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eDNA Surveillance of Asian Carp on the St. Croix and Mississippi Rivers

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Summary Findings

St. Croix Falls and Lock and Dam No. 1

- 50 samples were collected from the St. Croix River on June 28, 2011 between St. Croix Falls, WI and Franconia, MN
- 50 samples were collected from the Mississippi River on June 29, 2011 between Lock and Dam No. 1 and the confluence with the Minnesota River (Pike Island)
- All samples were screened for bighead carp, silver carp and black carp
- **All samples tested negative for bighead carp**
- **All samples tested negative for black carp**
- **All samples tested negative for silver carp on the Mississippi River**
- **22 samples tested positive for silver carp on the St. Croix River**

Acknowledgments

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Tim Schlagenhaft, Minnesota Department of Natural Resources
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Background and Methods

Objective

To delimitate the invasion front of bighead carp (*Hypophthalmichthys nobilis*), silver carp (*H. molitrix*), and black carp (*Mylopharyngodon piceus*) in the Upper Mississippi and St. Croix rivers by screening surface water samples for the target species' environmental DNA (eDNA). Samples for bighead and silver carp were collected from the reaches downstream of the dam at St. Croix Falls, Wisconsin on the St. Croix River and Lock and Dam No. 1 in Saint Paul, Minnesota on the Mississippi River, locations thought to represent the upper distribution limit for both species. While the dam at St. Croix Falls represents a barrier to upstream expansion of Asian carps, carp can get through the lock at Lock and Dam No. 1.

eDNA technology

Traditional monitoring methods for aquatic organisms are often not able to detect target species at low abundances due to inherent logistical difficulties of aquatic systems. By detecting the genetic trail left behind by a target organism rather than the physical specimen, environmental DNA technology allows for a more sensitive detection of rare species (Jerde et al. 2011). DNA of an organism remains suspended in the water after it is shed or sloughed, which can then be collected in a water sample and captured on filter paper. The total DNA of the sample that is captured on the filter paper can subsequently be extracted. Using species-specific markers, target DNA is amplified from the extracted material and visualized with fluorescent dye, providing sensitive evidence of a species' presence.

Methods

As determined by the Asian carp working group (see the acknowledgments for participating members), a series of 50 2-liter samples were collected from a 4.3 mile stretch of the St Croix river below St. Croix Falls dam and a 3.6 mile stretch of the Mississippi River below Lock and Dam No. 1 (100 in total, plus 6 additional control samples). The number of samples chosen was based on experience gained from previous sampling efforts in the Chicago Area Waterway System (CAWS; Jerde et al. 2011). The placement of each sample was determined through collaboration between Lindsay Chadderton of the

Nature Conservancy and Tiffany Hickox of Environmental DNA Solutions using aerial imagery software (Google Earth), with sample locations marked on a map for use in the field. Sampling sites were selected to ensure coverage, while focusing on the areas where DNA accumulation and/or carp presence was most likely, based on previous sampling experience in the CAWS. The sampling plan was refined in the field according to the conditions and included both samples collected in transect across the river and targeted sampling in areas where eDNA might accumulate (eddies, backwaters, snags, etc.).

Samples were collected and filtered largely according to the protocols described in Mahon et al. (2010), but with the following variations:

- Prior to sampling on the Mississippi River, the boat in use was not sterilized using a 10% sodium hypochlorite solution. This was deemed acceptable because its slip was located only a few river-miles downstream of the sampling reach and the flow rate of the river was high enough to consider this water a non-contaminating source. The boat in use for the St. Croix River sampling effort was appropriately cleaned.

- From the St. Croix Falls dam downstream to Taylor Falls, samples could only be collected from the shore because this stretch of river is not accessible to boats. Samples were collected around a series of access points on either side of the river, targeted to habitats likely to promote accumulation of surface floating material. One control sample was taken during this sampling process to ensure no contamination occurred in the transportation process. That is, a 2 liter sample bottle prefilled with de-ionized water was taken down to the river, opened and resealed, dunked, and then returned to the cooler. Ice was added to the cooler immediately following the shore-based sampling and the cooler remained with the vehicles during the rest of the St. Croix River sampling.

- One transportation control sample was collected for every 19 samples, or one control per cooler (6 control samples total). These bottles were randomly selected from the cooler, and were prepared with de-ionized water before traveling to the sampling location.

- According to the suggestions in an EPA-led audit (Blume et al. 2010), disposable, one-time-use collection bottles and filter funnels were used to minimize any chance of cross-contamination. Samples of the equipment were tested before use in the field to ensure similar functionality and lack of contamination. The disposable 300-ml funnels were assembled with one piece of the appropriate filter paper using forceps in a UV-sterilized hood prior to sample collection. Each funnel was individually sealed in a plastic bag that was only removed just prior to its use. Because new funnels were used for

each sample, no filtration of de-ionized water as an equipment control was necessary. Similarly, no positive equipment controls samples were necessary, as in use by the USACE only, due to the lack of any inhibiting residual hypochlorous acid from the cleaning procedure.

- No central vacuum system was available to filter samples and this was substituted by portable electric vacuum pumps.

- The filtered samples were stored in the available freezer below 0˚C until they were transported on ice to Environmental DNA Solutions' laboratories in Granger, IN. The samples were stored at -20˚C while awaiting processing.

Environmental DNA processing methods are adapted and modified from those used by Mahon et al. 2010.

- To increase the sensitivity and objectivity of the screening process, qPCR was used for silver and bighead carp analyses rather than PCR and gel electrophoresis. Species-specific markers (Jerde et al. 2011) were each stringently tested using SYBR Green qPCR for sufficient sensitivity of the assay and for specificity against similar species (common carp, goldfish, grass carp, black carp and either bighead carp or silver carp for the respective species). Each sample was tested in triplicate for silver carp and bighead carp, using the appropriate positive and negative controls for each step. All presumptive positives were confirmed by dot blot hybridization using a highly specific probe to further demonstrate that the amplified DNA was in fact from the target species (Curry et al. 2008). The probes underwent similar validation and specificity testing as the markers.

- Probe-specific qPCR was used to screen for black carp (*M. piceus*). The probe and marker were stringently tested for sensitivity and specificity against similar species (common carp, goldfish, grass carp, bighead carp, and silver carp). This marker has not been field validated and was included as research only. All samples were tested in triplicate and included the appropriate positive and negative controls for each step.

Results

Figure 1: Reach of Mississippi River sampled (approx. 3.6 miles), including all sampling points.

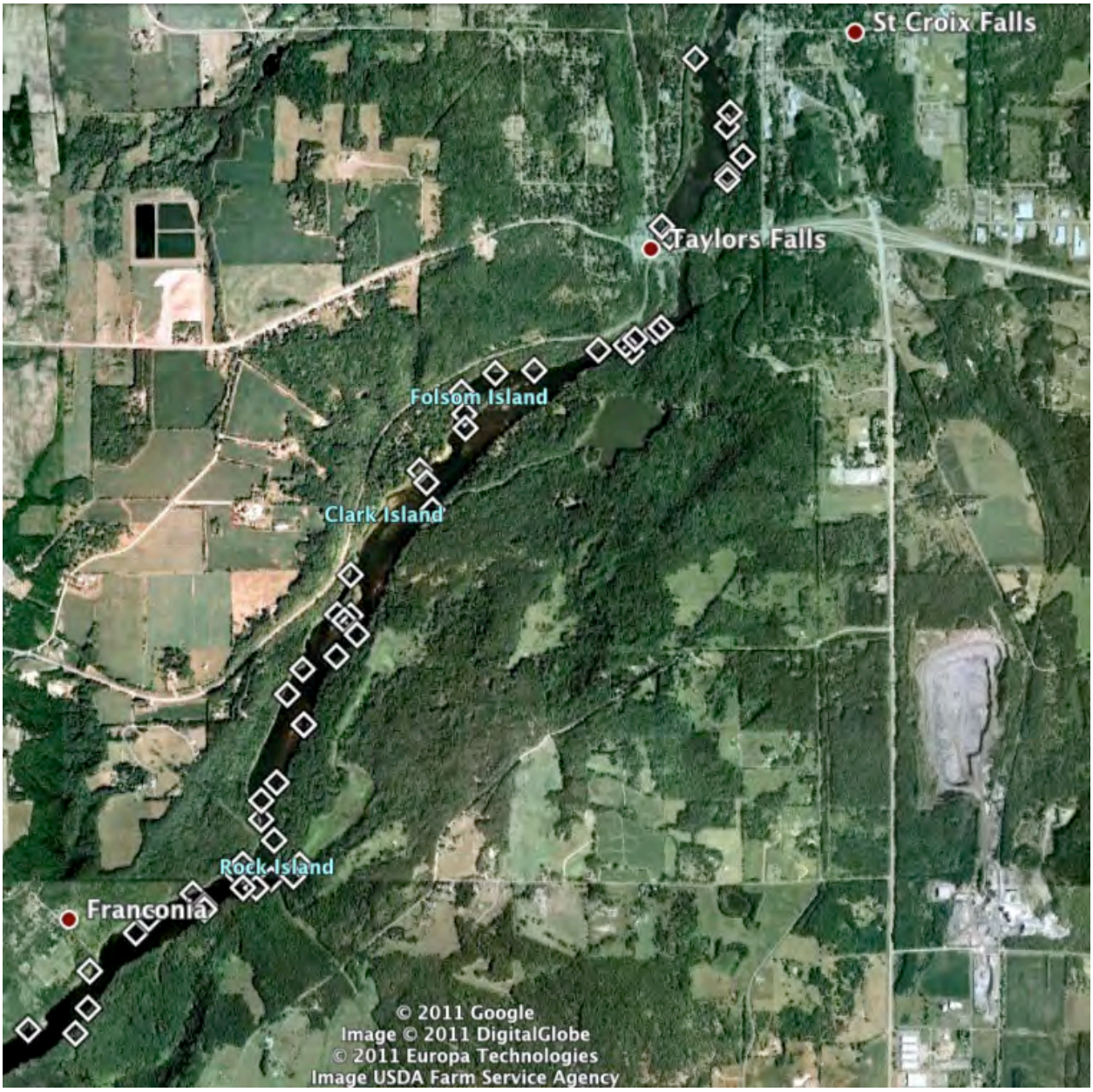

Figure 2: Reach of St. Croix River sampled (approx. 4.3 miles), including all sampling points.

Figure 3: Results from the Mississippi River. No samples tested positive for any of the target species (bighead, silver, black carp).

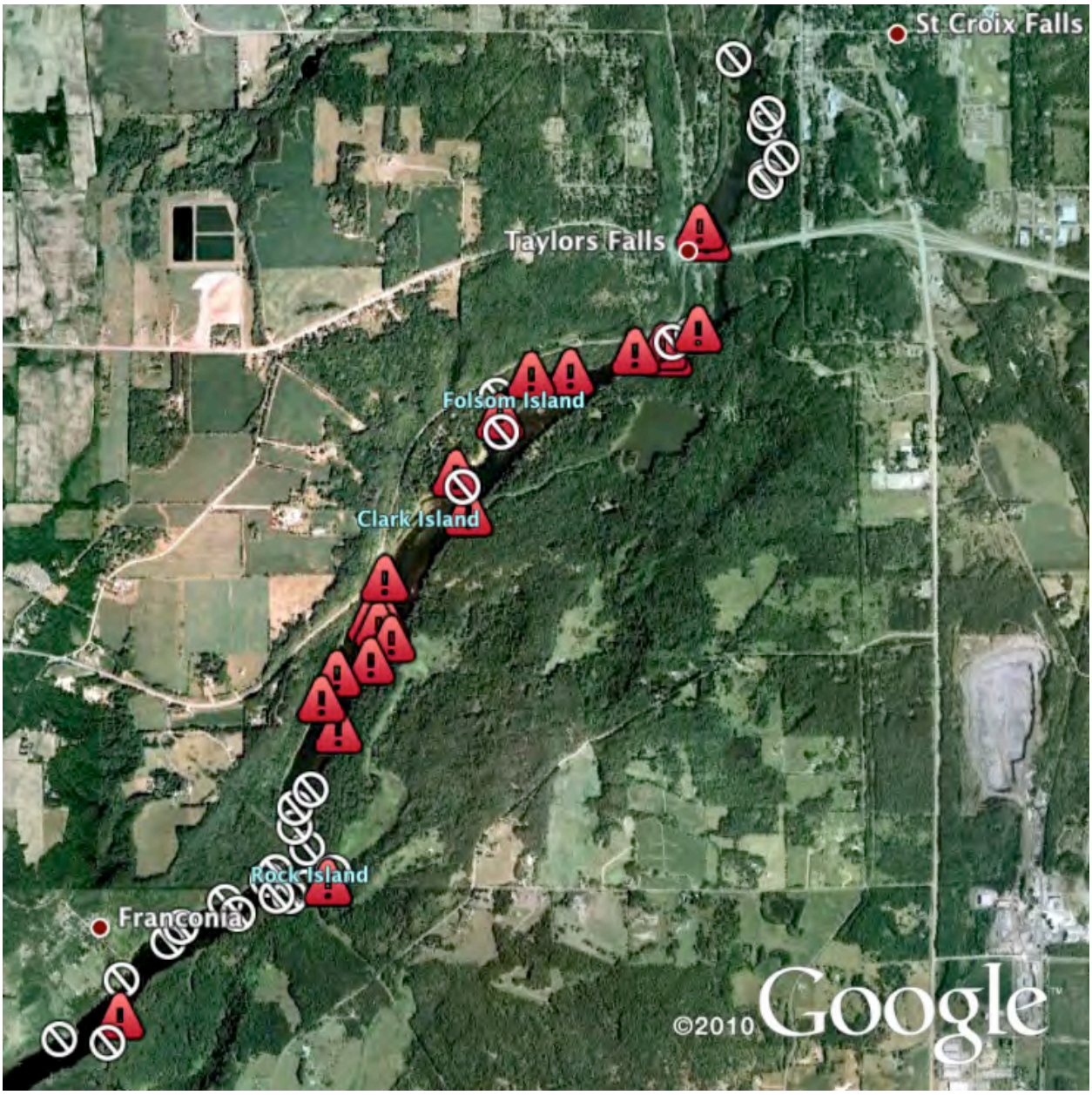

Figure 4: Results from the St. Croix River. Red caution icons represent positive results for silver carp. White circle icons indicate negative results. No sample tested positive for bighead carp or black carp.

Silver	Bighead	Black	River	Latitude	Longitude	Date Collected
0	0	0	St. Croix	45 24.502	-92 38.823	Jun 28, 2011
0	0	0	St. Croix	45 24.463	-92 38.835	Jun 28, 2011
0	0	0	St. Croix	45 24.311	-92 38.834	Jun 28, 2011
0	0	0	St. Croix	45 24.325	-92 38.833	Jun 28, 2011
0	0	0	St. Croix	45 24.376	-92 38.772	Jun 28, 2011
0	0	0	St. Croix	45 24.653	-92 38.964	Jun 28, 2011
+	0	0	St. Croix	45 24.149	-92 39.068	Jun 28, 2011
+	0	0	St. Croix	45 24.178	-92 39.099	Jun 28, 2011
+	0	0	St. Croix	45 23.892	-92 39.101	Jun 28, 2011
0	0	0	St. Croix	45 23.882	-92 39.125	Jun 28, 2011
0	0	0	St. Croix	45 23.860	-92 39.206	Jun 28, 2011
+	0	0	St. Croix	45 23.825	-92 39.219	Jun 28, 2011
+	0	0	St. Croix	45 23.843	-92 39.244	Jun 28, 2011
+	0	0	St. Croix	45 23.829	-92 39.355	Jun 28, 2011
+	0	0	St. Croix	45 23.771	-92 39.614	Jun 28, 2011
+	0	0	St. Croix	45 23.763	-92 39.771	Jun 28, 2011
0	0	0	St. Croix	45 23.710	-92 39.908	Jun 28, 2011
+	0	0	St. Croix	45 23.647	-92 39.896	Jun 28, 2011
0	0	0	St. Croix	45 23.607	-92 39.892	Jun 28, 2011
+	0	0	St. Croix	45 23.487	-92 40.076	Jun 28, 2011
0	0	0	St. Croix	45 23.453	-92 40.049	Jun 28, 2011
+	0	0	St. Croix	45 23.376	-92 40.025	Jun 28, 2011
+	0	0	St. Croix	45 23.191	-92 40.356	Jun 28, 2011
+	0	0	St. Croix	45 23.079	-92 40.410	Jun 28, 2011
+	0	0	St. Croix	45 23.076	-92 40.356	Jun 28, 2011
+	0	0	St. Croix	45 23.062	-92 40.381	Jun 28, 2011
+	0	0	St. Croix	45 22.924	-92 40.549	Jun 28, 2011
+	0	0	St. Croix	45 22.853	-92 40.611	Jun 28, 2011
+	0	0	St. Croix	45 22.767	-92 40.544	Jun 28, 2011
0	0	0	St. Croix	45 22.603	-92 40.654	Jun 28, 2011
0	0	0	St. Croix	45 22.552	-92 40.716	Jun 28, 2011

Silver	Bighead	Black	River	Latitude	Longitude	Date Collected
0	0	0	St. Croix	45 22.438	-92 40.665	Jun 28, 2011
0	0	0	St. Croix	45 22.329	-92 40.676	Jun 28, 2011
+	0	0	St. Croix	45 22.339	-92 40.586	Jun 28, 2011
0	0	0	St. Croix	45 22.370	-92 40.562	Jun 28, 2011
0	0	0	St. Croix	45 22.304	-92 40.737	Jun 28, 2011
0	0	0	St. Croix	45 22.305	-92 40.790	Jun 28, 2011
0	0	0	St. Croix	45 22.349	-92 40.824	Jun 28, 2011
0	0	0	St. Croix	45 22.254	-92 40.945	Jun 28, 2011
0	0	0	St. Croix	45 22.287	-92 40.994	Jun 28, 2011
0	0	0	St. Croix	45 22.217	-92 41.171	Jun 28, 2011
0	0	0	St. Croix	45 22.176	-92 41.223	Jun 28, 2011
0	0	0	St. Croix	45 22.068	-92 41.409	Jun 28, 2011
+	0	0	St. Croix	45 21.963	-92 41.416	Jun 28, 2011
0	0	0	St. Croix	45 21.892	-92 41.466	Jun 28, 2011
0	0	0	St. Croix	45 21.901	-92 41.658	Jun 28, 2011
0	0	0	St. Croix	45 22.371	-92 40.792	Jun 28, 2011
0	0	0	St. Croix	45 22.498	-92 40.717	Jun 28, 2011
+	0	0	St. Croix	45 22.960	-92 40.413	Jun 28, 2011
+	0	0	St. Croix	45 23.022	-92 40.330	Jun 28, 2011
0	0	0	Mississippi	44 54.895	-93 11.982	Jun 29, 2011
0	0	0	Mississippi	44 54.886	-93 11.961	Jun 29, 2011
0	0	0	Mississippi	44 54.760	-93 11.944	Jun 29, 2011
0	0	0	Mississippi	44 54.724	-93 11.987	Jun 29, 2011
0	0	0	Mississippi	44 54.718	-93 12.002	Jun 29, 2011
0	0	0	Mississippi	44 54.698	-93 12.057	Jun 29, 2011
0	0	0	Mississippi	44 54.697	-93 12.090	Jun 29, 2011
0	0	0	Mississippi	44 54.906	-93 12.146	Jun 29, 2011
0	0	0	Mississippi	44 54.917	-93 12.149	Jun 29, 2011
0	0	0	Mississippi	44 54.852	-93 12.137	Jun 29, 2011
0	0	0	Mississippi	44 54.828	-93 12.128	Jun 29, 2011
0	0	0	Mississippi	44 54.825	-93 12.128	Jun 29, 2011

Silver	Bighead	Black	River	Latitude	Longitude	Date Collected
0	0	0	Mississippi	44 54.805	-93 12.117	Jun 29, 2011
0	0	0	Mississippi	44 54.647	-93 12.096	Jun 29, 2011
0	0	0	Mississippi	44 54.523	-93 12.045	Jun 29, 2011
0	0	0	Mississippi	44 54.484	-93 11.997	Jun 29, 2011
0	0	0	Mississippi	44 54.506	-93 11.946	Jun 29, 2011
0	0	0	Mississippi	44 54.432	-93 11.873	Jun 29, 2011
0	0	0	Mississippi	44 54.330	-93 11.687	Jun 29, 2011
0	0	0	Mississippi	44 54.234	-93 11.436	Jun 29, 2011
0	0	0	Mississippi	44 54.201	-93 11.423	Jun 29, 2011
0	0	0	Mississippi	44 54.114	-93 11.446	Jun 29, 2011
0	0	0	Mississippi	44 54.008	-93 11.509	Jun 29, 2011
0	0	0	Mississippi	44 53.823	-93 11.394	Jun 29, 2011
0	0	0	Mississippi	44 53.764	-93 11.437	Jun 29, 2011
0	0	0	Mississippi	44 53.745	-93 11.413	Jun 29, 2011
0	0	0	Mississippi	44 53.738	-93 11.358	Jun 29, 2011
0	0	0	Mississippi	44 53.742	-93 11.249	Jun 29, 2011
0	0	0	Mississippi	44 53.720	-93 11.185	Jun 29, 2011
0	0	0	Mississippi	44 53.694	-93 11.082	Jun 29, 2011
0	0	0	Mississippi	44 53.653	-93 11.039	Jun 29, 2011
0	0	0	Mississippi	44 53.636	-93 10.909	Jun 29, 2011
0	0	0	Mississippi	44 53.674	-93 10.865	Jun 29, 2011
0	0	0	Mississippi	44 53.608	-93 10.753	Jun 29, 2011
0	0	0	Mississippi	44 53.622	-93 10.705	Jun 29, 2011
0	0	0	Mississippi	44 53.589	-93 10.646	Jun 29, 2011
0	0	0	Mississippi	44 53.596	-93 10.626	Jun 29, 2011
0	0	0	Mississippi	44 53.656	-93 10.417	Jun 29, 2011
0	0	0	Mississippi	44 53.726	-93 10.243	Jun 29, 2011
0	0	0	Mississippi	44 53.710	-93 10.291	Jun 29, 2011
0	0	0	Mississippi	44 53.817	-93 09.020	Jun 29, 2011
0	0	0	Mississippi	44 53.842	-93 08.958	Jun 29, 2011
0	0	0	Mississippi	44 53.904	-93 09.041	Jun 29, 2011

Silver	Bighead	Black	River	Latitude	Longitude	Date Collected
0	0	0	Mississippi	44 53.820	-93 09.145	Jun 29, 2011
0	0	0	Mississippi	44 53.881	-93 09.325	Jun 29, 2011
0	0	0	Mississippi	44 53.838	-93 09.425	Jun 29, 2011
0	0	0	Mississippi	44 53.768	-93 09.507	Jun 29, 2011
0	0	0	Mississippi	44 53.828	-93 09.005	Jun 29, 2011
0	0	0	Mississippi	44 53.686	-93 09.941	Jun 29, 2011
0	0	0	Mississippi	44 53.700	-93 10.018	Jun 29, 2011
0	0	0	Cooler Blank			Jun 28, 2011
0	0	0	Cooler Blank			Jun 28, 2011
0	0	0	Cooler Blank			Jun 28, 2011
0	0	0	Cooler Blank			Jun 29, 2011
0	0	0	Cooler Blank			Jun 29, 2011
0	0	0	Cooler Blank			Jun 29, 2011

Conclusions and Suggestions

The discovery of silver carp eDNA in more than 40% of the samples taken in the St. Croix River suggests that silver carp are already present in this reach of the river. Due to the high percentage of positive samples, it is recommended that a smaller number of samples should be collected to corroborate this result. We did not detect any silver carp DNA in the Mississippi River below Lock and Dam No. 1; however, this reach is only about 30 miles upstream from the confluence of the St. Croix and the Mississippi Rivers whereas the St. Croix Falls dam is about 50 miles upstream. Hence silver carp could be expected to be present in the Upper Mississippi as well. Our failure to detect silver carp DNA may have been caused by high water flows as the river was at flood stage during the collection of samples, and/or the habitats and water velocities in this reach may have limited the accumulation of DNA. It is recommended that a second sampling effort is undertaken in the reach between Lock and Dam No. 1 and Pike Island during lower flows with increased replicates, and that an additional set of samples is collected in the pool above Lock and Dam No. 1, assuming conditions are more conducive to DNA accumulation.

The lack of positive results for bighead and black carp do not necessarily indicate that they are not present. The high water levels during sample collection may have reduced the potential to collect eDNA of these species, which tend to be deeper in the water column (Kolar et al. 2007). To ensure the best use of available funds, we would suggest that testing continues to include sampling for bighead carp, but not black carp as we are not aware of any demonstration that this species can be detected from the field using eDNA.

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