



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2013 Aquatic Invasive Species Research Center Sub-Project Work Plan

Date of Report: July 26, 2017

Date of Next Status Update Report: January 30, 2018

Date of Work Plan Approval: July 9, 2015

Sub-Project Phase 1 Completion Date: Jun 30, 2019

Project Completion Date: June 30, 2019

Does this submission include an amendment request? N

SUB-PROJECT TITLE: Aquatic Invasive Species Research Center Sub-Project 3: Attracting carp so their presence can be accurately assessed

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Location: Statewide

Total ENRTF Sub-Project Budget:

ENRTF Sub-Project Appropriation: \$682,969

Amount Spent: \$230,327

Balance: \$452,642

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

Appropriation Language:

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

I. SUB-PROJECT TITLE: Attracting carp so they can be measured.

II. SUB-PROJECT STATEMENT: The Sorensen lab group is currently developing a scheme to prevent adult bigheaded (invasive) carp from migrating upstream from the lower Mississippi River in numbers sufficient to create a self-sustaining population in Minnesota waters. This scheme relies on deterring adult carps from moving through lock and dam structures by developing acoustic deterrents that can be added to locks while developing an understanding of carp behavior and water flows sufficient to guide changes in gate operations to create water velocities that can hold carp back without affecting other fishes or dam scour. This scheme relies on having extremely accurate and precise information on the abundance of adult invasive carps in the immediate vicinity of the locks and dams because altering gate operation needs to be as strategic and efficient as possible. Information on the abundance of invasive carp could of course, also eventually be used by the DNR for possible removal efforts. Our ongoing work also shows that while current monitoring technologies for carps are all extremely poor (unquantifiable), measurement of the DNA released by fish (eDNA) has excellent potential if problems associated with its current inability to measure scattered carp located even modest distances away from sample points because of rapid dilution and degradation could be solved. eDNA alone is also limited because it cannot provide information on carp sexual maturity, information of critical importance at the invasion front. This proposal will attempt to remedy these deficiencies by developing new techniques to cause predictable aggregations of adult invasive carps to facilitate their accurate measurement using a combination of measurement techniques that include eDNA and pheromones, the latter of which could provide information on fish maturity to compliment the former. Research examines the potential of using sexual and feeding cues to cause aggregations. We examine both the possibility of using live sterile carp releasing sexual cues (“Judas fish”) and sex pheromones to locate and drive aggregations. Food and food chemicals will also be tested. They have promise because carps have unique food preferences that differ from native fishes. Research uses common carp locally to develop concepts with additional, complimentary studies of Bigheaded carp planned out of the state where such test are possible. While several approaches will be examined initially, the project will be modified to focus on the most promising attributes if appropriate. A possible second phase of this project could explore implementation of the most promising option(s) in 2018.

III. SUB-PROJECT STATUS UPDATES:

Sub-Project Status as of *January 31, 2016:*

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

Sub-Project Status as of *July 31, 2016:*

Work is ahead of schedule. Water samples collected for eDNA and pheromone evaluation were completely analyzed and a baiting scheme perfected. Experiments conducted last summer to test whether food and pheromones could be used as attractants to drive common carp aggregation have now been analyzed; both were highly successful. In one experiment, we were able to attract a third the population of mature common carp to a specific location within a lake using food while measuring carp abundance using both eDNA and a sex pheromone with a level of sensitivity, precision and accuracy previously unseen. Pheromone-releasing Judas carp were also attractive. A third study successfully measured common carp mating pheromones in waters near mating carp. Finally, a pilot study using food to attract Bigheaded carp was completed in Illinois with the University of South Illinois as collaborators. Whether this behavior enhanced our ability to measure them using

eDNA or pheromones (as shown with carp) is presently being evaluated. In sum, experiments are promising and work is ahead of schedule and we likely will be able to determine whether food stimuli or pheromones are most promising for use in invasive carp control by the next report when an amendment with a possible rebudget may be requested.

Sub-Project Status as of January 31, 2017:

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

Amendment request April 12, 2017

Research is proceeding well and is on schedule but some field work remains for next fall (because of an early snowfall in 2016) and laboratory work still needs to be done on sound as a deterrent (our ENRTF2014 funding for sound ends June 30 2017). An amendment is thus being requested at the request of the LCCMR to accommodate new sound work using funds from Activities #1 and #2 which are on (or under) budget. Phase III funds will supplement funds from Activities #1 and #2. This new project (Activity #3) will test the effects of using an enhanced sound gradients associated with an air curtain on carps as well as the effect of lights combined with this sound. This lab work will also address the effects of this optimized sound system on two or more native fishes which will likely include bass. This amendment to ENRTF2013 accompanies a related amendment to Sorensen's ENRTF2012 project to study changes to gate operations at Lock and Dam #4; by pairing these projects, a new coordinated, 2-year activity has been created. The new Activity #3 is described below along with a brief update for Activity #1 and Activity #2. A 5-month extension is also requested for Activities #1 and #2 to complete fall field work and analyze these data by June 2018 (which is within the period of time authorized for ENRTF201306A). This extension also coordinates reporting times for all activities. Specifically, the amendment seeks to move funds accordingly:

Activity 1:

Decrease personnel from \$209,171 to \$204,195
Decrease professional and technical services and repairs from \$22,003 to \$14,056
Decrease Equipment/tools/ supplies from \$94,900 to \$45,719

This \$62,105 total surplus from Activity #1 will be moved to create a new Activity #3

Activity #2:

Decrease personnel from \$124,176 to \$86,453
Decrease Professional/Technical Services and Contracts from \$3,000 to \$2,000
Increase Equipment/Tools/Supplies \$14,750 to \$19,104 because more eDNA analyses are needed.
Decrease travel from \$8,500 to \$5,942

This \$36,927 total surplus from Activity #2 will be moved to create a new Activity #3

Activity #3 (new)

The combined \$99,032 surplus from Activities #1 and #2 above will be re-budgeted to support the first year of a new Activity #3 along with \$182,968 of unspent funds from Phase III of this project. Activity #3 will start July 2017 and finish June 2019. The transfer of these reserve funds will be reflected in an update to the overall workplan and budget. This new laboratory project will address the ability of an optimized sound (developed as part of ENRTF2014) to deter carp when combined with an air curtain and with a light system using customized equipment developed by Fish Guidance Ltd. (FGS). Effects of the system on at least two native fishes (likely to be bass and trout) will also be addressed, thereby completing all laboratory work originally proposed in 2016 (117-D) to develop a carp deterrent for possible field deployment for a total cost of \$282,000 (less than that proposed in 2016). We will also be available to assist the MN DNR with field assessment. Funds will be budgeted as follows for two years:

Personnel: \$195,000 for 3 weeks summer salary for Peter Sorensen, support Clark Dennis working on this project while working on his PhD, 60% time for a laboratory technician, temporary, and undergraduate help.

Professional/Technical Services, Contracts and Repairs: \$51,000- includes \$30K to lease sound equipment custom-manufactured by Fish Guidance Systems Ltd. (we have been leasing this sound and air curtain system since 2016—it includes numerous state-of-the-art technologies this company has been developing since the 1990s), statistical help (2K), fish shipping (6K) and equipment shipping (2K), and repair (2K), lab space in the MAISRC Containment Lab (6K), etc. (3K)

Equipment/Tools/Supplies: \$23,000 for fish, fish food, tags, and lab supplies for the tanks.

\$4,000 MN travel for workshops and meetings sponsored by the DNR and USFWS on carp

\$9,000 Out of state travels for meetings to present results and learn about new approaches with sound by federal researchers (there is quite a bit of research starting up now on sound in WI, IL and MS by the USGS, USFWS and USACE), and for travel for Dr. Dan Zielinski (Great Lakes Fishery Commission but formally a U of MN postdoc who started this project and has great expertise) and other fish sound experts (ex. Dr. Andy Turnpenny) to visit us and provide essential advise

Amendment Approved by LCCMR 4/18/2017

Sub-Project Status as of July 23, 2017:

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

Overall Sub-Project Outcomes and Results:

IV. SUB-PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: To develop reliable and practical ways of using sexual stimuli to reliably find and /or attract carp to induce aggregations so their presence can be measured, or alternatively they could be trapped and removed.

Description: Sexual stimuli are powerful motivators of adult fish behavior, especially carps that aggregate and spawn in groups just a few times of the year. Living in turbid (dark), turbulent (noisy) waters that are expansive and often deep, the carps have evolved extremely well developed, chemical detection systems and sex pheromones (chemical cues that pass between members of the same species and mediate reproductive synchrony). All carp (common and bigheaded) are scramble spawners (i.e. they spawn in open waters and rely on males finding and locating females). The Sorensen team has already determined that the carps rely on hormonally-based sex pheromones to locate each other, some of which (sex steroids) they release both immediately prior to, and others (F prostaglandins) during spawning and which they detect with extreme sensitivity (grams in billions of liters of water) and sensitivity. We also already know how to artificially induce both female sexual behavior and sex pheromone release using hormone implants. The ability to locate or cause sexually-active invasive carps to aggregate and spawn has enormous practical implications because spawning groups are presently unpredictable but if controlled they could either be assayed (to guide prevention efforts) or perhaps removed by management agencies. Activity #1 will examine the possibility that sex pheromones released directly via pumps or by hormone-treated fish could reliably locate and/or cause spawning aggregations that we could then measure using various tracking systems, eDNA measurement and sex pheromone concentration. We will examine the possibility that synthesized compounds or the suite of compounds released by hormone-treated (and ideally sterile) carps equipped with tracking devices (“Judas fish”) could be useful to drive or alternatively locate aggregating adult carp. We will examine these possibilities both in common carp in local lakes where proof-of-concept studies are both relevant and possible, and then with bigheaded carps out-of-state with the USGS where these species can be studied in the wild. We will initially test whether we can measure induced aggregations using eDNA (which is sensitive and species-specific), as well as with sex pheromones (which could describe gender and maturity) as well as traditional techniques such as radio-tags. Field work will be complimented by laboratory studies as appropriate. We take an iterative approach in which a variety of possibilities will tested first but if some are found to be more promising than others, we likely will focus on them to eventually identify a single set of optimized approaches that might be pursued as a possible second phase of the overall project in river(s). This research will be conducted in parallel with work on feeding cues (Activity #2) and with the assistance of (and in collaboration with) the USGS for Bigheaded carps to improve efficiency.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 287,470
Amount Spent: \$ 147,658
Balance: \$ 139,812

Activity Completion Date: January 31, 2018

Outcome	Completion Date	Budget
1. Establish a pheromone baiting and tracking system in a lake for common carp that might also be used bigheaded carps.	Jan 2016	\$70,000
2. Complete sample collection for common carp sex pheromones and eDNA in a lake and conduct initial analyses.	July 2016	\$65,000
3. Determine to what extent sexual stimuli (Judas fish and/or sex pheromones alone) can reliably induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds.	Jan 2017	\$86,000

4. Identify specific approaches by which sex stimuli might be used to induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds that can be measured.	July 2017	\$82,000
5. Final report that describes a recommended scheme for using food-based and/or sex based attractant system that can reliably induce carp aggregations and then measure them using eDNA, sex pheromones and/or other techniques (matches Outcome #5 in Activity #2)	June 2018	\$46,574

Activity Status as of January 31, 2016:

We have established a system to add a pheromone to a lake, track fish and then sample waters which can be used for pheromone and/or eDNA analyses. We decided to initially focus on using free-ranging hormone-treated fish to release pheromone as these also function as a sexually active and attractive “Judas carp.” This method (versus adding pheromone using a pump) appears promising and likely to work to attract carp. Because food baiting worked very well as a stationary bait, we will develop food (and not pheromones) as stationary bait and Judas fish as an alternative to locate and track moving fish (see Activity 2 below). In our pheromone system, female common carp are captured and implanted with the hormone, prostaglandin F2 α (PGF2 α), and released into the lake while males are tracked in their vicinity. PGF2 α evokes normal female sexual activity and sex pheromone release in carp, and attracts male carp under laboratory conditions. Water samples can then be taken near PGF2 α -implanted females. This method was initially tested in Long Lake, New Brighton with radio-tagged males in the summer of 2015. PGF2 α -implanted females and males were tracked for three 24-h cycles over a period of six days and aggregations as well as surface spawning activity noted. We are currently analyzing the tracking data to test whether the PGF2 α -implanted Judas carp induced any kind of aggregation in the lake. We are using tracking locations and proximity of radio-tagged carp as a measure to test for induced aggregations and/or attraction and are collaborating with Dr. Meggan Craft, Assistant Professor, Veterinary Population Medicine, University of Minnesota. Water samples have yet to be completely analyzed but preliminary results are equivocal – this approach may be best for finding moving males using radio-tags only. Plans for the next steps are still being formulated but likely we may explore releasing the prostaglandin directly by placing implanted fish into cages and then conduct more detailed water analyses for eDNA and pheromones.

Activity Status as of July 31, 2016:

We completed sample collection for common carp sex pheromones and eDNA in a lake and have analyzed all of these samples and shown that pheromones have great promise for use both controlling and measuring carp. The possibility of using pheromones released by Judas fish to induce aggregations of male common carp has also been successfully tested. Pilot studies for bigheaded (silver)carp have also been completed in another pond in Illinois with Southern Illinois University (we are collaborating with them instead of the USGS as they have more available test ponds); these samples await analysis. A brief synopsis of each completed experiment follows.

Using pheromones to measure the presence of common carp. In an experiment (details described in Activity #2), we used cracked corn (food) to induce common carp to aggregate in the fall 2015 in Lake Steiger, Victoria, MN and collected water samples near the food-induced aggregation to measure pheromone and eDNA, to determine if these compounds could serve as accurate and highly sensitive population indices. We measured the female sex pheromone, prostaglandinF2 α (PGF2 α) using high-resolution mass-spectrometry to confirm gender. Analyses of prostaglandin F2 α (and eDNA) showed both to be at or below the detection limit in the absence of food attractant and outside but increased dramatically within the aggregation and reached final concentrations of 106 ng/ml and 590 copies/ml, respectively. Data analysis is nearly complete and a manuscript is being prepared.

Determining whether common carp pheromones be measured in lake waters. We collected water samples from spawning aggregations of common carp in Rice Lake (New Brighton, MN, n =3), Lino Lake

(New Brighton, MN, n=2), Wasserman Lake (Victoria, MN, n=2) and Staring lake (Eden Prairie, MN, n=1) lakes in late 2015. Initial analyses of water samples using liquid chromatography and mass spectrometry demonstrates the presence of 15keto-ProstaglandinF2 α (a sex pheromone released by spawning females). This is the first time fish sex pheromone has been measured in natural waters (Figure 1-1), the result has been published (Sorensen and Johnson 2016) and we will now determine if we can measure pheromones released by male common carp this summer in aggregation experiments.

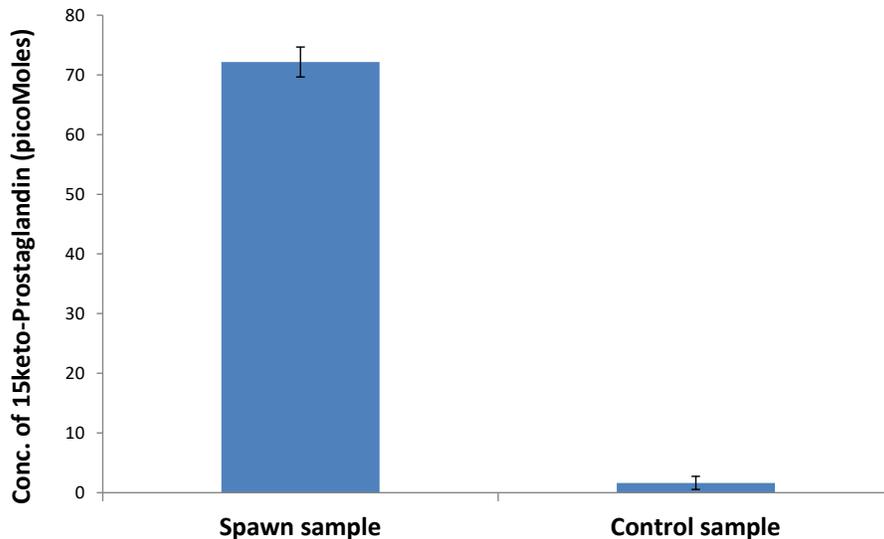


Fig. 1-1. Concentration of 15-Keto-Prostaglandin measured in lake water collected from spawning aggregation (spawn sample) in Wasserman Lake, MN. Spawning aggregations typically consisted of 3-4 males and 1-2 females. Control samples were collected 100 meters away from the aggregation. Water samples were analyzed using Applied Biosystems (Foster City, CA) 4000 QTRAP hybrid, triple-quadrupole, linear ion trap mass spectrometer.

Testing whether pheromone-releasing Judas carp can attract males.

During the summer 2015, we released 3 PGF2 α -implanted females in Long Lake, New Brighton. The lake had 9 radio-tagged males. We also released 3 blank capsule-implanted females as controls. PGF2 α -implanted and control females, and males were tracked for three 24-h cycles over a period of six days (treatment phase) and aggregations as well as surface spawning activity were noted. Implanted females and males were also tracked during the “spent” phase, 2 weeks after all the PGF2 α was released from the capsule. We used social network analysis to analyze the tracking data. Initial social network analysis is demonstrating that, PGF2 α implanted Judas carp can attract males and induce an aggregation. Analyses have yet to be completed to determine the densities (and reliability) of these aggregations.

Experiments this summer and fall will now explore whether and how sex pheromones can be pumped into ponds of male common carp to attract them and also if this might also work for silver carp, and if so, to what extent. Depending on final results, and whether we remain under budget, 2017 studies will then focus on pheromones or food (see activity #2) so attractants that can be used to aid in population measurement. An amendment with possible re-budget may be requested.

Activity Status as of January 31, 2017:

Sex pheromone experiments continue to show promise. Data analysis is nearly complete on our Judas fish experiments which tested the F prostaglandin sex pheromone. This work demonstrated that we can attract and induce aggregations of adult male common carp in an open lake. This has never been shown in vertebrate before. A draft manuscript has been written. A final experiment testing pheromone plume size is planned. We plan to have this experiment and accompanying manuscript complete by July. Meanwhile, during fall 2016, we conducted pheromone-release experiments in a pond (0.52 ha area) at the golf course of the University of Minnesota using adult male common carp. Fifteen male common carp were electrofished from Lake

Wasserman, Carver County, MN and stocked into a University of Minnesota golf course pond after being implanted with pit tags and radio-tags. A PIT-tag array system was established with two antennas fixed at opposite ends of the pond and monitored for baseline distribution. After this pre-test period, the whole spawning pheromone was tested: holding water of a prostaglandinF2 α (PGF2 α)-implanted carp (implanted with 0.4g/kg PGF α) was released for 40 min (with a flow rate of 0.5l/min) at daybreak. In a second experiment, PGF2 α alone was added to see whether this single component might be as effective. Water samples were collected for both eDNA and sex pheromone analysis and initial results are promising. However, only a single trial could be run and we were unable to run fall experiments in Illinois with silver carp because the weather changed (it snowed). Initial results are nevertheless promising: we saw strong attraction to the whole pheromone and smaller, but seemingly notable responses to the PGF2 α . (Fig. 2-1). This experiment will be repeated this spring to see if we can replicate the result and if so, publish. A similar, final experiment is also planned for silver carp with collaborators at Southern Illinois University. Together, when completed these experiments will complete the project.

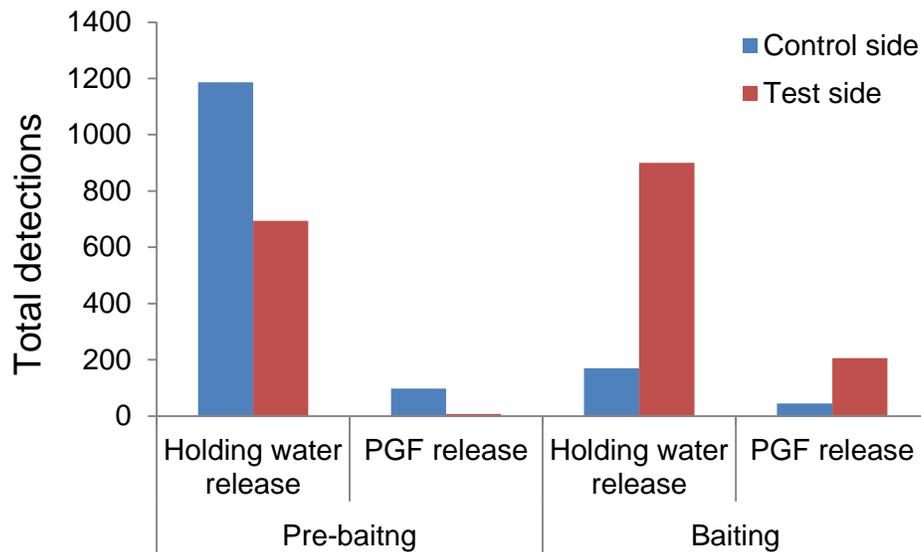


Figure 1-2: Total detections of pit-tagged male common carp during the pre-baiting and baiting phases at the control and test sides. Two baiting conditions were tested: holding water of PGF2 α -implanted carp (holding water release i.e. the whole pheromone) and PGF2 α (PGF release; i.e. a component of the pheromone).

Activity Status as of July 31, 2017:

Sex pheromone experiments continue to look quite promising and three approaches for using pheromones have now been identified which vary by species. Final testing and data analysis are now underway. These approaches include: 1) Using pheromone (prostaglandin) implanted free-swimming Judas fish to induce aggregations; 2) pumping synthesized pheromone into waterways; 3) pumping the pheromone produced by pheromone-implanted fish water into lakes (a more natural solution). These approaches and progress are summarized below:

1. Judas fish. We have completed a Judas fish study with pheromone-implanted common carp. Analyses show promise for use in smaller lakes with low-to-moderate numbers of common carp as Judas Fish strongly induce localized aggregation/ social network formation (Fig 1-3). However, while effective, this approach does not allow us to manipulate carp distribution precisely enough for either removal or census using eDNA sampling. This scenario is exemplified by Fig 1-3 which shows radiotagged common carp in Long Lake which have been induced to aggregate in dynamic social groups by adding a pheromone (Prostaglandin F2 α)-implanted Judas female carp to the lake (Fig 1-3a) and then a lack of aggregation after the common carp no longer had pheromone (i.e. it ran out; Fig 1-3b). These data are now undergoing final analyses for submission as peer-review manuscript. Use of pheromone-implanted Judas fish for silver (invasive) carp looks to be even more challenging than for common carp because our studies in Illinois ponds showed silver carp to not survive surgeries associated with pheromone implantation. (data not shown). Data will be fully analyzed for the next (and final report) in a year. If reasonable, we might examine this phenomenon more closely in the lab to examine how close carps will aggregate and why.
2. Pumping synthetic prostaglandin F2 α pheromone into targeted areas in waterways. We completed initial studies in which we pumped prostaglandin F2 α into ponds containing adult male common carp. Moderate attraction was observed in 2016 but experiments ended with an early snowfall so must now be repeated. We will conduct at least one more experiment to test this strategy late this summer/early fall with common carp. We may also attempt some lab work to look at the species-specificity for conspecific odor in carp in the laboratory to see if we can extend this work by allowing full synthetic mixtures to be identified and added. A full report will be available in a year.
3. Pumping holding water of pheromone (PGF2 α)-implanted carp into targeted areas in waterways. Our previous LCCMR-funded work (Lim et al. 2011, 2012 J. Chem. Ecol.) showed that pheromone (PGF2 α)-implanted carp release a more complete pheromone than PGF2 α alone and was more effective at attracting conspecifics. We have now completed two studies in which we pumped water from prostaglandin F2 α -implanted common carp or silver carp into ponds. An initial experiment with common carp went very well and we found we could attract male common carp and measure their eDNA release (Fig. 1-4a,b). Tests of this technique using implanted silver carp were less promising because as with Judas fish, they did not survive the surgery for long (they are extremely sensitive) so this technique may not be well suited to the latter species. This experiment will be repeated with common carp this summer/fall and then evaluated for application and publication.

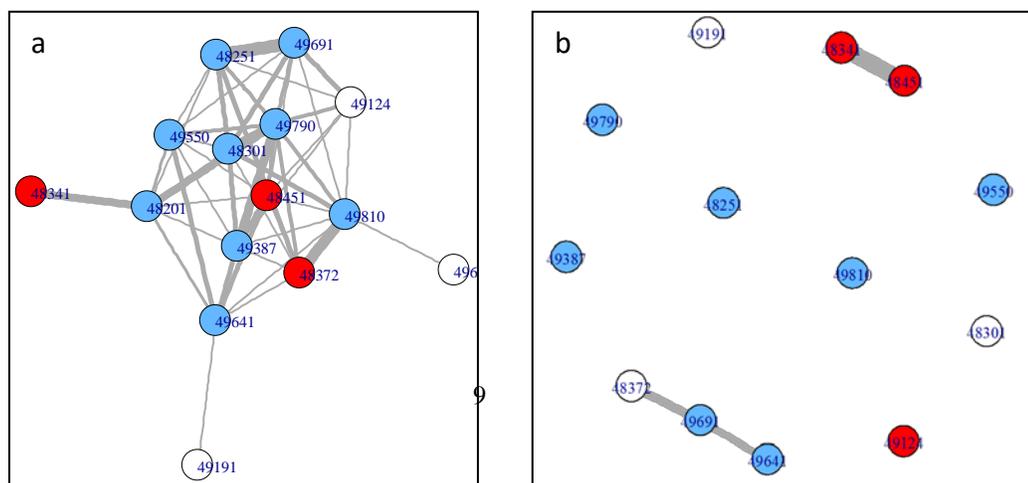


Figure 1-3. Interaction maps using social network analysis to evaluate aggregated male common carp (●), treated female common carp (●), and untreated female common carp (○) during the PGF2 α -treated phase (panel A) and the PGF2 α -spent phase (panel B). Numbers represent the unique radiotag number for each carp.

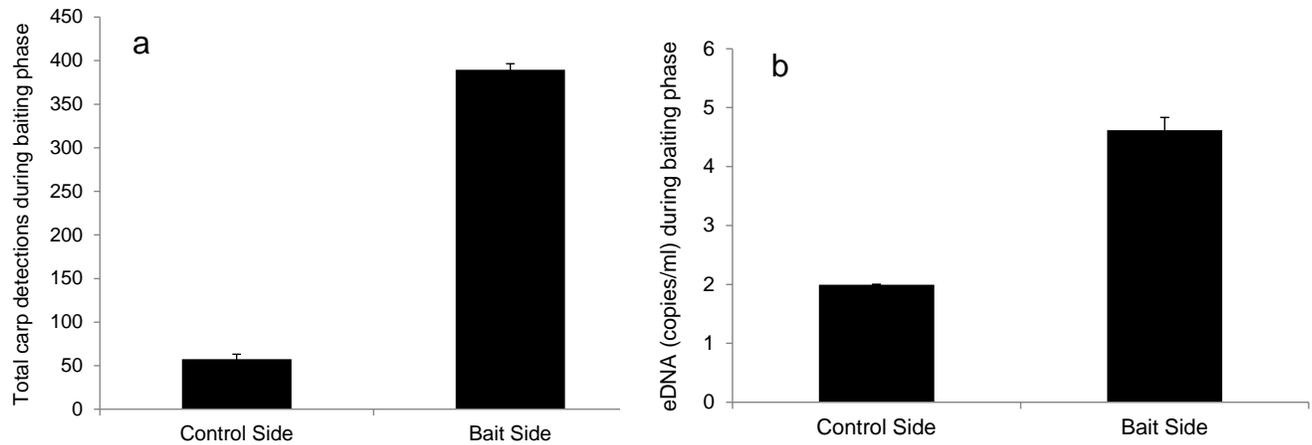


Figure 1-4.: a) Total number of detections of pit-tagged male common carp (Mean ± SD) during the pheromone-baiting phase at the control and bait sides in ponds. b) eDNA measurements (Mean ± SD) during the baiting phase at the control and bait sides from our initial experiments which will be repeated. Fig 4-1a is a reanalysis of figure 2-1.

Activity Status and Final Report June 30, 2018:

ACTIVITY 2: To develop reliable and practical ways of using feeding stimuli to induce carp to aggregate so their presence can be measured, or alternatively they could be trapped and removed.

Description: In addition to sexual stimuli, food stimuli are also extremely powerful motivators of adult fish behavior. Living in turbid (dark) waters that are expansive, the carps have evolved extremely well-developed senses of smell and taste which they use to detect and locate their highly specialized diets, which are comprised of tiny particles located either in the sediments (common carp) or open water (bigheaded carps). The bigheaded carps are planktivorous, unlike any native fish, making their feeding behavior an attribute that could be targeted in their control. Further, adult carps feed most of the year. This activity will examine the possibility that feeding cues might be used, perhaps in addition to sexual cues (Activity #1), to predictably and reliably cause aggregations of fishes that we can then measure in the field. We will examine the possibility that either food or a synthesized compound or compounds released by known food items could be useful to drive aggregations of carps. We will first test food stimuli in common carp in a Minnesota lake where proof-of-concept studies are both relevant and possible. Next, food stimuli will be tested on an established population of bigheaded carps outside of Minnesota in a field-scale study that is not possible in state, as no such populations currently exist. We will also test whether we can measure any aggregations we induce using eDNA (which is sensitive and species-specific), as well sex pheromones (which could indicate gender and maturity), and other traditional techniques such as radio-telemetry and sonar. We take an iterative approach in which some

possibilities maybe determined to be less promising than others in certain species, to eventually arrive at a single set of optimized approaches that might be pursued as a second phase of the overall project in the river. This field research will be conducted in the summer and in conjunction with a related (supporting) laboratory component during the academic year which is supported by ENRTF2012 (it's Activity 6) for the first 2 years and a project with the United States Geological Survey (USGS). Activity #2 will be conducted together with work on sexual cues (Activity #1) and in collaboration with the USGS to conserve time and funds.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 113,499
Amount Spent: \$ 82,669
Balance: \$ 30,830

Activity Completion Date: January 31, 2018

Outcome	Completion Date	Budget
<i>1. Establish a food baiting and tracking system in a lake for common carp that might also be used for bigheaded carp.</i>	Jan 2016	\$34,000
<i>2. Develop a baiting strategy using feeding stimuli to induce aggregations of common carp that can be measured.</i>	July 2016	\$29,000
<i>3. Determine to what extent feeding stimuli (food and/or its odor) can reliably induce aggregations of common carp and bigheaded carp in lakes and/or ponds that can be measured.</i>	Jan 2017	\$43,000
<i>4. Identify specific approaches by which food stimuli might be used to induce aggregations of common carp and/or bigheaded carp in lakes and/or ponds. that can be measured.</i>	July 2017	\$36,000
<i>5. Final report that describes a recommended scheme for using food-based and/or sex based attractant system that can reliably induce carp aggregations and then measure them using eDNA, sex pheromones and/or other techniques (matches Outcome #5 in Activity #1)</i>	June 2018	\$8,426

Activity Status as of January 31, 2016:

We have established a system to add food to a lake, track fish and then sample waters which can be used for pheromone and/or eDNA analyses. We have also tested it and collected water samples for both eDNA and pheromone to measure fish density. Results appear promising. Our strategy involves using a fixed baiting site to which bait is added on a regular basis while fish are tracked using radio-tags and water samples taken for both eDNA and pheromone analysis. PIT tags tracking systems did not appear promising. We tested this approach using radio-tags in the early fall of 2015 and results are promising. Briefly, using electrofishing gear, 30 common carp were captured, radio-tagged following established procedures and released in late summer in Lake Steiger (Victoria, MN). This experiment was conducted in September-October 2015 when water temperatures were relatively warm (15°C) and carp were still actively feeding. All of the radio-tagged carp were first tracked for 3 days on a 24-h cycle to determine their pre-baiting distribution in the lake. During each tracking cycle, carp were located twice, once during the day (9.00h to 15.00h) and once during the night (21.00h-3.00h). We tracked all the carp from a small boat using a loop antenna. Carp locations were bi-angulated by measuring two bearings that will be evaluated using computer software (LOAS® 4.0; Ecological Software Solutions, CA, USA). Based on the pre-baiting distribution of the carp, we next selected a location in the northeastern part of the lake that was rarely used by the tagged carp (i.e. a low activity area) so we could use it as a food baiting station. The food baiting experiment had 2 phases – the control and the baiting phases, which were separated by 3 days. During the control phase, empty mesh bags (control) were placed on the lake bottom at the food baiting location and attached to an anchored float. We lifted the empty bags after every 6 h and tracked all the radio-tagged carp on a 24-h cycle for 7 days. During the baiting phase, we filled the bags with cracked corn, a preferred food

of the common carp. Corn-filled mesh bags (2 bags each weighing 25 kg) were also lifted after every 6 h, weighed (using a portable balance) and re-filled once in a 24-h cycle with a known amount of fresh corn. We also tracked all the radio-tagged carp on a 24-h cycle for 7 days during the baiting phase. Tracking cycles and procedures were similar to as described during the pre-baiting distribution. An automated stationary data logger (Advanced Telemetry systems, Isanti, MN) was placed near the baited site to record the locations of the radiotagged carp within 150 meters of the baiting site, during both the control and the bait phases. We are currently analyzing the tracking data to test whether food (cracked corn) caused any aggregation of common carp in the lake water. We also collected water samples to measure eDNA and pheromones at the baiting site and the analysis is currently underway. Results however look promising; we are ahead of schedule. More sophisticated experiments will likely be conducted next year to address different species and/or temperatures. This might involve a re-budget, which might include changes in personnel (the student who had expressed interest in this as part of a Ph.D. has withdrawn).

Activity Status as of July 31, 2016:

We have now both developed a strategy using food to induce aggregation in common carp and shown that these aggregations can be measured using pheromones (also see activity #1 for the pheromone measurement) in combination with eDNA (i.e. we are slightly ahead of schedule). A promising pilot study using silver carp was completed in Illinois. Some of the methodological details for inducing aggregations of common carp using food in Lake Steiger (MN) were described in the December report so they are not all reported here; however, we have now completed analysis of these data so that is. Briefly, during this experiment we tracked 30 radio-tagged mature common carp for 2 weeks on a 24h cycle. The food baiting experiment had 2 phases – the control (no food) and the baiting (cracked corn bait) phase. Radio-tracking data showed a high number of tagged carp ($n=18$) at the food station during the baiting phase, almost twice the number of carp during the control phase ($n=8$). Further, while the total number of detections during the control period was only 200, it increased three-fold to 600 during the baiting phase. During the baiting phase, the night-time activity was higher with a large number of detections of tagged carp than during the day (an average of 300 detections during the day versus an average of 500 detections during the night). Overall activity at the food station was highest on day 5 and day 6 of the baiting phase. Thus, baiting with cracked corn was highly successful and induced a stable aggregation of common carp in a lake. Further, eDNA measurements (briefly reported in activity #1) increased from 0 to 590 copies/ml with aggregation while the concentration of prostaglandin F₂ α also increased. W Finally, we recently conducted a pilot food baiting experiments in the silver carp in experimental ponds at the Southern Illinois University, Carbondale, IL. We established a pit-tag array system in these ponds and used Spirulina as bait to induce an aggregation of silver carp. Although these data are not yet fully analyzed, it is clear that silver carp distribution shifted. eDNA and pheromone values are still be analyzed. Common carp experiments are now complete and a manuscript is in preparation. We plan to return to Illinois this fall to repeat these experiments and try to enhance food attraction. Depending on results, a final set of experiments using food to attract bigheaded carp is planned.

Activity Status as of January 31, 2017:

Food experiments continue to go well. The data from our earlier lake experiment with common carp are now almost completely analyzed and clearly show that we can strongly attract carp and measure aggregations using both eDNA and pheromones. A first draft of a publication is now complete. Meanwhile, we conducted food-baiting experiments with silver carp in the outdoor ponds located at Southern Illinois University, Carbondale. Adult silver carp ($n=30$) were electrofished from Big Muddy River, Illinois and stocked into a pond (0.04 ha area). All of these silver carp were then pit-tagged and a pit-tag array system was established using two antennas fixed at opposite ends of the pond. A pit tag system recorded the baseline distribution of the carp for two days. Based on the initial distribution (pre-baiting phase), one side of the pond was then designated as the control (the side with higher frequency of carp occurrence [to be conservative]), while the other side was designated as the test

side. Spirulina (a favorite food of silver carp [Claus and Sorensen in press]) was next added to the test side (2 oz. of powdered spirulina mixed in 2L of pond water) for 10 min. During the time of Spirulina release (baiting phase), the total number of carp detections was much higher at the test side than at the control side (Fig 2-2). Water samples were also collected from both the test and control sides for eDNA and pheromone analyses (results pending). We plan to repeat these pond experiments during the spring of 2017. No additional work with common carp is needed so the project should be complete by year's end.

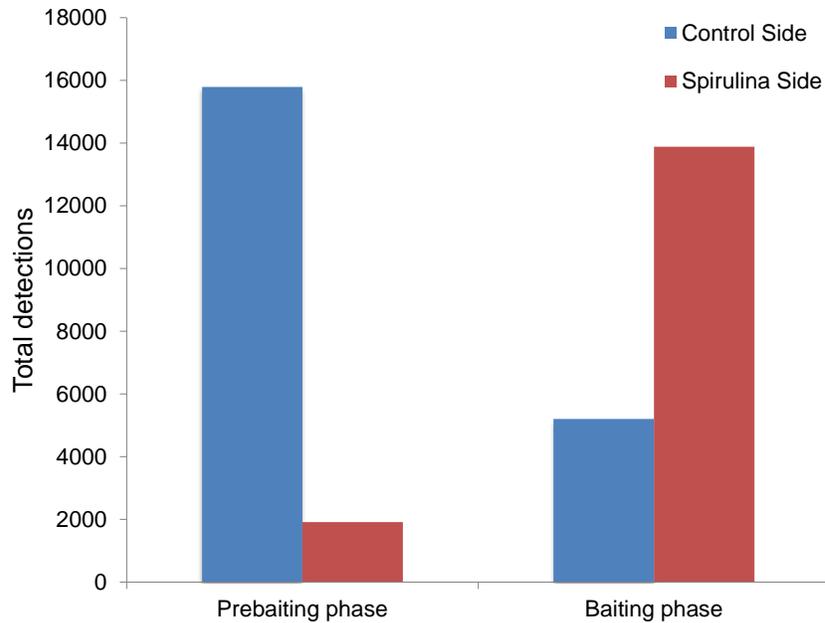


Figure 2-1: Total detections of pit-tagged silver carp during the pre-baiting and baiting phases at the control and spirulina (test) sides.

Activity Status as of July 23, 2017:

Food experiments continue to look especially promising. Two approaches have been identified, one of which (#2), is extremely promising for both common and silver carps. These approaches include: 1) Attracting carp to food odor (alone); and 2) Training carp to come to food bait in a target section of a waterway. Initial tests of these options showed that it took 2-3 days to attract fish to food (releasing odor), meaning that adding food odor alone (Option #1) has little promise because of the large amounts of odor and time needed, so we have focused on Option #2, training carp by adding food in predictable ways for several days. A large food training experiment (Experiment 2A) was completed in 2015 with common carp and data analysis/ manuscript writing is almost complete; it shows great promise for this species and we summarize it below. Two promising experiments for silver carp have also been completed for food in Illinois and we summarize them below too (Experiment 2B). Data will be fully analyzed by the next scheduled report.

Experiment 2a: Training common carp to come to food bait in a targeted area of a lake. A definitive and highly promising experiment was completed using common carp in Lake Steiger, MN. This technique allowed us to measure carp in waters where they were previously not measurable because of low density. This is an excellent and novel technique to attract large numbers of carp for removal or census, especially outside of their spawning season. In this experiment, we attracted common carp to a target area in the lake using cracked corn as bait, and fish distribution was monitored using radiotelemetry. We also measured environmental DNA (eDNA) and sex pheromone (prostaglandin F2α, PGF2α – as a way to determine fish gender) within the induced aggregation. Fig 2-2 shows that detections of radiotagged carp at the baiting site increased over time along with an increase in

eDNA copy number and PGF2 α concentration. Our study shows that an integrated approach of using food stimulus in combination with eDNA and pheromone measurements is highly useful in inducing and measuring the aggregations of invasive species, such as common carp. These data are now being prepared for a high quality peer-reviewed publication.

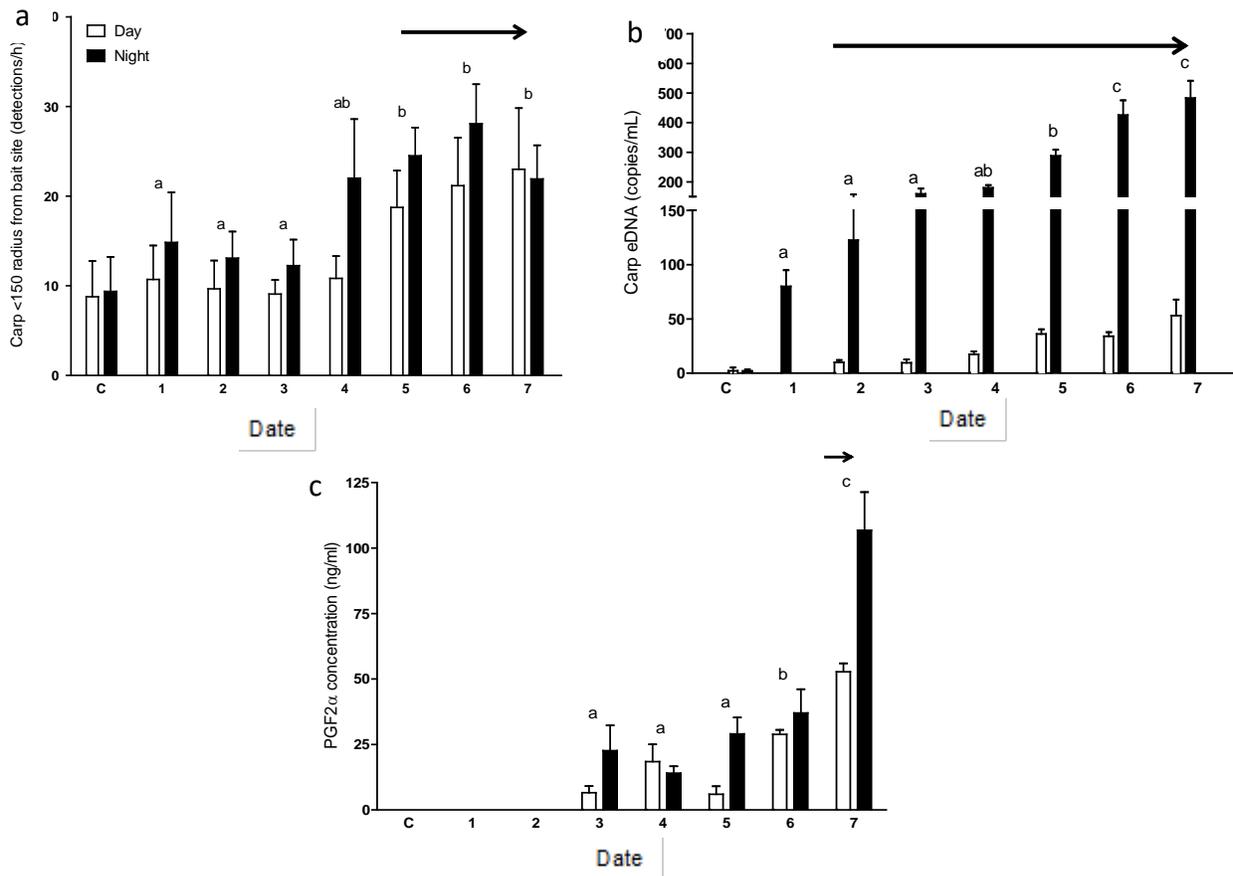


Fig 2-2. Measurements at the bait site during the control and baiting phases showing a) Hourly detections of radiotagged carp (<150 m radius from the bait site) by stationary receiver (Mean \pm SD); (b) eDNA concentrations measured in the water at the baiting site (copies/mL, Mean \pm SD, N = 3 sample replicates); (c) Prostaglandin (PGF2 α) concentrations (ng/mL, Mean \pm SD, N = 3 assay replicates) measured in the water at the food baiting site show that adult females are present. C indicates the average across the control phase and 1-7 indicates the baiting phase. Arrow indicates the period during which the nighttime measurements were higher than the daytime periods (Two-way ANOVA, $P < 0.05$). Different alphabets indicate significant differences across dates (One-way ANOVA, $P < 0.05$).

2b) Training silver carp to come to food bait in a targeted area: Initial pilot experiments tested spirulina as a bait for silver carp in outdoor ponds in Illinois and were repeated in 2017. We found that silver carp were attracted

to one side of the pond using spirulina bait in both years (see January report for 2016 data; Fig. 2-1). More recently, we analyzed the pilot 2016 data which showed that that eDNA technique worked well to measure these carp (Fig 2-3-2). Armed with this success, this experiment was repeated in the summer of 2017 in the outdoor ponds (n = 2) located at Southern Illinois University, Carbondale. The experimental design was similar to the food-baiting experiments conducted in 2016. Briefly, adult silver carp were electrofished from the Big Muddy river, Illinois and stocked into two ponds (each 0.04 ha area). Each pond had 15-20 adult pit-tagged carp with a pit-tag array system at opposite ends of the pond. This pit tag system recorded the baseline distribution of the carp for two days. Based on the initial distribution (pre-baiting phase), one side of the pond was then designated as the control (the side with higher frequency of carp occurrence [to be conservative]), while the other side was designated as the baiting side. Spirulina (a favorite food of silver carp [Claus and Sorensen 2017 J. Chem. Ecol.]) was next added to the baiting side (2 oz. of powdered spirulina mixed in 2L of pond water) for 10 min, for 2 consecutive days in each pond (Fig 2-3). Water samples were collected from both the baiting and control sides for eDNA analyses. We are presently in the process of extracting the eDNA samples for final analysis Preliminary analyses of the pit-tagged fish do suggest that spirulina was able to attract silver carp to a targeted area (carp detections were approximately three times higher at the baiting side (or spirulina side) than at the control side during the baiting phase (Fig. 2-4)). Field data collection is now complete and data will be fully analyzed by the time of the next report. Time permitting, the timing of fish training may be examined in laboratory experiments that would inform management and complement these valuable studies that greatly enhance the utility of eDNA measurement techniques at the invasion front.

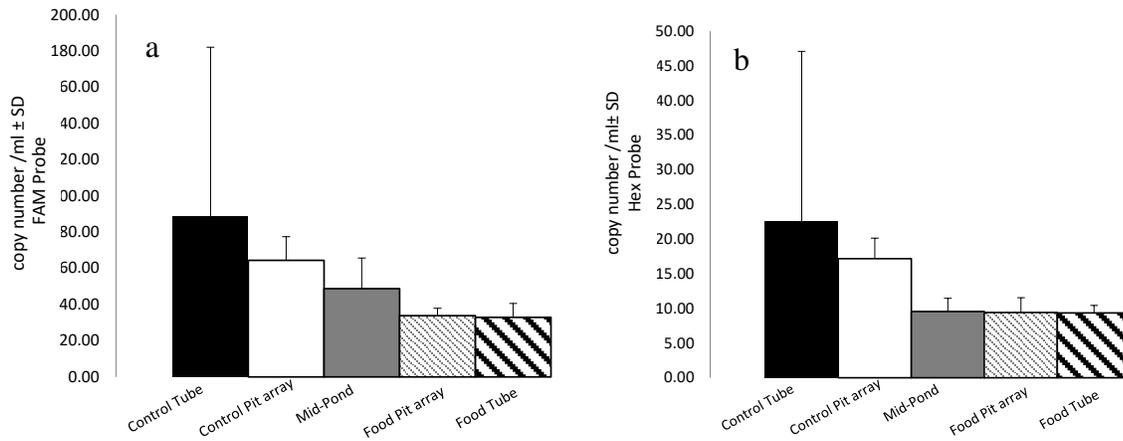


Figure 2-3 Whole pond eDNA results during the pre-baiting (control) phase of 2016 using both the a) FAM and b) HEX qPCR probes for silver carp in Illinois.

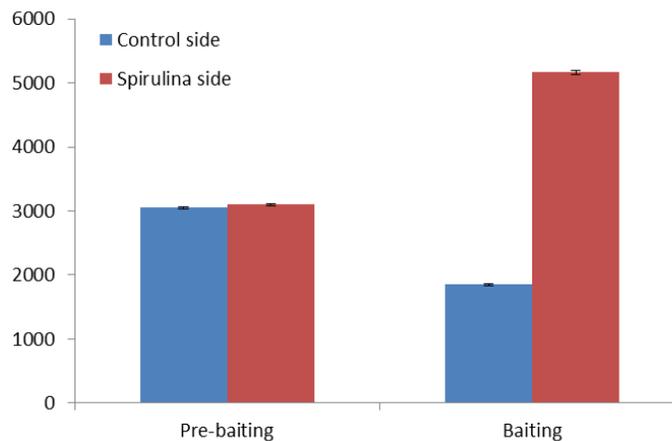


Figure 2-4: Total detections of pit-tagged silver carp during the pre-baiting and baiting phases at the control and spirulina (test) sides during the summer 2017.

Activity Status and Final Report: June 30,2018

ACTIVITY 3: Determining if and how a sound-bubble system can be combined with light in the laboratory to deter carp while examining potential impacts to native fishes.

Description: In addition to developing new ways to monitor bighead and silver carp in Minnesota waters, the Sorensen research team has been developing a deterrent system to prevent invasive carps from passing through key locks and dams. This proposed deterrent scheme has two key components: i) modifying existing spillway gate operations to block carp passage without further disrupting native fish (funded in ENRTF2012, and ENRTF2014); and ii) developing sound systems that can be added to locks to specifically deter carp. Together, a system (that might also include fish predators) that uses all of these components together with monitoring, appears to have great promise to stop carp in the Mississippi River; however, more laboratory development is still needed to identify the best type(s) of sound and system. Briefly, the Sorensen team has been testing types of sound deterrents in the laboratory (ENRTF2014) and has recently discovered that one sound, of several tested, has great and unexpected promise. It is a sweeping sound developed by Fish Guidance Systems Ltd. (FGS) which this company has developed over the course of 20 years and developed customized equipment to produce. We believe it would be wise to take advantage of this expertise and test it systematically and directly on invasive carps (which has not been done before). Further, our pilot lab tests now suggest this sound might also be enhanced by adding air curtains and lights, and native fish should be tested. Ongoing work is now examining the importance of temporal patterning of this sound through June 2017 (ENRTF2014) and has been more successful than we had expected and an extension would be enabling. Much of this work is best done in the lab. A new lab-based Activity #3 is proposed here to continue this important and extremely successful work as originally described in a March 2016 proposal and recommended for funding by the LCCMR using re-budgeted funds from ENRTF2012 as well as Phase III of this project, to enable proof-of-concept work in the lab. All proposed lab-based components of the March 2016 proposal (117-D) will be addressed, albeit in a modified order that is more efficient. Briefly, we will first explicitly examine the effect of adding an air curtain (which modifies the sound gradient) to the best sound to stop all species of carp. Next, we will test adding lights (also recommended by FGS) – an easy and inexpensive step that often works for fish in the field. Third, we will determine the possible impact this optimized deterrent system might have on at least two species of native fishes. Lastly, we analyze all data and assist the DNR and USACE with their work (ex. a possible feasibility study). All experiments proposed for this activity #3 will be conducted in the newly renovated MAISRC laboratory on the St. Paul Campus. Extending the FGS lease on their sound custom-built sound equipment will cost approximately \$30K. The results of these laboratory studies will provide immediate recommendations and guidelines for possible field testing.

Summary Budget Information for Activity 3:

ENRTF Budget: \$282,000
Amount Spent: \$0
Balance: \$282,000

Activity Completion Date:

Outcome	Completion Date	Budget
1. <i>Test an air curtain with sound to improve its ability to stop carps in the laboratory.</i>	January, 2018	\$73,000
2. <i>Test whether strobe lights enhance the efficacy of a sound-bubble system to stop carp in the laboratory</i>	June, 2018	\$72,000
3. <i>Test whether a sound-bubble-strobe light system impacts movement of at least 2 native fishes in the laboratory.</i>	January, 2019	\$69,000

4. Analyzing data from the lab assembling it into a Final Report for the LCCMR, MN DNR and USACE.	June, 2019	\$68,000
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Activity Status as of 1/30/2018:

Activity Status as of 7/30/2018:

Activity Status as of 1/30/2019:

Final Report Summary of 6/30/2019:

V. DISSEMINATION:

Description: The principal investigator will give at least one scientific and one public talk a year. The research associates and graduate student will give at least one scientific talk a year. We also aim to publish at least one peer-reviewed publication a year. Information will also be disseminated through MAISRC.

Status as of January 31, 2016:

Data are still be analyzed so we have not disseminated the findings yet.

Status as of July 31, 2016:

We gave one talk and published one peer-reviewed article:

Sorensen, PW and Johnson, N (2016). Theory and application of semiochemicals in nuisance fish control. Journal of Chemical Ecology DOI 10.1007/s10886-016-0729-4

Eichmiller JJ, Ghosal R and Sorensen PW. (2016) Detection of carp by environmental DNA and measurement of pheromones in induced aggregations. Minnesota Chapter of American Fisheries Society, Duluth, MN.

Status as of January 31, 2017

We gave a talk:

Sorensen, Zielinski (2016) The solution for Invasive carp. MAISRC Showcase.

Status as of July 31, 2017:

Sorensen gave a talk on carp deterrents that included these data at the Mississippi River Invasive Species Task Force meeting in Oklahoma, July 18, 2017.

Final Report Summary January 31, 2018:

VI. SUB-PROJECT BUDGET SUMMARY:

***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.**

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Explanation
Personnel:	\$333,347	1 P.I. (Sorensen, 2wks a year) to provide overall direction; 1 full-time Research Associate to serve as project manager (1/3 time) and conduct research on pheromones (Activity 1; 1/3 time) 1 part-time Research Associate (approximately 1/3 time) to conduct eDNA research (Activities 1 and 2); 1 part-time graduate student (approximately ½ time) to conduct food attractant research (Activity 2); 1 technician (approximately half-time) to provide technical help with all field research; Undergraduate help (part-time) to provide additional field help.
Professional/Technical Services and Contracts:	\$25,003	Professional advise form the University Statistics Clinic, Assess to mass spectrometry equipment at the University Mass Spectrometry Center (pheromones); Assess to University Sequencing center (eDNA); Misc. repairs to field and lab equipment. (no contracts)
Equipment/Tools/Supplies:	\$109,650	Radio and PIT Tags for locating carps, radio-tracking gear, field supplies, pheromones, eDNA supplies, ELISA reader for pheromone analysis, computer for field tracking data.
Capital Expenditures over \$5,000:	\$0	
Travel:	\$32,000	Travel to local field sites for common carp attractant studies (mileage); travel to field sites outside of Minnesota for Invasive bigheaded (Asian) carp studies (mileage, daily food, attend hotel); local meetings to share findings and acquire technical knowledge of local interest (ex. DNR); participate in national meetings to share and acquire information with Asian carp experts as appropriate
Other:	\$0	
TOTAL ENRTF BUDGET:	\$500,000	First Phase

Explanation of Use of Classified Staff: n.a.

Explanation of Capital Expenditures Greater Than \$5,000: n.a.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 5.25 FTEs (approx.)

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds

Non-state			
U.S.G.S.	\$117,700	\$49,700	Supports half of the salary of the graduate student during the academic year (only) when they are working on feeding cue identification (Activity 2).
State			
ENRTF2012 (activities 3,4,6)	\$713,849	\$447,849	Supports laboratory work and related, (supportive) eDNA work by the research associate doing the eDNA. Also supports about half of the salary of the graduate student during the academic year when they are working feeding cue identification in collaboration with the USGS.
TOTAL OTHER FUNDS:	\$831,549	\$497,549	

VII. SUB-PROJECT STRATEGY:

A. Sub-Project Partners: The U.S.G.S. is working with the Sorensen team to identify feeding cues for the silver and bigheaded carps. In a concurrent (but supporting) project the U.S.G.S. (Little team) is supporting the academic work of the graduate student and are assisting with test fish acquisition and field site support (to which we provide supplies). The MN DNR has been supportive of the project and has provided an older electrofishing boat at low cost (\$5500) to the Sorensen lab that we should be able use to catch carps, although some repairs and updating likely will be needed (yet to be determined). The DNR may fund additional components in the future and is providing permits and other assistance. The U.S. Army Corps of Engineers has been broadly supportive of our work by providing research sites and other research opportunities for us.

B. Sub-Project Impact and Long-term Strategy: It is the long-term goal of this project and related projects by the Sorensen team to produce a sustainable prevention strategy for invasive carps that also promotes the well being of native ecosystems and fishes in the Misississippi River. All effective AIS prevention programs have both a detection and a response component. This particular project develops the detection component while other work on lock and dams are the response component. We plan to have this strategy developed (after a possible Phase 2) within several years at which time it would be transferred to the MN DNR and its partners for implimentation

C. Spending History:

Funding Source	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13	M.L. 2013 or FY14
ENRTF M.L. 2009 Chp.143, Sec. 2, Subd. 6d.		300,000			
Ramsey Washington Metro Watershed District: \$207,600		207,600			
ENRTF M.L. 2012, Chp. 264, Art.4, Sec. 3 (for the MAISRC)				2,000,000	
ENRTF M.L. 2014, Chp. 226, Sec. 2, Subd. 04a					\$854,000

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): N/A

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH PROPOSAL: See attached research proposal

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than 1/31/2016, 7/31/2016, 1/31/2017, and 7/31/2017. A final report and associated products will be submitted between January 31 and March 31, 2018.