes can be predicted with high confidence by collection of 50 water samples using eDNA collection and extraction methods optimized for detection and a point-intercept sampling scheme. The relationship can be described as follows:

Carp density (individuals/ha) = 173*(positive samples/50) – 1.57

We also found that carp can be detected with 95% confidence in lakes with 50 individuals/ha or more with approximately 10 water samples. This enables managers to quickly and inexpensively assess carp populations in lakes that they manage. These results are an important advance in the field of eDNA research, fisheries science, and invasive species. Our results also indicate that eDNA holds considerable promise for assessing native fish populations in Minnesota .



ACTIVITY 5: Testing whether carp can be located using 'Judas fish': a new behavioral tool to locate aggregating invasive fish so they might be tracked and/or removed.

Description: The Judas fish technique (tracking a few individual animals to find other members of their group) will be developed as a means to locate low numbers of Asian carp in Minnesota waters. This technique has been used with great success in other locations (ex. tropical islands to find/remove feral goats). The first step will be developing means to sterilize carp in collaboration with a veterinarian. Later, we will develop means to track them using radio-transmitters in rivers. Common carp will be our primary model but we will include work on Asian carps in the laboratory. Dr. Sorensen (1.0 mo/year) will lead this 2-year start-up program, for which additional funding will requested from the ENRTF starting in 2015 to further develop the technique so that it can made useful in carp control in open rivers.

Summary Budget Information for Activity 5:

ENRTF Budget: $189,043
Amount Spent: $189,043
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Determining whether silver carp shoal (in the lab)	2014	0
2. Determining whether sex steroids can be used to masculinize silver carp in the lab	2014	$89,573
3. Determining whether masculinized (sterile) silver carp find conspecifics using pheromones (proof of Judas fish concept)	2015	
4. Surgical means to vasectomize common carp and silver carp developed with the help of a vet	2015	$91,660

Activity Status as of *June 30, 2013*

Although this activity has not officially started, some preliminary research with a veterinary surgeon has been completed. This study has shown that while male common carp will be difficult to sterilize using tubular ligation (because their sperm ducts are short and hard to get to), male silver carp will be much easier because they have long sperm ducts. Of course, adult silver carp are not available in Minnesota, but we do have juvenile fish in our laboratory that can be tested. Thus, while we continue to attempt to sterilize common carp, we will now attempt to develop the Judas fish technique using silver carp in the laboratory. First, we will test whether silver carp shoal (form small groups); the Judas fish can only work if this is the case. Simultaneously , we will develop protocols to masculinize juvenile silver carp using sex steroid treatments (to provide fish that behave like sexually mature fish whose social behavior can be studied in the laboratory and /or released [if sterilization is not feasible]). If successful, we will then test whether masculinized (and sterile) silver carp find conspecifics using sex pheromones (a proof of concept of the Judas fish technique in the laboratory). Outcomes have been changed accordingly.

Activity Status as of *April 1 2014*

Experiments have now conclusively demonstrated that juvenile silver carp shoal (aggregate) in the laboratory and that group size does not influence the nature of this behavior: the Judas fish concept has potential to be used to locate and control this species. In our experiments, juvenile silver carps were placed into circular tanks in groups ranging from 2 to 20 while their behaviors were noted using overhead low-light cameras. Nearest neighborhood distance (NND) was then measured every 15-sec for 15-min trials, and 8-9 trials were performed for each group size of fishes. The NND was then used to develop a statistical model that tested and predicted the probability of distribution. This model was based on the assumption that each individual time points for a particular trial are independent and identically distributed. We found that silver carp shoal strongly ($P<0.05$) irrespective of the number of

fish. Interestingly, larger groups of 4, 8, 12 and 20 fishes split into smaller shoaling units (~2-3 fishes). This work will be expanded to bighead carp and mixed species aggregations if we can acquire the fish required. Work continues with sterilization techniques in common carp and is promising: we now have had vasectomized common carp in the lab for two months and their behavior seems normal. Other experiments planned for the summer of 2014 will determine how to masculinize juvenile carp and test whether sexual receptivity and pheromone (sexual and conspecific) release might enhance the probability of shoaling.

Activity Status as of *June 30, 2014*

The overarching goal of our study is to determine if, and how, we can use "Judas" Bigheaded carp (radio-tagged carp) to locate aggregations of invasive carp (both Bigheaded [the "Asian carps" of greatest concern, *Hypophthalmichthys* spp.] and common carps) so they can be counted and, if possible, removed. We now know that Bigheaded carp shoal so the technique has promise. Our next step is to determine if we can enhance the tendency of these fish (which would be immature or sterilized) to aggregate by making them sexually-receptive and attractive using hormones. Because working with adult Bigheaded carps is extremely difficult, work has employed goldfish as a model in initial pilot studies. This is reasonable because goldfish are also carps, hormone systems are extremely conserved in vertebrates, and goldfish exhibit a full range of behaviors in the lab where they also can easily be housed. These initial experiments are now complete and show promise. Recent work has focused on androgenic steroids (steroids which enhance masculinity) and how they might be used to drive sexual behavior (such as seeking females) in carps. In a now complete, as part of a 6- month experiment, we exposed female goldfish to one of three androgen treatments: i) a natural androgen (11ketotestosterone [11KT] plus testosterone [T]) in implanted capsules; ii) a synthetic and inexpensive androgen (methyltestosterone [MT]) in capsules; and iii) MT in a bath (a much easier and more practical way to expose fish in the lab – but not the field). Normal, untreated males and females were used as controls. Behavioral tests which paired treated fish with sexually-active females, electrophysiological studies of olfactory sensitivity to female sex pheromones, and hormone analysis via blood sampling were conducted on a quasi-monthly basis. While olfactory masculinization was complete within 6 weeks in all three experimental groups, behavioral responses to females lagged in intensity (time taken to spawn and number of spawning behaviors). Specifically, while male behavioral responses were measurable at 6 weeks, it took them 3 months to reach the level exhibited by untreated control males at the start of the experiment for both the 11KT+T fishes and MT bath. After this, responsiveness of all implanted fish declined while that of MT bath fish increased to eventually match that of untreated males (which also increased with time). The MT capsule treatment had little effect. Thus, 11KT+T capsules have promise for use in sterilized wild carps but one would need to wait three months and levels may not equal those of wild, spawning males which would continue to spawn and become more receptive during this time. A manuscript is in preparation. In the meantime, we have started several experiments as follow-ups to if, and how, we might deploy this set of techniques in Asian carps and if alternatives might exist to drive sexual receptivity. First, we are now treating Silver carps to determine if we can masculinize them as we did the goldfish. MT bath treatments are being used and these fish will be tested after several months with sets of spawning stimuli. If reasonable, we will also examine the effects of long-term steroid treatments independent of experience to further ascertain how competitive masculinized Asian carps could be in the wild (for monitoring) if implanted in advance of spawning. Second, we are also exploring the alternative possibility that instead of masculinizing carps we might instead feminize them using prostaglandin $F_{2\alpha}$ implants. This treatment could be cheaper, easier and safer than using androgenic sex steroids. Once again, we are using goldfish in pilot studies before moving to Silver carp. By year's end we should be able to identify the most promising technique for use in Judas Silver carp.

Activity Status as of *December 31, 2014*

Analysis of our experiment (outcome 1) which sought to determine whether sex steroids could be used to fully masculinize male carp is now complete and a manuscript has been submitted to the journal

Hormones and Behavior. Final and complete analysis of these data which used the goldfish as a model clearly demonstrate that while sex steroid implants that use 11ketotestosterone and testosterone masculinize carps and can be used in laboratory studies of spawning, they probably do not have the ability to be truly useful in the field. These data demonstrate that while implants can fully masculinize olfactory sensitivity to sex pheromones and sexual arousal, they do not fully masculinize sexual drive as reflected by courting and spawning even after 6 months. Thus, while this technique will allow us to proceed with proof-of-concept studies of Asian carp spawning in the lab, it's unlikely that it would be very useful in Judas fish released into the wild where these fish would have to compete with normal males. Accordingly, we are now focused on conducting experiments designed to determine if we can fully feminize sterile fish instead (this is a new, additional objective/outcome). This new experiment is going very well. Briefly, we are using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) implants (a treatment that evokes normal female sexual activity and sex pheromone release) to induce female-typical behaviors (feminization) in carp. To choose the correct $PGF_{2\alpha}$ dose, we first conducted experiments on goldfish, one of the best understood and an excellent model to investigate hormonal system in fish. We implanted female goldfish with different doses of the $PGF_{2\alpha}$ to find out the optimal dose that will be non-toxic to the carps and also will induce sex-typical courtship behaviors in the implanted fish for 28 days. After determining the optimal dose, we conducted experiments to determine the levels of sex-typical behavior in the implanted fish and compared these to $PGF_{2\alpha}$ –injected fish which previous experiments have already shown to exhibit complete and normal levels of female activity. We have found that the implanted fish show normal female typical courtship behavior and spawning activity with mature males for 3 weeks. Pilot work has started using PGF treated bighead and masculinized Bighead males in flow-through tanks to determine if and how they locate each other using pheromones (Outcome 3). A range of environmental conditions will be tested (water velocities, temperature, addition of putative priming sex pheromones) to optimize the spawning behaviors. After optimizing the trial conditions, feminization experiments will be carried out with $PGF_{2\alpha}$ –implanted bigheaded carp. Work with shoaling behavior is nearing completion (it shows carp shoal strongly at multiple group sizes) and now being analyzed and written up for publication (Outcome 1). Work is also underway with a veterinarian, Dr. Amy Kizer, to sterilize common and now immature bighead carps (Outcome 4). We are exploring complete gonadectomy. This work is going well and is very promising and we expect to continue this work and expand it to proof of concept studies on sterilized/feminized Judas fish in the field as part of a new proposal now being developed for ENRTF2013 that will be submitted through MAISRC this winter.

Activity Status as of *June 30, 2015*

Experiments have now demonstrated that masculinized bigheaded males locate each other using pheromones (Outcome 3). Using a two choice maze, we observed the attraction behavior of silver carp towards odors released by prostaglandin F2α implanted conspecifics. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment evokes normal female sexual activity and sex pheromone release in implanted carps. We observed that masculinized (sterile) silver carp were strongly attached to the water of PGF implanted conspecifics ($P<0.05$) but not towards the water of PGF implanted bighead and common carp ($P>0.05$) (Fig.4-1). Masculinized silver carp are also attracted to synthetic mixtures of PGF metabolites mimicking the ratios measured in implanted water of silver carp (-8Molar concentration); thus, masculinized silver carp use sex pheromones to locate mature females and Judas fish technique should be useful. A manuscript is in preparation.

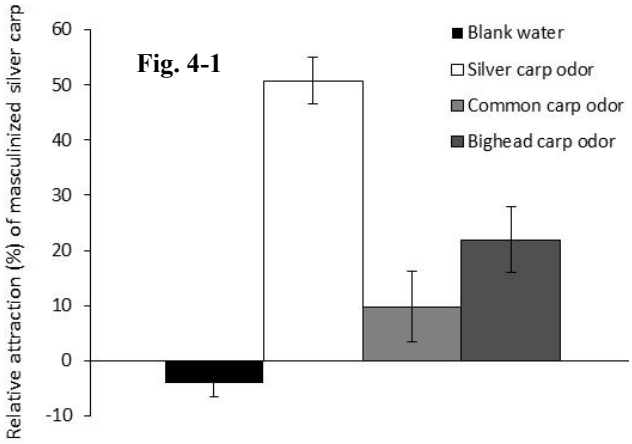


Figure 4-1. Attraction of masculinized silver carp to pheromones.

Work is also underway with a veterinarian, Dr. Amy Kizer, to develop-sterilization procedures for male common and bigheaded carps (Outcome 4). We are pursuing two options. First, we are examining vasectomy as way to produce sterile Judas fish. Although male common carp showed regeneration of their testis after a year following both tubular ligation and gonadectomy (removal of testis), we have since discovered that the anatomy of bigheaded carps is different and may support complete gonadectomy. We have offered to pursue this option this fall with the USGS. Simultaneously, we are also now examining the possibility of inserting an anesthetic cartridge into vasectomized Judas carps that could be opened automatically by timer after a few months before they could possibly regenerate their testes (and which would kill them). Eugenol appears to have promise and a local company (ATS) is designing an automated, timed release mechanism. By December 2015 we will have answers to both approaches when we will re-budget, close this project and prepare a final report. Its results will be used as part of a new ENRTF2013 project (subproject #3) at that time.

Activity Status as of *December 31, 2015*

We have been pursuing two options that might allow us to sterilize carp so we can adopt the Judas fish method for bigheaded carps. Both show promise. For our first option, we have been examining both vasectomy and gonadectomy as ways to produce sterile male Judas fish. This work has been done in collaboration with a veterinarian, Dr. Kizer. Although initial experiments were discouraging because we found that male common carp (our model) completely regenerated their sperm ducts and testes within a year of removal (apparently from fragments of testes), recent re-evaluation suggests promise for male silver carp whose reproductive tract is unique and might permit effective gonadectomy. Briefly, while the sperm and urinary ducts of common carp merge before exiting the body-- complicating complete gonadectomy-- the sperm ducts of silver carp run directly and independently of the urinary ducts to the cloaca, so complete gonadectomy might be possible. We plan to explore this possibility in a future collaboration with the USGS laboratory in Missouri. For our second option, we have been exploring the possibility that we might be able to implant carp with an anesthetic cartridge that could be emptied into the body cavity using an automated/integrated timer. We recently identified eugenol (the active component of clove oil) as a candidate because it has been approved by American Veterinary Medical Association for use as a fish anesthetic and is safe for human use. In recently completed experiments, we found that at a dose of 1mg/ body weight, eugenol would kill both common carp and bighead carp within one hour (Table 1). We are now working with Advanced Telemetry Systems (Isanti, MN) to develop the automated cartridges to release this compound.

Table 1: Different doses of Eugenol tested on common carp, goldfish and bighead carp

Species	Sample size	Eugenol dose	Mortality	Time to mortality
Common carp	4	0.5 mg/g	No	NA
Common carp	4	1 mg/g	Yes	1 hour
Common carp	4	2 mg/g	Yes	1 hour
Goldfish	2	1 mg/g	No	NA
Goldfish	2	2 mg/g	Yes	24 hours
Bighead carp	2	1 mg/g	Yes	1 hour
Bighead carp	2	2 mg/g	Yes	1 hour

Final report summary: December 31, 2015 In summary, this project showed that the Judas fish technique has promise for use with male bigheaded carp. First, we demonstrated that both juvenile bighead and silver carp shoal strongly (especially the former); i.e. these fish are social and Judas fish should be useful in the field as they would find other conspecificis. Second, we discovered that while we can completely feminize male carps using prostaglandin implants and that they will release attractive pheromones, we cannot fully masculinize females using androgen implants; i.e. if we create Judas fish we should do so by feminizing male carp. Third, we have found that we can sterilize carp for at least a few months and very likely we can create anesthetic capsules using eugenol to insert into them that could then euthanize them. The MN DNR has expressed great interest in this work which we plan to continue using other funding. Judas Fish techniques will be addressed as part of the 2013 ENRTF Subproject 3 "Attracting carp so their presence can be measured."

ACTIVITY 6: Developing food attractants for silver carp that can be used to induce aggregation and control them: a new biochemical tool.

Description: This project will identify and develop chemical food attractants for Asian carp that could be used with the poison nanoparticles presently being developed by the USGS laboratory in LaCrosse, WI. It will be supervised by Dr. Sorensen (1.0 mo/yr) and employ a graduate student from 2013-2015 when additional funding is being requested from the ENRTF to develop this concept further by examining pheromones, sound and other sensory stimuli to manipulate the distribution of carps.

Summary Budget Information for Activity 6:

ENRTF Budget: $102,934
Amount Spent: $102,934
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget

1. Determine if chemicals released by specific food items stimulate ingestion behavior and determine the chemicals' identity	2014	
2. Determine what sensory system mediates these responses and if the same chemicals that mediate ingestion are also attractive.	2015	$49,125
3. Evaluation of known algal metabolites as feeding stimulants	2015	40,000
4. Final characterization of the olfactory activity of metabolites	2016	6,809
5. Characterization of behavioral activity, Final report	2017	7,000

Activity Status as of *June 30, 2013*

Although this project has not officially started, we have done some preliminary work using juvenile silver carp which are now being held in our laboratory. A graduate student, Aaron Claus, has been accepted into the program and started with the help of federal funds (USGS Missouri). He is finding that silver carp are good laboratory models and prefer to eat specific types of cyanobacteria; this work has promise. The outcomes have been changed slightly to accommodate new pilot data that shows that Asian carps have specialized feeding organ known as the epibranchial organ that stimulates food ingestion and is chemosensitive.

Activity Status as of *April 1 2014*

Laboratory experiments using juvenile bighead and juvenile silver carp have demonstrated that ingestion behavior (buccal pumping) in both of species of bigheaded carps is largely mediated by food-related chemicals. Experiments have shown that high rates of buccal pumping are stimulated in these species when highly filtered food extracts are added to their aquaria, which although less intense than the activity stimulated by food itself, were large and highly significant ($P<0.01$). Experiments also suggest that certain food items and their extracts are much more stimulatory than others: the responses are extremely specific. Indeed, of nine items tested, the activity of dried cyanobacteria *Spirulina platensis* was especially strong. Work also showed that amino acids explain part, but not all, of this activity. Upcoming work will examine the chemosensory system that is responsible for this behavior and the chemical identity of the cues (Dr. Hoye from the Chemistry Department will be helping us). The USGS is a partner in this work and is funding related work. A manuscript describing some of these data is in preparation. In conclusion, food attractants appear to have the potential to be used in bigheaded carp control.

Activity Status as of *June 30, 2014*

Experiments to test characterize the role of feeding cues in food recognition and ingestion (buccal pumping) in Bigheaded carps have been completed and confirmed that both Bighead and Silver carp have nearly identical behaviors and preferences. Studies have also now shown that while initial recognition of food is driven by chemical stimuli, responses (buccal pumping) is self–reinforcing as responses to food chemicals decline within 4 minutes of exposure in both species, while these persist for at least 8 minutes when food particles are physically present. Chemical baits might thus be best accompanied by particles if they are to attract the most fish and drive consumption. Analyses of the amino acid constituents of a favored algal food have also been completed and we now know that 17 L-amino acids are present and that this mixture can explain about a quarter of the food's potency. Initial work with the epibranchial organ (a highly specialized organ in Asian carp buccal cavity) suggests that while it likely can explain food consumption and is highly chemo-sensitive. We hypothesize that unique feeding stimuli may be present in Asian carp algal food that might allow them to be targeted. The graduate student working on this project (Aaron Claus) met with his committee and now plans to continue this work as part of a PhD that would be eventually supported as part of ENRTF 2013 (an

option articulated in original work plan). The budget for this project will also need to be adjusted and reconciled at the next update to reflect the increased amount of technician time needed for this student because of inherent needs and the fact a full time Center technician may no longer be available to assist with research. Algal food is now being chemically characterized with the active assistance of natural products chemist (Professor T. Hoye) while Mr. Claus writes a Ph.D. proposal.

Activity Status as of *December 31, 2014*

All major objectives of this work plan have now been met. An experiment has determined that the sense of smell is the primary sensory modality that discerns chemical food stimuli in both silver and bighead carp. In this experiment, the noses of carp were temporarily occluded with inert material while their feeding responses (buccal pumping) were noted. Buccal pumping slowed greatly, but not completely, suggesting that olfaction mediates food recognition while taste (the eipbranchial organ) likely mediates ingestion. This is important because it allows us to now focus on studies of the olfactory system to identify key food stimuli which our previous work (see above) has already suggested to be unique algal metabolites. The graduate student responsible for this work (Aaron Claus) is now writing these findings up for a manuscript and as his Masters thesis before he starts Ph.D. research. Meanwhile, we have published an article in the *Journal of Experimental Biology* on the unique internal taste system of Asian carp (the epibranchial organ) and how could its unique function could also be targeted as a means to stimulate ingestion and deliver poisons. The USGS has expressed a strong interest in all of this work because they are developing algal attractants to capture and sample bigheaded carps, and because they wish to use these feeding chemicals to make the poisoned baits they are developing more likely to be eaten. Chemicals (vs extracts) are of special interest because they could be targeted, quantified, and save money. There has also been special interest in our determining the chemical identity of these feeding stimuli for Bigheaded carps for which the USGS has provided some matching funds. Accordingly, we now propose to focus on this particular task (identifying the actual chemicals responsible for food recognition and ingestion) using some funding remains because we finished under budget. This important but slow, methodological work, which requires about 20 h a week and will take three years. Initial work would focus on testing promising algal chemicals including known biogenic sulfides and geosmin. Secondarily, we would focus on isolating chemical components from algae found to be of special importance to these species with the help of a chemist (Dr. Hoye). This would proceed as isolation and fraction step followed by identification and testing. It would be achieved with the help of the USGS and we anticipate that this laboratory work will be supplemented by field work with the USGS and other agencies as well as a new proposal being submitted to MAISRC this winter for ENRTF2013 funding. The later work would likely proceed in two steps (only the first of which is described here) as final definitive structure identification and application of feeding metabolites would require using a modern chemistry lab in 2017-2018 after compounds have been isolated. This work would be Aaron Claus's Ph.D. dissertation.

Activity Status as of *June 30, 2015*

Olfactory ablation experiments (also discussed in the December 2014 report) have clearly demonstrated that food chemicals are discerned by the olfactory system (sense of smell) of Bigheaded carps. This has recently been confirmed by electro-olfactogram recording (EOG) which has shown that the Bigheaded carp olfactory system is sensitive to food extract and many putative food chemicals which it and plankton release (see below). A manuscript encompassing this work is found in Aaron Claus's recent defended MS thesis and is now being prepared for submission to the journal *Physiology and Behavior*. Meanwhile, an open circular tank attraction assay has shown that the same suites of chemicals that stimulate buccal-pharyngeal pumping behavior are also strongly attractive (see figure 6-1 below, n=11) to these carp species.

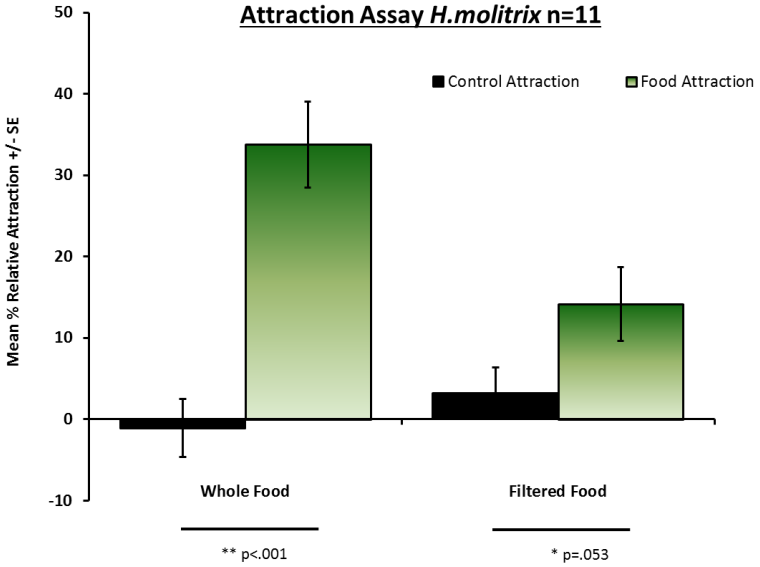


Figure 6-1 Attraction assay

Work has started to test algal metabolites. Initial olfactory recording experiments are complete and data indicates that Dimethylsulfoniopropionate (DMSP) is smelled by bighead carp at micromolar and higher concentrations while Dimethyl sulfide (DMS) is not. Geosmin and MIB were also smelled, but responses are low (see figure 6-2 below, n=6).

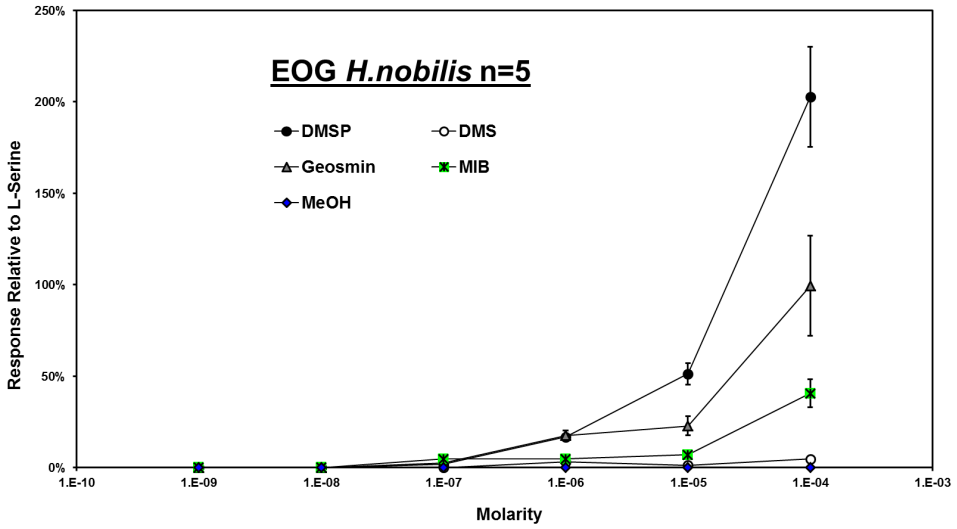


Figure 6-2 EOG responses to algal metabolites.

A buccal-pharyngeal pumping behavior assay of DMSP has also shown that this chemical is unfortunately not promising, at least on its own (see figure below) so we will start to explore additional chemicals that have been identified by others and are commercially available (Table below). Bio-assay guided fractionation would be the next option if needed.

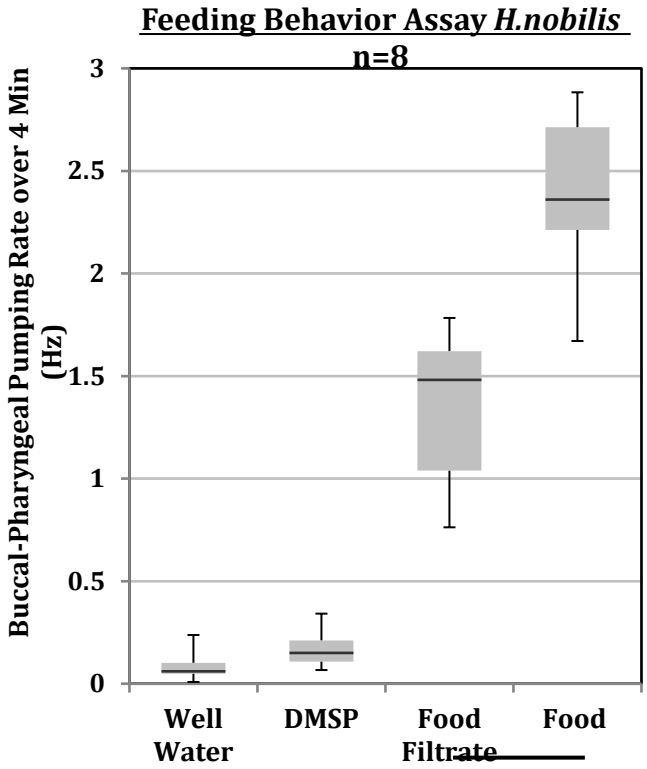


Fig. 6-3. Behavior assay

Table 6-1. Known Algal metabolites we will test.

Odorant	Class	Biological Source	MW
β-cyclocitral	Nor-carotinoid	Phytoplankton	152.23
E2,E4 decadienal	PUFA derivative	Cyanobacteria	152.23
β-Ionone	Nor-carotinoid	Phytoplankton	192.30
Linoleic acid	Polyunsaturated fatty acid	Phytoplankton	280.45
Eicosapentaenoic acid	Polyunsaturated fatty acid	Phytoplankton	302.45
Sucrose	Carbohydrate	Cyanobacteria	342.30
Trehalose	Carbohydrate	Cyanobacteria	378.30

The balance of this activity was adjusted to account for a cost share of graduate student (Aaron Claus)'s salary with a companion USGS grant. This resulted in a one-time increase in the balance.

Activity Status as of *December 31, 2015*

As planned, we continued tests of algal metabolites as olfactory feeding stimulants using electro-olfactogram (EOG) recording on bighead carp. We considered all 6 compounds described previously (Table 6-1) as well as 10 L-amino acids and two additional fatty acids salts. Several free fatty acids (β-cyclocitral, E2,E4 decadienal, eicosapentaenoic acid) were found to be insoluble in water so of no interest, at least as possible long distance olfactory stimulants. Similarly, β-lanone was not commercially available and was not tested. We were, however, able to acquire three fatty acid salts (lineoleic acid sodium salt, octanic acid sodium salt, and butyric acid sodium salt) for initial proof-of-concept studies. These have not been tested before in fish and are abundant in some algae; two were found to be highly active olfactory stimulants and thus of likely biological relevance (Figure 6-4). In

addition, we acquired and tested 10 L-amino acids, common constituents of fish feeding; these were also found to be strong olfactory stimuli although not as potent (physiologically) as fatty acid salts (Figure 6-5). Both carbohydrates (sucrose and trehalose) were soluble but were not stimulatory.

Figure 6-4. EOG responses to the three fatty acid salt at concentrations of 10^{-4} Molar.

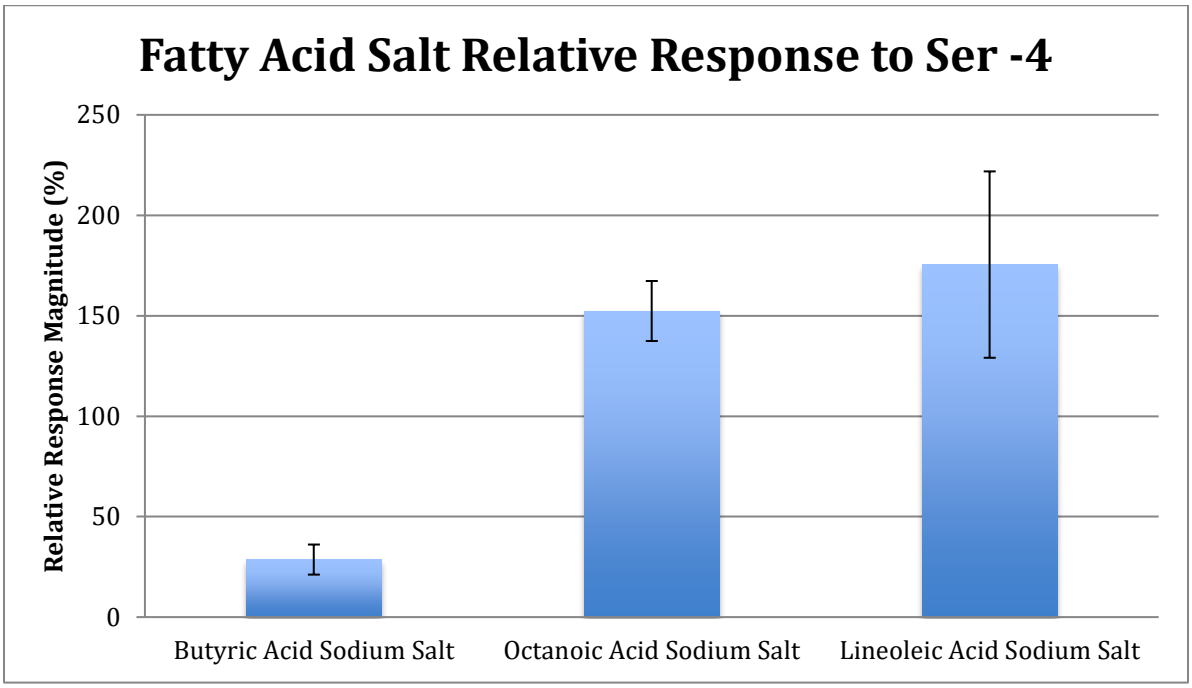
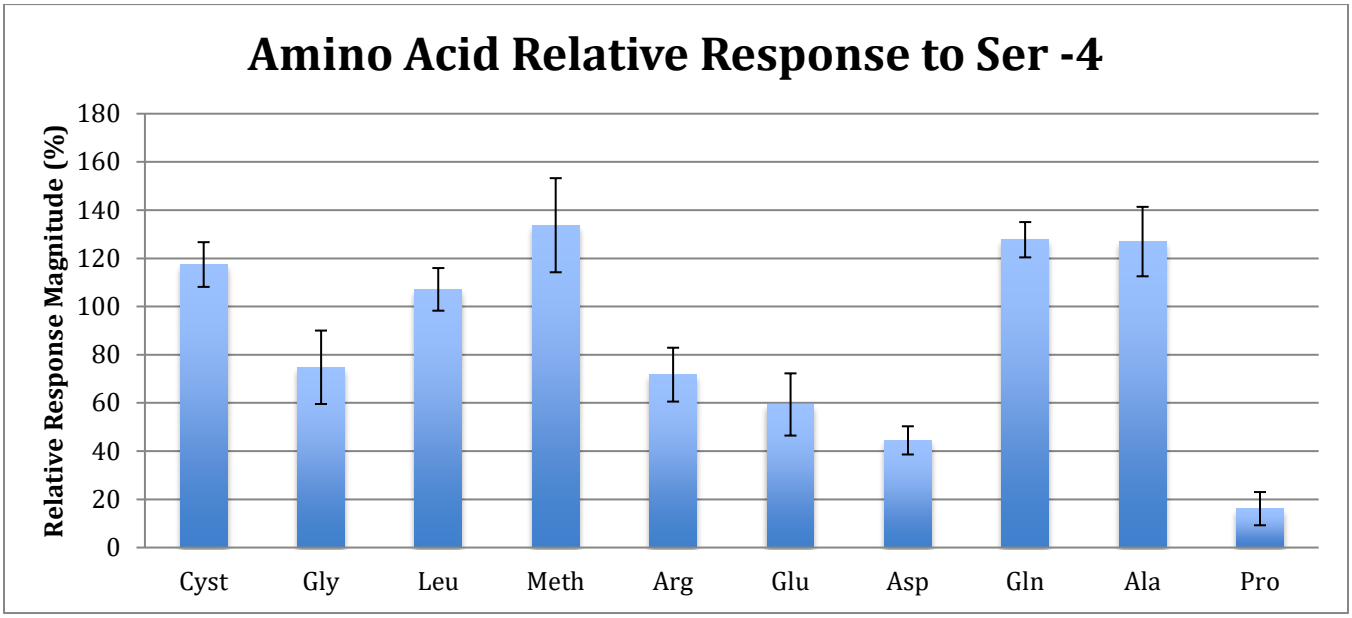


Figure 6-5. EOG responses of 10 amino acids at 10^{-4} Molar.



Mr. Claus has now completed both his MS thesis and a high quality manuscript which will soon be ready for submission to the *Journal of Chemical Ecology*. He has, however, decided not to continue for a Ph.D. Accordingly, we will continue these important studies with the help of a technician instead of a Ph.D. student. We will continue to examine algal metabolites as olfactory stimulants for bighead carp (as initially proposed) and then test the most promising ones as feeding stimulants (likely as attractants and as stimulants) that might explain the bioactivity of whole food. Fatty acid salts would be a special focus but we will also review the literature for other promising candidates. In six months we will re-evaluate plans depending on progress and may, if reasonable, re-engage a new graduate student to give the project focus and an educational component.

Activity Status as of *June 30, 2016.*

Progress identifying food stimulants is good. As discussed in the last update, we evaluated all experiments on feeding attractants and stimulants for the Asian carps. Work to date has clearly demonstrated that both bigheaded and silver carps are stimulated to perform buccal-pharyngeal pumping (BPP) in response to aqueous extracts of various components of their diet, including algae. Spirulina (*Arthrospira spp*.) is the most stimulatory of the algae tested in both carp species and it accounts for well over half the activity of the entire food mixture we have been feeding them (Figure 6-6). We have shown that most BPP activity is stimulated by olfactory cues (their sense of smell) and not their taste system (epibranchial organ). Attraction has not yet been demonstrated. A literature search has shown that the biochemistry of Spirulina is already extremely well described because of its role in the food industry. This makes it highly suitable for experiments and possible application in the field to attract wild Asian carps. Spirulina has been analyzed over half a dozen times and is known to contain proteins and free amino acids, fats and fatty acids, carbohydrates, vitamins, phycocyanin, a few minerals and several other minor compounds. Several of these are novel and have not been examined in fish behavior – so have special promise for carp. It is reasonable to now focus on these compounds (rather than trying to identify new ones) to determine whether and how they are used as olfactory food stimulants and attractants. Initial tests of the olfactory sensitivity of silver carp using electro-olfactogram recording (EOG) have clearly shown that they detect amino acids and somewhat surprisingly, fatty acids (Figure 6-7). We do not know whether these classes of compounds elicit any behavioral activity.

We propose to spend the next six months examining olfactory sensitivity and specificity to these classes of compounds to identify those that are most important, and then test their behavioral role first with BPP. Fatty acids will be of focus if they prove to be important. The first part of 2017 will then be spent examining their roles as attractants and writing a publication (perhaps for Fish Physiology and Biochemistry) and LCCMR report, and finally making recommendations to the USGS for possible application. Ms. Grace Van Susteren, a Researcher 1, will perform this work under the direction of Dr. Sorensen. We expect to complete all work June 2017.

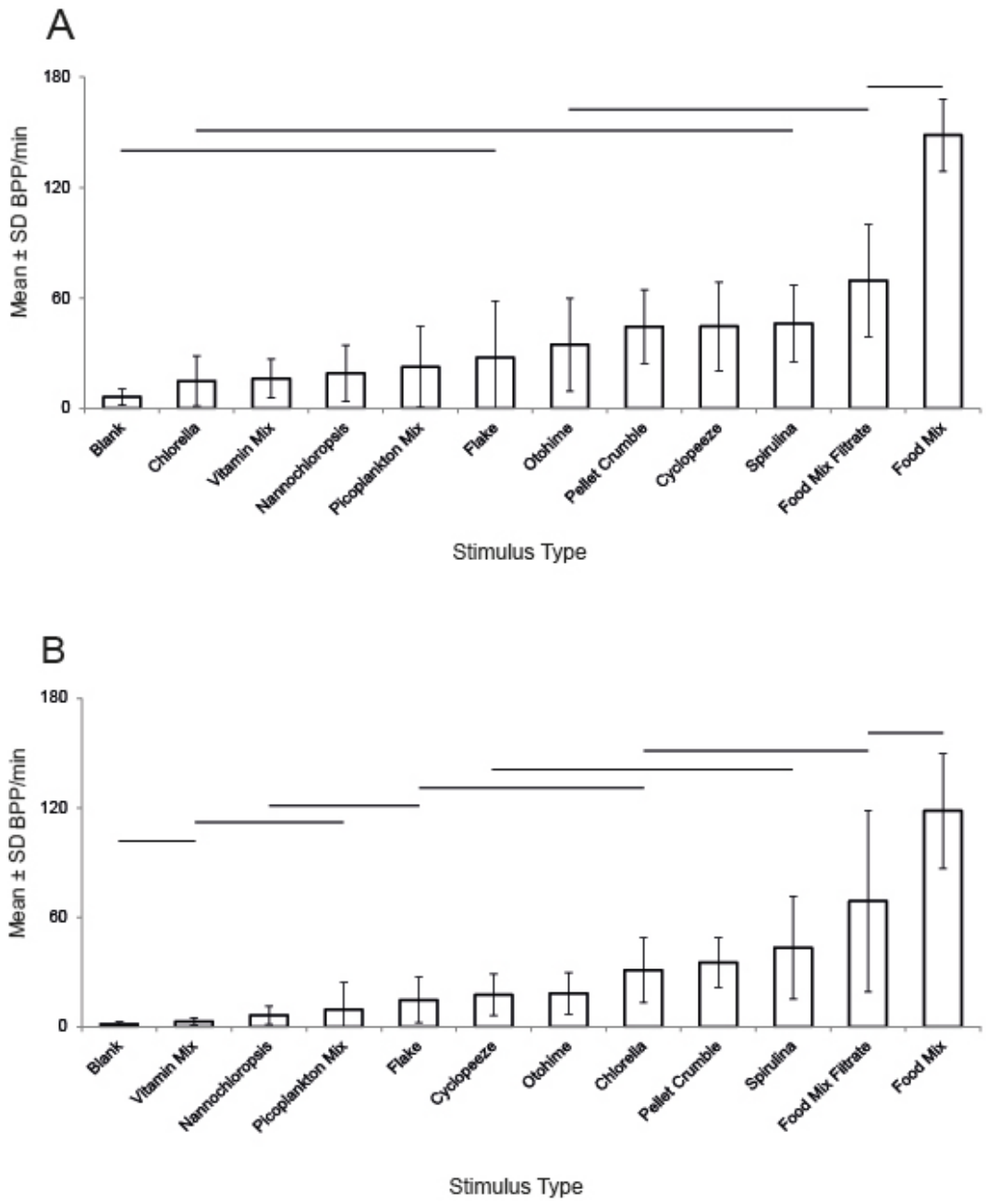


Figure 6-6. Mean BPP response to food component filtrates for silver (A) and bighead carps (B). Note that Spirulina is the most potent and has a very important role. Its chemical composition is also well characterized and it is commercially available for study and application.

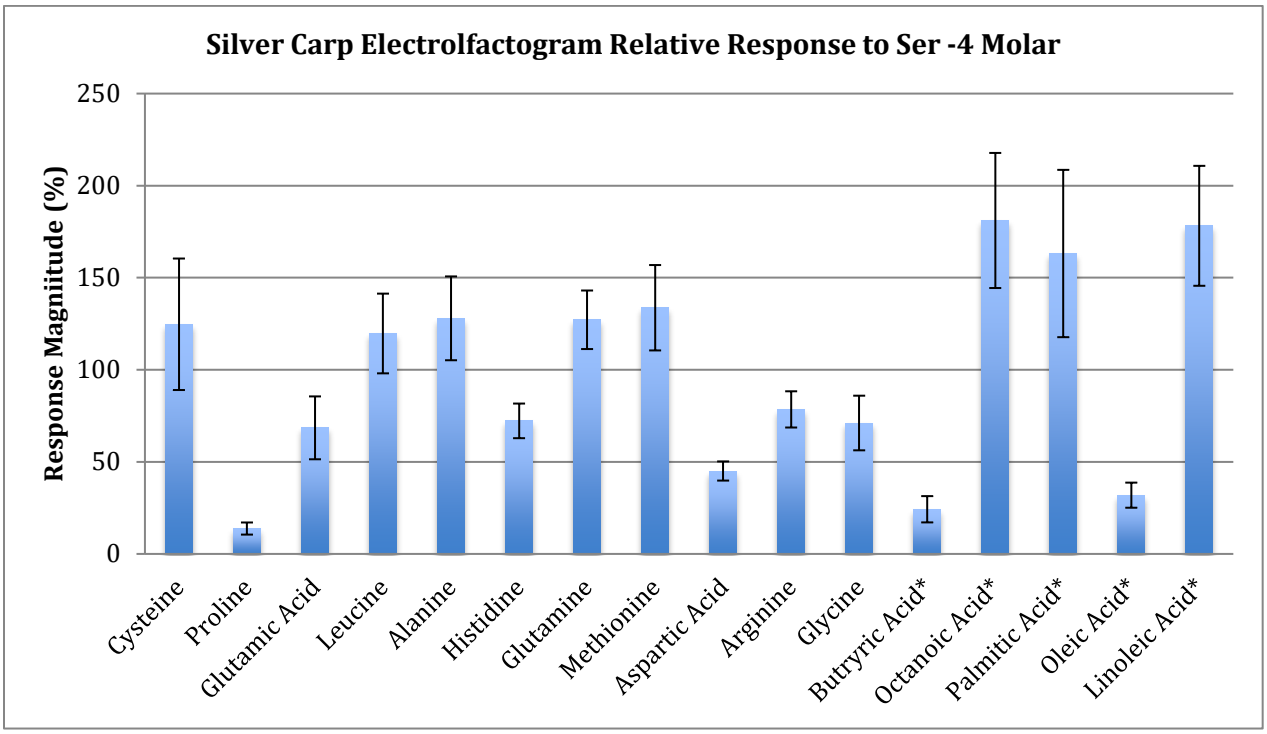


Figure 6-7. Amino acid and fatty acid salt relative response to serine standard ± standard deviation. Compounds measured at -4 molar. N=7. Standard amino acid abbreviations used. * Fatty acids are all Fatty Acid sodium salts.

Activity Status as of December 5, *2016*

Our work characterizing feeding stimulants is going well, and both olfactory and behavioral work are promising. It suggests that these fish use complex mixtures of common algal metabolites including fatty acids to identify food. This is a novel finding. New experiments are now planned to address this final complete question of mixture composition and will take 6 months to complete using the part-time help we now have (Ms. Grace Van Sustren). This work is being completed in collaboration with the USGS. Understanding the complexity and nature of food stimulants for this unique filter-feeding fish should allow us to design novel food attractants and stimulants to use their control and monitoring in rivers. Briefly, Ms. Van Sustren has now fully characterized the olfactory (EOG) activity of all commercially available and soluble metabolites of Spirulina (*Arthrospira spp.*), the most promising feeding stimulant for bigheaded carps She (we) have shown that these carps detect a wide variety of compounds in very low concentrations including amino acids, fatty acids, vitamins, and polyamines – this phase of the study is complete (Fig 6-8). Further, dose-response analyses using olfactory (EOG) recording on especially promising compounds, and found that certain fatty acids including linoleic fatty acid salt, are detected at nanomolar concentrations -- a concentration that should be biologically relevant and useful to carp control (Fig. 6-9). Other compounds whose dose-response were evaluated include various amino acids, polyamines, and vitamins (data not shown). EOG mixture experiments were also performed for fatty acids to characterize the independence of their olfactory receptor(s) and we have found that, they seem to share receptor sites (although there is still some element of independence) (Fig. 6-10). This is important because it will allow us to substitute certain common components for others Behavior work can now proceed.

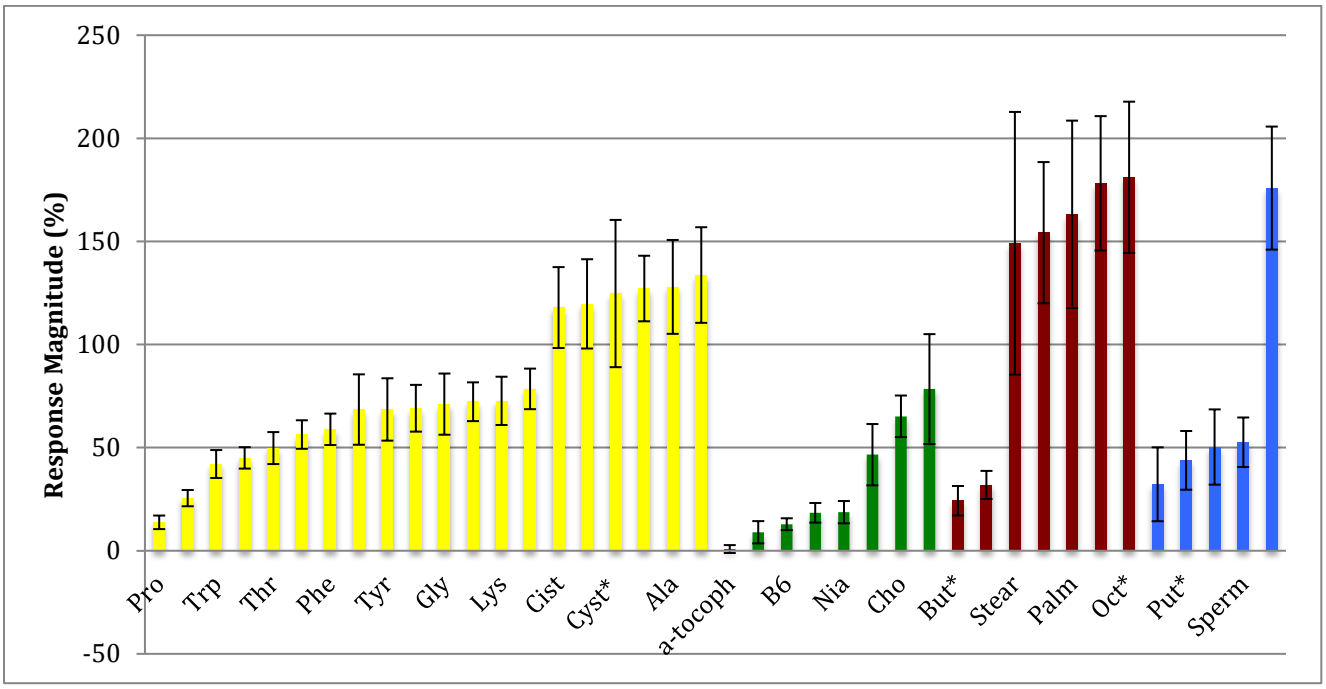


Figure 6-8. Final summary EOG figure for bighead carp. Common abbreviations are used. Amino Acid (Pro-Meth), Vitamin (a-tocph – Thi), fatty acid salt (But – Oct), and Polyamine (Norsperd – AgmSS) response to Serine -4 M standard ± standard deviation. Compounds measured at -4 Molar. n=7 for all compounds except Cys (n=6) and a-tocoph (n=4).

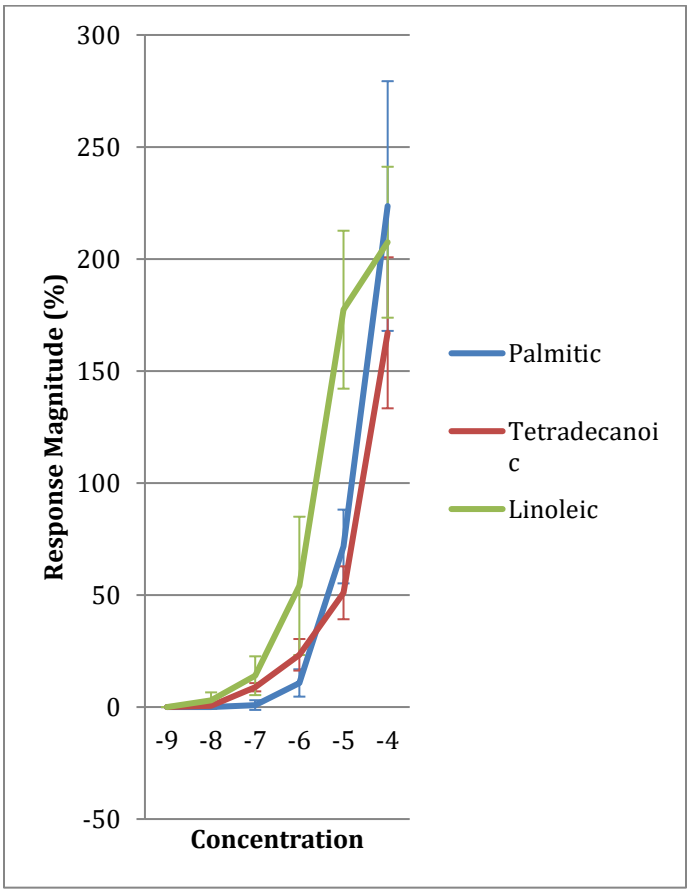


Figure 6-9. Silver Carp dose-response EOG curves to three key fatty acids. Responses shown relative to q standard of -4M Serine (n=6.

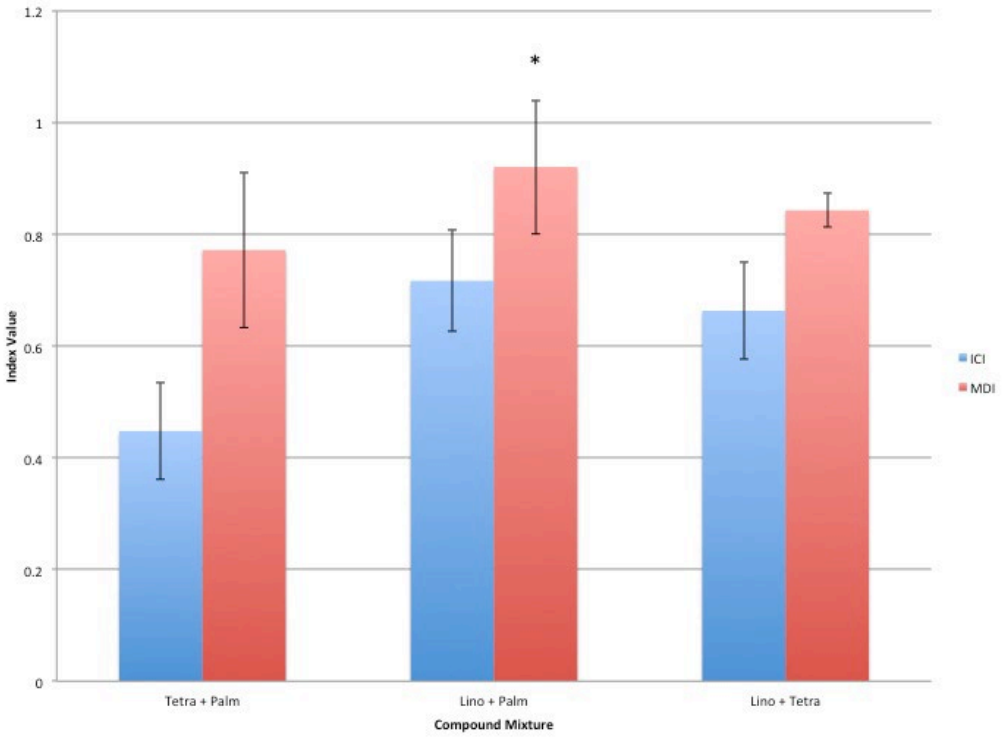


Figure 6-10. Results of an EOG mixture experiment that characterized olfactory receptor independence to key fatty acids. Mean ICI and MDI values of fatty acids ± standard deviations. A ICI score of 0.5 suggests shared receptors; a MDI score suggests completely independent receptors. The Fatty acid salts tested were Tetradecanoic, Palmitic, and Linolei (n=5). Data were compared to 0.5 and 1,0 respective.

We have also started examining the behavioral activity compounds by monitoring buccal pumping activity (BPP) as used by Claus previously. Fatty acids continue to be interesting, and show promise for Linoleic fatty acid salt (Figure 6-11). Behavioral activity is noted to this fatty acid (first time for any fish) although responses are not nearly as large as the whole algae (Spirulina) filtrate suggesting mixtures are important. This is novel for a filter feeding fish. We now propose to spend the next six months examine the behavioral role of these compounds via BPP and also using both attraction studies. We will examine both the effects of odorant concentration and then mixture using BPP. Once a uniquely stimulatory mixture is identified we will examine it ability to attract. Ms. Grace Van Susteren, a Researcher 1, will perform this work under the direction of Dr. Sorensen. We expect to complete all work June 2017 along with a comprehensive data report (that could be the basis of an eventual peer-reviewed publication) and recommendations for possible applications to the USGS.

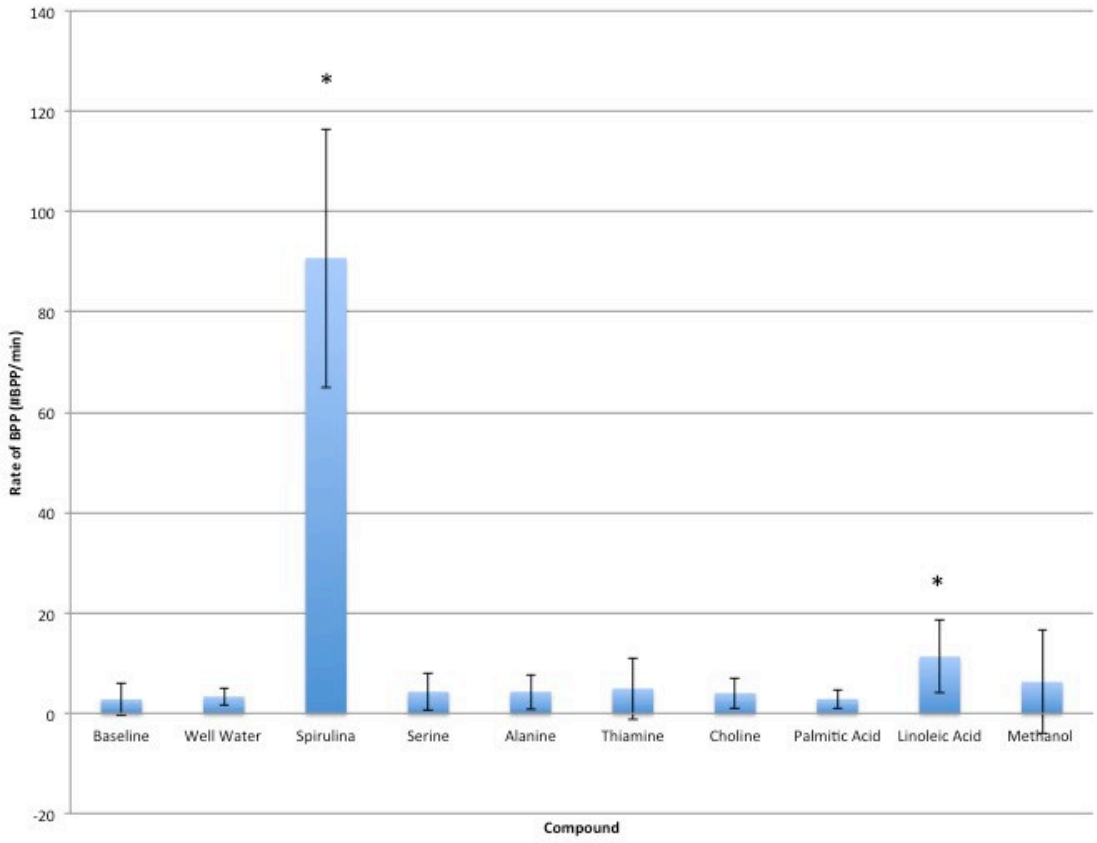


Figure 6-11. Mean behavior (BPP rate) during the first 3 minutes of silver carp to individual compounds of special interest at concentrations found in Spirulina (thus they varied) . Data were winsorized for extreme values. N=10 for all compounds except Baseline (N=71), Well water (N=8) and Methanol (N=8). Asterisks denote significant responses (vs control). Note that fatty acid is more stimulatory than amino acids, traditional food stimuli for all fish studied to date.

Final Status Report as of *June 30, 2017*

Activity #6 on feeding stimulants for invasive (Asian) is now complete. In the last 6 months we published a manuscript (Claus and Sorensen 2017) and clearly established that those chemical feeding stimulants for silver carp that stimulate buccal pumping (BPP) are complex mixtures comprised of amino acids, fatty acids, and other compounds). We confirmed that amino acids stimulate buccal pumping responses (BPP- i.e. food recognition and sampling) and synergize each other's activity when tested as a mixture but could still not account for all of the activity of spirulina (i.e. other still unknown compounds exist). Fatty acids, which we previously hypothesized might elicit large BPP responses, were found to have only moderate to low activity (Fig 6-13). Clearly, other stimulants of BPP activity await discovery and have great potential for carp control – and should be examined in future work. Meanwhile, a new assay was developed to test for attraction using a flowing rectangular glass tank. It works well. This assay has clearly shown that spirulina odor itself and the amino acids it contains are both highly attractive (Fig. 6-14). Initial work currently being analyzed also demonstrates that fatty acids are likely attractants since fish have demonstrated visibly obvious search behavior. Attraction could be used in baiting and carp removal. This work has been repeated in the field as part of ENRTF2013 and is now being prepared for publication.

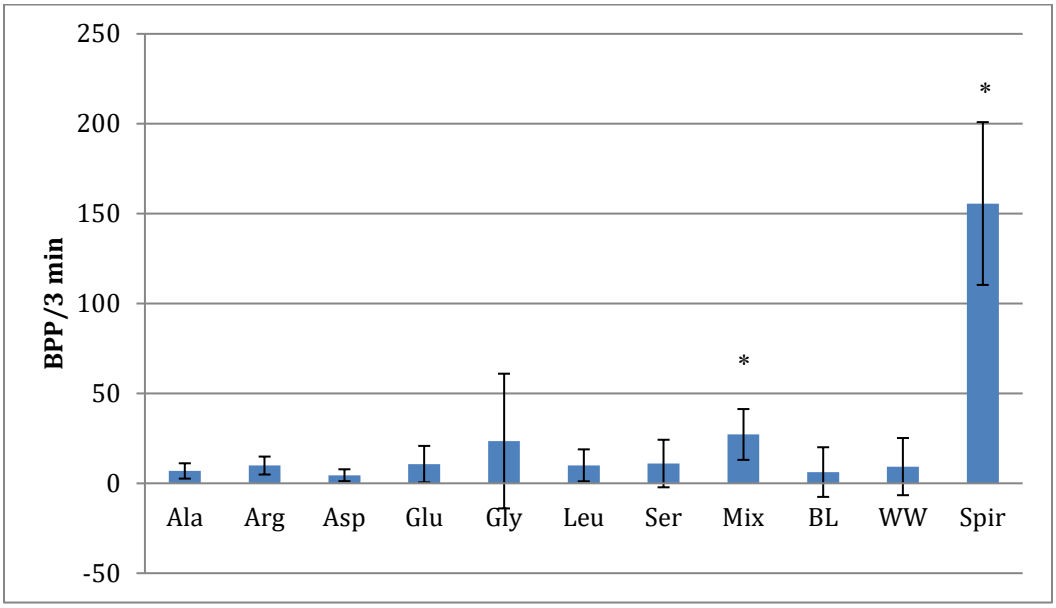


*Fig. 6-12 Average BPP responses of carp to individual amino acids or mixtures of these compounds ± standard deviation. The Y axis is total number of buccal pumps (BPP) in first three minutes of trial. All compounds tested (including mixture) were at 7*10^-4 Molar. Statistical analysis is a one way ANOVA between well water and each compound.*

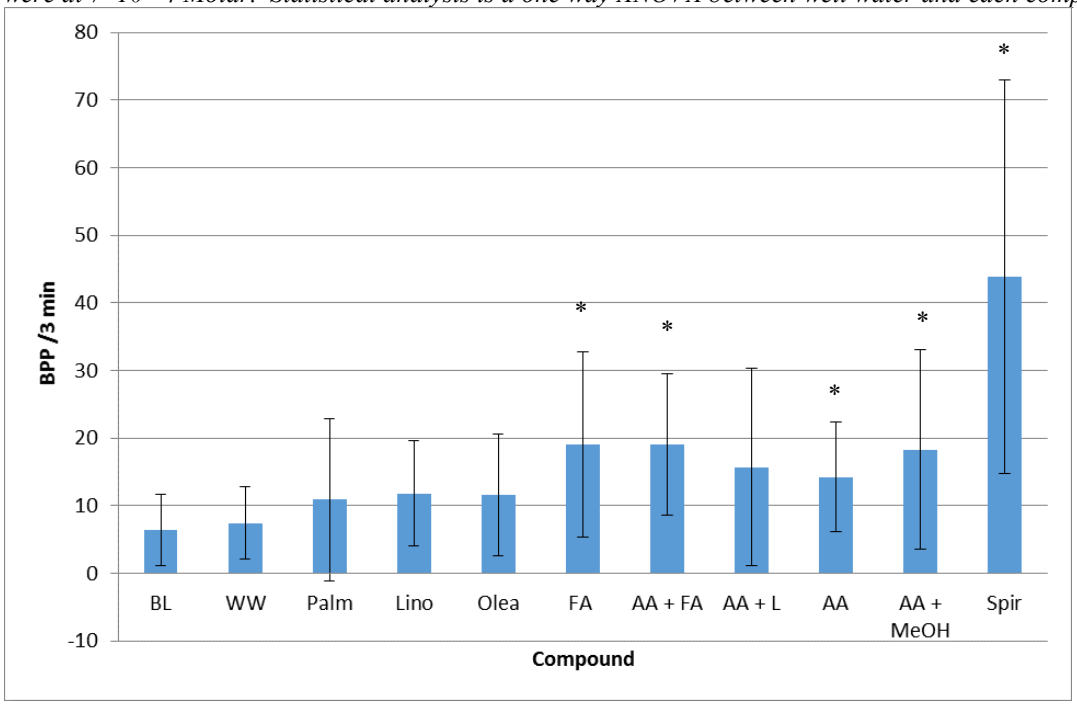


*Fig. 6-13. Average BPP response to fatty acids, a mixture of fatty acids, mixtures of amino acids + fatty acid mixtures ± standard deviation . Y axis is total number of buccal pumps in first three minutes of trial. All fatty acids tested (including mixture) at 3*10^-5 Molar (M). Amino acid mix is 7*10^-4 M. Concentrations of amino acids + fatty acids are additive and not scaled for concentration: AA+ FA and AA+Lino is at, 7.3*10^-4M. Statistical analysis is a one- way ANOVA between well water control and each compound.*

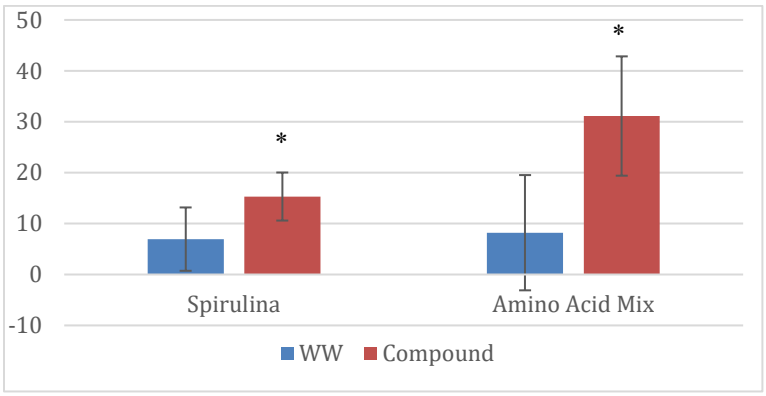


*Fig. 6-14. Attraction (%) of silver carp towards spirulina filtrate and an amino acid mix (7*10-4M) relative to well water control ± standard error in flowing lab tank. Significance for each compound vs it's own well water control tested via one-tailed T-test.*

In summary, Activity 6 has led to the discovery that chemical cues play a strong and important role attracting bighead and silver carp to their food and then stimulating them to consume it by buccal pumping behavior. This work can, and is, being used to attract bigheaded carp in the field for removal and measurement. Other work by the USGS is using it for toxin development. Spirulina is an important planktonic food for both carp species but differences in preferences between species are evident. Much of the activity of chemical feeding stimulants is detected by the olfactory system but the taste system, especially via the internal epibranchial organ, has a role too. Novel unaccounted stimulants exist. Most recently we found that these cues can also serve as attractants and that unique combinations of chemicals are involved which if identified could be extremely useful. A manuscript has been published and a second manuscript is now being prepared.

ACTIVITY 7: Screening for VHS in Minnesota waters

Description: VHS, a highly contagious (invasive) virus that kills fishes, has been found in Wisconsin and could invade Minnesota waters. To date, Nick Phelps at the University Veterinary School has been screening fish in local waters for this AIS but base funding has disappeared. We will use ENRTF funding to extend this program for two years while he looks for additional support. The DNR will collect the samples without charge.

Summary Budget Information for Activity 7:

ENRTF Budget: $24,047
Amount Spent: $24,047
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Test and report on VHS in local water in 2012	2013	$11,298
2. Test and report on VHS in local waters in 2013 and 2014	2014	$12,066

Activity Status as of *June 30, 2013*

Fish from 14 water bodies have been tested for VHS and no evidence of VHS has been found. Fish from another 8 water bodies will tested prior to June 30, 2013. All results have been posted on the Minnesota Veterinary Diagnostic Laboratory's fish health website (http://www.vdl.umn.edu/ourservices/vhs/) and updated on the MDNR's Lake Finder website (http://www.dnr.state.mn.us/lakefind/index.html). To date, Minnesota remains free of VHS; however, the threats of introduction remain a concern for the State's fish health managers.

Activity Status as of *April 1 2014*

In year one of the project, 1,982 fish from 19 lakes and rivers were tested for VHSV. During the first half of year two, 1,358 fish from 14 lakes and rivers have been tested for VHSV. All results have been negative and posted on the Minnesota Veterinary Diagnostic Laboratory's fish health website (http://www.vdl.umn.edu/ourservices/vhs/) and updated on the MDNR's Lake Finder website (http://www.dnr.state.mn.us/lakefind/index.html). While Minnesota remains free of VHS, the threats of introduction remain a concern for the State's fish health managers. In the spring of 2014, about 15 additional bodies of water will be tested.

Activity Status as of *June 30, 2014*

In the spring of 2014, 1,212 fish from 15 bodies of water were tested for VHSV. Testing for six locations is complete, with all results negative and posted on the Minnesota Veterinary Diagnostic Laboratory's fish health website (http://www.vdl.umn.edu/ourservices/vhs/) and updated on the MDNR's Lake Finder website (http://www.dnr.state.mn.us/lakefind/index.html). Testing from the remaining nine locations is still in progress as of June 30 and will be updated prior to the final report on July 31, 2014. While Minnesota remains free of VHS, the threats of introduction remain a concern for the State's fish health managers.

Final Report Summary *August 1, 2014*

Viral hemorrhagic septicemia virus (VHSV) is a highly invasive and pathogenic virus of more than 30 fish species in the Great Lakes region. While the virus has yet to invade Minnesota, it has been detected in all Great Lakes and inland waters of Michigan, New York, and Wisconsin. Early detection of this devastating disease in Minnesota is critical for rapid intervention and management. To that end, this project used highly sensitive diagnostic tools to survey lakes and rivers in Minnesota for VHSV. The locations and species included in the survey were selected in partnership with the MN DNR based on introduction risk, susceptibility, popularity, and geographic distribution. From July 1, 2012 to June 30, 2014 a total of 4,552 fish from 36 bodies of water, eight of which were sampled each year, were negative for the virus. While Minnesota remains free of VHSV, the threats of introduction remain a concern for fish health managers. This study has informed future surveillance strategies, risk assessment, and improved confidence in current management approaches.

Dissemination: Routine updates of the VHSV survey results were made publicly available on the MN Veterinary Diagnostic Laboratory website and integrated into the MN DNR Lake Finder website. This project has been used to inform MN DNR detection and prevention strategies for VHSV.

ACTIVITY 8: Ascertaining whether an enhanced bubble curtain could deter Asian carp movement into small tributaries in a practical manner; immediate installation of sound deterrents in the Mississippi River

Description: With funding from ENRTF in 2009, the University of Minnesota previously developed an enhanced new bubble curtain design that reduces up- and down-stream movement of the invasive common carp by 70-80% (result under peer-review). The primary advantage of this new technology is that it is very practical and inexpensive: a simple industrial blower connected to PVC pipes with holes drilled in a specific manner (that costs less than $2000) can stop about 75% of all common carp. This technology also has the potential to be taxon-specific because it is based on sound and hydrodynamic fields generated by the bubbles and additionally will work safely and efficiently in shallow waters. Because the silver and bighead carp (Asian carps) are just as (if not more) sensitive to sound as the common carp, this technology could have great potential for stopping these new, highly invasive species in the hundreds of small tributaries to Minnesota's large rivers that are too expensive and

difficult to protect otherwise (i.e. using electrical or mechanical barriers) . This 1-year study will test the possibility that bubble curtains could be deployed to meet this specific need.

In continuation of MAISRC efforts to use sound deterrents to control movement of bighead and silver carp in Minnesota's rivers, and response to the recent report of late-stage Bigheaded carp embryos being found in Mississippi River Pool 9, the MAISRC proposes to immediately purchase and install underwater transducers at Lock & Dam #8. The aim is to complete this work before the spawning season and before July 1, when a 2014 ENRTF appropriation is anticipated to become available to continue this work and conduct associated research.

Summary Budget Information for Activity #8:

ENRTF Budget: $128,891
Amount Spent: $128,891
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Evaluate the effectiveness of an enhanced bubble curtain system to impede silver carp movement	2013	$
2. Evaluate the effectiveness of an enhanced bubble curtain system to impede bighead carp movement	June 30, 2014	$59,192
3. Purchase and install underwater transducers at Lock & Dam #8 to impede Bigheaded carp movement	July 31, 2014	$69,700

Activity Status as of *April 1, 2014*

Work is well underway. To facilitate progress, we tested the effects of sound alone, the primary sensory cue produced by bubble curtains, on common carp and bigheaded using speakers as they are much easier to run than bubble curtains. These experiments demonstrated that carp are strongly repelled by sound in a directional manner associated with local acoustic particle motion. Based on these results we have built two experimental setups to test how bubble curtains might now be used at low cost to deflect bigheaded carp movement in controlled manners. We have also PIT tagged both silver and bighead carp as a PIT tag antenna system is used to monitor movement in the experimental setup. Initial experiments with silver carp are highly promising and will complete within several weeks. Tests with bighead carp completed by July.

Activity Status as of *June 30, 2014*

Initial proof-of-concept experiments to determine the ability of relatively simple, enhanced bubble curtains to divert both Bigheaded carp species (*Hypophthalmichthys* spp.) from entering small tributaries using optimized bubble curtain (curtains with optimized aperture sizes and orientation) are complete and show promise. Tests were conducted using a split passage experimental channel in large tanks that employed an enhanced bubble curtain as a deflection screen (a more favorable configuration than as a cross-stream barrier). These bubble curtains produced broad spectrum sounds (100Hz-3000Hz) with a peak sound pressure level of 143dB (ref 1 μPa) at 300Hz, which closely corresponds to the most sensitive hearing region of both species of Bigheaded carps. During control periods (no bubbles), Silver carp (total length=23.6±3.5cm, mass=120.0±41.5g [mean ± SD]) preferred to swim in the downstream direction averaging 100 and 25 passages through the outside and inside channels, respectively. However, once the bubble curtain was activated, passage of Silver carp was reduced by 86% through the outside (blocked) channel. Similarly, Bighead carp (total length=28.0±4.4cm, mass=215.0±102.5g) preferred to swim downstream during controls, averaging 110 and 20 passages

through the outside and inside channels. Once the bubble curtain was activated, it reduced passage of Bighead carp by 89%. Because of the relatively low cost to build and install this structures and the lack of alternatives, such results suggest bubble curtains can be an effective control mechanism for diverting all species of invasive carps form entering low-head tributaries, especially when reduction, not total elimination (which generally seems reasonable), of movement is the goal. We believe this technology may also have uses in the main river and should be publishable. Specifically, we would like to test it in the lock systems.

We also finished analyzing the data from earlier experiments using underwater speakers to test how common and Bigheaded carps navigate through (and avoid) an aversive sound stimuli, and if responses are directional. In these experiments we coupled measurements of the local sound field (particle motion and sound pressure) with videos of carp swimming in a square enclosure while subjected to complex, broad frequency sound signals (derived from underwater boat motor recordings). These experiments showed carp actively avoid the sound source in a highly directional manner associated with local acoustic particle motion, which has not been previously shown in the literature. We believe these data are also publishable.

We continue to work with the US Army Corps of Engineers (USACE) to install an acoustic transducer array in the lock chamber at Lock and Dam #8 near Genoa, WI. Although installation has been delayed by several administrative complications (the need for MOUs with the University to both acquire lock design plans, and then use federal land for experiments, flooding), barring renewed flooding, installation is expected by mid-July (i.e. only small delay). Because the river is still in flood and is still cold, it is highly likely that the benefits of this activity will be realized this year. The USACE has granted us permission to use their design plans and completed an environmental review of the installation design, and is presently in the process of completing a real estate agreement. Quotes have also been received from both a commercial diving contractor (J.F. Brennen, La Crosse, WI - $9,800) and electrician (Poellinger Electric, La Crosse, WI - $4,600), and we only await final approval from the USACE (the real estate agreement) before actual installation can commence. All necessary equipment including transducers, amplifiers, signal splitter, microcontroller, data logger, storage shelter, and attachment supplies have been purchased. Notably, these expenditures were made after 5/30/2014, so the cost of these expenditures ($65,000) is not reported in this specific report. The total cost of equipment and contractor services is also likely to be approximately $10,000 lower than originally expected, while we now need to continue working in July to finish installation and associated experiment. A re-budget is requested to address these issues. Given this dynamic situation, we also ask that Activity 8 be extended through the summer to December 31 2014 when we will write a final report and reconcile accounting to close the activity as part of the regular update.

Activity Status as of *December 31, 2014*

On July 28, 2014 we installed an array of underwater transducers to the downstream face of the downstream lock chamber miter gates at Lock and Dam #8 (Genoa, WI). Commercial divers from J.F. Brennan Company mounted the transducers to the gates and secured cables underwater, while Poellinger Electric provided electrical service to the site. University personnel installed an amplifier, signal transformer system, and storage shed. Custom microcontrollers were designed and built to operate and monitor the transducers. A magnetic switch attached to the gates triggers the transducers to play sound only when the downstream gates are in use, and remain on until closed. Playback signals are derived from boat motor sounds and filtered to contain frequencies outside of the hearing range of most native fish, yet correspond to the most sensitive hearing range for Silver and Bighead carp (500-1500 Hz). Sound mapping downstream of the lock chamber indicated the system projects a sound field with a peak sound pressure level of 177 dB with a spectral sound pressure level of 155 dB at 600Hz. The signal is 60-70 dB above background and extends up to 100 m from the lock chamber, and thus easily detected by carps. Although we cannot determine the actual effectiveness of the system to deflect carp passage because we lack a monitoring system (something we strive to address with new Outdoor Heritage funding through DNR), the deterrent system is being operated using the

best available knowledge and future improvements will be made based on field scale experiments in the auxiliary lock chamber at Lock and Dam #1 (ENTRF 2014). Recently, we turned the system off when the locks froze and were winterized. The system will be reactivated next spring. WI DNR and local residents have been highly supportive. No significant increase in adult carp abundance or reproduction has been noted above this system although sampling effort has been limited. The system will continue operation until Dec 2016 (following the guidelines of the MOU which now exists between the University and USACE), at which time its performance will be reviewed and recommendations for future installation/operation will be made.

Final Report Summary *December 31, 2014*

This activity installed the first sonic deterrent system in a lock system and clearly demonstrated that enhanced bubble curtains and sound alone can function as behavioral deterrents with potential to selectively control the movement of fish with high sensitivity to sound including the invasive carps. Due to their low cost, ease of installation, safety, and taxon-specific effects, we believe bubble curtains hold great promise for protecting the many low head tributaries connecting with the Mississippi River. In this particular study we investigated the effectiveness of a bubble curtain as a deflection screen which directed carp away from one channel into another and found this approach (vs. blocking) to be especially promising. Using a split passage experimental channel we determined that common carp, silver carp, and bighead carp passage could all be diverted away from a specific channel in the laboratory with a success rate of 82-90%. This rate was approximately 10-15% higher than we noted earlier with a design that simply blocked. It also used $1/10^{th}$ of the air flow rate. A description of these promising results is presently being prepared for publication. In addition to demonstrating the diversion functions more efficiently than blocking, we also demonstrated in a different experimental design (that was not formally one of our outcomes/objectives) for the first time in either a freshwater or invasive fish that carps detect and respond to sound in directional manners and thus sound could be used in directional and predictable manner to divert. It is very possible that sound alone produced by speakers could be highly effective in the natural world, especially if sound is engineered correctly. This is important because air curtain use is limited to shallower waters because of possible deflection by water currents, need to produce highly pressurized air, and limited sound pressures generated. The results of this work are also being prepared for publication. Part of our ENTRF 2014 grant continues this line of research by evaluating the response, or lack thereof, of native, non-hearing specialist species, lake sturgeon (*Acipenser fulvescens*) and brown trout (*Salmo trutta*), to an acoustic deterrent to quantify the species specific differences. Our findings from the bubble curtain and directionality response studies have been presented at the 2014 International Conference on Engineering & Ecohydrology for Fish Passage in Madison, WI and the 2014 Upper Midwest Invasive Species Conference in Duluth, MN. Applications of these technologies should be explored and hopefully soon will be.

ACTIVITY 9: Develop Solutions to Address Weaknesses in Lock and Dam #4 and then Optimize its Gate Operation

Description: This activity seeks to identify potential weaknesses (scenarios by which carp might swim through the lock and dams) in Lock and Dam #4 and then optimize gate operation to block Bigheaded carp. Lock and Dam #4 is of special interest because it maintains higher velocities than other dams, is ideally situated far from the invasion front, and is located just upstream of Lock and Dam #5 so the two systems can be used together. This concept was proposed to the LCCMR in spring of 2016 who judged it to have merit and asked that the project proceed by rebudgeting ENRTF2012 and 2013 to accommodate this need rather than create entire new project with new funds. This work will proceed in several steps: 1) development of a 3-dimensional statistical model (computational fluid dynamics [CFD] model) to calculate velocities in and around Lock and dam #4 under a variety of operational conditions and river discharges; 2) acquisition of field measurements of velocities near the dam and use them to validate the CFD model; 3) development and then implementation of a new computational tool to

search through 3-D velocity fields to identify specific weaknesses (i.e. swimming pathways) for Bigheaded carps and 4) pairing this information with swimming performance data to determine how best to block carp passage without causing undue scour ('optimization') and having minimal effects on native fishes (Sturgeon and Trout). Results will be used in collaborative work with the USACE to develop new gate operation plans that optimally block Bigheaded carps throughout the Mississippi River while minimizing scour and which we fully expect the USACE will consider and then deploy.

Summary Budget Information for Activity 9:

ENRTF Budget: $93,832
Amount Spent: $93,832
Balance: $0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Develop and validate CFD model of Lock and Dam #4	December, 2017	$40,000
2.Identify weakness at Lock and Dam #4 and develop solutions to optimize gate operation based on Bigheaded carps swimming ability (report)	June, 2018	$53,832

Activity Status as of 6/15/2017:
Work has not yet started (as planned).

Activity Status as of 12/31/2017:

CFD modeling of Lock and Dam #4 is now complete. We used the same modelling scheme used for Lock and Dam #8 but combined two steps to allow faster and earlier calculations of fish passage using Fortran code. Nine flow conditions have now been modeled (examples shown in Figs 9-1 and 9-2). Initial runs demonstrated slightly higher fish passage through open gates than expected, so final adjustments to the model are now being made using data from Lock and Dam #8 to cross-check. Validation will be completed this January and then presented to the USACE. We are on schedule to have recommendations for optimized operating conditions by this June.

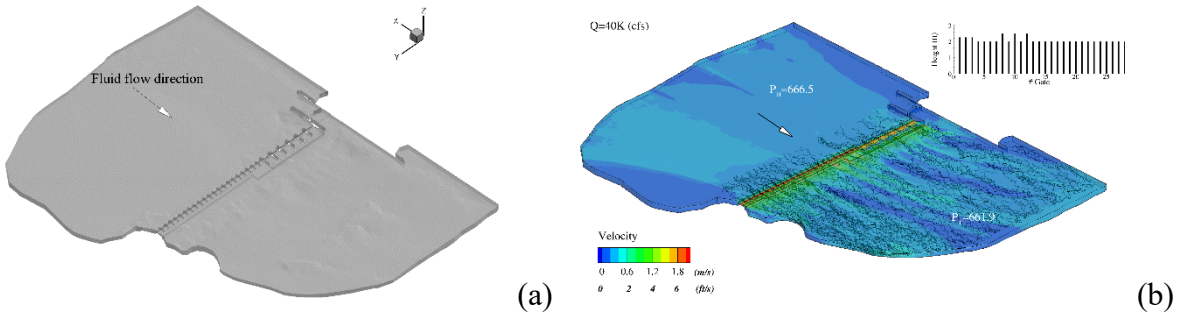


Fig.9-1 *(a) Solid structure for LD#4 and (b) simulations of fluid flow and fish passage through the L&D#4 at Q=40,000 cubic ft/sec. The graphic in the right hand corner describes gate operating conditions regulation and the height of roller and tainter gates*

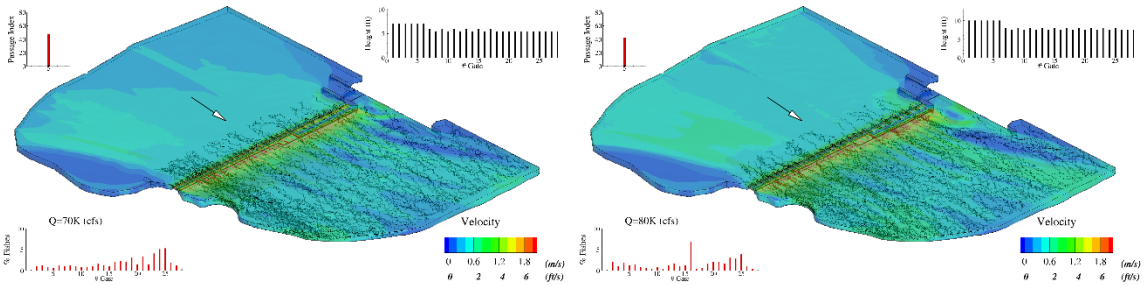


Fig.9-2 *Contours of velocity flows in the computational region around L&D#4 for current gate configurations for* $Q = 70K\ (left)$ *and* $80K\ (right)\ cfs$. *Gate operations are shown in the top right.*

Final Report Summary of 6/30/2018:

Modeling of carp passage at Lock and Dam #4 was completed and has identified solutions to reduce Silver and Bighead Carp passage by about 50% at this structure that do not either impact scour, impact navigation or safety. This set of solutions was shared these with the U.S. Army Corps of Engineers (USACE) which now summarize. Briefly, after validating our earlier computational fluid modeling (CFD), we performed agent-based modeling for five flow regimes (*Q=20,40,60,80,90 cfs*) at Lock and Dam #4 and then developed possible solutions to reduce carp passage by adjusting spillway gate heights in manners that fit USACE preferences as communicated to us in a winter meeting. This included the option of leaving blocks of spillway gates closed at times of low flow. Gates were adjusted in 0.5 ft intervals and we focused ion gates nearest the control structure to make changes easier. In our simulations we considered five sizes of carp: $S_i = 0.6, 0.7, 0.8, 0.9, \text{and } 1.0\ (m)$ based on the silver carp population from the Wabash River (the only published dataset of carp size). Except at times of open river (very rare at this location), we consistently able to reduce passage of both Silver and Bighead carp at least 50% (Figure 9-3; Table 9-1) at all flow conditions. Similar drops were seen in passage for the native Lake sturgeon. A complete report with tables of suggested, new operating conditions and their consequences was shared with the USACE.

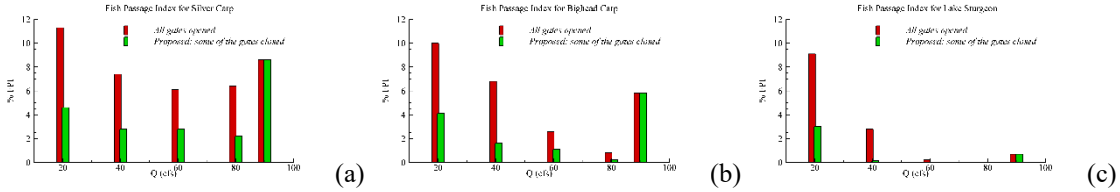


Fig. 9-3. *Fish Passage Index for (a) Silver Carp, (b) Bighead Carp, and (c) Lake Sturgeon at different flow rates, Q. Red = gates at present operating conditions and green = some of the gates closed (modified conditions).*

Table 9-1. *Population passage estimates at Lock and Dam #4 for Silver (SC), Bighead (BC) Carp and Lake Sturgeon (LS) under existing (all gates open) and modified gate operations at different flows.*

Q(cfs)	All gates opened			Some of the gates closed		
	SC	BC	LS	SC	BC	LS
20	11.3	10	9.1	4.6	4.1	3
40	7.4	6.8	2.8	2.8	1.6	0.15
60	6.1	2.6	0.2	2.8	1.1	0.03
80	6.4	0.8	0.02	2.2	0.25	0
90	8.6	5.8	0.7	8.6	5.8	0.7

Final Report Summary as of 6/30/2018:
In conclusion, our computational model provided recommendations for new gate operating protocols for Lock and Dam #4 that could block at least an additional 50% of all invasive carps passing through this key structure in ways acceptable to the USACE. Further, because these changes could be paired with similar changes in Lock and Dam 5 and #8 (ENRTF 2014), an overall 90% reduction is possible. Because the number of carp passing through these structures is already likely very low, this would be very significant. The USACE has adopted our recommended changes for Lock and Dam #8 and we are hopeful they will give similar consideration to recommendations for Locks and Dams #4 and #5.

V. DISSEMINATION:

Description: Findings will be disseminated by annual public workshops organized by the newly forming center, eDNA reports to the DNR, the Center's web site, collaborative meetings with our advisory boards, and DNR, and via peer-reviewed publication and student theses.

Status as of *June 30, 2013:*
The eDNA report (Activity #3) was provided to DNR, disseminated to stakeholders, and was posted to the MAISRC and the USGS websites. The citation is as follows:

Amberg, J,J., McCalla, S.G., Miller, L,Sorensen., P., Gaikowski, M. 2013. Detection of environmental DNA of bigheaded carps in samples collected from selected locations in the St. Croix River and in the Mississippi River. U.S. Geological Survey Open-File Report 2013-1080, 52p.

The DNR and the MAISRC collaborated on a press advisory, release, and event. Initial findings of eDNA marker research (Activity #4) were disseminated at the Minnesota Chapter of the American Fisheries Society meeting in St. Cloud in 2013. All results for VHS testing (Activity #7) have been posted on the Minnesota Veterinary Diagnostic Laboratory's fish health website (http://www.vdl.umn.edu/ourservices/vhs/) and updated on the MDNR's Lake Finder website (http://www.dnr.state.mn.us/lakefind/index.html).

Activity Status as of *April 1, 2014*

Several manuscripts are planned or are underway, specifically for Activities 4, 5, and 6. Researchers also presented at the 2014 Minnesota American Fisheries Society meetings and Dr. Sorensen has participated in several presentations and conferences, including the March 2014 Minnesota AIS Summit. Future presentations include the State of Waters Conference in May and the Upper Midwest Invasive Species Conference in October.

Activity Status as of *June 30, 2014*

Dr. Sorensen, Dr. McCartney, and Dr. Newman gave an invited talk on the MN AIS Research Center to the State of Water conference in Brainerd, MN in March 2014.

Dr. Dan Zielinski and Peter Sorensen gave invited talks on carp barrier technologies (Activity #8) to the International Fish Passage Conference in Madison Wisconsin in May 2014

Dr. Zielinkski and Dr. Sorensen's ENRTF- funded work on bubble curtains has been published as Zielinski, D.A., V.R. Voller, J. Svendsen, M. Honzo, A. F. Mensinger and P.W. Sorensen. 2014. Laboratory experiments demonstrate that bubble curtains can effectively inhibit movement of common carp. Environmental Engineering. 67: 97-103. http://dx.doi.org/10.1016/j.ecoleng.2014.03.003

A talk by Dr. Phelps, Galatowitsch, and McCartney to representatives from counties around the state is planned for August, as are talks at the Upper Midwest Invasive Species Conference in October.

Activity Status as of *December 31, 2014*

Dr. Sorensen gave invited talks on invasive carp at: The Asian Carp Forum (St. Paul;), MAISRC Showcase (St. Paul), Upper Midwest Invasive Species Conference (Duluth), The Water Resources Conference (St. Paul) and the International Society for Chemical Ecology. He also gave a webinar on Judas fish.

Dr. Eichmiller gave an invited talk at the American Fisheries conference on eDNA and another at the Upper Midwest Invasive Species Conference (Duluth), and the MAISRC Showcase. She also gave a webinar on eDNA.

Dr. Ghosal gave an invited talk at the Upper Midwest Invasive Species Conference (Duluth), The International Society of Chemical Ecology, and the MAISRC Showcase.

Dr. Zielinski gave an invited talk at the Upper Midwest Invasive Species Conference (Duluth) and the MAISRC Showcase. He also gave a presentation to the local Engineers certification group.

Mr. Claus gave an invited talk at the Upper Midwest Invasive Species Conference (Duluth) and the MAISRC Showcase.

Dr. Phelps, Dr. Galatowitsch, and Dr. McCartney gave a webinar to the Association of Minnesota Counties and all gave invited talks at the MAISRC Showcase.

We published the following two peer-reviewed articles:

Eichmiller, J. J., Bajer, P.G., & Sorensen, P.W.. (2014) The Relationship between the Distribution of Common Carp and Their Environmental DNA in a Small Lake." *PloS one* 9.11 (2014): e112611.

Hansen, A., Ghosal, R., Caprio, J., Claus, A. W., & Sorensen, P. W. (2014). Anatomical and physiological studies of bigheaded carps demonstrate that the epibranchial organ functions as a pharyngeal taste organ. *The Journal of Experimental Biology*, *217*(21), 3945-3954.

Additional communications:

Two new e-newsletters have been published, providing stakeholders the opportunity to learn more about the Center's work.

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. 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, 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 in Winter 2015.

Activity Status as of *June 30, 20*15

We published one article in a peer-reviewed journal (Eichmiller, J. Miller, L. and P.W. Sorensen. Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. *Molecular Ecology Resources*. doi: 10.1111/1755-0998.12421). Four presentations were delivered to the MN American Fisheries meetings in Brainerd, one of which won best student award. A master thesis was awarded to Aaron Claus for his work on Activity 6. He continues as a Ph.D. student.

Activity Status as of *December 31, 20*15

Three presentations were delivered at MAISRC showcase.

Activity Status as of *June 30, 2016*

Dr. Sorensen attended the meeting of the Mississippi River Aquatic Nuisance Species Task Force and presented many of these findings. A presentation was made at the MN Chapter of the American Fisheries Society.

Three articles were published:

Eichmiller, Jessica J., Sendréa E. Best, and Peter W. Sorensen. (2016) Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Environmental Science & Technology* 50: 1859-1867.

Ghosal, Ratna, and Peter W. Sorensen. (2016) Male-typical courtship, spawning behavior, and olfactory sensitivity are induced to different extents by androgens in the goldfish suggesting they are controlled by different neuroendocrine mechanisms. *General and Comparative Endocrinology* 232: 160-173.

Ghosal, Ratna, Peter X. Xiong, and Peter W. Sorensen. (2016) Invasive Bighead and Silver Carps form Different sized shoals that readily intermix. *PloS one* 11.6 (2016): e0157174.

Activity Status as of *December 5, 2016*

Dr. Sorensen attended the meeting of the Mississippi River Aquatic Nuisance Species Task Force and presented many of these findings. A presentation was made to MAISRC Showcase. Two peer-reviewed article was published:

Eichmiller, J. J., Hamilton, M. J., Staley, C., Sadowsky, M. J., & Sorensen, P. W. (2016). Environment shapes the fecal microbiome of invasive carp species. *Microbiome*, *4*(1), 44.

Sorensen, P.W. and N.S. Johnson 2016. Theory and application of semiochemicals in invasive fish control. Journal of Chemical Ecology 42: 692-715.

Activity Status as of *June 30, 2017*

An article was published.
Claus, A. W. and Sorensen, P. W. (2017). Chemical cues which include amino acids mediate species-specific feeding behavior in invasive filter-feeding Bigheaded Carps. *Journal of chemical ecology*, *43*(4), 374-38

Activity Status as of *December 27, 2017*

An article was submitted to a peer-reviewed journal to be considered for publication. We made two presentations to the USACE and one to the MN DNR on gate operations.

Activity Status as of *August 15, 2018*

Two presentations were given at the Minnesota chpater of the American Fisheries Society and nn article was published: Zielinski, D., Voller, V.R. and P.W. Sorensen. 2018 A physiologically inspired agent-based approach to model upstream passage of invasive fish at a lock-and-dam. Ecological Modeling 382: 18-21.

Final Report Summary: *Aug 15, 2018*

In sum, this project resulted in at least 11 peer-reviewed scientific publications, 1 non-reviewed publication, 1 Webex presentation, 2 press releases, an e-newsletter, several TV interviews, a web site, 27 scientific presentations and about half a dozen presentations to lay audiences.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget: *This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report. See the Sub-Project Budget document for an up-to-date project budget, including any changes resulting from amendments.

Budget Category	$ Amount	Explanation
Personnel:	$942,054	
Professional/Technical Services and Contracts:	$217,357	USGS contract to analyze eDNA samples, divers to install transducers, electrician to power transducers, vet contract for sterilizing carp, lab equipment service, DNA sequencing
Equipment/Tools/Supplies:	$148,170	Lab and field supplies for eDNA, radiotags, etc
Capital Equipment over $3,500:	$87,451	qPCR machine, autoclave, -80C freezer, underwater transducers, FastPrep-24, plate reader
Travel Expenses	$39,968	$28,021 in Minnesota; $297 for a non-employee to consult on a project; $11,650 outside of Minnesota to bring samples to the USGS, collect samples,

		recruit new faculty, and for 4 investigators to attend national meetings to present results of research
Other:	$565,000	$565,000 for renovation and repair of AIS holding facilities
TOTAL ENRTF BUDGET:	$2,000,000	

Explanation of Use of Classified Staff: *n.a.*

Explanation of Capital Expenditures Greater Than $3,500:
A large -80C freezer is needed to store water samples for eDNA analysis for activities 3 and 4.
An autoclave to sterilize vials for genetics analysis.
A qPCR machine to process eDNA samples.
Underwater transducers @ $8,200/ ea
FastPrep-24 and/or plate reader @ $10,000

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:

Scientific director: 0.75
Administrative director: 2.0
Scientists (Sorensen): 0.25
Postdoctoral associate: eDNA: 3.0
Postdoctoral associate: Judas fish: 2.0
Postdoctoral associate: barrier: 1.0
Graduate student: 2.0
Fish technician: 0.5
Fish pathologist:0.05

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:
USGS lab (LaCrosse, WI): 2.0

B. Other Funds (related projects that can synergize this one):

Source of Funds	$ Amount Proposed	$ Amount Spent (12/1/14)	Use of Other Funds
Non-state			
National Science Foundation	$234,000	$155,121	Radio-tags for Judas fish
USGS	$259,146	$150,813	Preliminary work with Asian carp
Riley Purgatory Bluff Watershed District	$2,728,771	$2,132,222	Preliminary work on Judas carp
State			
Clean Water Legacy Funds	$1,800,000	$538,362	Startup for Center
TOTAL OTHER FUNDS:	$5,,021,917	$2,976,518	

VII. PROJECT STRATEGY:

A. Project Partners:
DNR (a full partner with whom the University will have a memoradum of understanding),

USGS (LaCrosse WI; and Columbia, MI), 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 infornation and useful techniques for their control to public agencies and the private sector.

C. Spending History:

Funding Source	M.L. 2005 or FY 2006-07	M.L. 2007 or FY 2008	M.L. 2008 or FY 2009	M.L. 2009 or FY 2010	M.L. 2010 or FY 2011
ENRTF (Accelerating plans for integrated control of common carp; Sorensen is PI)		550,000			
ENTRTF (Novel barriers for invasive species of fish, Voller PI, Sorensen Co-Pi with Mensinger and Honzo)			300,000		

VIII. ACQUISITION/RESTORATION LIST: n.a.

IX. MAP(S): Entire state of Minnesota

X. RESEARCH ADDENDUM:

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted not later than June 30, 2013; December 31, 2013, June 30, 2014, December 31, 2014, June 30, 2015, December 31, 2015, June 30, 2016, and December 31, 2016. A final report and associated products will be submitted no later than between June 30 and August 15, 2018 as requested by the LCCMR.

Environment and Natural Resources Trust Fund

Project Title: Aquatic Invasive Species Research Center
Legal Citation: M.L. 2012, Chp. 264, Art.4, Sec. 3:
Project Manager: Peter Sorensen.
Organization: University of Minnesota – Minnesota Aquatic Invasive Species Research Center
Project Budget: $2,000,000
Project Length and Completion Date:: 6 Years, June 30, 2018
Date of Report: August 15, 2018

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	*Activity #1: Establishing the Administrative Structure of an AIS Cooperative Research Center at the University (2012, 2013)*			*Activity #2: Establishing dedicated holding facilities for AIS*		
BUDGET ITEM	**Activity 1 Budget**	**Amount Spent as of 6/20/16**	**Activity 1 Balance**	**Activity 2 Budget**	**Amount Spent as of 6/20/16**	**Activity 2 Balance**
Personnel (Wages and Benefits) - Total	**$357,863**	**$357,863**	**$0**			**$0**
Scientific Director- Professor: $87,046 salary, $29,038 benefits (33.7% fringe rate) Total FTE .67 (Act 1)						
Associate Director- Professional & Admin: $150,560 salary, $50,699 benefits (33.7% fringe rate) Total FTE 1.88 (Act 1)						
Aquatic technican (Civil Service): $32,021, $8,499 (27.4% fringe rate) Total FTE 0.8 (Act 1)						
Sorensen- Professor: $5,642 salary, $1,959 benefits (33.7% fringe rate) Total FTE .05 (Act 3)						
Research Associate: $39,844 salary, $14,057 benefits (33.7% fringe rate) Total FTE .75 (Act 3)						
Undergraduate Student: $13,894 salary, $30 benefits (0% fringe rate) Total FTE .56 (Act 3)						
Aquatic technican (Civil Service): $705 salary, $180 benefits (27.4% fringe rate) Total FTE .02 (Act 3)						
Sorensen- Professor: $2500 salary, $900 benefits (33.7% fringe rate) Total FTE .02 (Act #4)						
Eichmiller- Post Doctoral Fellow: $137290 salary, $35285 benefits (20.75% fringe rate) Total FTE 3.43 (Act #4)						
Undergraduate Student: $13747 salary; $92 benefits (2% fringe rate) Total FTE .55 (Act #4)						
Aquatic Technician (Civil Service): $8158 salary, $1211 benefits (27.4% fringe rate) Total FTE 0.20 (Act #4)						
Sorensen Professor: $10,125 salary, $2464 benefits (33.7% fringe rate) Total FTE 0.08 (Act #5)						
Ghosal- Post Doctoral Fellow: $101,372 salary, $19889 benefits (20.75% fringe rate) Total FTE 2.53 (Act #5)						
Undergraduate Student: $11559 salary, $100 fringe; (0% fringe rate) Total FTE .46 (Act #5)						
Aquatic Technician (Civil Service): $11,715 salary, $2798 benefits (27.4% fringe rate) Total FTE .29 (Act #5)						
Sorensen- Professor: $9516 salary, $3211 benefits (33.7% fringe rate) Total FTE .08 (Act #6)						
Claus- Graduate Student: $50,293 salary, $35,904 benefits (37% tuition, 9% fringe rate) Total FTE 2.29 (Act #6)						
Undergraduate Student: $2250; (0% fringe rate) Total FTE 0.09 (Act #6)						
Aquatic Technician (Civil Service): $4501 salary, $1291 benefits (27.4% fringe rate) Total FTE .11 (Act #6)						
Phelps- Assistant Professor: $2925 salary, $1002 benefits (33.7% fringe rate) Total FTE .03 (Act #7)						
Zielinski- Post Doctoral Fellow: $46270 salary, $9918 benefits (20.75% fringe rate) Total FTE 1.03 (Act #8)						
Aquatic Technician (Civil Service): $2500 salary, $920 benefits (27.4% fringe rate) Total FTE .06 (Act #8)						
Professional/Technical Services and Contracts - Total	**$5,727**	**$5,727**	**$0**	**$0**	**$0**	**$0**
Services- office & gen oper. (printing/duplication, mailing, etc.)	$1,730	$1,730	$0			$0
Services- lab & medical (data storage, sequencing, biochemistry, microscopy, veterinary services (ACTIVITY 5) etc.)			$0			$0
Professional Services & contracts- (fees or honoraria for guest lecturer and speakers; contract with USGS's Midwest Environmental Science Center to analyze eDNA samples; contract with electrician for Activity 8, contract with divers for Activity 8)			$0			$0
Repairs- lab & field (qPCR contract, vehicle, EFL holding facility, or printer, other equipment)	$3,997	$3,997	$0			$0
Rental- (PLEASE LIST DETAILS SUCH AS: fish test pond rental from USGS in MO)			$0			$0
Equipment/Tools/Supplies - Total	**$9,547**	**$9,547**	**$0**	**$0**	**$0**	**$0**
Supplies- office & gen oper. (paper, software, folders, brochures, ink, displays, shipping, etc)	$3,187	$3,187	$0			$0
Supplies- lab & field (primer, extraction kits, reagents, filters, bottles; anesthesia, fish, fish food, aquaria, hormones, electrodes; tubing, air stones; gloves, bags; PVC, electrical wire, plastic mesh, motors, pit tags, shipping, etc)	$332	$332	$0			$0
Equipment- non capital lab & field SUCH AS: computers, printer; flasks, dissecting tools; accelerometer; surgical equipment, heat block, vortex, mini centrifuge, pipetters, incubators, high speed centrifuge; or trap nets, seine nets, dip nets; equipment rack, MP3 player and signal splitter, wireless alarm system, shipping and sensors (for the transducer work in Activity 8, etc)	$6,028	$6,028	$0			$0
Capital Expenditures Over $5,000 - Total	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**
(List specific items. Add rows as needed.)						
Cap expenditures over $5,000: (-80C freezer $8,265.00, autoclave $X, qPCR $30,405.36; FastPrep and/or plate reader $10,000 for Activity 4; portion of 5 x underwater transducers for Activity 8 ($8,200 ea), shipping, etc)			$0			$0
Travel - Total	**$6,033**	**$6,033**	**$0**	**$0**	**$0**	**$0**
(*Specify types of travel expenses, e.g., mileage, lodging, meals. Per diems are not allowed.)*						
Travel - MN (mileage, rental for conferences, sample collections, mtgs, etc.)	$6,033	$6,033	$0			$0
Travel - Domestic (mileage, conferences, mtgs, etc.) travel to out of state conferences to present results of research			$0			$0
Travel- Non employee (generally not accepted but specifics could be proposed for LCCMR consideration)			$0			$0
Other - Total	**$0**	**$0**	**$0**	**$565,000**	**$565,000**	**$0**
Alterations & Renovations- Hodson Hall and portion of costs for new well, security, and plumbing and electrical repairs in Engineering and Fisheries			$0	$565,000	$565,000	$0
Telecommunications (generally not accepted, but specifics could be proposed for LCCMR consideration). Conference calls			$0			$0
Research-specific utilities (when needed at a ROC e.g. for a research pond; specifics required for LCCMR approval); electricity to power transducers at Lock & Dam #8 (approx. cost 1 of 3 years)			$0			$0
COLUMN TOTAL	**$379,170**	**$379,170**	**$0**	**$565,000**	**$565,000**	**$0**

Activity #3: Establishing and implementing eDNA as a molecular technique to assess the presence of Asian carp in large Minnesota Rivers (2012, 2013, 2014, 2015)			*Activity #4: Determining the ability of two approaches to measure environmental DNA (eDNA) to reliably quantify the abundance of invasive common carp in Minnesota lakes (2012, 2013, 2014)*			*Activity #5: Testing whether carp can be located using "Judas fish:" a new behavioral tool to located aggregating invasive fish so they might be tracked and/or removed (2012,2013,2014)*			*Activity #6: Developing food attra carp that can be used to induce a control them: a new biochemical 2014)*	
Activity 3 Budget	**Amount Spent as of 6/15/17**	**Activity 3 Balance**	**Activity 4 Budget**	**Amount Spent as of 11/15/16**	**Activity 4 Balance**	**Activity 5 Budget**	**Amount Spent as of 12/31/15**	**Activity 5 Balance**	**Activity 6 Budget**	**Amount Spent as of 6/15/17**
$45,174	**$45,174**	**$0**	**$184,884**	**$184,884**	**$0**	**$160,022**	**$160,022**	**$0**	$87,378	**$87,378**
$151,000	**$151,000**	**$0**	**$11,880**	**$11,880**	**$0**	**$1,039**	**$1,039**	**$0**	**$1,053**	**$1,053**
		$0	$0	$0	$0	$387	$387	$0	$193	$193
$1,000	$1,000	$0	$10,205	$10,205	$0	$652	$652	$0	$860	$860
$150,000	$150,000	$0			$0			$0		
$0		$0	$1,675	$1,675	$0	$0		$0		
		$0			$0			$0		
$8,670	**$8,670**	**$0**	**$58,548**	**$58,548**	**$0**	**$23,771**	**$23,771**	**$0**	**$11,940**	**$11,940**
		$0	$0		$0	$0		$0	$0	
$8,670	$8,670	$0	$53,772	$53,772	$0	$23,771	$23,771	$0	$11,940	$11,940
		$0	$4,776	$4,776	$0			$0		
$0	**$0**	**$0**	**$48,597**	**$48,597**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**
		$0	$48,597	$48,597	$0			$0		
$2,373	**$2,373**	**$0**	**$5,957**	**$5,957**	**$0**	**$4,211**	**$4,211**	**$0**	**$2,563**	**$2,563**
$100	$100	$0	$3,422	$3,422	$0	$1,779	$1,779	$0	$1,162	$1,162
$2,273	$2,273	$0	$2,535	$2,535	$0	$2,432	$2,432	$0	$1,401	$1,401
		$0			$0			$0		
$0	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**
		$0			$0			$0		
		$0			$0			$0		
		$0			$0			$0		
$207,217	**$207,217**	**$0**	**$309,866**	**$309,866**	**$0**	**$189,043**	**$189,043**	**$0**	**$102,934**	**$102,933**

ctants for silver ggregation and ool (2012, 2013,	Activity #7: Screening for VHS in Minnesota Waters (2012, 2013)			Activity #8: Ascertaining whether an enhanced bubble curtain could deter Asian carp movement into small tributaries in a practical manner (2013)			Activity #9: Develop Solutions to Address Weaknesses in Lock and Dam #4 and then Optimizing its Gate Operation			
Activity 6 Balance	**Activity 7 Budget**	**Amount Spent as of 12/15/15**	**Activity 7 Balance**	**Activity 8 Budget**	**Amount Spent as of 12/15/15**	**Activity 8 Balance**	**Activity 9 Budget**	**Activity 9 revised Budget**	**Amount Spent as of 6/15/17**	**Activity 9 Balance**
$0	**$3,927**	**$3,927**	**$0**	**$59,608**	**$59,608**	**$0**	**~~$91,200~~**	**$93,641**	**$93,641**	**$0**
$0	**$20,120**	**$20,120**	**$0**	**$14,487**	**$14,487**	**$0**	**$0**		**$0**	**$0**
$0			$0			$0				$0
$0	$20,120	$20,120	$0			$0				$0
$0			$0	$14,487	$14,487	$0				$0
$0										
$0			$0			$0				$0
$0	**$0**	**$0**	**$0**	$13,048	**$13,048**	**$0**	$0		**$0**	**$0**
$0			$0			$0				$0
$0	$0		$0	$8,646	$8,646	$0				$0
$0			$0	$4,402	$4,402	$0				$0
$0	**$0**	**$0**	**$0**	**$38,781**	**$38,781**	**$0**	**$0**		**$0**	**$0**
$0			$0	$38,781	$38,781	$0				$0
$0	**$0**	**$0**	**$0**	$2,967	**$2,967**	**$0**	**~~$2,632~~**	**$191**	**$191**	**$0**
$0			$0	$1,070	$1,070	$0	**~~$1,000~~**	$191	$191	$0
$0			$0	$1,600	$1,600	$0	**~~$1,632~~**	$0		$0
$0			$0	$297	$297	$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
$1	**$24,047**	**$24,047**	**$0**	**$128,891**	**$128,891**	**$0**	**$93,832**		**$93,832**	**$0**

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND

TOTAL BUDGET	TOTAL Amount Spent as of 6/15/17	TOTAL BALANCE
$990,056	**$992,497**	**$0**
$205,306	**$205,305**	**$1**
$2,310	$2,310	$0
$32,837	$32,836	$1
$164,487	$164,487	$0
$5,672	$5,672	$0
$0	$0	$0
$125,524	**$125,524**	**$0**
$3,187	$3,187	$0
$107,131	$107,131	$0
$15,206	$15,207	-$1
$87,378	**$87,378**	**$0**
$87,378	$87,378	$0
$26,736	**$24,295**	**$0**
$14,566	$13,757	$0
$11,873	$10,241	$0
$297	$297	$0
$565,000	**$565,000**	**$0**
$565,000	$565,000	$0
$0	$0	$0
$0	$0	$0
$2,000,000	**$2,000,000**	**$0**