

Great Lakes Region Environmental DNA

2021 Annual Report





Contents

Program Overview	3
Whitney Genetics Lab	4
Upper Mississippi River Basin	7
Chicago Area Waterway System	9
Ohio River Basin	10
Great Lakes Basin	11
Missouri River Basin	13

Program Overview

Cover: A La Crosse FWCO biologist and fisheries technician prepare tubes for water sample collection during an invasive carp eDNA sampling event on the Mississippi River. Kyle Von Ruden/USFWS

Left: Whitney Genetics Lab staff use thermocyclers to detect invasive carp DNA in eDNA samples.

This report was compiled from contributions of Lower Great Lakes, Alpena, Green Bay, Ashland, LaCrosse, Carterville, Columbia, Missouri River & Great Plains Fish and Wildlife Conservation Offices; Whitney Genetics Lab; Northeast Fish Technology Center; and Bozeman Fish Health Center.

Aquatic nuisance species (ANS) pose a major threat to aquatic ecosystems worldwide. Once established, ANS can have large negative impacts on native ecosystems, recreational activities, and economics associated with these systems. Understanding ANS population densities in aquatic systems can help managers make decisions to prevent or limit the negative impacts of ANS. Environmental DNA (eDNA) monitoring is a tool that can be used in conjunction with other sampling methods to provide helpful information on species detection, range expansion, and population trends through the detection of DNA in water.

One of the largest ANS threats in the United States are bighead and silver carp (also referred to herein as invasive carp). Imported into the United States in the early 1970s for water quality and fish production purposes, they soon became established in the lower Mississippi River Basin. Today, bighead and silver carp are established in portions of the Mississippi River, Ohio River, and Missouri River basins. To detect these species sooner, the US Army Corps of Engineers and Notre Dame University developed and refined eDNA detection methods from 2009-2013. In 2013, the U.S. Fish and Wildlife Service took over the bighead and silver carp eDNA monitoring program.

This bighead and silver carp eDNA work is guided by a Quality Assurance Project Plan (QAPP) to ensure consistent and reliable collection of data, proper data management, and timely communication

of results. The QAPP is regularly reviewed to improve program outputs, interpretation, and communication. This detailed, technical document can be found on the Whitney Genetics Laboratory (WGL) projects page¹.

The detection and surveillance program is a year-round effort. The USFWS in collaboration with partners, identifies sampling locations for bighead and silver carp eDNA. Water samples are collected by trained staff from Fish and Wildlife Conservation Offices (FWCOs) and sent to WGL for analysis. Results are shared with state and tribal partners prior to publicly posting results. This allows management agencies time to interpret results and formulate further actions if needed. Results from 2021 and previous years are located on the USFWS eDNA dashboard².

The USFWS Region 3 eDNA program engages in a variety of work beyond bighead and silver carp. This includes developing field and lab methodology for other ANS, research to improve detection and efficiency, advancing native species detection, and exploring multi-species techniques. Much of this work is collaborative with other agencies, universities, and USFWS labs.

¹ <https://www.fws.gov/office/whitney-genetics-laboratory/what-we-do/projects-research>

² <https://fws.maps.arcgis.com/apps/dashboards/52b22abe9c4d4575ad-fe851a946f444d>

Midwest Fisheries Center Whitney Genetics Laboratory (WGL)

Goals

- Process eDNA monitoring samples for the early detection and monitoring of invasive carp in the Great Lakes and Mississippi River basins
- Work with the USFWS Region 3 Fisheries Program as well as external partners to develop and implement new genetic tools for early detection and monitoring of ANS
- Provide genetic support to the USFWS Region 3 Fisheries Program to meet our conservation goals for threatened and endangered fish and aquatic species

Highlights

- In 2021, WGL staff processed nearly 10,000 eDNA monitoring samples collected for bighead and silver Carp monitoring in the upper Mississippi River and Great Lakes basins
- WGL worked with staff from the Alpena FWCO to re-initiate a field validation of recently developed grass carp eDNA markers
- WGL collaborated with staff from the USFWS Northeast Fishery Center and all four Great Lakes FWCOs to initiate a pilot study to use eDNA metabarcoding for the early detection of ANS in the Great Lakes

Work Summary

Table 1. Bighead and silver carp eDNA samples processed in 2021

Great Lakes	Upper Mississippi River	CAWS	Ohio River
Lake Michigan n = 1752	Pool 8 n = 1650	May n = 440	Muskingum n = 176
Lake Huron n = 297	Pool 13 n = 440	September n = 440	
Lake Erie n = 2920	Pool 14 n = 1056		
Lake Superior n = 110	Pool 16 n = 176		

Environmental DNA Sample Analysis
Since 2013 the USFWS has worked with partners to use eDNA detection data as a monitoring and early detection tool for invasive silver and bighead carp. All samples are analyzed for the presence of carp eDNA with three marker sets: silver carp only, bighead carp only, and non-specific invasive carp. The non-specific invasive carp marker set can detect either bighead carp or silver carp but is not specific enough to say which of these species is present. If both species-specific markers are detected in a water sample, it is reported under the “Bighead AND Silver” category. 2021 was the

first sampling year that the non-specific invasive carp detections were reported publicly.

After greatly reduced sampling in 2020 due to COVID, full monitoring activities resumed in 2021. Approximately 10,000 water samples were screened for invasive carp eDNA. Highlights for this work in 2021 included: repeated sampling in Pool 8 of the Mississippi River to coincide with U.S. Geological Survey and Minnesota Department of Natural Resources Modified Unified Method sampling events; coordinated sampling with U.S. Geological Survey to compare eDNA

collection methods; follow-up sampling in the Milwaukee River; and use of a multiplexed PCR marker (multiple markers in a single reaction) to increase lab efficiency.

Validating a Field Sampling Protocol for Grass Carp eDNA Monitoring
WGL staff worked with the Alpena FWCO, the U.S. Geological Survey Upper Midwest Environmental Sciences Center, the states of Michigan and Ohio, and Michigan State University to field validate newly developed grass carp eDNA markers and optimize an eDNA sampling strategy for grass carp. This involved collecting water samples in multiple habitat types of the Sandusky River several times throughout the year to determine the optimal time of year, habitat type, and number of samples to collect to increase the likelihood of detecting grass carp eDNA. This information will be used to develop an occupancy model for grass carp eDNA in the Sandusky River which will begin to form the basis for a Great Lakes grass carp eDNA monitoring program.

Early Detection and Monitoring Study
The Northeast Fishery Center (NEFC) and WGL are participating in a pilot study to genetically identify ichthyoplankton samples by using a metabarcoding method developed for ethanol decants from larval tows. A total of 80 samples were sent to both NEFC and WGL this year, with this study serving to identify logistical constraints in the field and laboratory processes as well as comparing the data obtained from samples prepared independently in different labs. Several logistical and sample processing challenges were identified and have been corrected and/or updated in the NEFC metabarcoding protocol for next year, in addition to the pilot data being shared via an ArcGIS Dashboard developed specifically for this project. The study is a first step towards being able to efficiently screen larval tow samples for aquatic invasive species and removing some of the burden of manually sorting samples.

Partnerships

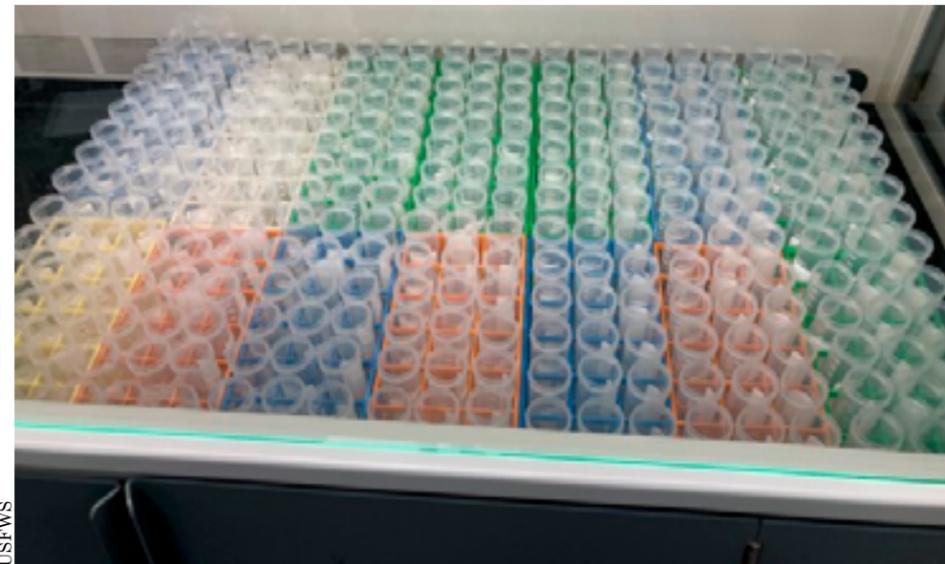
Topeka Shiner eDNA Monitoring
The La Crosse FWCO, Minnesota Department of Natural Resources, and Region 3 Ecological Services Program are partnering on Topeka Shiner surveillance. The Topeka Shiner is an endangered minnow inhabiting streams, oxbows, and other off-channel habitats. Habitat occupancy is unknown in many areas. FWCOs in Regions 3 and 6 as



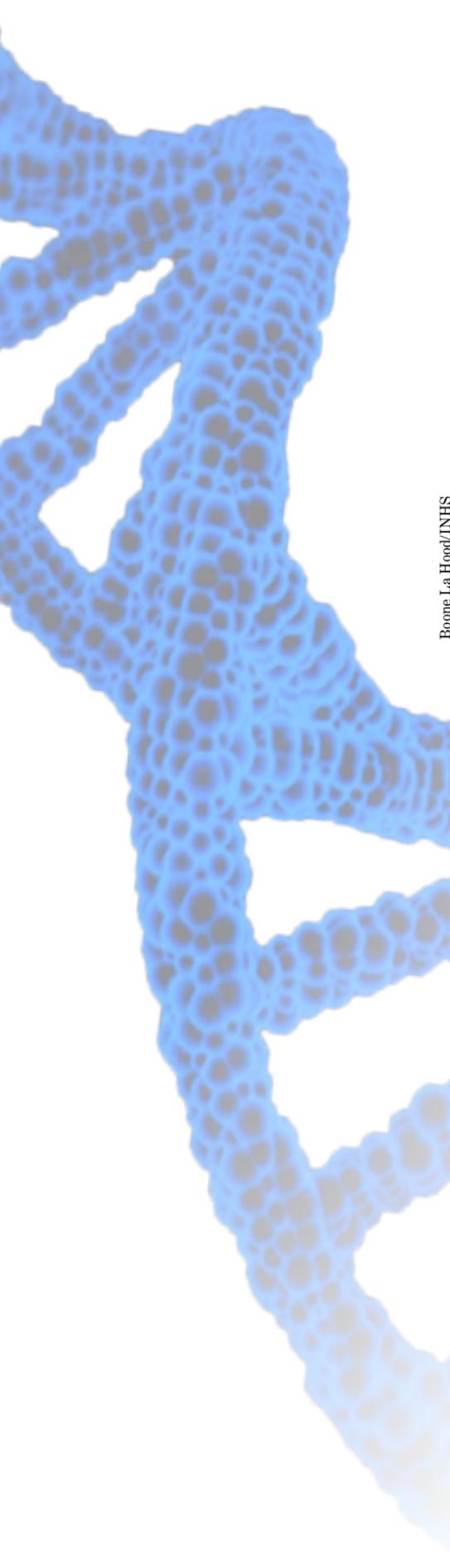
WGL staff are collaborating with the Alpena FWCO and others to develop an eDNA occupancy model for the Sandusky River, Ohio that will begin to inform early detection and monitoring efforts for grass carp in Great Lakes tributaries. Staff from the Alpena FWCO collected water samples from the Sandusky River on four occasions in 2021. Water samples were tested for the presence of grass carp eDNA.



Topeka Shiners are small floodplain minnows that can be difficult to detect with traditional sampling gears. WGL staff is working with La Crosse FWCO, USFWS Ecological Services and State Partners to evaluate the use of eDNA as an additional monitoring tool for the species.



Environmental DNA collection tubes dry overnight in a hood prior to DNA extraction.



Boone La Hood/INHS

Staff worked with the Illinois Natural History Survey to use qPCR to conduct species ID for larval bighead and silver carp collected in light traps in the Upper Mississippi River.

well as many partner agencies conduct annual monitoring surveys to document the species distribution in historic and restored ranges. Traditional surveys are time and labor intensive and may fail to capture and document rare species. In 2021, we validated an eDNA marker to efficiently screen oxbows and other habitats to determine if Topeka Shiners are present. We also worked with our partners to refine our Topeka Shiner eDNA field sampling methods in Minnesota and Iowa oxbows. This information will help USFWS and state partners prioritize habitats for sampling efforts.

What does that mean?
 qPCR: Quantitative Polymerase Chain Reaction, a method that measures the amplification of targeted DNA in real-time during a PCR reaction. This method looks for one particular species DNA in a sample.
 Metabarcoding: A method that aims to resolve taxonomic identifications by simultaneously sequencing all organisms in a sample using a single or multiple fragments of genetic code that can discriminate distinct species. This method looks for multiple species DNA present in a sample.

Larval Fish Genetic ID and Native Larval Fish Metabarcoding
 WGL staff partnered with staff at the Illinois Natural History Survey to identify larval fish captured upstream of Lock and Dam 19 in the Upper Mississippi River during light trap surveys. 1,552 samples that had been identified as silver or bighead carp visually were genetically verified using the qPCR assays developed for the invasive carp program. The results of this study were published by La Hood et al. (2021) in the North American Journal of Fisheries Management. WGL is now preparing to metabarcode an additional 1,415 samples from this project for species identification. The information gained from these data are expected to help determine which native species are spawning at similar times as bighead, grass, and silver carps. Currently, little is known about which native species spawn at similar times to the invasive carp, and this is a first step towards understanding which of our native species may be negatively impacted by overlapping spawn times.

Upper Mississippi River Basin

Goals

- Monitor for silver and bighead carp eDNA in targeted backwaters within pools immediately upstream of the Intensive Management Zone of the Upper Mississippi River
- Inform managers of potential trends in invasive carp presence and provide data to support the prioritization of new backwaters to target with traditional capture methods
- Refine detection probability and optimal sampling design for eDNA monitoring in the Upper Mississippi River

Highlights

- USFWS staff collected 3,432 samples across five Mississippi River Pools
- USFWS La Crosse FWCO collaborated with the U.S. Geological Survey to conduct paired sampling to compare collection and processing methodologies between agencies
- Positive detections in Pool 13 and Pool 14 were noticeably higher than previous years of sampling

Work Summary

Table 2. Upper Mississippi River Water Samples Collected

Pool	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Pool 8	1500	1434	2	30	0	8	26	150
Pool 13	400	354	3	30	2	11	0	40
Pool 14	960	895	4	43	0	18	0	96
Pool 16	160	124	1	27	0	8	0	16

*Field blanks are a quality control measure and are not included in total collected or results

Mississippi River eDNA Monitoring
 USFWS staff from the La Crosse FWCO conducted spring and fall sampling in Pools 13, 14, and 16. For each event in Pool 13, 264 samples were collected in the spring across 3 backwater sites and 176 in the fall across two sites. The positive detection rate for the spring event was 18.75% (all four eDNA marker types detected) and 14.2% (excluding the non-specific invasive carp eDNA detections for comparison with past data), and the fall positivity rate was 0.6% (only silver carp eDNA was detected). Positive detections in the recent years were 0-1.2% in Pool 13. Pool 13 and 14 samples were collected to inform managers of

which are consistently higher detection rates than past events in this pool where positive sample rates were 0-1.45%. In Pool 14, 528 samples were collected across six backwater sites and there were 8.75% positive detections (bighead only, silver only, and non-specific invasive carp eDNA marker types detected) in the spring and 4.8% positive detections in the fall. If the non-specific invasive carp eDNA detections are removed from the total (to compare with previous years where non-specific invasive carp eDNA detections were not reported), the positive rates were 5.8% and 3.9%



Staff from the La Crosse FWCO and WGL in the field collecting eDNA samples (left). La Crosse FWCO biologist decants and preserves eDNA water samples in the mobile processing trailer (right).



potential trends or shifts in the bighead and silver carp population front adjacent to the Intensive Management Zone.

In addition to regular monitoring, 88 samples were also collected from one backwater site in Pool 16 in both the spring and fall. These samples are collected as part of an effort to consistently refine and learn about invasive carp eDNA detectability. The backwater targeted by this sampling hosts a real-time telemetry receiver, which detects the presence of acoustically tagged bighead and silver carp. Environmental DNA positive detections can then be compared with the confirmed presence or absence of tagged invasive carp at the time of sampling.

Modified Unified Method Support and eDNA Sampling Method Comparison
USFWS staff from the La Crosse FWCO conducted three sampling events in Pool 8 in March, July, and October. For each event, 550 eDNA samples were collected across five backwater sites. Samples in March and October were collected in the week immediately preceding the Modified Unified Method (MUM) for invasive carp removal conducted by the Minnesota Department of Natural Resources and the U.S. Geological Survey Columbia Environmental Research Center. eDNA sampling informed Minnesota Department of Natural Resources of potential bighead and silver carp presence and those data were compared to actual capture data from the MUM to infer detectability of silver and bighead carp in Pool 8. The results from both eDNA sampling events match very closely with observations and captures of these two species during the MUM events. In addition to supporting the MUM, eDNA collections in Pool 8 at all three times were conducted in coordination with the U.S. Geological Survey Upper Midwest Environmental Sciences Center to compare sampling and processing methods between agencies. The goal of this effort was to use the data to refine eDNA methodologies to determine best practices based on eDNA program goals, as well as more clearly define the advantages and limitations of each method.

Sampling in Support of Extraction Study by Whitney Genetics Laboratory
In support of a laboratory study by WGL, La Crosse FWCO collected experimental samples from Boston Bay in Pool 18 and from one backwater site in Pool 8. These samples were used as part of a research project to compare extraction methods and reagents and determine the best

combination for detection of bighead and silver carp eDNA.

Partnerships

Minnesota Department of Natural Resources
La Crosse FWCO staff worked with Minnesota Department of Natural Resources to select sites for Pool 8 eDNA collection based on desired MUM locations and habitat characteristics conducive to bighead and silver carp seasonal congregation and eDNA detection.

U.S. Geological Survey
La Crosse FWCO worked with U.S. Geological Survey's Upper Midwest Environmental Sciences Center and Columbia Environmental Sciences Center to coordinate eDNA sampling related to MUM activities and method comparison sampling. Through effective communication and open collaboration, USFWS and U.S. Geological Survey were able to work side-by-side during consolidated sampling efforts to achieve each agency's program goals.

Whitney Genetics Laboratory
WGL provided staff and equipment, on multiple occasions, to assist the La Crosse FWCO with eDNA sample collection and field processing during times of staffing shortages and equipment malfunction.



USFWS biologists assist the Minnesota Department of Natural Resources process fish during the October 2021 MUM event in Mississippi River Pool 8. The USFWS collected eDNA samples prior to the event.

Minnesota DNR

Chicago Area Waterway System (CAWS)

Goals

- Sample for bighead and silver carp eDNA in targeted areas of the CAWS to maintain vigilance and complement other ongoing monitoring efforts above the Electric Dispersal Barrier

Highlights

- USFWS staff collected 880 samples upstream of the Electric Dispersal Barrier
- Positive detections were few and consistent with previous sampling years

Work Summary

Table 3. CAWS Water Samples Collected

Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Lake Calumet	600	598	0	1	0	1	0	60
Little Calumet River	200	199	0	1	0	0	0	20

*Field blanks are a quality control measure and are not included in total collected or results

CAWS eDNA Sampling
USFWS staff from the La Crosse FWCO conducted spring and fall sampling above the Electric Dispersal Barrier in the CAWS. For each event, 330 samples (300 samples plus 30 field blanks) were collected in Lake Calumet and 110 (100 samples plus 10 field blanks) were collected in the Marine Services Marina on the Little Calumet River. In the spring, there were zero positive eDNA detections at both sites. In the fall, there were 0.6% positive detections (silver only and non-specific invasive carp eDNA marker types detected) or 0.3% (excluding the non-specific invasive carp eDNA detections for comparison with past data) in Lake Calumet. There was a 1% positive detection rate (silver carp only DNA marker type detected) in the marina. The detection rate in Lake Calumet is slightly higher than spring 2021 and fall 2019 events which had zero detections, however it is lower than the 2.2% positive rate observed in spring 2019. The detection rate in the Little Calumet River site is slightly higher than past sampling events in that area which have all had zero positive detections. Although this is the first time that USFWS has detected eDNA in the marina, the detection rate is low and the site harbors boats with ballast water compartments and is next to a landfill which hosts thousands of gulls. The lack of prior detections in addition to no captures or observations during an intensive fall physical sampling effort lead by Illinois Department of Natural Resources, indicates that the positivity

may have likely resulted from secondary vector contributions to the system.

Partnerships
Multiple FWCOs across Region 3
La Crosse FWCO used the CAWS eDNA sampling events as opportunities to train staff from the Carterville FWCO that are new to the eDNA program. The QAPP provides a thorough description of the eDNA process, however in-person training is also an essential part to ensuring all steps of the collection and centrifuging process are completed consistently and accurately. Later that fall, with the knowledge and experience gained, the Carterville FWCO was able to confidently conduct their first solo eDNA sample collection event in nearly three years, with the goal of reinvigorating the eDNA monitoring program in the Ohio River Basin.

Monitoring and Response Work Group
USFWS works closely with the Monitoring and Response Work Group to time eDNA sampling to compliment intensive physical sampling efforts lead by the Illinois Department of Natural Resources. Given the proximity to Lake Michigan and the downstream connectivity to large populations of invasive carp and secondary vectors of invasive carp DNA, the syncing of these two monitoring methods allows physical capture and observation data to support and help interpret eDNA results to gauge the likelihood that positive eDNA detections are the result of a live invasive carp threat versus a secondary vector contribution to the system.

Ohio River Basin

Goals

- Re-establish the Cartersville FWCO eDNA program following a three-year hiatus
- Acquire training in eDNA sample collection and processing for the field crew
- Collect water samples for eDNA analysis to aid in the detection of silver and bighead carp in areas where their presence is unknown

Highlights

- Maintenance checks were performed, and all equipment was cleared for use
- Three Cartersville FWCO staff members participated in training events led by the La Crosse FWCO
- With the assistance of a staff member from the Alpena FWCO, the Cartersville FWCO collected 176 samples from two sites on the Muskingum River in Ohio

Work Summary

Table 4. Ohio River Basin Water Samples Collected in the Muskingum River

Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Beverly	80	80	0	0	0	0	0	8
Devola	80	79	0	0	0	0	1	8

*Field blanks are a quality control measure and are not included in total collected or results

Muskingum River eDNA Sampling

With the assistance of a staff member from the Alpena FWCO, staff from the Cartersville FWCO collected 176 samples from two sites on the Muskingum River, a tributary of the Ohio River. Sites were located upstream of the Devola Dam, including the mouth of Rainbow Creek, and downstream of the Luke Chute Dam. Within the Ohio River, silver and bighead carp have been captured upstream of the confluence of the Muskingum and Ohio rivers. However, they have not been reported in the Muskingum River upstream of the Devola Dam, which is the dam nearest the confluence with the Ohio River. The Tuscarawas River, a tributary of the Muskingum River, has a gated

connection to the Ohio and Erie Canal and the Great Lakes watershed. In this system, eDNA sampling is being used as an early-detection technique to alert managers to any changes in the presence of silver and bighead carp upstream of the Devola Dam.

Partnerships

Ohio Department of Natural Resources Cartersville FWCO staff collaborated with the Ohio Department of Natural Resources to determine access sites and local water conditions to help prioritize and identify potential eDNA sampling locations.



Cartersville FWCO staff cleaning gear following sampling (left). Cartersville FWCO staff preparing eDNA samples to be shipped to WGL for analysis (right).



Sampling on the Muskingum River in Ohio was conducted upstream of the Devola Dam in the east and downstream of the Luke Chute Dam in the west (top), blue circles indicate sample sites. Yellow circles indicate the locations at which water samples were collected for eDNA analysis near the Devola Dam (middle) and the Luke Chute Dam (bottom). Eighty-eight samples were collected at each site.

Great Lakes Basin

Goals

- Use eDNA methodologies to aid in the detection of bighead and silver carp at low densities and further detect their spread into previously uninhabited areas
- Use eDNA methods to field validate a grass carp genetic marker and optimize an eDNA sampling strategy for grass carp in the Great Lakes
- Test the feasibility and repeatability of a high throughput rapid-response program for larval fish based on metabarcoding analysis

Highlights

- USFWS staff collected 4,885 samples across 13 sites within the Great Lakes Basin; a large number of these samples (2,218) supported the grass carp field validation and optimization study conducted in the Sandusky River, Ohio
- No positive detections for bighead or silver carps in lakes Superior or Huron; Lake Ontario was not sampled
- Two positive detections, one bighead carp and one non-specific invasive carp, in the Milwaukee Estuary; no live invasive carps captured after joint response effort with Wisconsin Department of Natural Resources
- One positive detection for bighead carp in the Sandusky River and no positive detections in follow up sampling
- Sampling efforts doubled in the eastern half of the Lake Erie

Work Summary

Table 5. Great Lakes Water Samples Collected by Lake

Lake Erie Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Buffalo River	90	90	0	0	0	0	0	10
Cattaraugus Creek	45	45	0	0	0	0	0	5
Tonawanda Creek	90	89	0	0	0	0	1	10
Presque Isle Bay	90	90	0	0	0	0	0	10
Sandusky River	2,017	2,016	1	0	0	0	0	201
Maumee River	320	320	0	0	0	0	0	32

Lake Huron Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Saginaw River	270	270	0	0	0	0	0	27

Lake Michigan Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
Grand River	506	506	0	0	0	0	0	56
Kalamazoo River	286	286	0	0	0	0	0	34
St. Joseph River	288	288	0	0	0	0	0	32
Milwaukee Estuary	322	320	1	0	0	1	0	28
Fox River	180	180	0	0	0	0	0	20

Lake Superior Location	Collected	Negative	Bighead	Silver	Bighead AND Silver	Invasive Carp	No Results	Field Blanks*
St. Louis River	100	100	0	0	0	0	0	10

*Field blanks are a quality control measure and are not included in total collected or results

Great Lakes eDNA Sampling

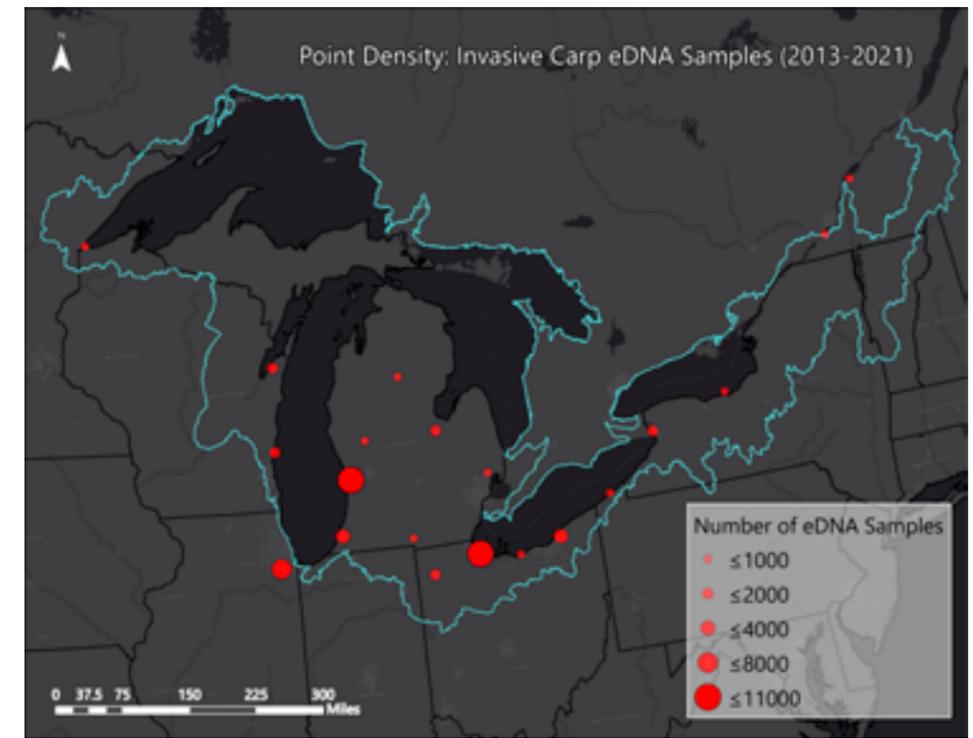
USFWS staff from the Alpena, Ashland, Lower Great Lakes, and Green Bay FWCOs each conducted multiple sampling events across 13 sites within the Great Lakes Basin throughout the 2021 season. The Alpena FWCO collected a total of 2,867 samples from the Sandusky and Maumee rivers in Lake Erie, and the Saginaw River in Lake Huron. The Ashland FWCO collected a total of 110 samples from the St. Louis River in Lake Superior. The Lower Great Lakes FWCO collected a total of 350 samples from the Buffalo River, Tonawanda and Cattaraugus Creeks, and Presque Isle Bay in Lake Erie, nearly double the number of samples taken in the 2019 season. The Green Bay FWCO collected a total of 1,752 samples from the Fox, Grand, Kalamazoo and St. Joseph rivers, and the Milwaukee Estuary in Lake Michigan.

Of all the samples collected in the Great Lakes Basin, three of them were positive for bigheaded carps. There was one detection for bighead carp in the Sandusky River; one detection for bighead carp in the Milwaukee Estuary, and one detection for non-specific invasive carp in the Milwaukee Estuary. Additional physical sampling yielded no invasive carp in the Milwaukee River. On the Sandusky River, water sampling was conducted as a repeat of the grass carp eDNA study following the initial detection and no other positives detections were recorded.

Partnerships

Multi-agency Grass Carp eDNA Validation

Alpena FWCO and WGL partnered with Ohio Department of Natural Resources, Michigan Department of Natural Resources, Michigan State University, the U.S. Geological Survey Upper Midwest Environmental Sciences Center and others from the Lake Erie Grass Carp Advisory Committee to field validate grass carp eDNA markers developed by WGL and optimize an eDNA sampling strategy for grass carp in the Sandusky River, Ohio. Grass carp have been captured in the Sandusky River and their known presence provided an opportunity to validate markers developed by WGL and to develop an eDNA optimization study. Water samples were collected from multiple habitat types in March, June, September, and November to determine optimal timing, habitat type, and number of samples to increase the likelihood of detecting grass carp eDNA. This information will be used



Point density of invasive carp eDNA samples (2013-2021).

to develop an occupancy model for grass carp eDNA in the Sandusky River, which will in turn begin to form a basis for a grass carp eDNA monitoring program.

Metabarcoding – USFWS Northeast Fishery Center

The aim of this project is to test the feasibility and repeatability of a high throughput rapid-response program for invasive larval fish and invertebrates based on metabarcoding analysis. Samples will be used to implement a workflow that includes both the USFWS WGL and Northeast Fisheries Center. The number of samples requested was limited in order to assess processing feasibility between labs, and to identify infrastructure or processing improvements necessary to scale up laboratory throughput. This study will also provide each participating FWCO an opportunity to evaluate detection data from their own samples, potentially contributing to other assessment objectives. This initial dataset can be used to inform future sampling plans developed to fit each FWCO's individual needs. It is envisioned that throughput will gradually increase over the next several field seasons to allow both the genetics laboratories and FWCOs to integrate the increased workloads and adapt processing protocols as necessary.

Wisconsin Department of Natural Resources Milwaukee River Response

Green Bay FWCO, in response to two positive detections for invasive carp eDNA in the Milwaukee River, partnered with the Wisconsin Department of Natural Resources to sample the Milwaukee and Kinnickinnic rivers. Over the course of two weeks, staff from both agencies used a combination of boat electrofishing and trammel nets attempting to capture a live invasive carp. No live bighead, grass, or silver carp were captured or observed during this extensive sampling event.

Illinois Natural History Survey Prairie Research Institute

The Ashland FWCO is collaborating with the University of Illinois, Illinois Natural History Survey Prairie Research Institute on a graduate research project to use eDNA to evaluate ANS fish species in Lake Superior. Detecting a new invasion can be challenging due to lower detection probabilities of small, unknown populations using traditional gears (e.g., fyke netting or electrofishing). The evolution of eDNA monitoring techniques have offered opportunities to increase the detection of rare species that may compliment traditional gears. Therefore, this study will assess differences in the fish communities using eDNA metabarcoding in comparison with fyke netting, electrofishing, and ichthyoplankton netting in the St. Louis River of Lake Superior during years 2021 and 2022.

Missouri River Basin

Goals

- Sample for bighead and silver carp eDNA in Missouri River tributaries with known invasive carp presence in North and South Dakota to determine the realistic eDNA detection probabilities, sample size requirements, and logistical and environmental limitations of using eDNA technology to detect bighead and silver carp presence
- If eDNA proves to be a viable tool based on optimization and preliminary sampling (Goal 1), apply results and knowledge to form a long-term, adaptive invasive carp eDNA monitoring strategy for the Upper Missouri River Watershed to monitor for evidence of bighead and silver carp presence in watersheds with unknown or no invasive carp occurrence

Highlights

- This pilot project consisted of two sampling events to test the viability of eDNA as an early detection tool in North Dakota and South Dakota. Despite low water, 500 samples were collected from three Missouri River tributaries in June and late August/early September
- Two USFWS regions partnered with state and academic interests to complete the sampling by drawing on Region 6 funding and staff, Region 3 expertise and training, and State knowledge of the systems.
- The Bozeman Fish Health Center has been able to successfully set up their eDNA processing capabilities as a result of this work

Work Summary

Missouri River Basin Water Samples Collected
USFWS staff from WGL, La Crosse FWCO, Great Plains FWCO, Missouri River FWCO, joined by observers from South Dakota Game, Fish and Parks collected 100 samples each in the Big Sioux River below Sioux Falls and below the Lake Vermillion spillway in the East Fork of the Vermillion River in June in South Dakota. In September, USFWS staff from multiple FWCOs collected 100 samples each in the confluences of the James, Vermillion, and Big Sioux rivers within the Missouri River Basin. The 500 samples were sent to Bozeman Fish Health Center for processing as part of a pilot study to help establish future sampling methodologies which will be aimed at detecting bighead and silver carp in areas of the Missouri River Basin where carp presence is unknown.

Bozeman Fish Health Center

As a secondary objective, the Bozeman Fish Health Center worked to establish their lab capabilities for processing eDNA samples. With the guidance of staff from WGL, the Bozeman Fish Health Center was able to successfully process all 500 samples collected as part of this pilot study and a final report of results is being developed. The Center added a separate invasive carp eDNA extraction room, reagent prep room, and additional equipment solely dedicated to invasive carp eDNA processing. The Center also developed station-specific Standard Operating Procedures (SOPs) from the QAPP (2020) and obtained specialized virtual and in-person training from WGL and others to enhance quality assurance and quality control standards. Staff from WGL reviewed all BFHC invasive carp eDNA SOPs and provided feedback on lab layout and

workflow plans. WGL staff also provided 50 invasive carp eDNA proficiency test samples.

Physical Capture to Verify Presence of Invasive Carp

Columbia FWCO staff were joined by Great Plains FWCO staff to deploy an electrified dozer trawl to sample for invasive carp in September in conjunction with the eDNA sampling. This sampling followed a day behind eDNA sampling to prevent contamination. This physical capture sampling is part of a larger effort by the Columbia FWCO to assess bighead and silver carp populations in the Missouri River Basin. The objective of that study is to quantify relative abundance, recruitment, growth, and mortality of bighead and silver carp in tributary confluences to inform management actions.

Partnerships

States of North Dakota and South Dakota

North Dakota Game and Fish and South Dakota Game, Fish and Parks provided information on invasive carp distributions, water access sites, and local water conditions to help prioritize and identify potential eDNA sampling locations within their respective states and provided staff to help collect and process water samples.

Multiple FWCOs and Fish Health Centers across Region 3 and 6

Staff from FWCOs in Wisconsin, North Dakota, South Dakota, and Missouri worked with staff at WGL in Wisconsin and Bozeman Fish Health Center in Montana to plan and implement a work plan to meet the identified goals. Individual roles are identified above, but the collaborative spirit of all involved demonstrates the ability to share knowledge and skills across regions and offices to accomplish lofty goals.

University of South Dakota

A graduate research project, coordinated through the University of South Dakota in cooperation with South Dakota Game, Fish and Parks, is being conducted in the same habitat areas to determine distribution and passage of in channel structures of invasive carp. The USFWS pilot work is supplementing the research data that will be used to help guide this graduate project.



September 2021 sampling was conducted in the confluences of the Vermillion near Vermillion, South Dakota (left), James near Yankton, South Dakota (right) and Big Sioux near Sioux City, Iowa (bottom) rivers. Yellow dots indicate eDNA sample collection sites (100 per river).



June 2021 sampling was conducted in the east fork of the Vermillion River near Canistota, South Dakota (left) and in the Big Sioux River below the Falls in Sioux Falls, South Dakota (right). Yellow dots indicate eDNA sample collection sites (100 per river).



Environmental DNA sampling at the Vermillion River site (left). Biologist holds a silver carp that jumped into the eDNA sample boat on the Big Sioux River (right).

March 2022



*eDNA sampling
at the James River
site near Yankton,
South Dakota.
Sam Stukel/
USFWS*