Project Title: eDNA Monitoring in the Upper Mississippi River

Geographic Location: Mississippi River (Pools 8, 13,14,16), Turkey River, Wisconsin River, Upper Iowa River, St. Croix River, Minnesota River

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Statement of Need: In the surveillance and 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 and tributaries 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 eDNA monitoring in Pools 8, 13, and 14 and select tributaries of the UMR to provide data to infer trends or changes in carp presence.
2) Continually work to refine optimal sampling design, sampling methods, and eDNA data interpretation

Project Highlights:

- USFWS collected 1,980 eDNA water samples across four Mississippi River pools and five tributaries
- Efforts in Pools 13 and 14 were reduce to allows sampling to occur in new areas further upstream
- The Wisconsin River yielded a high rate of positive detections during spring sampling

Methods:

USFWS staff from the La Crosse FWCO conducted spring sampling in Pools 13 and 14. Two backwater in Pool 13 and one in pool 14 were targeted and 80 samples collected per backwater. This was a reduced effort in these pools compared to previous years and was implemented due to the increased detection of invasive carp DNA in pools 13 and 14 in 2021, which also coincided with increased observation in pools above the IMZ. Therefore, some of the sampling capacity

previously dedicated to these pools was shifted upstream to monitor for emerging populations higher in the watershed. A handful of sites were maintained in Pools 13 and 14, however, to maintain the long-term eDNA dataset in those areas and continue to monitor trends in eDNA presence over time.

Three sampling events were conducted in Pool 8 in March, July, and October. The spring event was comprised of 300 samples across 3 backwaters (ice conditions prevented the sampling of two additional sites) and fall event was comprised of 500 samples collected across 5 backwaters and were conducted as part of regular monitoring, while the summer sampling event was comprised of 100 samples collected from a single backwater. The spring event was conducted a bit earlier than normal so that it could immediately precede the Modified Unified Method for Invasive carp removal conducted by the Minnesota Department of Natural Resources (MUM) and the U.S. Geological Survey Columbia Environmental Sciences Center. By doing this, eDNA sampling informed MN DNR of potential Bighead and Silver carp presence and those data were compared to actual capture data from the Modified Unified Method to infer detectability of Silver and Bighead carp in Pool 8. A portion of the samples from the spring and fall events and the entirety of the summer event in Pool 8 were conducted in coordination with the U.S. Geological Survey Upper Midwest Environmental Sciences Center to compare sampling and processing methods between agencies. This is an ongoing study 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.

In both spring and fall, USFWS collected 80 samples and 8 field blanks from the Credit Island backwater in Pool 16. 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.

USFWS worked with the states of Iowa, Wisconsin, and Minnesota to identify tributaries of interest for eDNA monitoring. Based on these discussions, five tributaries were selected for surveillance and sampled for Invasive carp eDNA. Sampling on all five tributaries occurred in May, when water temperatures were sufficient to suggest that Invasive carp, if present, may have entered tributaries to attempt spawning activities. These included the Turkey River (IA), Wisconsin River (WI), Upper Iowa River (IA), St. Croix River (MN/WI), and Minnesota River (MN). In each tributary, 100 samples and 10 field blanks were collected from a single site, except for the St. Croix River where 200 samples and 20 field blanks were collected across two sites. Based on the results in May, the Wisconsin River was sampled a second time, in October, compare detections across seasons.

All sample collection and processing procedures followed the 2022 Quality Assurance Project Plan (USFWS 2022). Field blanks were taken in addition to regular monitoring samples. Field blanks are a quality control measure and are not included in reported results. 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.

Results and Discussion:

In pool 13, the rate of positive eDNA detections was 20.6% (Bighead only, Silver only, Bighead AND Silver, and non-specific Invasive Carp marker types detected) and pool 14 was 1.25% (Silver only) (Figure 1). When comparing the detection rates for the three sampled backwaters to the results of the previous spring, the positive detection rates for all sites were lower in 2022 (28% in pool 13 and 13.75% in pool 14 in 2021), however the pool 13 rate was still relatively high compared to historic rates indicating that carp presence may be similar to 2021.

In Pool 8, the positive detection rate in the spring was lower than the previous spring (<1% overall with 2 of 3 sites positive in 2022 and 4% overall with four of five sites positive in 2021) (Figure 2). Captures and observations of invasive carp during the MUM were also lower than the previous spring, which is consistent with eDNA results. Water temperature may have played a large part in the decrease in the detection rate between years. The average water temperature was 11°F lower in 2022 (36°F) than in 2021 (47°F) during the week of sampling. Past telemetry data shows that invasive carp typically start moving into backwater areas in the spring when water temperatures are around 45°F. Both MUM and eDNA data indicate that carp presence in the sample locations was lower from the previous year, which corroborates with telemetry trends elsewhere. The positive detection rate in the fall was slightly lower than the previous fall (<1% overall with two of five sites positive in 2022 and 1.6% positive with three of five sites positive in 2021). Consistent with previous telemetry and eDNA detection data from other pools, these data indicate that invasive carp are likely congregating in these backwater habitats in the spring and not as much in the fall. This suggests that timing of removal and other sampling efforts for invasive carp in backwaters of pool 8 could be more successful in the spring versus the fall, but only if environmental conditions are also appropriate for congregation of these species.

In the spring, in the Credit Island backwater in Pool 16, there were 25% positive detections, and, in the fall, there were zero detections (Figure 3). The spring results had a lower positive rate than the previous year (40% in 2021) but were still noticeably higher than historical rates. The fall results were consistent with the previous fall which also had zero detections. When compared to telemetry detections, the eDNA data match well as far as more detections occurring in the spring than in the fall. At the time of the spring sampling, there were two Silver carp present and those two Silver carp and one hybrid had been present regularly for several weeks leading up to

sampling. At the time of the fall sampling one Silver carp was present and had been for several weeks prior to the event. A hybrid carp was also present, but only fleetingly on the day of sampling and was not present in the weeks leading up to the event. Although the total number of carp present at the time of sampling is unknown, based on tagged fish alone, it seems that the length of stay for individuals versus number of individuals present may be important to consider when attempting to gauge carp presence using eDNA detections.

Two of the five tibutaries sampled had positive eDNA detections. The Turkey, Upper Iowa, and Minnesota River sites all had zero eDNA detections. The Wisconsin River had a positive detection rate of 20% (Bighead only, Silver only, Bighead AND Silver) (Figure 4). These results are supported by observations of Invasive carp at this site around the time of sampling as well. The second sampling event which occurred in October, had just 2% positive detections (Silver only). This suggests that invasive carp congregate in this area in the spring, likely as part of attempted spawning activities, and are less present in the fall. This is supported by telemetry data from other Mississippi River tributaries and these data could be used to monitor for carp presence over time since tagged invasive carp are not abundant in this tributary or the connecting pool. Of the two sites sampled in the St. Croix River, both resulted in positive detections with an overal positive detection rate of 6% (Figure 5). One of the sites, located near in Bayport, MN is a site frequented by a small number of telemetry tagged Invasive carp in the area.

Tables and Figures:

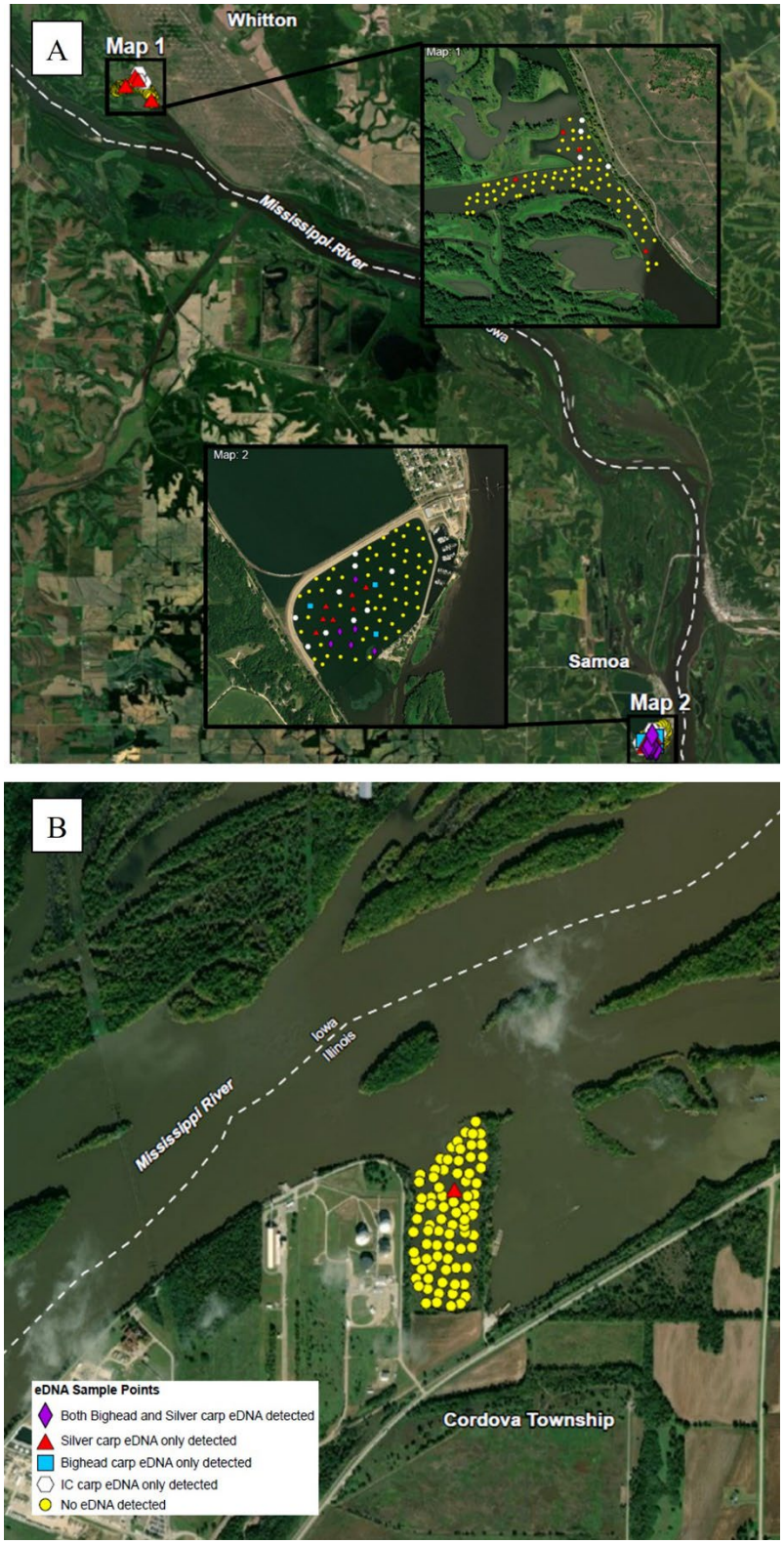

Figure 1. *Detection results for Invasive carp eDNA sampling in Pool 13 (A) and Pool 14 (B) of the Upper Mississippi River in spring 2022.*

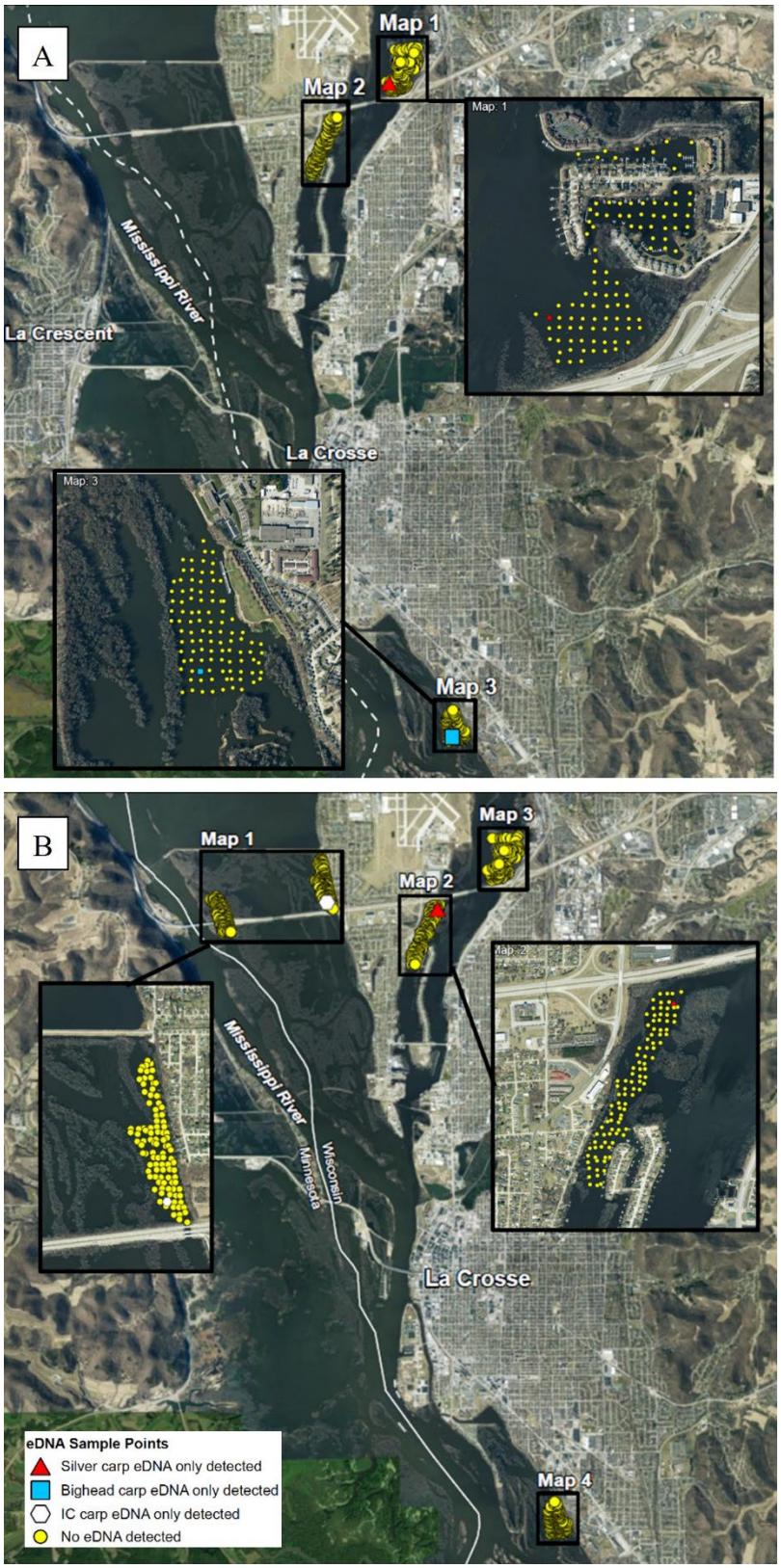

Figure 2. *Detection results for Invasive carp eDNA sampling in Pool 8 of the Upper Mississippi River in spring (A) and fall (B) 2022.*

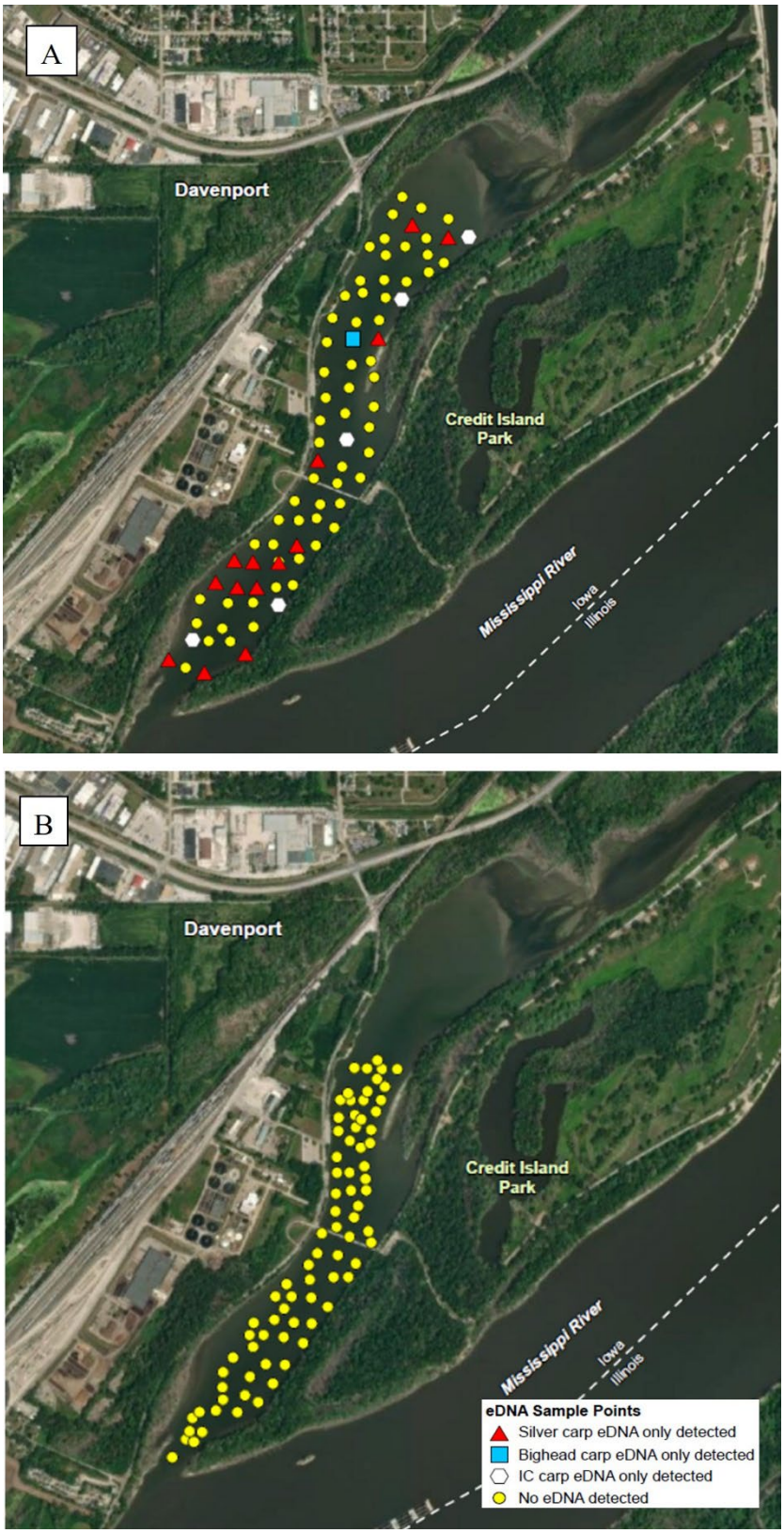

Figure 3. *Detection results for Invasive carp eDNA sampling in the Credit Island backwater in Pool 16 of the Upper Mississippi River in spring (A) and fall (B) 2022.*

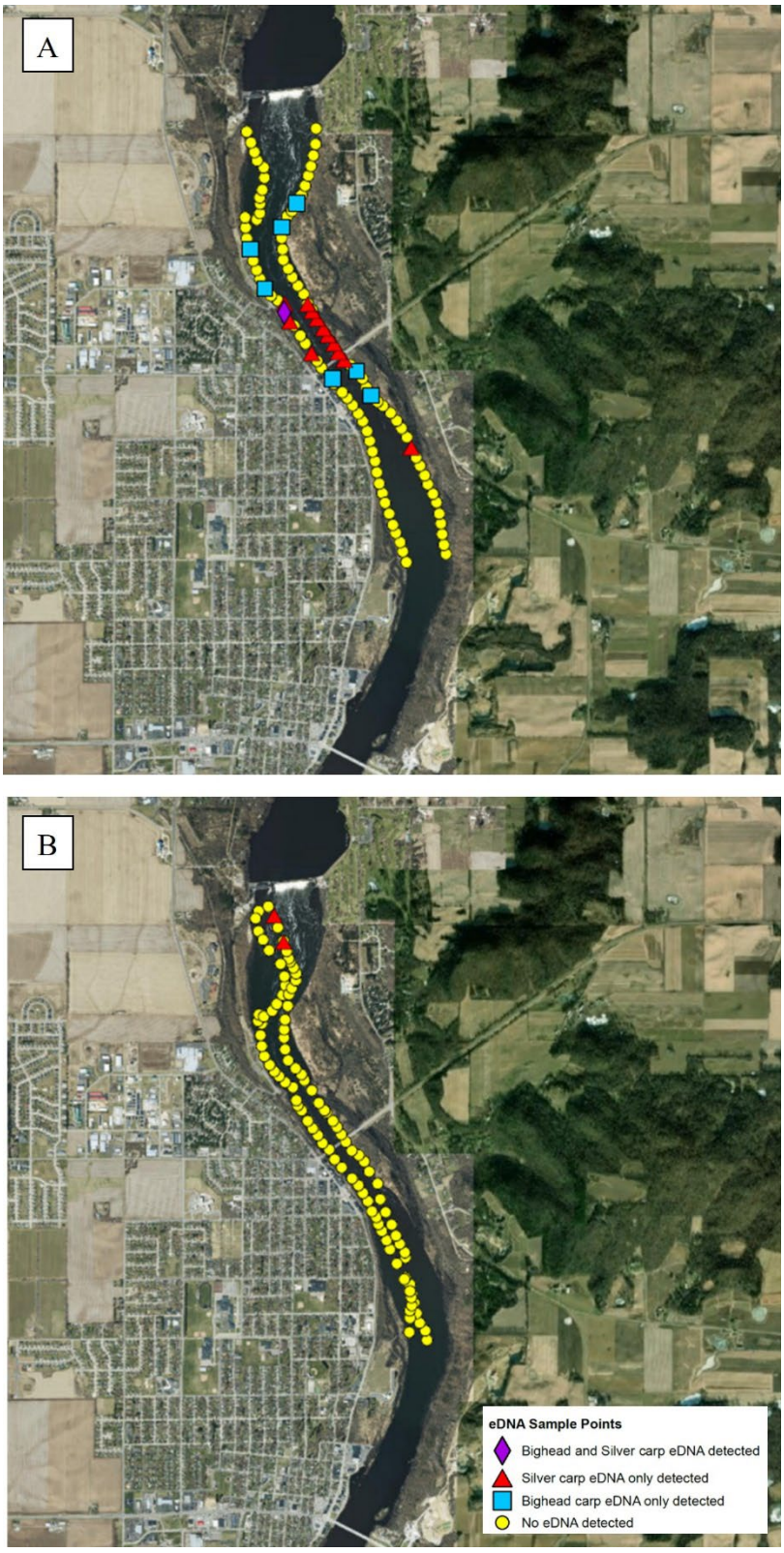

Figure 4. *Detection results for Invasive carp eDNA sampling in the Wisconsin River spring (A) and fall (B) 2022.*

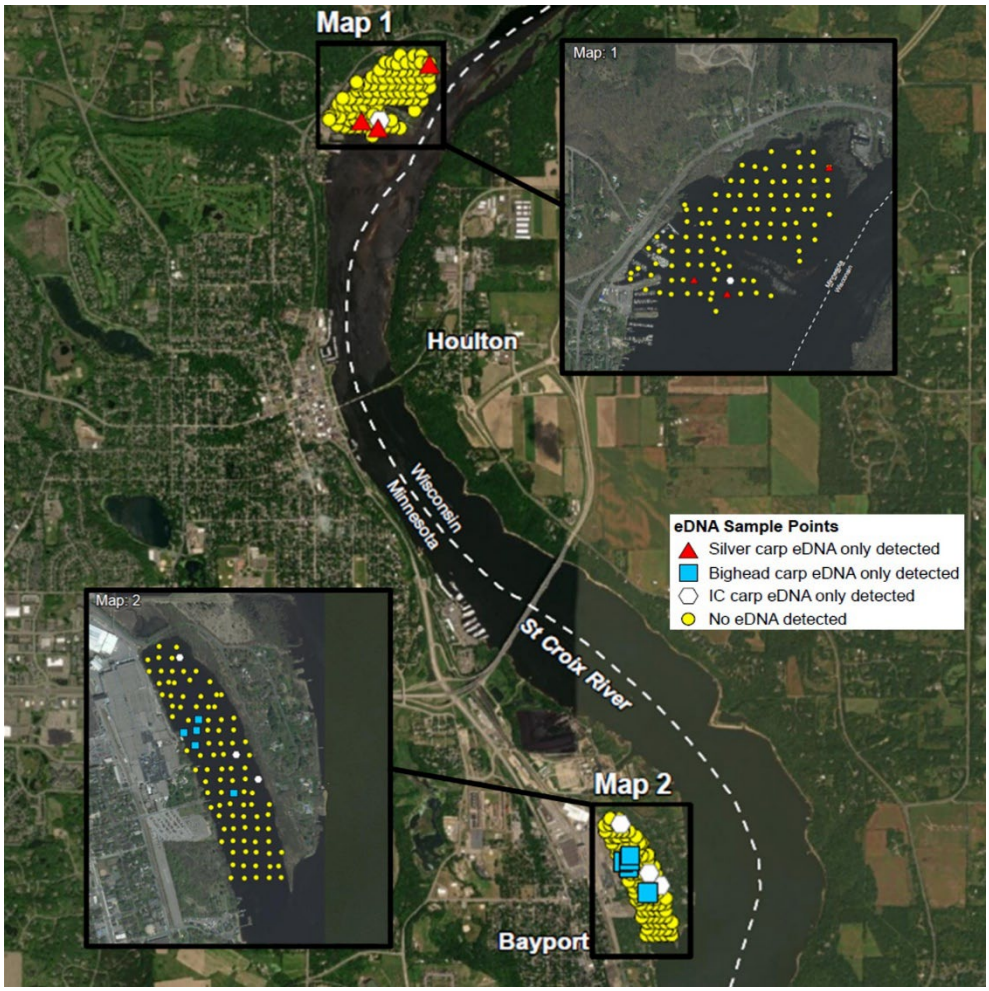

Figure 5. *Detection results for Invasive carp eDNA sampling in the St. Croix River in spring 2022.*

Recommendation:

USFWS recommends that eDNA efforts in Mississippi River Pools 13 and 14 continue at the reduced level to maintain a long term dataset if those data are useful to state and federal partners. This would include continued sampling at two sites in Pool 13 and one site in Pool 14. Sampling at the Credit Island in Pool 16 should continue to continually add to a comparison data set between telemetry and eDNA detections. eDNA monitoring in Pool 8 in the spring should as requested by state partners and be used to advise potential trends and changes in carp presences. Sampling this pool in the fall should be reconsidered or ended since the data in the spring are more valuable for removal efforts. eDNA sampling in the Wisconsin River will be repeated and focus on the spring when carp may be tavelling up to the first barrier for spawning activities. USFWS recommends removal or tagging efforts in this area as 2022 sampling indicated the site as a likely location of Invasive carp congregation in the spring. Sampling in the Upper Iowa and Turkey rivers should follow suit with the Wisconsin River and samples should be relocated in 2023 from the confluences with the Mississippi river to below the first barriers in the hopes of capturing carp presence in the spring, as fish may travel up these tributaries for spawning

activities. Sampling in the Minnesota River and St. Croix River will also be repeated in the spring, as requested by Minnesota DNR, to monitor for carp presence and spring congregation in off-channel and backwater habitats. These data could assist state partners with future removal efforts.

References:

US Fish and Wildlife Service (USFWS). 2022. Quality assurance project plan eDNA monitoring of bighead and silver carps. Midwest Region, Bloomington, Minnesota. Available: https://www.fws.gov/sites/default/files/documents/eDNA-QAPP-2022-Whitney-GeneticsLab.pdf