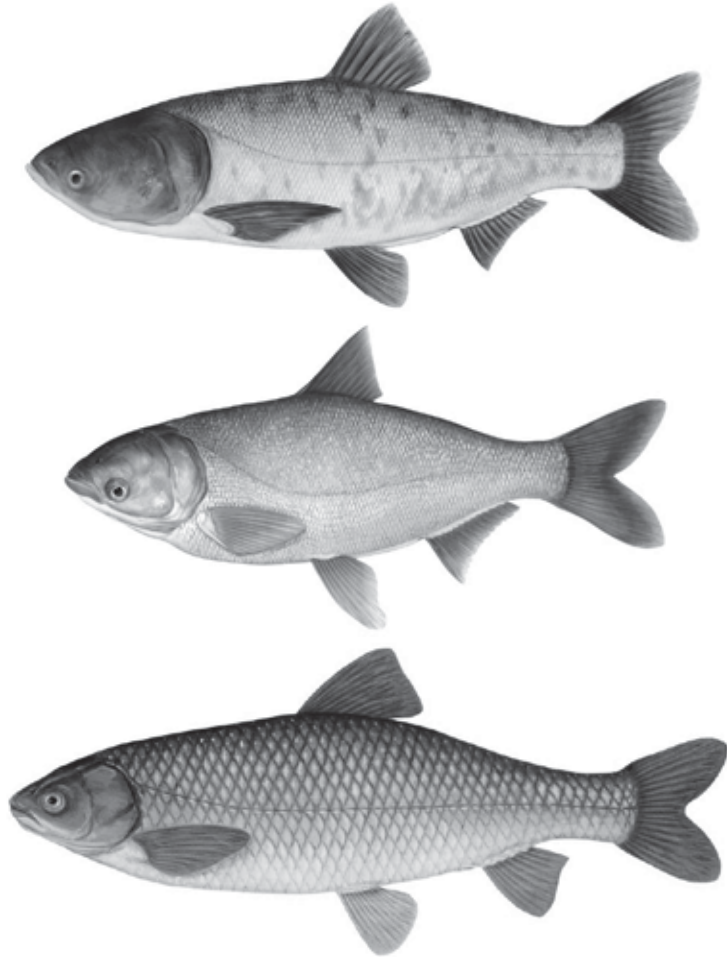


INVASIVE CARP SAMPLING REPORT
JANUARY – DECEMBER 2017
MINNESOTA DEPARTMENT OF NATURAL RESOURCES
DIVISION OF FISH AND WILDLIFE
SECTION OF FISHERIES



UPPER MISSISSIPPI RIVER, POOLS 1-5
LOWER ST. CROIX RIVER, BELOW ST. CROIX FALLS

March 20th, 2018

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Introduction

Bighead Carp *Hypophthalmichthys nobilis*, Silver Carp *H. molitrix*, Grass Carp *Ctenopharyngodon idella*, and Black Carp *Mylopharyngodon piceus* (hereafter collectively referred to as Invasive Carps) are invasive species currently found in the United States. These species were introduced into the United States during the early 1970's as aids in fish aquaculture operations (Henderson 1976). Subsequently, large flood events allowed these species to escape into the Mississippi River drainage, where they began reproducing and spreading (Freeze and Henderson 1982). Invasive Carps have migrated up the Mississippi River, and adjoining tributaries, quickly establishing populations in newly invaded areas. In Minnesota, Bighead and Grass carp have been collected in the Mississippi, Minnesota, and St. Croix rivers while Silver Carp have only been captured in the Mississippi and St. Croix rivers (Figure 1). Black Carp have never been collected in Minnesota or Wisconsin waters. Currently, there is no evidence of Invasive Carp reproduction in Minnesota waters.

Invasive Carps have the potential to devastate local ecosystems by competing with native planktivores and overcrowding other native species. With high fecundity and the ability to populate new areas quickly, Invasive Carps can reach high abundances, sometimes comprising most of the fish biomass in certain systems (MICRA 2002). Invasive Carps have a voracious appetite, and coupled with their large size (>70 pounds), have the ability to consume large amounts of food by filtering zooplankton, phytoplankton, and organic particles out of the water column (Jennings 1988; Smith 1989; Voros 1997). If Invasive Carp populations establish in Minnesota, native planktivores such as Paddlefish *Polyodon spathula*, Bigmouth Buffalo *Ictiobus cyprinellus*, Gizzard Shad *Dorosoma cepedianum*, and the larval stages of many other native fishes may be in direct competition with Invasive Carps for food resources. Evidence from the Illinois River suggests that competition with Invasive Carps resulted in reduced

condition factors for Bigmouth Buffalo and Gizzard Shad (Irons et al. 2007). Worldwide, introductions of Invasive Carps have led to declines in fish species diversity and abundances of commercially desirable species (Spatura and Gophen 1985; Petr 2002).

With the continuing progression of Invasive Carps up the Mississippi River, Minnesota waters are threatened by a potential invasion. A better understanding of the current status of individual Invasive Carp and populations in Minnesota will allow for more effective efforts to prevent their spread and/or eradicating them. Standard fish sampling assessments have been ongoing in Minnesota's major rivers and have the potential to catch Invasive Carps. However, the gears and methods used in the standard assessments are not the most efficient methods for capturing Invasive Carps. The purpose of this sampling effort is to use gears more specific to use carp-specific gears and techniques to monitor all life stages of Invasive Carps and associated native fishes in Minnesota waters.

Objectives

- Detect and monitor all life stages of Invasive Carps to:
 - o Inform management efforts in Minnesota; and
 - o Provide information for Upper Mississippi River managers on carp population changes in the presence front.
- Monitor native fish species that may be affected by the establishment of Invasive Carps.

Sampling Sites

In the Mississippi River, standard Invasive Carp sampling occurred in approximately 89 km of water from St. Anthony Falls Lock and Dam in Minneapolis, MN to Polander Lake in Pool 5A 5 near Winona, MN. In the St. Croix River, standard effort was focused on an 83 km

stretch from the dam near Taylors Falls, MN to the confluence with the Mississippi River near Prescott, WI.

Sampling Methods

Gears, methods, and targeted locations were derived from personal communications with biologists who have been sampling Invasive Carps (V. Santucci, Illinois Department of Natural Resources, personal communication; J. Lamer, Western Illinois University, personal communication) and conducting research on the most efficient gears to sample Invasive Carps (M. Diana, Illinois Natural History Survey, personal communication), literature review of sampling techniques and habitat preferences (Lohmeyer and Garvey 2009; Williamson and Garvey 2005; Dettmers et al. 2001; DeGrandchamp et al. 2007; Kolar et al. 2007; DeGrandchamp et al. 2008; Wanner and Klumb 2009; ACRCC 2012), and experience from prior field seasons. Sampling information for Invasive Carps included in this report took place between January 1, 2017 and December 31, 2017.

Commercial Fishing

Commercial fishermen were contracted to target Invasive Carp with gill nets and seines. Minnesota Department of Natural Resources (MN DNR) personnel accompanied contracted commercial fishermen to direct sampling locations and monitor efforts. The number of fish caught by species was estimated during gill netting operations and total weight harvested was requested from the commercial fishermen for both gill netting and seining operations.

Invasive Carp Acoustic Tagging and Tracking

In Minnesota, Statute 84D.05, Subdivision 1 stated “A person may not possess, import, purchase, sell, propagate, transport, or introduce a prohibited invasive species.” In 2017, the

legislature passed and the governor signed an amendment to this statute: *Subd. 1a. Permit for invasive carp. The commissioner may issue a permit to departmental divisions for tagging bighead, black, grass, or silver carp for research or control. Under the permit, the carp may be released into the water body from which the carp was captured. This subdivision expires December 31, 2021.* As part of the permitting process, DNR fisheries developed a protocol to characterize and minimize potential risk while maximizing the amount of information gained (See MN DNR “Permit for DNR Divisions to tag and release invasive carp”). For further information regarding the tagging and tracking procedures, please see the permit issued by the Department of Natural Resource’s Division of Ecological and Water Resources.

Based on the tagging results, MN DNR staff will gain a better understanding of movement patterns and habitat preferences, while posing a very low risk to native fish populations or risk of increasing Invasive Carp populations. Other states have already begun work of this nature in riverine environments and have shown significant results and ability to remove additional fish with this tagging method. This information will be used to inform sampling and removal efforts.

The DNR was permitted to tag one or two Invasive Carp at a time with acoustic tags. The DNR utilized both passive telemetry (a stationary receiver array already in place) and active tracking (using a portable receiver) to determine preferred habitats, longitudinal movement patterns, depth preferences, and specific locations for capture efforts.

The state of Minnesota has a total of 88 receivers from above the Coon Rapids Dam to Lock and Dam 5 in the Mississippi River, from the Mississippi River confluence at Prescott, WI to Taylor’s Falls in the St. Croix River, and from the Mississippi River confluence to the County Road 6 bridge north of Delhi, MN, in the Minnesota River (river mile 209). 66 receivers are

maintained by the East Metro office, eleven are maintained in the Minnesota River by the Hutchinson office (from river mile 18.7 to river mile 209), and eleven are maintained by the Lake City office in the Chippewa River and Pools 4 and 5 of the Mississippi River. In addition, the U.S. Fish and Wildlife Service maintains seven receivers in Minnesota waters and 47 additional receivers that extend downstream to Pool 19 near Keokuk, IA. Additional receivers are maintained by other states and universities that include, but are not limited to, 11 receivers maintained by the Missouri Department of Conservation from Pool 19 to the confluence with the Ohio River.

By tagging one or two Invasive Carp, we hope to capture additional Invasive Carp if they are present. Recapture actions will be taken, including the use of commercial fishermen, when tagged fish are in jeopardy of being un-trackable due to tag life nearing completion, leaving the passive array footprint, or to support removal of other conspecifics. The DNR will take all reasonable measures to ensure all tagged fish are tracked and their locations known through active tracking and an extensive passive tracking network. Comprehensive removal efforts will be employed to remove tagged and un-tagged fish from Minnesota waters.

The impacts of releasing wild-caught Invasive Carp back into the wild have been considered and are believed to be minimal when compared to the potential information gained from this project. As outlined in this report, MN DNR maintains an extensive monitoring and removal program to ensure populations are adequately sampled and document if reproduction is occurring in Minnesota waters to provide accurate information for Upper Mississippi River managers on carp population changes in the presence front. Finally, Minnesota is remaining conservative with only one or two fish permitted to be tagged and released at a time, with all other Invasive Carp euthanized.

Pool 2 Stable Isotope Analysis

With the financial support of the Minnesota Environment and Natural Resources Trust Fund (ENRTF) from the Legislative-Citizens Commission on Minnesota Resources (LCCMR), during the 2017 field season samples were collected from Pool 2 of the Mississippi River for Carbon (C^{13}) and Nitrogen (N^{15}) stable isotope analysis. The purpose of this project is to use stable isotope analysis to examine the aquatic food web within Pool 2 and provide baseline trophic data before Invasive Carp establishment. All Invasive Carp caught were to be euthanized and processed according to state sampling protocols and a pelvic fin clip was taken to determine trophic niche overlap with native fish to better understand the potential for management of native fish to control the spread of Invasive Carp and retain biological diversity.

Our goal was to collect 30 samples per species/species group in lower Pool 2. The sampling area will focus downstream of River Lake and extend to Lock and Dam 2 near Hastings. Samples were dried (using Cabela's Pro Series Dehydrator), stored in vacuum seal bags, and frozen. Samples were originally sent to the University of Minnesota for pre-processing and analysis; however, the mass spectrometer had problems which could not be repaired in a timely fashion. As a result, the contract with the University of Minnesota was nullified and samples were returned to the MN DNR. After completing a competitive bidding process, MN DNR contracted the pre-processing and analysis of these samples with Cornell University and are awaiting results at the time of publication.

Baseline samples were collected from zebra mussels, crayfish, snails, phytoplankton, zooplankton, invertebrates, detritus, leaves, and submerged aquatic vegetation during the summer (May-July). For mussels only zebra mussels were sampled, with 30 samples collected. Crayfish and snails (dominant species of each) were collected from hand picking or grab samples

with 30 samples collected. A 63 micrometer plankton tow net and 500 micrometer plankton trawl net were used to collect phytoplankton and zooplankton samples. Zooplankton (Cladocerans and Rotifers) were allowed to evacuate their gut contents in refrigerated water, separated, and dried. The most abundant species of primary consumer invertebrates were dried and analyzed (which will likely include a Scrapper/Shredder (nymph mayfly), a Collector/Gatherer (Chironomid), and a Filter Feeder (nymph caddisfly, Hydropsychid)). In addition, 30 samples were collected for each of the 3 most abundant predatory invertebrate species. Thirty samples of detritus and leaves were collected throughout the sampling season. Submerged aquatic vegetation (curly-leaf pondweed, Eurasian watermilfoil, and Vallisneria) were collected and the samples will be pooled based on the most abundant species per site.

Fish were collected using a variety of gears with length and weight information collected for all sampled individuals. Fish sampling focused primarily during the fall (August-October). Predator fish species included Flathead Catfish, Channel Catfish, Sauger, and Walleye. Prey species included Gizzard Shad [*both large (individuals 1 year old or older, > 280 mm) and small size individuals (young of the year individuals or < 280 mm)], Emerald Shiners, Bluegill [both large (>90 mm) and small size (< 90 mm) individuals], Silver Redhorse, Shorthead Redhorse, and Freshwater Drum. For Gizzard Shad, age-at-length determinations were based on previous research conducted by East Metro Fisheries staff from Pool 2. Other species analyzed included all Invasive Carp (Silver, Bighead, or Grass Carp) and Paddlefish, as well as 30 Common Carp, Bigmouth Buffalo, and Smallmouth Buffalo. Fish, excluding Invasive Carp, had a fin clip taken, and released alive. For all fish species (except Bluegill, Gizzard Shad, Paddlefish, and Invasive Carp) individuals sampled were tallied using length frequency bins to

best achieve a wide range of lengths to determine if differences exist in trophic signature among size classes for each species.

Larval Trawling

Larval trawling was conducted in the Mississippi River Pools 2 and 3 and the St. Croix River to target early life stages of Invasive Carps. Eight standardized sites were sampled in Pool 2 (Figure 2), the St. Croix (Figure 3), and Pool 3 (Figure 4). A bow mounted ichthyoplankton net (0.75 m x 3 m) consisting of 500 um mesh was pushed near the surface into the current so that the velocity of the water entering the net is between 1.0 to 1.5 m/s. At sampling locations where no water current exists (e.g. backwaters), sampling occurred towards a random direction that allowed for a complete sample to be taken in a relatively linear path. A mechanical flow meter was placed in the mouth of the net to determine the volume of water sampled. In 2017, water quality measurements were collected for all samples including surface temperature, dissolved oxygen, conductivity, and pH. A total of eight locations were sampled in each standardized system with two, 5-minute pushes conducted at each location. In Pool 2, Pool 3, and the St. Croix River, sampling sites were located in the following macro habitats: 4 main channel, 2 side channel, and 2 backwater locations in each system. For all samples, contents were placed in containers labeled with sample location, name of the water body, and date, and preserved. For preservation, samples were placed into 10% buffered formalin for 24-48 hours and then the formalin was removed and replaced by 90% alcohol. All samples were sifted to remove all excess material, with only eggs and fish kept. Fish and eggs were examined to determine if any Invasive Carp species were collected and to identify specimens to the lowest possible taxonomic level. Samples were also sent to an external researcher for verification and to create a reference collection of the species caught for future reference. Sampling site locations, sampling dates,

gear description, effort, habitat type (main channel border, backwater, wing dike, etc.), water depth, and crew details were recorded for each site.

Buffalo Tagging

This study will provide information on population dynamics and movements of Smallmouth Buffalo and Bigmouth Buffalo in Pool 2 of the Mississippi River. From the literature and previous experience, Bigmouth Buffalo are often found with Silver and Bighead Carp. As a result this species is being studied to serve as a surrogate for tagging additional Bighead or Silver Carp.

Beginning in the spring of 2015, Buffalo sampled in the Mississippi River Pool 2 have been collected primarily from large mesh gill net and seine commercial fishing operations, as well as standard large mesh gill nets, trammel nets, and electrofishing have been tagged. Buffalo are tagged externally with a yellow Floy t-bar tag, along with a secondary mark by removing one pelvic fin ray. This allows recaptured Buffalo to be identified for as long as the tags are retained (>4 years) and to assess tag retention. Pelvic fin rays are being used for ageing purposes to determine variation in movement patterns by age as well as validate ageing techniques by re-ageing recaptured fish using the original fin when the fish was tagged and the fin clip when the fish is re-captured in subsequent years. To date, no known studies have validated Buffalo ageing techniques.

As one of the United States' most prolific and valuable freshwater commercial fisheries, it is also imperative that fisheries managers develop management plans and quotas to ensure populations are sustainably harvested and do not become overfished. Further, Buffalo are native to the United States, occupying a distinct ecological niche that may ultimately be filled by

Invasive Carp species should Buffalo populations become overfished. Bighead and Silver Carp have adverse effects on all life stages of native fish species because they feed on plankton, the primary food source of several adult fish (Irons et al. 2007), all larval fish (Schrank et al. 2003), as well as all mussel species (Kolar et al. 2007) creating cascading trophic effects throughout the food web. The full impacts of Invasive Carp, should they become established in Minnesota, cannot be well documented without this biological data from commercial fish species.

Electrofishing

Electrofishing occurred in a variety of habitats including backwaters, side channels, main channel borders, and over wing dikes. Sampling locations consisted of 8 standardized sampling locations in Pool 2 (Figure 2) and the St. Croix River (Figure 3), and all other sampling events occurred at non-standardized locations in the aforementioned habitats at the discretion of the sampler. Standardized sampling locations were selected based on habitats Invasive Carps are likely to occupy and are 1/3 mile (500 meters) in length. At these set sampling locations, all observed fish were collected, identified, measured and weights and ageing structures were taken from fish included in the age and growth analysis. If positive identification was not possible, voucher specimens were kept, labeled, and preserved in 90% ethanol for later identification. At non-standardized sampling sites, fish were identified in the water and only fish needed to collect ageing structures and Invasive Carps were collected. This reduced unnecessary processing time and allowed for greater sampling effort. Sampling site locations, sampling dates, gear description, effort, habitat type (main channel border, backwater, wing dike, etc.), water depth, and crew details were recorded for each electrofishing run.

Trap Netting

Trap netting was conducted on Pool 2 of the Mississippi River in 2017 using standard and mini-fyke nets. Trap netting was not conducted in the St. Croix River this field season. The mini-fyke nets consist of a double frame (27 in. x 39 in.), 4 hoops (2 ft.), a single throat, and a 25 ft. lead, with a square mesh size of 0.125 in. throughout. All fish were identified and enumerated in the field.

Seining

A small 35 foot seine was proposed to be used to sample shallow water habitats for young fish from June through September on Pool 2 and the Minnesota River; however no small seine hauls were completed in 2017 due to high waters and high juvenile fish catch in trap nets. The seine measure 35 ft. long and 6 ft. deep with 3 ft. square bag (3 ft. x 3 ft. x 3 ft.) located at the center of the net, consisting of "Ace"-type nylon netting 1/8 in. mesh, with a mudline.

Fish Tagging Efforts

Currently several species of fish in the Mississippi River Pool 2 and the St. Croix River are tagged according to study guidelines as part of ongoing tagging studies when encountered. These species included Flathead Catfish *Pylodictis olivaris*, Channel Catfish *Ictalurus punctatus*, Smallmouth Buffalo *Ictiobus bubalus*, and Bigmouth Buffalo in Pool 2. In the St. Croix River, Lake Sturgeon *Acipenser fulvescens*, Muskellunge *Esox masquinongy*, White Bass *Morone chrysops*, Flathead Catfish, and Channel Catfish are being tagged. In both Pool 2 and the St. Croix River, Paddlefish are also tagged.

Age and Growth Analysis

In 2017, age and growth analyses were limited to Smallmouth Buffalo and Bigmouth Buffalo. Bigmouth Buffalo are native planktivores that may be in direct competition with Bighead and Silver Carp. Smallmouth Buffalo, as well as Bigmouth Buffalo, are commercially important and a better understanding of these species will be useful to determine effects from commercial fishing and/or the presence of Invasive Carp. For the previously mentioned species, lengths, weights, and ageing structures were collected as follows: for fish less than 300 mm, up to 5 individuals in each 10 mm length group and for fish 300 mm and greater up to ten individuals in each 25 mm length group. For Smallmouth and Bigmouth Buffalo, pelvic fin rays were extracted and compared. During the 2015, 2016, and 2017 field seasons, nearly 4,000 Smallmouth and Bigmouth Buffalo (1,882 Smallmouth Buffalo and 2,182 Bigmouth Buffalo) have been tagged with Floy tags and their pelvic fins were removed for ageing and to validate ageing analyses using re-captured fish in the future as part of another study. Fin rays were dried and cut using a low-speed isomet saw. Two independent readers counted each opaque band as an annulus under a dissecting microscope, using both reflected and transmitted light sources. If counts differed between readers, the readers re-examined the structure independently a second time. If readings differed the second time, the readers conferred until a consensus was reached. The results of this study are presented in an annual MN DNR tagging report.

Gill and Trammel Netting

Gill netting and trammel netting occurred during multiple sampling events on each system. Large mesh gill nets of depths from 8 to 24 feet and lengths of 150 to 300 feet with square mesh sizes of 4 to 6 inches were used to target adult Invasive Carps. Trammel nets with outside wall square mesh sizes of 14 inches and inner square mesh sizes of 4 inches were also

used to target adult Invasive Carps. Experimental gill nets 250 feet in length and 6 feet deep consisting of 50 foot complements of net with square mesh sizes 0.75, 1, 1.25, 1.5, 2 inches were used to target juvenile Invasive Carps. Nets were set either short-term or overnight, with short-term sets favored when water temperatures were greater than 60° F. All fish caught were identified and measured.

Invasive Carp Blood Hormone Analysis

November 29 through December 1, 2016 Minnesota DNR personnel traveled to Illinois to work with Western Illinois University researcher Dr. Jim Lamer along with commercial fishermen to track acoustically tagged Silver and Bighead Carp in Pools 17 through 20. In addition to gaining experience tracking tagged Invasive Carp, MN DNR staff were able to collect 61 blood samples to determine if hormone levels in blood could be used to determine the sex and maturity of fish without lethal analysis. Ten males and ten female Silver and Bighead Carp were collected and nine male and ten female Grass Carp were collected. Approximately 10 ml of blood was drawn for plasma hormone analysis in the field and placed in a Vacutainer tube with heparin. Surgical incisions were made in the field to validate sex and reproductive status of individuals. Blood samples were immediately put on ice and centrifuged within 12 hours. Plasma was extracted from centrifuged samples and plasma was then placed in a deep freezer until analysis.

Plasma sex steroids, including estradiol, testosterone, and 11-ketotestosterone, regulate spawning behavior and gonad development in many fish species. Concentrations of these sex steroids follow predictable cycles that have been used to accurately determine sex and reproductive status of many fish species. This research will help determine if estradiol, testosterone, and vitellogenin could be used to determine sex and reproductive condition in

Silver, Bighead, and Grass Carp. If this proves to be effective, it will also be used to test temporal changes in sex steroid concentrations to identify potential spawning dates.

Analyses of these samples were analyzed by Dr. Joshua Lallaman at St. Mary's University in Winona, MN. Overall differences in mean plasma hormone concentrations between sex and reproductive condition will be tested using a 2-Factor ANOVA and a stepwise-discriminant function analysis will be used to determine if sex and spawning condition could be correctly classified by hormone concentrations. If this research shows potential for determining sex and maturity, Invasive Carp caught in Minnesota will also be sampled in this manner for confirmation of this method.

Results and Discussion

Sampling Results

In total, 90 days were spent sampling between January and December 2017 on the Mississippi River Pool 1, 2, 3, 4, and 5, and the St. Croix River with gears appropriate for sampling Invasive Carps (Table 1)(Figure 5). A greater amount of effort was focused on Pool 2 and the St. Croix River, because Invasive Carps were found above Lock and Dam 2 on the Mississippi River in 2014 and due to the finding of multiple Bighead Carp at the Allen S. King Plant discharge on the St. Croix in 2015. In 2017 there was also an increase in field sampling off the Minnesota River due to a Bighead Carp captured near Redwood Falls in June 2017 and previous captures.

In 2017, a total of 3 Bighead Carp were caught in Minnesota waters and Wisconsin boundary waters (Table 2). One mature female Bighead Carp was collected near the confluence of Pool 3 and the St. Croix River at Point Douglas by commercial fishermen on March 10, 2017. In addition, the first Silver Carp captured on the St. Croix River was collected in this commercial

seine haul at Point Douglas. On March 10, 2017 and April 2, 2017 in two separate seine hauls, two mature male Silver Carp were caught at Point Douglas. Though this is the first capture of Silver Carp on the St. Croix River, these fish were found very close to the confluence with the Mississippi River and Silver Carp have been captured further upstream on the Mississippi River in 2014 when two were caught in Pool 2 in Lower Grey Cloud Slough. On April 11, 2017 in a contracted commercial gill net one mature male and one mature female Grass Carp were caught in Pool 2 in Lower Grey Cloud Slough.

On June 4, 2017 a mature female Bighead Carp was shot by a bowfisherman in a small gravel pit off the Minnesota River near Redwood Falls, MN. This represents the furthest upstream this species has ever been observed in the Minnesota River. The bowfisherman called the MN DNR Invasive Carp reporting phone number and the fish was picked up on June 5th. As a result of this capture, MN DNR staff worked with land owners to prepare a plan to survey the property and subsequently sampled these pits using over 3,000 feet of large mesh gill nets on June 9th.

A larger, collaborative effort took place on June 16th. DNR fisheries crews from East Metro and Hutchinson offices set another 3,000 feet of large mesh gill nets in the pits, electrofished within the pits and nearby sections of the Minnesota River, and set 12 hoop nets in the Minnesota River near Granite Falls, MN. The U.S. Fish and Wildlife Service collected 66 eDNA samples in the pits and electrofished on the river near Granite Falls, MN. No additional Invasive Carp were caught in these efforts and all eDNA samples collected tested negative for Invasive Carp DNA. Otoliths were shipped to Dr. Greg Whitledge at Southern Illinois University of microchemistry analysis. Otolith microchemistry results suggest that this fish was born in an environment inconsistent with the Mississippi, St. Croix, or Minnesota rivers and was most

recently also inhabiting existing areas inconsistent with the Minnesota River. Based on these results, we are still unsure where this fish lived and how long it had been within these gravel pits.

Lastly, on July 28, 2017 a Bighead Carp was caught by MN DNR staff at the Allen S. King plant on the St. Croix River as part of routine monitoring of this area. This was the first Invasive Carp to be tagged with an acoustic transmitter and released in Minnesota. Preliminary results of this tagging can be found below.

Contracted commercial fishermen were hired to use large mesh gillnets and seines to sample in the Mississippi River in Pools 2, 3 near Red Wing, Pool 4 near Lake City, and Pool 5A near Winona, and in the St. Croix River from Lake St. Croix to the confluence with the Mississippi River near Prescott, WI. Contracted commercial fishermen set approximately 57,600 feet of gill nets during eight days of effort and conducted four seine hauls between January and December 2017. Gill nets were set short term (2-3 hours) and fish were chased towards the net with boats, typically in large backwater areas. In 2017, nine regular commercial fishing operations were also monitored for the presence of Invasive Carp.

Larval trawling was conducted for 143 total trawls during 10 days by the Invasive Carp fisheries personnel. All samples were sifted by Invasive Carp fisheries personnel and all samples with fish larvae or eggs are preserved and have been sent to Colorado State University for expert analysis to determine the species caught and their respective number.

Both random and standardized electrofishing sampling was conducted on Pool 2 of the Mississippi and the St. Croix rivers. A total of 2,078 minutes of “on time” over 29 days were spent electrofishing between January and December 2017. In 2017, 5 standardized electrofishing sites were sampled once for a total of 115 minutes. Random electrofishing was used to monitor

for Invasive Carp and for collection of individuals for age and growth and stable isotope analyses.

Trap netting was conducted using fyke nets for a total of thirty net nights on Pool 2 of the Mississippi River. All fish were counted and measured in mini fyke nets, except Emerald Shiners *Notropis atherinoides* and Common Carp *Cyprinus carpio* due large numbers captured.

Gill nets and trammel nets set by MN DNR personnel were often used to sample behind wing dikes and in smaller side channel and backwater areas where it wasn't feasible for commercial fishermen to target with their larger operations. In 2017, a total of 33,400 feet of gill and trammel nets were set in Pool 2 and the St. Croix River during 24 days, with most net sets being short-term sets (2-5 hours).

Numerous unique or rare native fishes worth mentioning were encountered during these sampling events. Of note for 2017, four Crystal Darters were collected on the St. Croix River near Taylor's Falls in June in one day, and the first confirmed collections of Brassy Minnow *Hybognathus hankinsoni* and Hornyhead Chub *Nocomis biguttatus* on the St. Croix River and Central Mudminnow *Umbra limi* and Silver Chub *Macrhybopsis storeriana* on Pool 2 for the Invasive Carp monitoring program. Also, from past experiences and in conjunction with tracking the tagged Bighead Carp, a large number of Paddlefish were caught and jaw and acoustic tagged as part of other MN DNR studies. A complete species list of species caught and observed on Pool 2 and the St. Croix River from January 2013 through December 2017 has been compiled (Table 3).

Determining if invasive carp seen in Minnesota are pioneering individuals or are indicative of a population is a key question for managers. While it is likely there are additional Invasive Carp present in the Minnesota waters of the monitored rivers, the level of effort

invested and resulting capture data support the hypothesis that the carp currently present are individual, wandering adults and not part of a population.

Invasive Carp Acoustic Tagging and Tracking

On July 28, 2017 during routine monitoring at the Allen S. King Plant on the St. Croix River, a Bighead Carp was caught by MN DNR staff in a large mesh gill net. The fish was then tagged using a VEMCO V16TP-6H (Vemco Ltd., Nova Scotia, 69 kHz) continuously transmitting acoustic tag containing sensors to measure pressure (depth) and temperature transmitting every 60 seconds on average (minimum transmission delay of 30 seconds, maximum delay of 90 seconds) and released. This fish was actively tracked using a VEMCO VR100 every day for a week after release, followed by actively locating the fish once a week every week until September 5, 2017. After September 5, 2017 the fish was located routinely until the last day in the field on November 20, 2017. In addition, this fish was routinely identified and data recorded by the passive VR2W receiver array in place, with the last VR2W downloaded on November 20, 2017 and the last detection using active tracking and the VR100 occurring on November 27, 2017. Details of when and where this fish were located can be found in Figure 6. Over this time frame, we received 30,388 data points from the VR2W array up until November 20 (115 days) and 319 data points from active tracking with the VR100 up until November 27 (122 days).

This fish was observed to range over an extent of 17.6 river miles from Stillwater, MN to the mouth of the Kinnickinnic River (Figure 6). Over the course of this first field season, several areas of recurring use were observed including near the King Plant during July and August, around Afton State Park in August and September, and Lake St. Croix in October, 2017 through February, 2018.

From the temperature and depth data, it appears this fish comes to the water's surface more often and inhabits a wider range of depths, 0 to 68.6 feet, than commonly believed (Figure 7) and tolerates temperatures ranging from 84 to 35.4 degrees Fahrenheit (Figure 8). On two occasions, the temperature the Bighead Carp was experiencing was colder than the Stillwater USGS gauge data, occurring on September 15 through 18 and again on September 24 through 26. During these times, the fish was inhabiting the area from the Kinnickinnic to Catfish Bar off Afton, MN. This time was associated with deep dives including the deepest dive observed over the study period to 68.6 feet. Before these colder periods, the fish was occupying habitats further upstream including close to Catfish Bar and even Lake St. Croix, inhabiting the area around Afton State Park during this period, and afterward returning back upstream to Catfish Bar and Lake St. Croix.

Recapture efforts occurred in November prior to ice up. Over 2 number days, 7250 feet of gillnet, 1 hour of electrofishing, and 12000 feet of large mesh commercial gill nets were deployed to recapture the fish. The fish was not recaptured. The Bighead Carp was suspended 20-30 feet below the surface which proved difficult to sample when the depth ranged from 50-60 feet deep in the area. Standard gill nets reach less than 25 feet from the surface or bottom and even commercial seines only reach 30-40 feet of water. From this experience, MN DNR has purchased additional 24 feet deep large mesh gill nets to better sample the Lake St. Croix and deeper St. Croix River habitats. The DNR is also exploring the purchase of a commercial sized purse seine. A second recapture effort will take place in spring 2018 after ice out.

From this tagged Invasive Carp, we have learned of additional areas where this fish has resided for prolonged periods of time including a potential overwintering site. Based on information from other areas tracking carp and historic sightings in Minnesota, the hypothesis

was that this fish would inhabit the King Plant discharge periodically with forays to Lake St. Croix and overwinter near a point where flow is constricted on the river with the most likely location being at Point Douglas, near Prescott, WI. From the tracking data collected, the fish was never observed within the King Plant discharge again despite continued monitoring within the discharge. From July to October, the fish was observed to inhabit a much wider spatial range but did exhibit some site fidelity, inhabiting several key locations for prolonged periods of time. These locations will be sampled extensively during the 2018 field season to determine if other Invasive Carp also use these areas and if this tagged Bighead Carp can reveal the locations of other Invasive Carp using the “Judas fish” technique. Finally, data from October through the last detection in November indicates this fish overwintered in Lake St. Croix, occupying deeper habitats than originally hypothesized. It is hypothesized that the fish will move to shallower water in the spring.

Based on the findings of 2017, tracking methods will be adjusted accordingly for 2018. MN DNR staff will continue to track tagged fish and analyze the data to increase sampling efficiencies.

Pool 2 Stable Isotope Analysis

During May, June, and July baseline samples were collected in Pool 2 to determine the bottom of the food chain and what important fish species consumed over the course of the summer. Baseline samples included algae, vegetation, detritus, phytoplankton, zooplankton, micro- and macro-invertebrates, crayfish, snails and mussels. In addition a few opportunistic samples of larval fish, fly larvae, and water scorpions were also collected. Overall, most species were collected as planned with several species absent from collection sites despite intense sampling (Table 4).

During August and September fish were collected and pelvic fin samples were collected to determine higher trophic levels. For all primary species, 30 samples were collected for each site to allow for comparison. Similar to baseline samples, most species were collected as planned with several species absent from collection sites despite intense sampling. As expected Bighead Carp, Silver Carp, Grass Carp, and Paddlefish were rare though several individuals were collected outside the study area (Table 5).

Mass spectrometry was contracted with the University of Minnesota for Carbon (C^{13}) and Nitrogen (N^{15}) stable isotope analysis. Due to problems with the mass spectrometer, the University of Minnesota could not complete analysis and samples were subsequently sent to Cornell University. Results are still pending.

Invasive Carp Blood Hormone Analysis

Overall, a discriminant function analysis was only able to categorize 50% of the samples correctly. This indicates that there was not good separation of genders based on the hormone data. There are some similar patterns in female data (all having high vitellogenin), but variable estradiol levels.

In general, the male data was very different between samples. Mature male Bighead Carp had high vitellogenin levels, whereas mature male Grass Carp had low vitellogenin levels. In Bighead Carp, immature males were the only group that completely separated from the mature males and female Bighead Carp. Gravid female Bighead Carp exhibited the widest range in estradiol values, while mature males showed the widest range in vitellogenin levels. In Grass Carp, mature males were the only group that completely separated from the immature males and female Grass Carp. Gravid female Grass Carp showed the widest range in both estradiol and vitellogenin levels. In Silver Carp, the majority of samples overlapped in hormone levels

between mature males and mature females. Male Silver Carp showed the greatest range in vitellogenin levels while females showed the greatest range in estradiol levels (Figure 9).

This project was conducted in the winter of 2016 when presumably hormone levels are low in both male and female Invasive Carp. The technique shows promise to determine sex and maturity from these three species; however, high variability among individuals only allows discrimination at approximately 50%. Based on this data, future prediction of sex and reproductive status from plasma samples alone would be difficult. Due to the timing of this preliminary study, it is likely that hormone levels were suppressed and would show a different pattern in spring and summer seasons when fish are undergoing physiological changes associated with spawning. Another round of validation samples would be beneficial to see how they would compare to late fall/winter samples collected in this study. It is possible there is too much variability in hormone concentrations between males and females and this technique would not be effective; however, we believe this warrants another trial. It would be of interest to conduct this study again in the summer to determine if sex and maturity can be better discerned during this time of year. In addition, recent research has shown that genetic markers have been developed to discern sex from fin clips from farm raised Silver and Bighead Carp in China (Liu et al 2018). Additional genetics samples will be collected from future caught fish in Minnesota to determine if this technique can be validated and used.

Recommendations

Continued monitoring and removal of Invasive Carp from Minnesota waters is recommended for the foreseeable future. This project is funded in part by the Minnesota Environment and Natural Resources Trust Fund through June 2020; however, it is recommended this project continue beyond that date to ensure Invasive Carp do not establish populations or if

they do, adequately document the effects of Invasive Carp to native fish populations. Further, it is recommended that Invasive Carp acoustic tagging continue and once the technique is proven it is recommended that the project expand the number and duration of tagged fish at liberty in Minnesota waters. At this time, per the permit, only two Invasive Carp will be at liberty at any given time.

Further age and growth analysis as well as population dynamics validation (including fecundity and recruitment) is recommended for commercially valuable Bigmouth and Smallmouth Buffalo, which may be in direct competition for food resources with Invasive Carps. In some states, current Invasive Carp population control efforts include increasing commercial fishing effort to decrease Invasive Carp abundance, although increased commercial effort in Minnesota would potentially negatively affect native species. Resource agencies would benefit from a greater understanding of the population dynamics of our commercially important native fishes. In addition to age and growth analyses, over 4,000 Bigmouth Buffalo and Smallmouth Buffalo have been tagged in Pool 2 during 2015, 2016, and 2017 as part of a study investigating movement, exploitation, age and growth, and other key population dynamics of these commercially important species. It is recommended that this tagging project continue to better understand movement patterns and approximate the numbers of individuals present in the Pool 2 of the Mississippi River via mark-recapture techniques.

Paddlefish are another native planktivore that may compete for food resources with Invasive Carps and therefore may be negatively affected. Currently, Paddlefish are a threatened species in Minnesota and populations across their range have suffered due to commercial navigation projects that impede movement and alter habitats, pollution, and overexploitation (Jennings and Zigler 2000). If Invasive Carps become established in Minnesota rivers, local

Paddlefish populations would be further stressed. Being a state threatened species, non-lethal means of studying Paddlefish populations are also recommended including continued tagging of encountered Paddlefish using jaw and acoustic tags. Further effort should also be used to encourage boaters to report any deceased paddlefish for age and growth analysis and other MN DNR offices should collect all deceased Paddlefish for analysis.

Acknowledgements

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Sampling and support from the U.S. Fish and Wildlife staff was crucial to the response that occurred in the gravel pits following the June 4th Bighead Carp capture. In addition, otolith microchemistry results were provided by Dr. Greg Whitley at Southern Illinois University.

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Tables

Table 1. Invasive Carp sampling summary for the Mississippi River Pools 1, 2, 3, 4, 5 and the St. Croix and Minnesota Rivers for January through December 2017. Number of Invasive Carp Captured represents the number of individuals caught by MN DNR, contracted commercial fishermen, or monitored commercial fishing. One Bighead Carp was also caught by a bowfisherman off the Minnesota River in June 2017.

Invasive Carp Sampling Summary January – December 2017			Days
Random Sampling Effort			
Gill/Trammel Netting	33,400	feet	24
Electrofishing	1,963	minutes	25
Trap Netting	30	net/nights	6
Standardized Sampling Effort			
Electrofishing	115	minutes	4
Larval trawling	143	trawls	10
Targeted Commercial Fishing Effort			
Gill Netting	57,600	feet	8
Seining	4	hauls	4
Monitored Commercial Fishing Effort			
Seining	9	hauls	9
Number of Invasive Carp Captured			
	6	fish	
Total Number of Days Sampled			90

Table 2. Invasive Carp caught from January through December 2017 in Minnesota and Wisconsin boundary waters.

Date	Species	Water Body	Location	River Mile	Length (mm)	Weight (grams)	Sex	Maturity	Capture Method	Age
3/10/17	Bighead Carp	St. Croix River	Point Douglas	812	1161	20000	F	M	Monitored Commercial Seine	10
3/10/17	Silver Carp	St. Croix River	Point Douglas	812	836	6100	M	M	Monitored Commercial Seine	9
4/2/17	Silver Carp	St. Croix River	Point Douglas	812	825	6050	M	M	Monitored Commercial Seine	7
4/11/17	Grass Carp	Pool 2	Lower Grey Cloud Slough	821	987	14000	F	M	Contract Commercial Gill Net	9
4/11/17	Grass Carp	Pool 2	Lower Grey Cloud Slough	821	941	10500	M	M	Contract Commercial Gill Net	9
6/4/17	Bighead Carp	Minnesota River	Near Redwood Falls	844+216	1210	2800	F	M	Bow Fisherman	11
7/28/17	Bighead Carp	St. Croix River	Allen S. King Plant	812+20	1086	1700	NE	NE	Gill Net	Unknown

Table 3. Species list for the Mississippi River Pool 2 and the St. Croix River from January 2013 through December 2017, including 78 native and invasive species.

Common Name	Genus Species	Pool 2	St. Croix River
American Eel	<i>Anguilla rostrata</i>	x	
Bighead Carp	<i>Hypophthalmichthys nobilis</i>	x	x
Bigmouth Buffalo	<i>Ictiobus cyprinellus</i>	x	x
Black Bullhead	<i>Ameiurus melas</i>		x
Black Crappie	<i>Pomoxis nigromaculatus</i>	x	x
Blackside Darter	<i>Percina maculata</i>	x	x
Blue Sucker	<i>Cypleptus elongatus</i>	x	x
Bluegill	<i>Lepomis macrochirus</i>	x	x
Bluntnose Minnow	<i>Pimephales notatus</i>	x	x
Bowfin	<i>Amia calva</i>	x	x
Brassy Minnow	<i>Hybognathus hankinsoni</i>		x
Brook Silverside	<i>Labidesthes sicculus</i>	x	
Brook Stickleback	<i>Culaea inconstans</i>	x	
Brown Trout	<i>Salmo trutta</i>		x
Bullhead Minnow	<i>Pimephales vigilax</i>	x	
Burbot	<i>Lota lota</i>		x
Central Mudminnow	<i>Umbra limi</i>	x	
Channel Catfish	<i>Ictalurus punctatus</i>	x	x
Common Carp	<i>Cyprinus carpio</i>	x	x
Channel Shiner	<i>Notropis wickliffi</i>	x	
Crystal Darter	<i>Crystallaria asprella</i>		x
Emerald Shiner	<i>Notropis atherinoides</i>	x	x
Fathead Minnow	<i>Pimephales promelas</i>	x	
Flathead Catfish	<i>Pylodictis olivaris</i>	x	x
Freshwater Drum	<i>Aplodinotus grunniens</i>	x	x
Gilt Darter	<i>Percina evides</i>		x
Gizzard Shad	<i>Dorosoma cepedianum</i>	x	x
Goldeye	<i>Hiodon alosoides</i>	x	
Golden Redhorse	<i>Moxostoma erythrurum</i>	x	x
Golden Shiner	<i>Notemigonus crysoleucas</i>	x	
Grass Carp	<i>Ctenopharyngodon idella</i>	x	
Greater Redhorse	<i>Moxostoma valenciennesi</i>	x	x
Green Sunfish	<i>Lepomis cyanellus</i>	x	x
Hornhead Chub	<i>Nocomis biguttatus</i>	x	x
Hybrid Sunfish	<i>Lepomis microlophus</i> x <i>L. cyanellus</i>	x	x
Iowa Darter	<i>Etheostoma exile</i>		x
Johnny Darter	<i>Etheostoma nigrum</i>		x
Lake Sturgeon	<i>Acipenser fulvescens</i>		x
Largemouth Bass	<i>Micropterus salmoides</i>	X	x
Logperch	<i>Percina caprodes</i>	X	x
Longnose Gar	<i>Lepisosteus osseus</i>	X	x
Mimic Shiner	<i>Notropis volucellus</i>	X	x
Mooneye	<i>Hiodon tergisus</i>	X	x
Muskellunge	<i>Esox masquinongy</i>	X	x
Northern Hogsucker	<i>Hypentelium nigricans</i>		x
Northern Pike	<i>Esox lucius</i>	X	x
Orangespotted Sunfish	<i>Lepomis humilis</i>	X	x
Paddlefish	<i>Polyodon spathula</i>	X	x
Pumpkinseed	<i>Lepomis gibbosus</i>	X	x
Quillback	<i>Carpiodes cyprinus</i>	X	x
Rainbow Darter	<i>Etheostoma caeruleum</i>		x
River Carpsucker	<i>Carpiodes carpio</i>	x	x
River Darter	<i>Percina shumardi</i>	x	x
River Redhorse	<i>Moxostoma carinatum</i>	x	x
Rock Bass	<i>Ambloplites rupestris</i>	x	x
Sand Shiner	<i>Notropis stramineus</i>	x	x
Sauger	<i>Sander canadensis</i>	x	x
Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>	x	x
Shortnose Gar	<i>Lepisosteus platostomus</i>	x	x
Silver Carp	<i>Hypophthalmichthys molitrix</i>	x	
Silver Chub	<i>Macrhybopsis storeriana</i>	x	
Silver Lamprey	<i>Ichthyomyzon unicuspis</i>	x	x
Silver Redhorse	<i>Moxostoma anisurum</i>	x	x

Table 3 (continued). Species list for the Mississippi River Pool 2 and the St. Croix River from January 2013 through December 2015, including 78 native and invasive species.

Common Name	Genus Species	Pool 2	St. Croix River
Skipjack Herring	<i>Alosa chrysochloris</i>	x	
Slenderhead Darter	<i>Percina phoxoccephala</i>	x	x
Smallmouth Bass	<i>Micropterus dolomieu</i>	x	x
Smallmouth Buffalo	<i>Ictiobus bubalus</i>	x	x
Spotfin Shiner	<i>Cyprinella spiloptera</i>	x	x
Spottail Shiner	<i>Notropis hudsonius</i>	x	
Spotted Sucker	<i>Minytrema melanops</i>	x	x
Tadpole Madtom	<i>Noturus gyrinus</i>	x	
Trout Perch	<i>Percopsis omiscomaycus</i>		x
Walleye	<i>Sander vitreus</i>	x	x
White Bass	<i>Morone chrysops</i>	x	x
White Crappie	<i>Pomoxis annularis</i>	x	x
White Sucker	<i>Catostomus commersonii</i>	x	x
Yellow Bullhead	<i>Ameiurus natalis</i>	x	
Yellow Perch	<i>Perca flavescens</i>	x	x

Table 4. Baseline specimens and number of samples collected for the Mississippi River Pool 2 Stable Isotope Analysis.

	3M Channel	Lower Grey Cloud	Nelson Mine	River Lake	Spring Lake	TOTAL
Algae	4	3	4	3	3	17
Bloodworms	3	3	3	3	2	14
Caddisfly	3	2	3	0	4	12
Crayfish	0	2	1	2	3	8
Damselfly	2	2	2	3	2	11
Detritus	3	3	4	3	3	16
Dragonfly	2	2	2	1	2	9
Fly larvae	0	0	0	1	0	1
Hexagenia	2	1	0	2	1	6
Larval fish	1	1	1	0	1	4
Leech	2	3	3	3	2	13
Mayfly	3	2	3	2	3	13
Phytoplankton	3	3	3	4	3	16
Predatory Caddisfly	2	2	2	0	2	8
Scud	3	4	3	3	3	16
Snail	3	3	3	3	3	15
Sow bugs	3	3	2	3	3	14
Stonefly	1	1	2	2	2	8
Vegetation	6	7	6	5	6	30
Water Boatmen	4	2	2	3	3	14
Water Scorpion	0	1	0	1	0	2
Whirlygig Beetles	1	3	1	3	1	9
Zebra Mussels	3	4	1	3	2	13
Zooplankton	2	3	3	3	3	14
TOTAL	56	60	54	56	57	283

Table 5. Fish species and number of samples collected for the Mississippi River Pool 2 Stable Isotope Analysis.

	3M Channel	Lower Grey Cloud	Nelson Mine	River Lake	Spring Lake	Other	TOTAL
Bighead Carp	0	0	0	0	0	2	2
Bigmouth Buffalo	6	6	6	6	6	0	30
Bluegill <90 mm	7	8	6	5	6	0	32
Bluegill >90 mm	6	5	6	7	6	0	30
Common Carp	14	6	6	8	6	0	40
Channel Catfish	6	6	7	6	5	0	30
Flathead Catfish	6	7	5	7	8	0	33
Freshwater Drum	10	7	6	6	6	0	35
Grass Carp	0	2	0	0	0	0	2
Gizzard Shad <280 mm	9	8	10	8	6	0	41
Gizzard Shad >280 mm	6	4	7	6	7	0	30
Paddlefish	0	2	0	0	0	1	3
Smallmouth Buffalo	6	6	6	6	6	0	30
Sauger	3	1	12	18	1	0	35
Silver Carp	0	0	0	0	0	2	2
Shorthead Redhorse	8	5	9	8	8	0	38
Silver Redhorse	6	6	7	6	6	0	31
Walleye	6	6	6	6	6	0	30
TOTAL	99	85	99	103	83	5	474

Figures

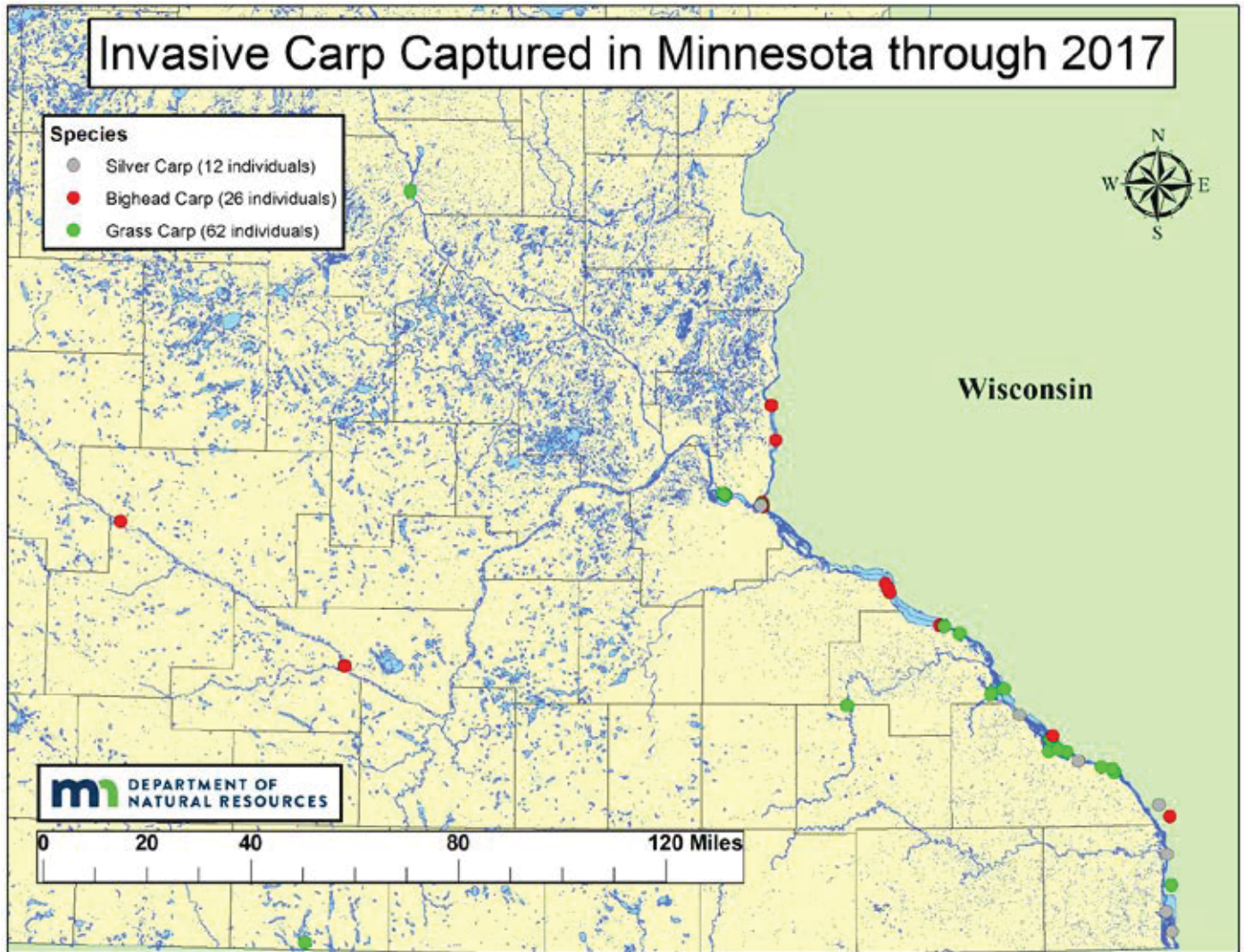


Figure 1. Locations of all known Invasive Carp captured in Minnesota waters through 2017.

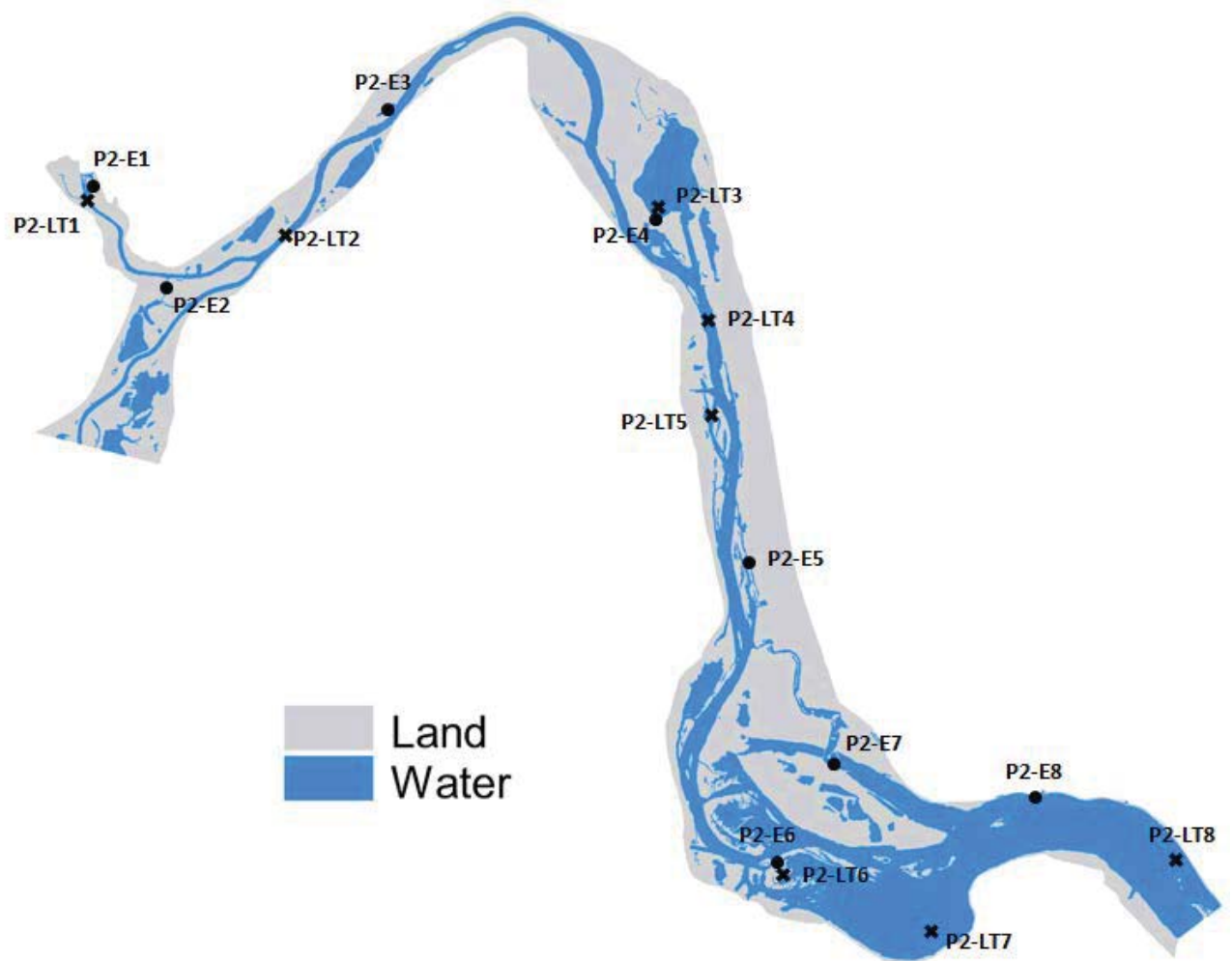


Figure 2. Standardized electrofishing (dark circle, E1 – E8) and larval fish trawling (dark cross, LT1 - LT 8) locations on Pool 2 (P2) of the Mississippi River.

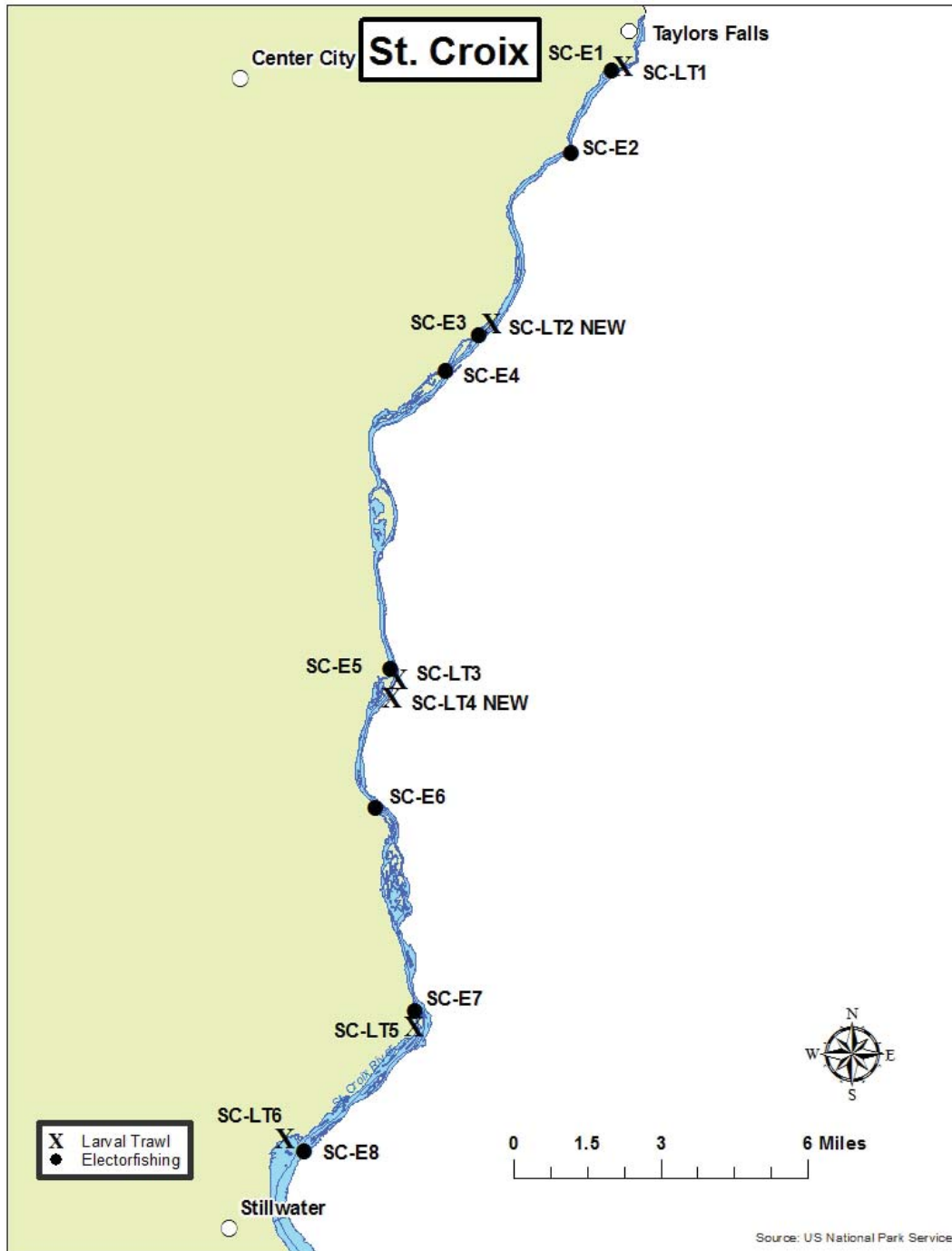


Figure 3. Standardized electrofishing (dark circle, E1 – E8) and larval fish trawling (dark cross, LT1 - LT 6) locations on the St. Croix River (SC). Sites SC-LT2 and SC-LT4 were moved in 2016 due to the site becoming too shallow to trawl.

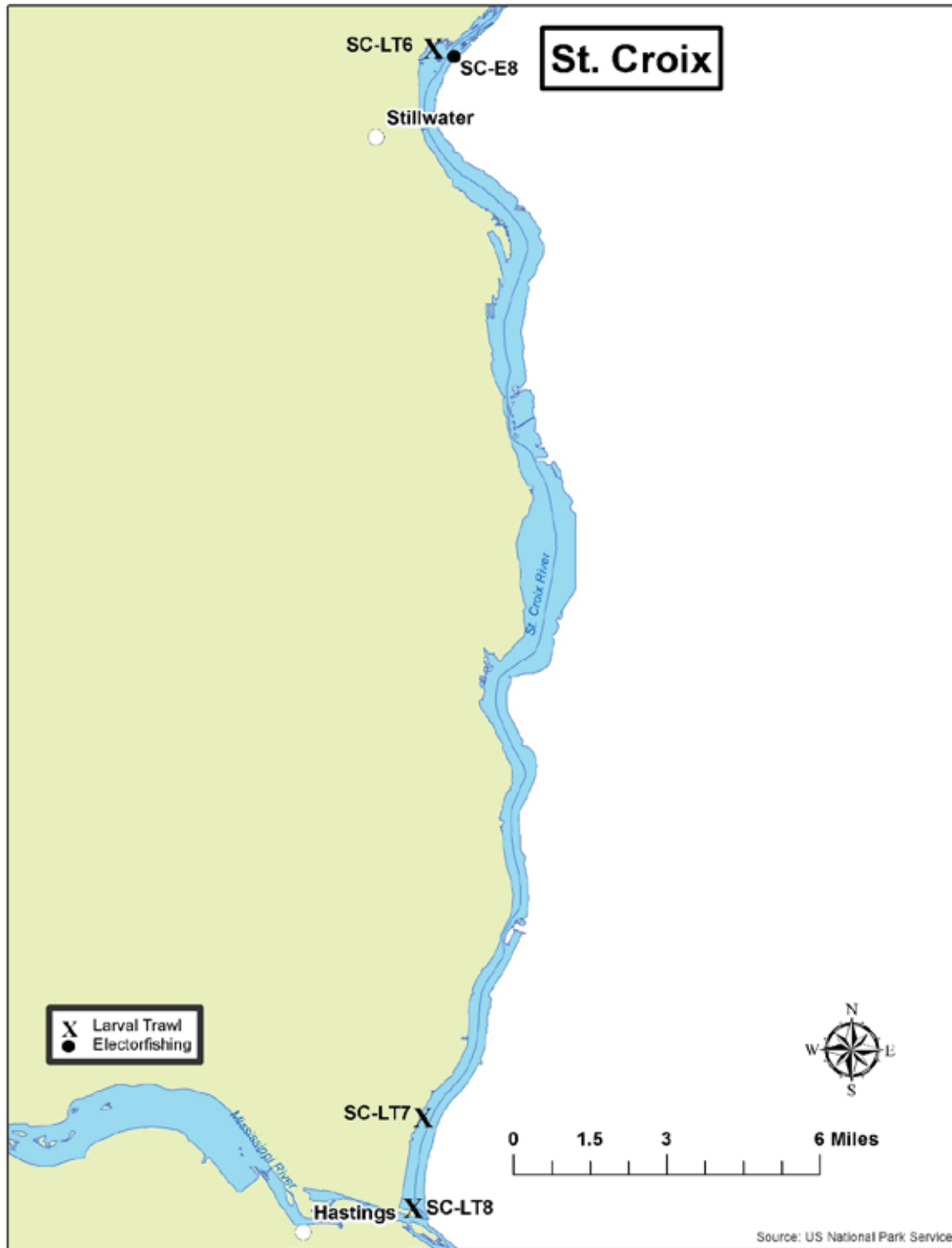


Figure 3 (Continued). Standardized electrofishing (dark circle, E8) and larval fish trawling (dark cross, LT 6 - LT 8) locations on the St. Croix River (SC).

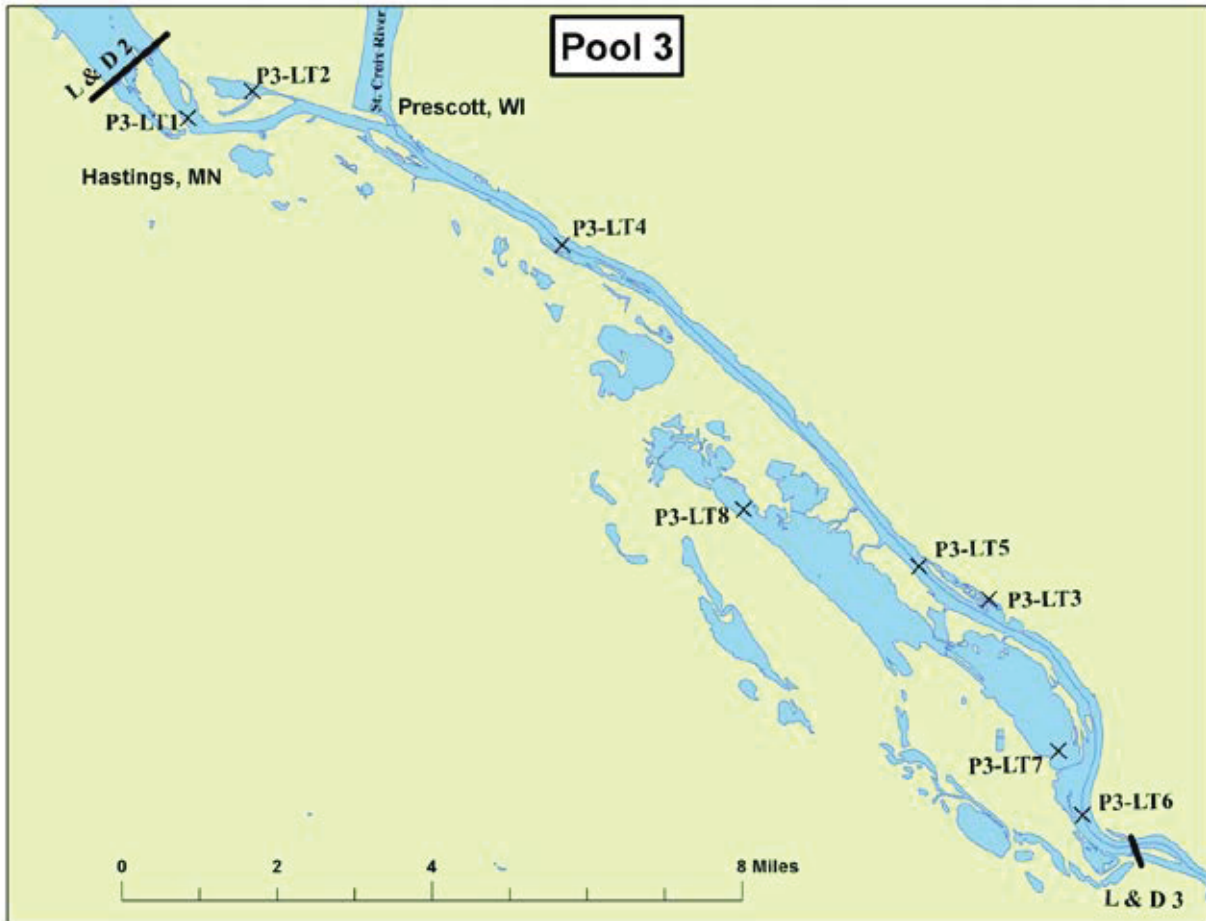


Figure 4. Standardized larval fish trawling (LT1-LT8) locations on Pool 3 (P3). Site P3-LT3 was moved in 2015 due to the site becoming too shallow to trawl.

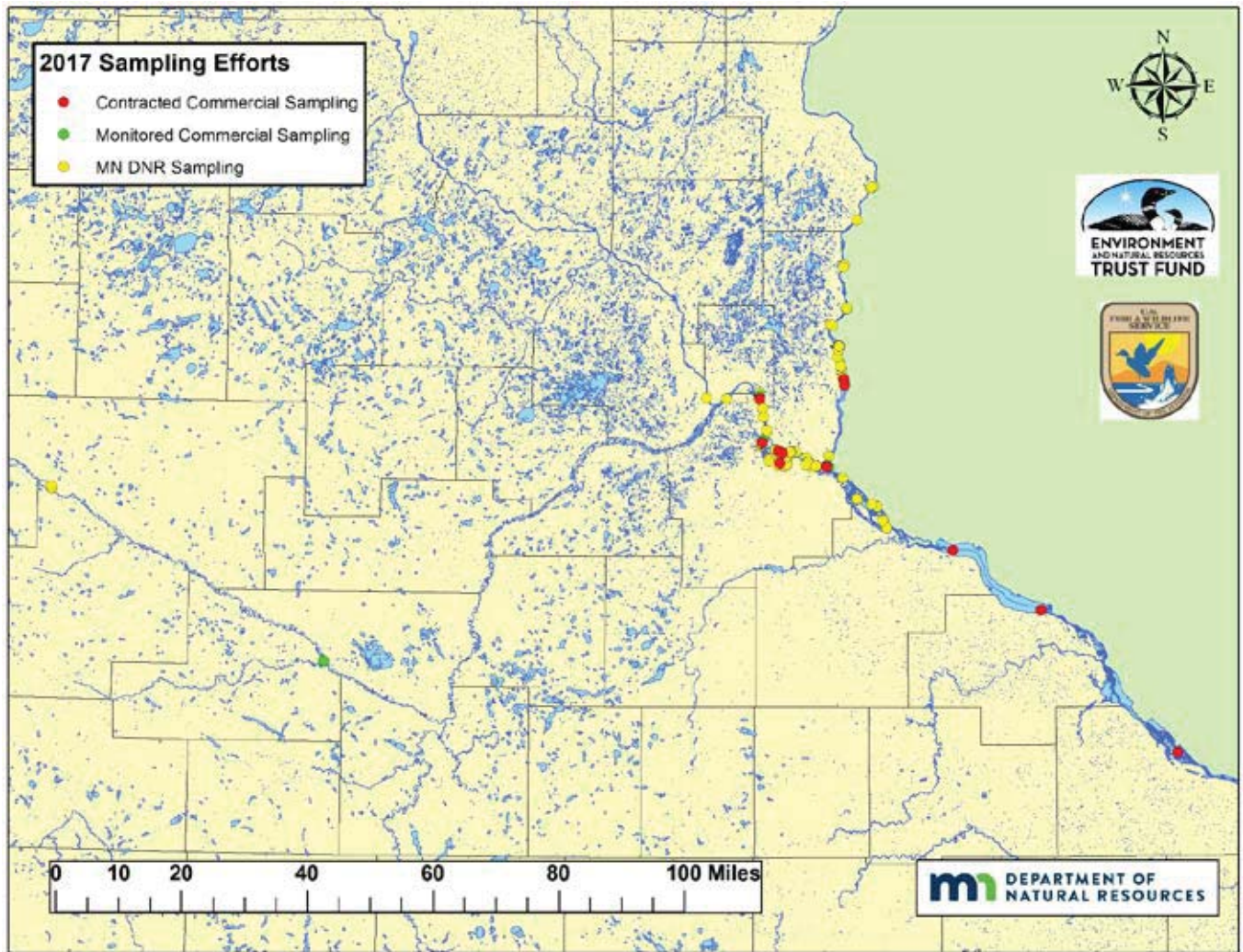


Figure 5. All sampling locations for contracted commercial sampling and MN DNR sampling on the Mississippi, St. Croix, and Minnesota Rivers during 2017.

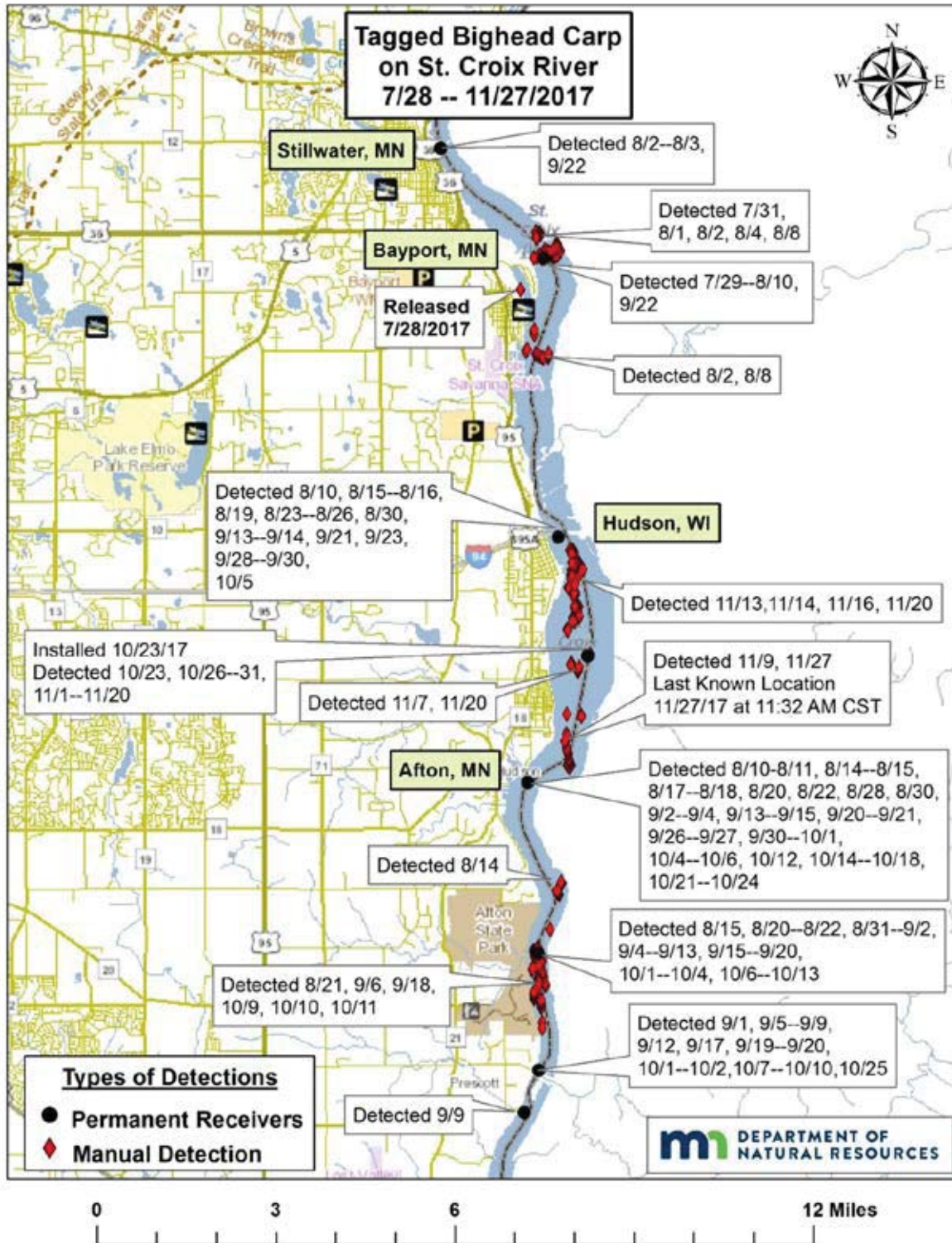


Figure 6. Movement patterns of tagged Bighead Carp from initial release through last manual detection on November 27, 2017.

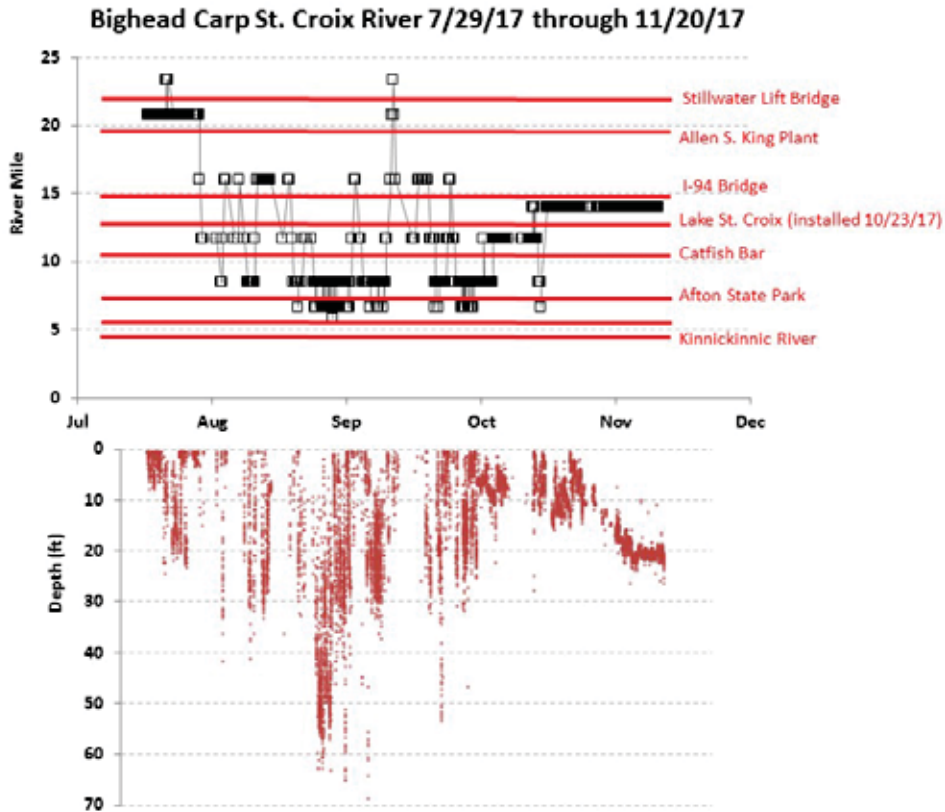


Figure 7. River Mile and Depth patterns of tagged Bighead Carp from initial release through last receiver download for 2017 on November 20, 2017.

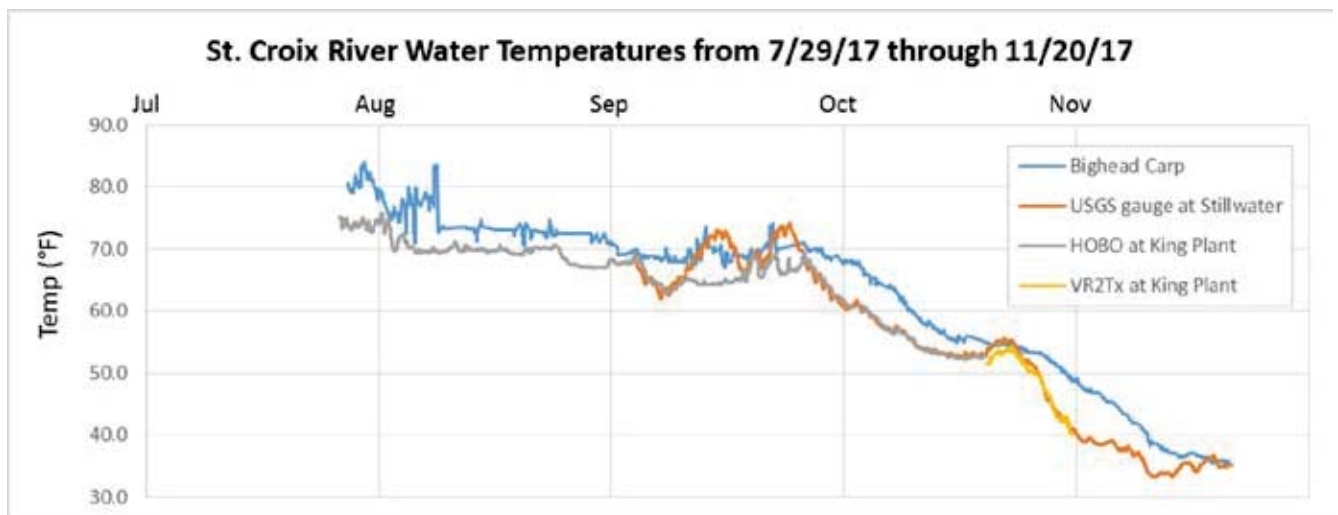


Figure 8. Temperature patterns of tagged Bighead Carp from initial release through last receiver download for 2017 on November 20, 2017. USGS gauge (05341550) data available from September 5 through November 20, 2017.

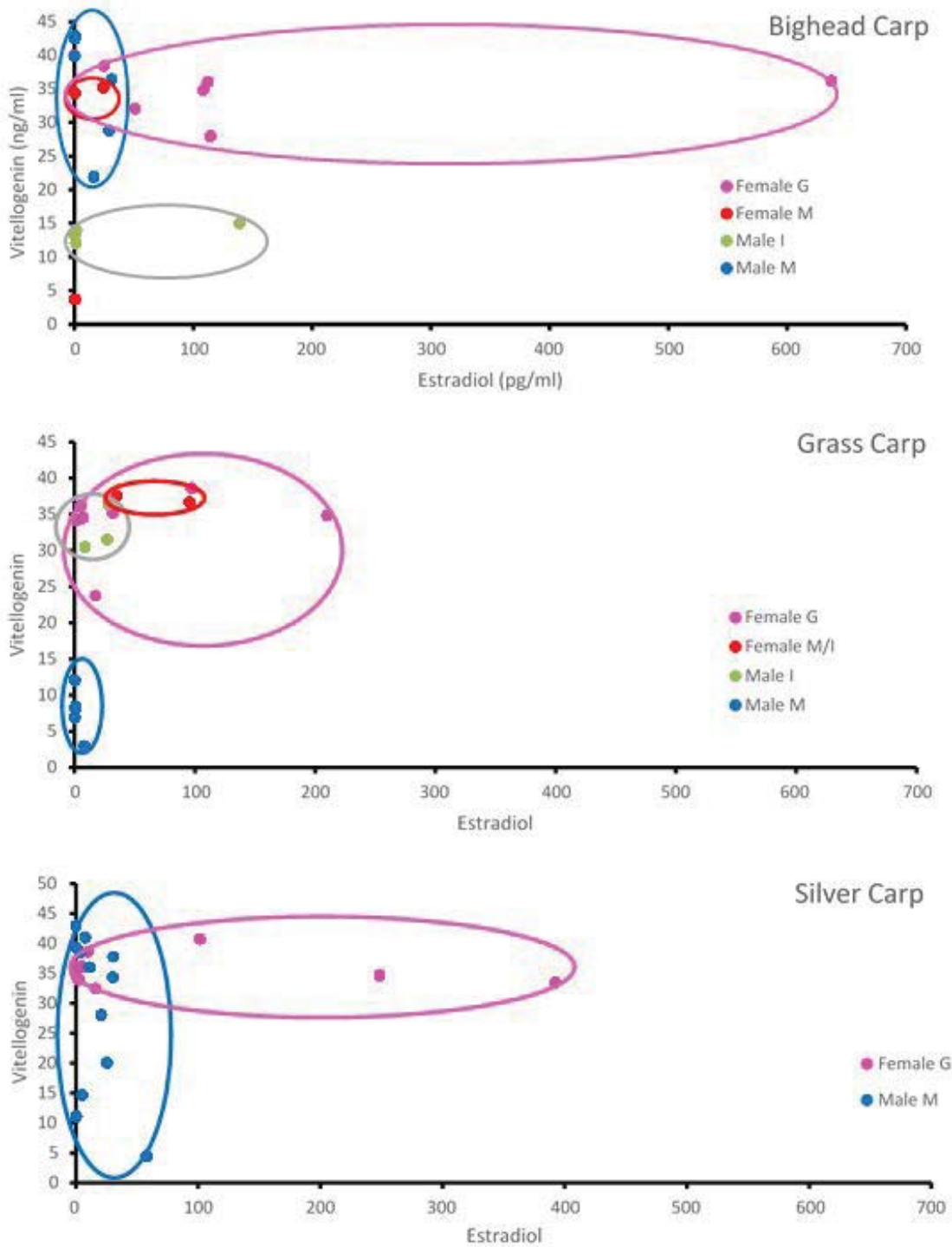


Figure 9. Analysis of vitellogenin and estradiol concentrations in carp plasma samples. Mature females should have both high estradiol & vitellogenin (upper right of graph), while mature males should have low levels of vitellogenin and estradiol (lower left of graph).

Field work and report by:

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