Project Title: eDNA Monitoring in the Upper Mississippi River

Geographic Location: UMR Pools 8, 10, 12, 13, 14, 17, and 19

Lead Agency: US Fish and Wildlife Service and US Geological Survey

Participating Agencies:

Statement of Need:
In the surveillance for detection of a species in areas where it is rare, using multiple detection methods provides a balanced and more complete monitoring program. Most efforts to monitor and remove Silver and Bighead Carp from the Upper Mississippi River (UMR) occur below Lock and Dam 15. Using eDNA upstream of this area as a long-term monitoring tool could provide early evidence to changes in the invasive carp presence in those pools where traditional capture gears are not heavily utilized and inform future redirection of effort. Recommendations from the latest research aimed at refining eDNA use for Bighead and Silver Carp DNA detection are being implemented in the UMR each year and annual eDNA results in the UMR contribute to better understanding and utilization of eDNA technology for this purpose. The eDNA program is intended to be adaptive and to look at trends of positive detections over time. Each year of collection may make these data more meaningful.

Project Objectives:
1) Continue long-term multi-year, multi-season eDNA monitoring in Pools 8-19 of the UMR to provide data on changes in carp presence
2) Provide eDNA monitoring support for MUM capture events in Pool 8
3) Refine detection probability and optimal sampling design of eDNA in the UMR between the USGS and the USFWS.

Project Highlights:
- USFWS collected 3,322 eDNA water samples across four Mississippi River pools
- USFWS and USGS began a collaborative study to compare collection and processing methodologies between agencies
- USFWS positive detections in Pool 13 and 14 were higher than previous years of spring sampling
- USGS collected 750 eDNA water samples across six Mississippi River pools
- USGS documented no evidence of increased detections in summer from previous years and low detections in fall compared to previous years
- USGS detections in Pool 8 decreased throughout the year at each sampling time point

Methods: USFWS

USFWS staff from the La Crosse FWCO conducted spring and fall sampling in Pools 13, 14, and 16. For each event in Pool 14, 480 samples were collected across six backwater sites. In Pool 13, 240 samples were collected in the spring across 3 backwater sites and 176 in the fall across two sites. Pool 13 and 14 samples were collected to inform managers of potential trends or shifts in the Bighead and Silver Carp population front adjacent to the Intensive Management Zone. In addition to regular monitoring, 80 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 DNA detectability. The backwater targeted by this sampling hosts a real-time telemetry receiver, which detects the presence of acoustically tagged Bighead and Silver Carp. eDNA positive detections can then be compared with the confirmed presence or absence of tagged invasive carp at the time of sampling. Finally, USFWS staff from the La Crosse FWCO conducted three sampling events in Pool 8 in March, July, and October. For each event, 500 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 (MN DNR) and the U.S. Geological Survey Columbia Environmental Research Center. eDNA sampling informed MN DNR 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. Data from all three Pool 8 events were used as part of an on-going study to compare sampling methodologies used by USFWS and USGS. All sample collection and processing procedures followed the 2021 Quality Assurance Project Plan (USFWS 2021). Field blanks were taken in addition to regular monitoring samples. Field blanks are a quality control measure and are not included in reported results (see QAPP for details on quality control steps). 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 species of the two. This is reported as a non-specific “Invasive Carp” detection. If both species-specific markers are detected in a water sample, it is reported under the "Bighead AND Silver" category.

Methods: USGS

USGS staff from the Upper Midwest Environmental Sciences Center (UMESC) conducted eDNA sampling as part of a regular multiyear study in Pool 19 (July only; 50 samples), Pool 17 (July only; 50 samples), Pool 13 (July and October; 50 samples July; 48 samples October), Pool 12 (July and October; 50 samples each), and Pool 10 (July and October; 50 samples July; 29 samples October). We collected 50 ml of water for each sample which were stored on ice and frozen until extraction in addition to field blanks in each pool. Water samples were centrifuged before DNA extraction using the IBI Scientific gMAX Mini Genomic DNA Kit (IBI Scientific,

Dubuque, IA). We used the AD qPCR multiplex reaction developed by Erickson et al. (2017) to amplify a Bighead Carp specific marker and a Silver Carp specific marker in the same reaction.

USGS staff also contributed to sampling in Pool 8 as part of both regular monitoring and supporting the MUM effort. Pool 8 sampling took place in March immediately before the spring MUM event (65 samples), April immediately after the spring MUM event (65 samples), July (99 samples), and October (144 samples representing 48 sampling points). The March, April, and July samples were collected by filtering 300-400 ml of water across a 1.2 micron PCE filter. The October samples were collected through three methods at each of the 48 points; a 50 ml water sample extracted through centrifugation, a 300-400 ml water samples filtered through a 1.2 micron PCE filter, and a 500 ml water sample filtered through a 5 micron PCE filter. There were 25 USGS sampling points collected in the same backwater as USFWS sampling in Pool 8. In April, USGS did not sample concurrently with USFWS but did sample on the same day. In July and October, USGS samples were collected concurrently with USFWS.

Results and Discussion: USFWS

For each the two events in Pool 14, there were 8.75% positive detections (Bighead only, Silver only, and Invasive Carp DNA categories detected) in the spring and 4.8% positive detections in the fall (Figure 1). If the non-specific invasive carp DNA detections are removed from the total (to compare with previous years where these detections were not reported publically), the positive rates were 5.8% and 3.9% which are consistently higher than past events in this pool where positive sample rates were 0-1.45%. In Pool 13, the positive detection rate for the spring event was 18.75% (all four DNA category types detected) or 14.2% (excluding the Invasive Carp DNA detections for comparison with past data), and the fall positivity rate was 0.6% (only Silver Carp DNA was detected) (Figure 2). Positive detections in the recent years were 0-1.2% in Pool 13, so the spring event, especially, was much higher than past events. Pool 13 and 14 samples were collected to inform managers of potential trends or shifts in the Bighead and Silver Carp population front adjacent to the Intensive Management Zone (IMZ). These eDNA results combined with increases in observed carp occurrence in pools even higher in the system suggest a potential increase in carp presence in these pools. In 2019, the river was flooded for an extended period of time, during which many of the dams that normally help to impeded upstream carp movement, were in open river conditions. This would have allowed unrestricted movement of carp upstream for prolonged periods. This may explain the reason behind the increase in carp DNA and observation above the IMZ.

At the site in Pool 16 in the spring, the rate of positive detections was 45% (Bighead Only, Silver only, and Invasive Carp DNA categories detected)(Figure 3). On the day of spring sampling, there were twelve tagged invasive carp (one Bighead x Silver hybrid and eleven Silver Carp) present at Credit Island and eighteen tagged individuals were present at various times in the

week prior. In the fall there were zero positive eDNA detections at the Credit Island site. On the day of fall sampling, there was only one tagged Silver Carp present. This individual was present during the week prior to sampling as was only one other individual, though very briefly. Although the total invasive carp presence is not known for either of these time points, the greater presence of tagged invasive carp in the spring is reflected in the eDNA detection data. This was the first year that these data were collected. Additional years of data will be needed to thoroughly analyze the relationship between tagged fish presence and the rate of DNA detection.

Similar to Pool 16, the results of Pool 8 eDNA sampling seemed to reflect the observed presence of invasive carp during the MUM events in spring (March) and fall (October) (Figure 4). In March, four of the five backwater sites were positive for invasive carp DNA. Sites with detections ranged from 1-13% positive and the site with the highest detection rate was also the site with the most captured and observed invasive carp during the MUM. The site with zero DNA detections was the only MUM site with no invasive carp observations. In the fall, observations of invasive carp matched fairly well with eDNA detection. Three of the five sites were positive for invasive carp DNA and ranged from 1-5% positive. During the MUM, invasive carp were observed in only one of the sites and none were captured. Overall positivity was lower in the fall compared to the spring and that was also reflected in the lower overall number of invasive carp observed in the fall MUM event compared to the spring, suggesting fewer carp were present at the targeted areas.

The spring and fall eDNA sampling events in Pool 8, and the third event which occurred in summer (July) were used as opportunities to conduct side-by-side sampling to compare the differing sampling techniques between USFWS and USGS. This study is on-going and future results and conclusions from these comparisons will be released in a formal report after the study is complete.

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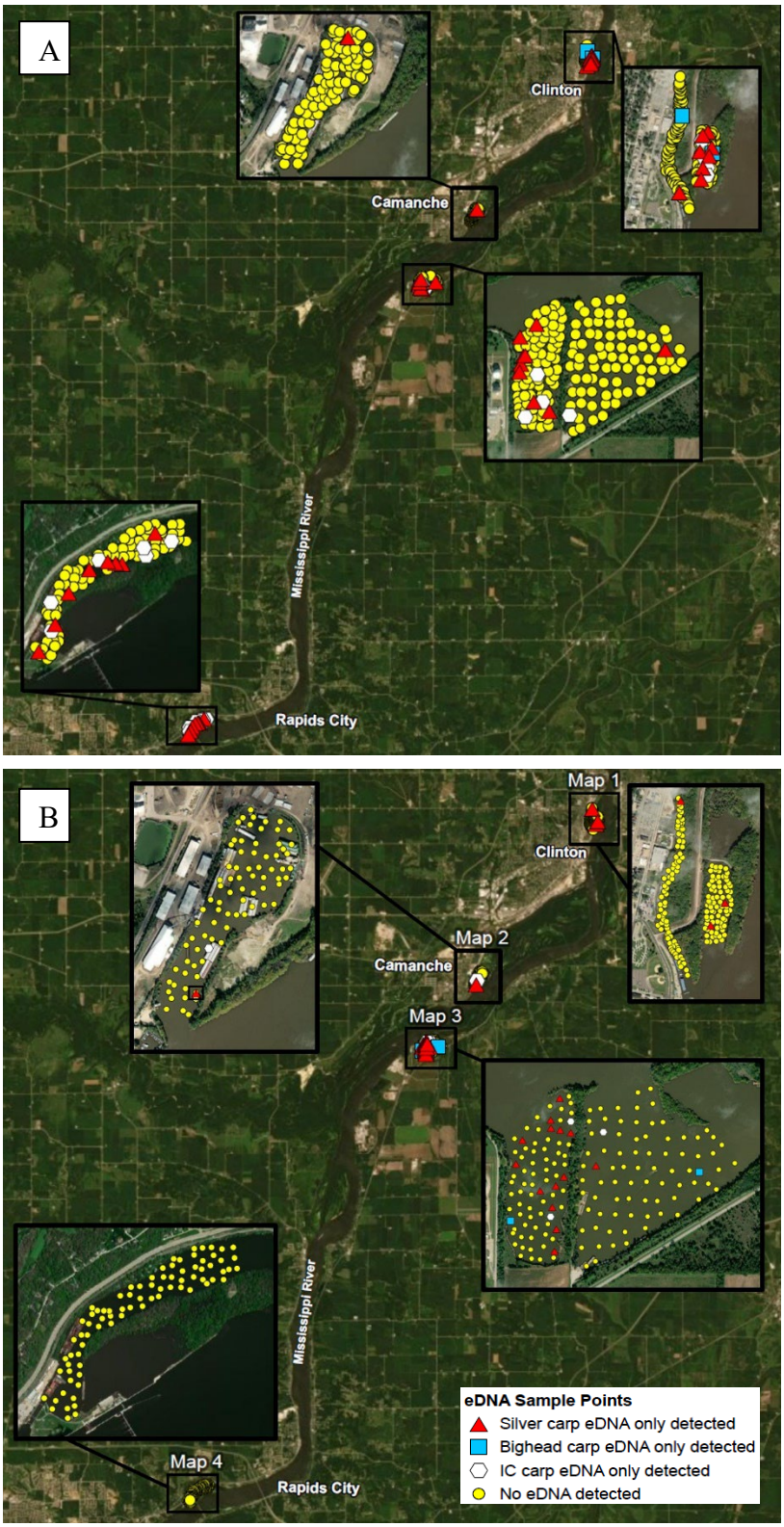

Figure 1. *Detection results for Invasive carp eDNA sampling in Pool 14 of the Upper Mississippi River in spring (A) and fall (B) 2021.*

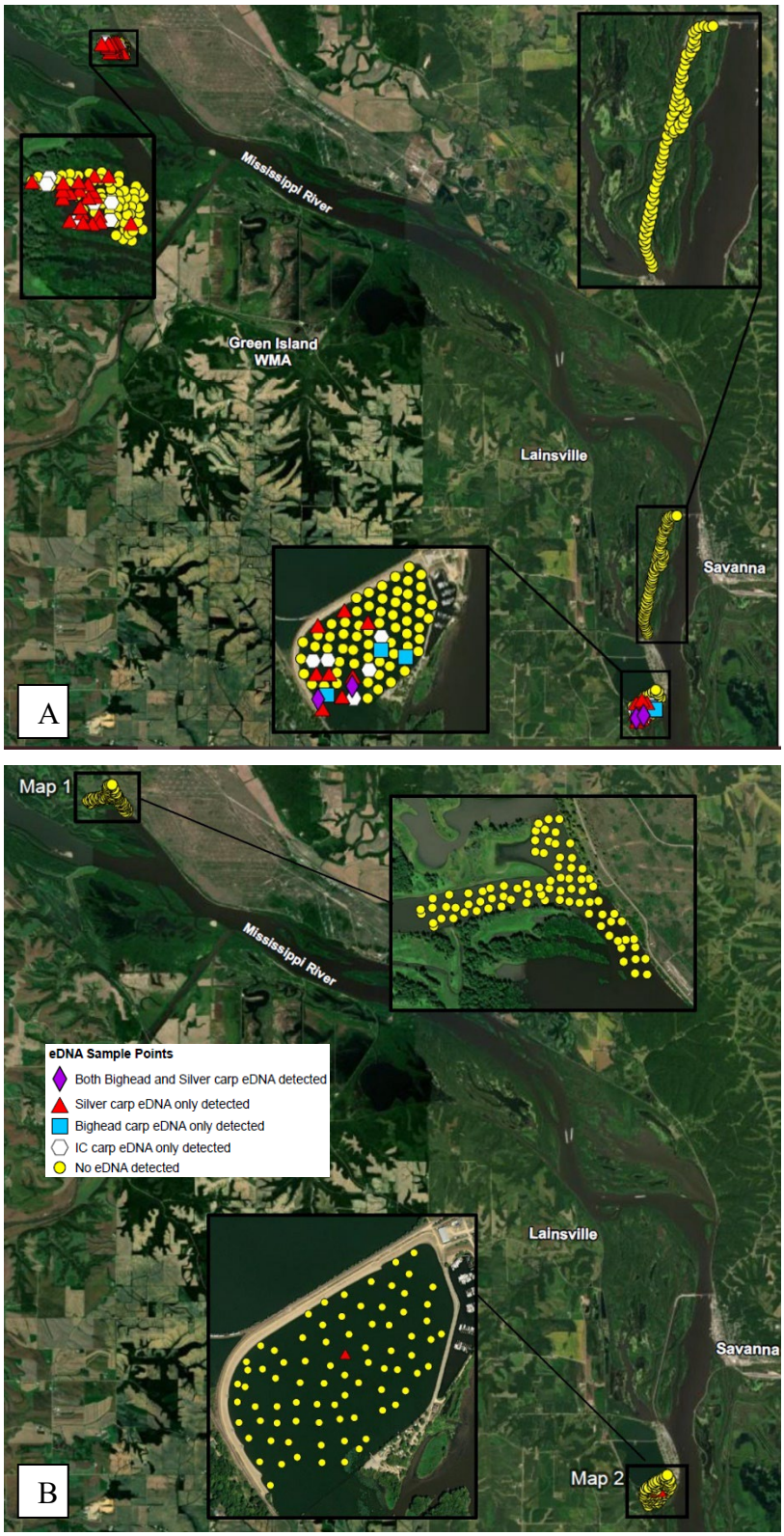

Figure 2. *Detection results for Invasive carp eDNA sampling in Pool 13 of the Upper Mississippi River in spring (A) and fall (B) 2021.*

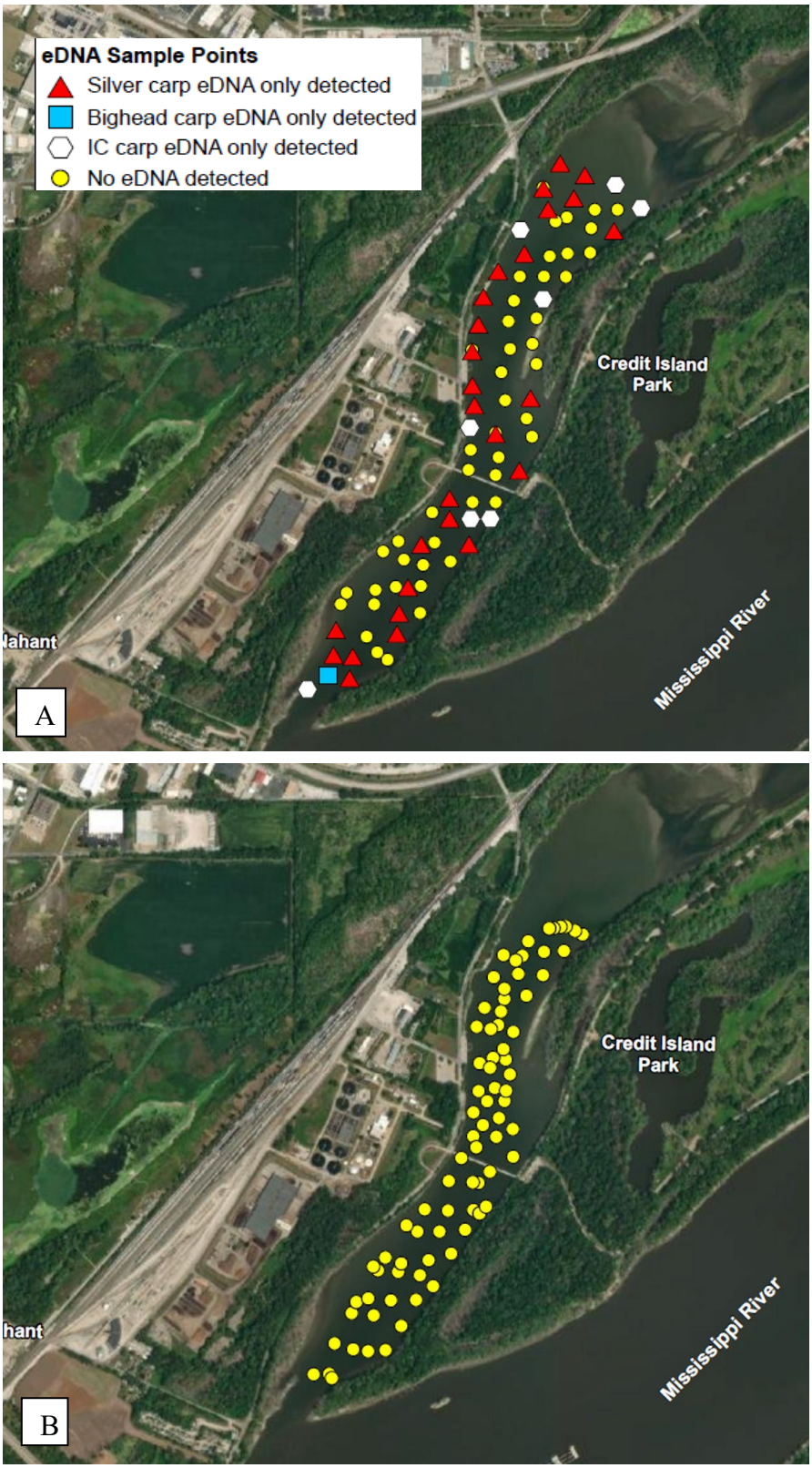

Figure 3. *Detection results for Invasive carp eDNA sampling in the Credit Island backwater in Pool 16 of the Upper Mississippi River in spring (A) and fall (B) 2021.*

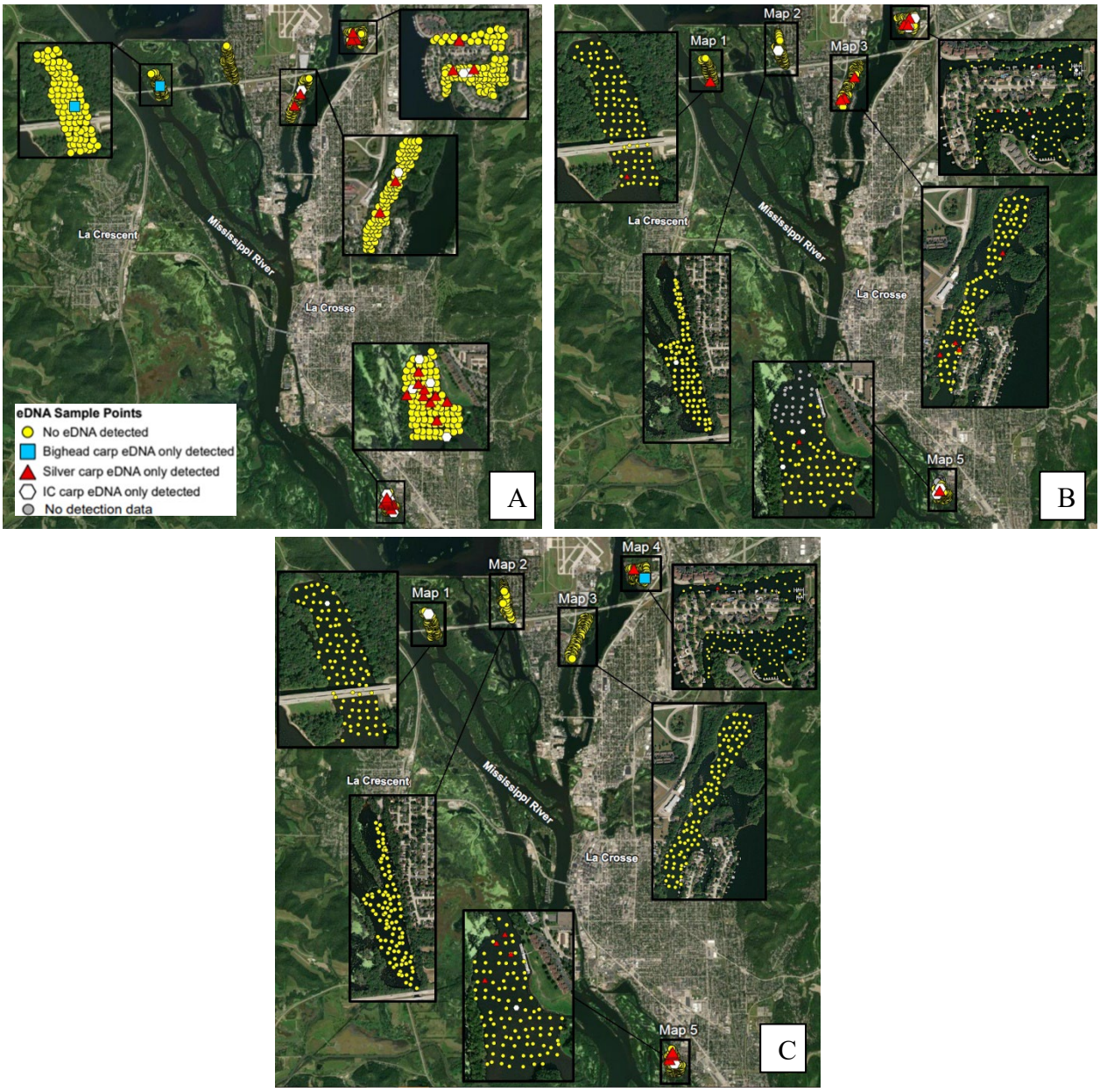

Figure 4. *Detection results for Invasive carp eDNA sampling in Pool 8 of the Upper Mississippi River in spring (A), summer (B), and fall (C) 2021.*

Results and Discussion: USGS

During the July sampling period, there were positive detections in Pool 19 (38% positive), Pool 17 (26%), and Pool 13 (2%) (Figures 5-6). There were no detections in Pools 12 and 10 (Figures

7-8). In Pool 19, 36% of detections were Bighead Carp and 32% were Silver Carp. In Pools 13 and 17, all detections were for both species. During the October sampling period, there were only positive detections in Pool 12 (4%; both for Bighead Carp) (Figure 7). No positive carp eDNA detections occurred in Pools 10 and 13 (Figure 6 & Figure 8). Overall, our results reflect similar to lower rates of detection compared to recent years for the same sampling time of year. For instance, in the summer sampling period, Pool 19 had the lowest percent of detection since 2016 and Pool 13 the fewest postive samples since 2015. The positive rate in Pool 17 in summer was similar to recent years (but note no sampling took place in summer 2020). Previous fall sampling in Pools 10, 12, and 13 have had lower rates of detection, which was consistent with fall 2021 results. The generally low rate of detections may be due to the lower water levels during 2021 which may have restricted carp movement overall during the summer and fall months.

The positive detection rates for Pool 8 were as follows: March – 27.7% positive; April – 20%; July – 2.7%, and October – 0.7% (Figures 9-10). This was consistent with both the MUM capture results and the overall low rate of detections across UMR pools. In October when we used three different water collection techniques, the single positive sample was from a 1.2 micron filter. As noted above, our comparison study with USFWS is still ongoing.

Table 1. *USGS detection results for Upper Mississippi River carp eDNA monitoring in 2021. N represents number of unique eDNA samples, BHC only represents number of samples positive for only bighead carp, SVC only represents number of samples positive for only silver carp, and Both represents number of samples positive for both species.*

Pool	Month	N	BHC only	SVC only	Both
19	July	50	3	1	15
17	July	50	0	0	13
13	July	50	0	0	1
	October	48	0	0	0
12	July	50	0	0	0
	October	50	2	0	0
10	July	50	0	0	0
	October	29	0	0	0
8	March	65	5	0	13
	April	65	0	0	13
	July	99	0	0	2
	October	144	0	1	0

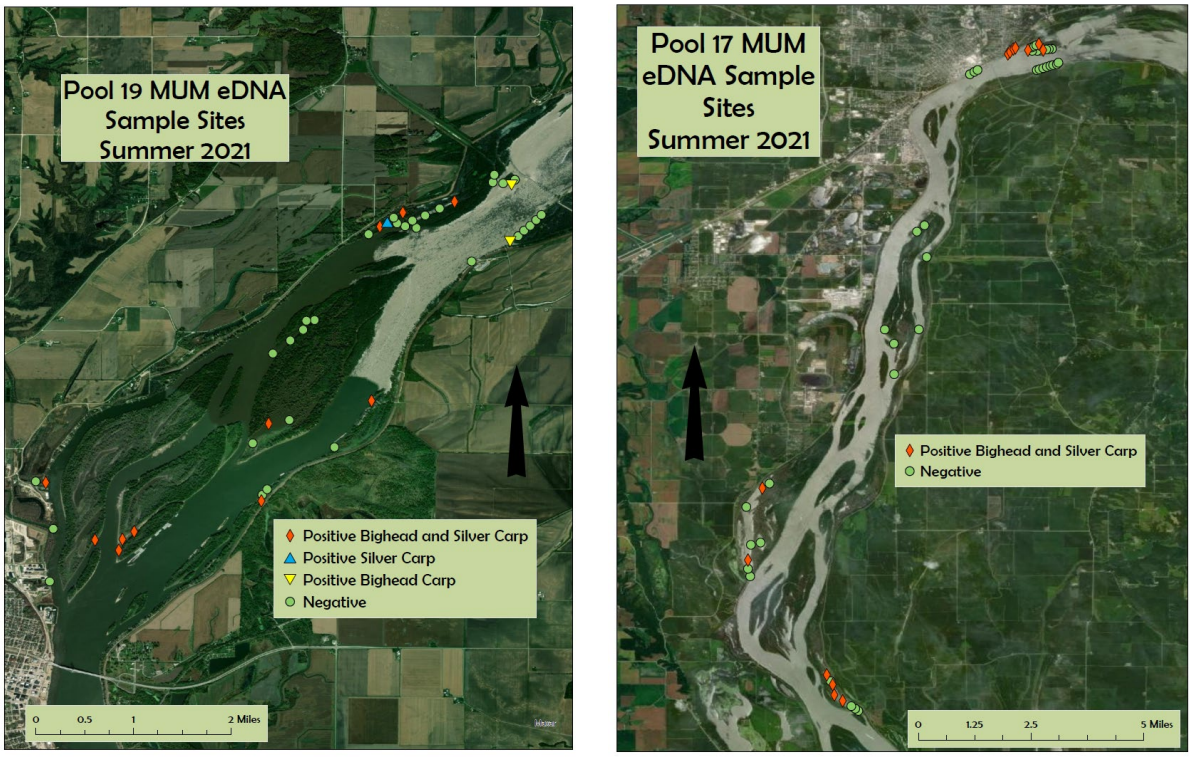

Figure 5. *Detection results for Invasive carp eDNA sampling in Pool 19 & 17 of the Upper Mississippi River in summer 2021.*

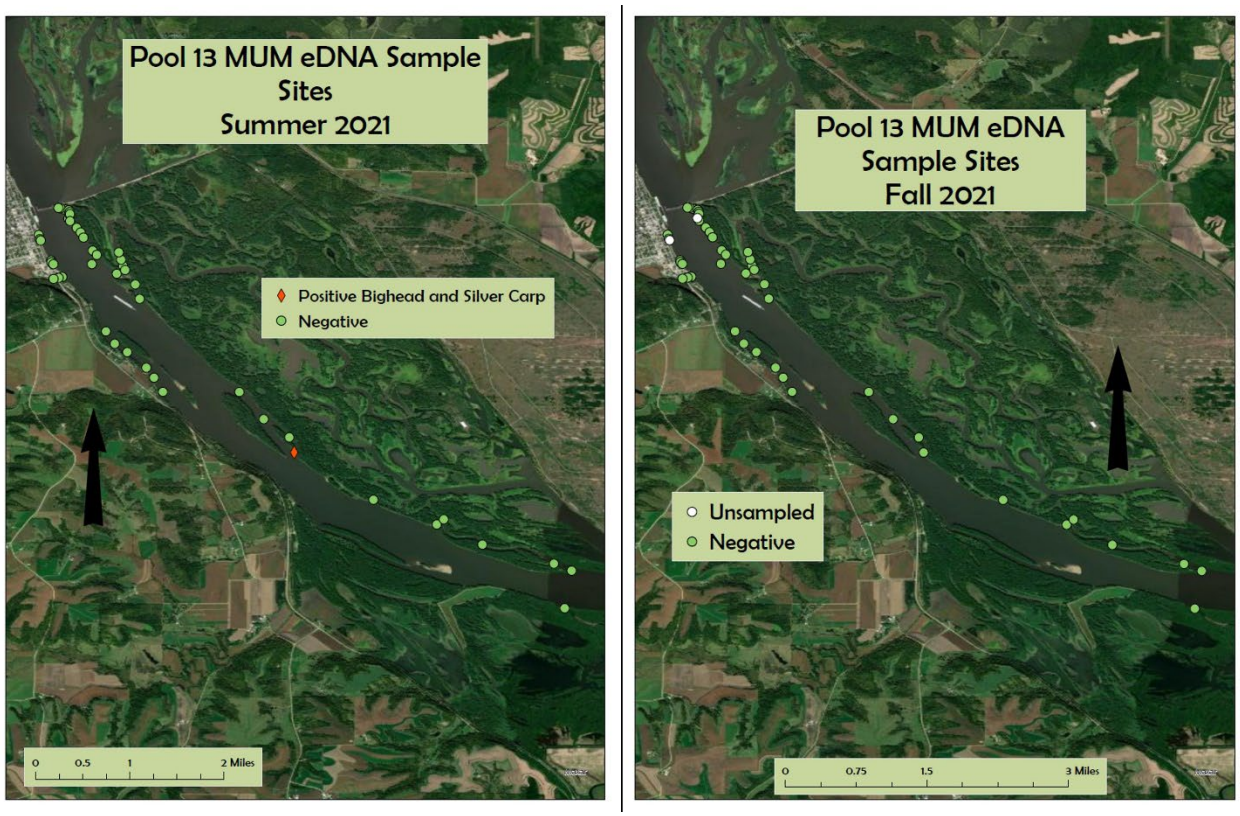

Figure 6. *Detection results for Invasive carp eDNA sampling in Pool 13 of the Upper Mississippi River in summer and fall 2021.*

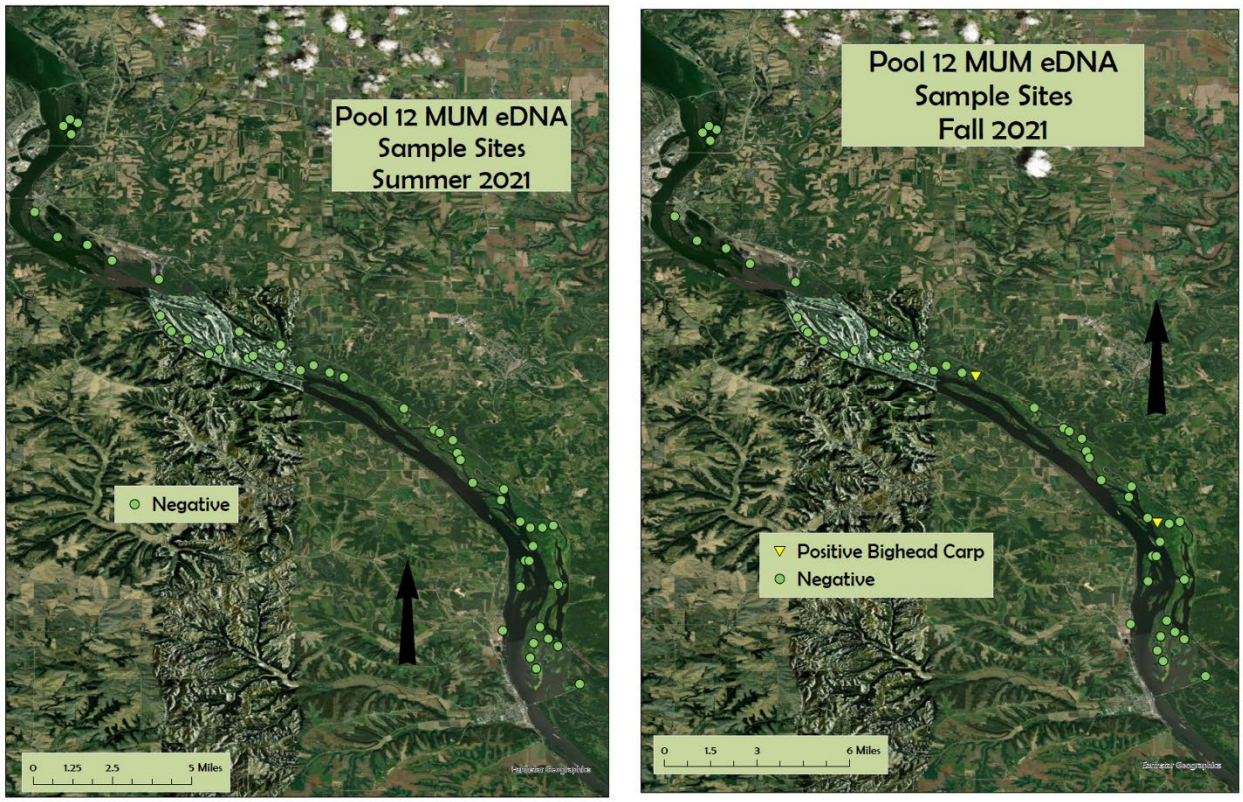

Figure 7. *Detection results for Invasive carp eDNA sampling in Pool 12 of the Upper Mississippi River in summer and fall 2021.*

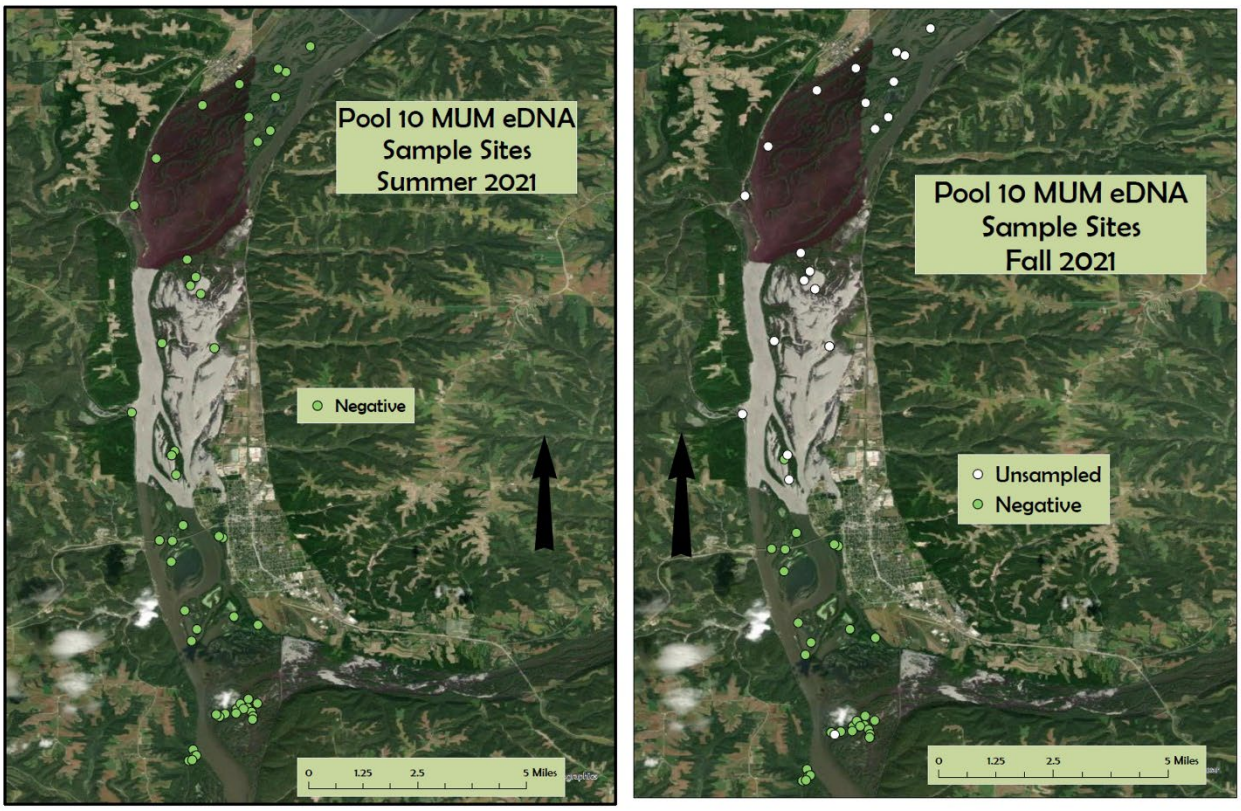

Figure 8. *Detection results for Invasive carp eDNA sampling in Pool 10 of the Upper Mississippi River in summer and fall 2021.*

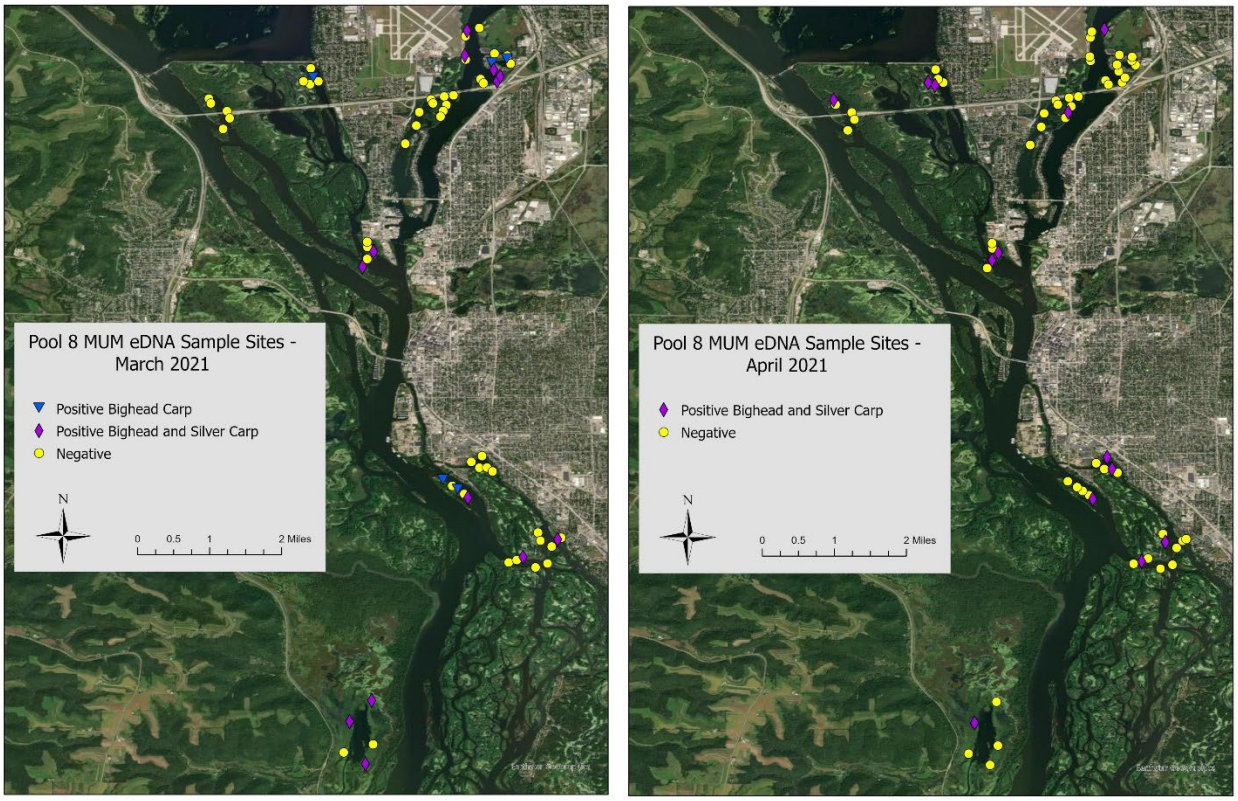

Figure 9. *Detection results for Invasive carp eDNA sampling in Pool 8 of the Upper Mississippi River in March and April 2021, representing before and after the spring MUM event.*

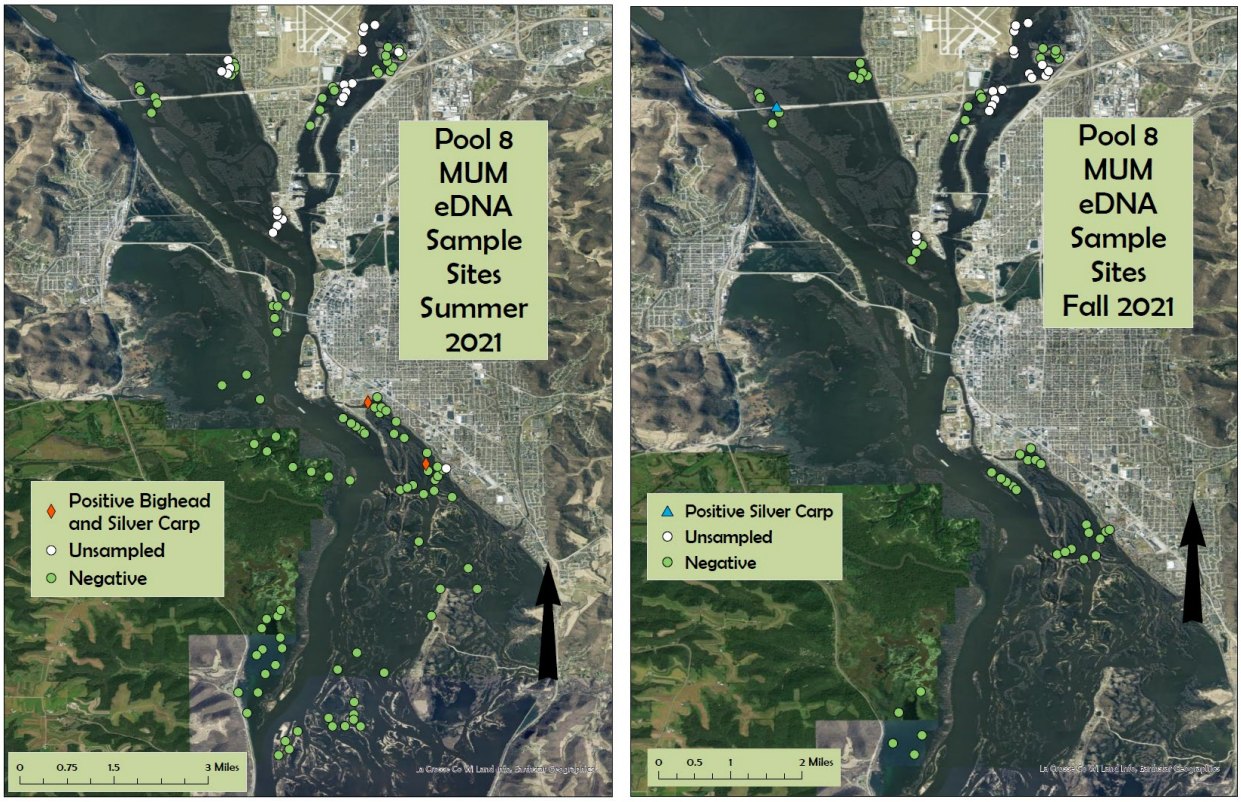

Figure 10. *Detection results for Invasive carp eDNA sampling in Pool 8 of the Upper Mississippi River in summer and fall 2021.*

Recommendation:

Given the inferred increase in invasive carp presence in the pools immediately above the IMZ based on eDNA detections and observations in 2021, USFWS recommends that eDNA effort in that area continue, but at a reduced level to maintain a long term data set. This will include continued sampling at 1-2 sites in Pool 13 and Pool 14 and the calibration site at Credit Island in Pool 16. USFWS will work with state partners to shift the majority of the remaining eDNA efforts further upstream in the basin. This shift in focus will help managers monitor emerging population establishment in the upper pools.

Additionally, it is recommended that USFWS and USGS continue to support future MUM efforts in Pool 8 and continue the inter-agency method comparison study.

Given the continued relatively low rate of detections in Pools 13 and upstream, USGS plans to continue summer and fall eDNA monitoring in Pools 8, 10, 12, 13, and maintain Pool 17 as a known site for carp detection. Additionally, USGS will continue to support Pool 8 spring MUM efforts through before and after sampling around the MUM event. USGS will coordinate with USFWS and state managers if additional sampling is needed in UMR and tributaries and that suit the USGS eDNA sampling design.

References:

Erickson, R.A., C.M. Merkes, C.A. Jackson, R.R. Goforth, J.J. Amberg. 2017. Seasonal trends in eDNA detection and occupancy of bigheaded carps. Journal of Great Lakes Research 43: 762-770.

US Fish and Wildlife Service (USFWS). 2021. Quality assurance project plan eDNA monitoring of bighead and silver carps. Midwest Region, Bloomington, Minnesota. Available: http://www.fws.gov/midwest/fisheries/eDNA/documents/QAPP.pdf . (February 2022).