Missouri River Basin

Define the Spatial Distribution and Population Demographics of Invasive Carp Populations and the Associated Fish Community in the Missouri River Basin

Project Title: Define the Spatial Distribution and Population Demographics of Invasive Carp Populations and the Associated Fish Community in the Missouri River Basin

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Participating Agencies:

South Dakota: South Dakota Department of Game, Fish, and Parks (SDGFP), East Dakota Water Development District (EDWDD), University of South Dakota (USD),

Nebraska: Nebraska Game and Parks Commission (NGPC), University of Nebraska-Lincoln (UNL),

Iowa: Iowa Department of Natural Resources (IADNR); Iowa State University (ISU),

Missouri: Missouri Department of Conservation (MDC),

US Fish and Wildlife Service - Columbia Fish and Wildlife Conservation Office

US Fish and Wildlife Service: Bozeman Fish Health Lab, Missouri River Fish, and Wildlife Conservation Office, and Great Plains Fish and Wildlife Conservation Office

Statement of Need: The USFWS and the Aquatic Nuisance Species Task Force, in collaboration with multiple stakeholders, released a national Invasive Carp Management and Control Plan (National Plan; Conover et al. 2007) to limit ecological and economic problems posed by these species. Despite tremendous progress towards achieving National Plan goals, there remains a great need to develop metrics to quantify the success of Invasive Carp management and inform control efforts, especially in the Missouri River Basin where funding for Invasive Carp research has been lacking. Defining the spatial distribution and demographics of Invasive Carp populations in the Missouri River Basin is fundamental to prescribing and assessing management actions as outlined in the National Plan Goals and Strategies related to prevention, containment and control, and extirpation. In addition, understanding the status and trends in abundance, size or age structure, maturity schedules, or fecundity of fish in a population are central to informed decision making.

Currently, more information on the abundance and distribution of Silver *Hypophthalmichthys molitrix*, Bighead *Hypophthalmichthys nobilis*, and Black Carp *Mylopharyngodon piceus* is needed to inform the strategic placement, development, and assessment of management actions across the Missouri River Basin as population assessments provide baseline population data to inform management decisions. Early detection sampling is used to detect new introductions and the spread of existing populations and can provide managers with critical information about the speed and mechanisms of spread. By detecting new populations early, actions can more effectively be implemented to control the population. Developing tools to assist with fish egg identification can help expedite the identification of range expansion. Monitoring provides empirical data about population changes over time and space, the ability to compare multiple populations, and a basis to evaluate the efficacy of management actions. Furthermore, historical and current information on select species and fish communities can identify species that may be negatively impacted by Invasive Carp and priority areas where Invasive Carp may be having a greater impact while providing metrics to measure the success of future management actions. These efforts may require long-term commitments of 3 to 10 years, depending on the complexity and scope of the situation.

To effectively guide efforts to manage and control Invasive Carp in the Missouri River Basin, managers must understand the factors influencing population dynamics. Examples of population variables that should be accounted for in management actions include numbers and locations of distinct populations within the basin, population sources and sinks, and movement into, out of, and within the basin. Technologies to answer questions about fish distribution and abundance are constantly advancing, and it would benefit managers to understand and implement emerging technologies that provide accurate and precise information. Environmental DNA (eDNA; presence/absence of DNA from the target species in the environment) is one example that is of interest to Missouri River Basin partners. The scope of this work and the depth of specialized knowledge will require a collaborative effort among partners to develop and implement an effective protocol.

The tasks outlined in this document are the initial development of Invasive Carp monitoring in the Missouri River and its tributaries. Collaborations between the U.S. Fish and Wildlife Service, the Missouri River basin States, universities, and other State partners will work towards the objectives listed below.

Project Objectives:

1. Determine the geographic extent (presence/absence) of Bighead, Silver, and potentially Black Carp throughout the Missouri River Basin to evaluate current barriers, prevent further range expansion, and identify potential control/removal opportunities (Agencies involved: SDGFP, NGPC, USFWS).

    a. Develop a Missouri River Basin Invasive Carp Genetics Team to increase understanding of environmental DNA (eDNA) as a tool for the detection and measurement of Invasive Carp populations, host informational webinars/workshops from experienced labs to provide education and learning opportunities for labs in the Missouri River Basin, and develop a standard framework for field collection, laboratory analysis, database development, and results communication.

b. Implement a strategy for information sharing on the methods needed to successfully analyze eDNA samples for Invasive Carp primers, coordinate efforts with USFWS Bozeman Fish Health Lab in Bozeman, MT & Whitney Genetics Lab in La Crosse, WI, to integrate methods with partners already using eDNA for detection of Invasive Carp.

c. Determine the feasibility and efficacy of eDNA analysis in these aquatic systems to detect the presence of Invasive Carp in water and/or sediment samples across various sized drainage areas.

d. Determine the presence/absence of Bighead and Silver Carp and investigate the feasibility of using eDNA for detecting Black Carp in the Missouri River and its tributaries concentrating above and below fish movement barriers to better understand Invasive Carp distributions.

2. Characterize spatial (tributaries longitudinally distributed in the Lower Missouri River) and temporal (seasonal and annual) patterns in the Silver and Bighead Carp population demographics (e.g., size structure and relative abundance) while developing standard operating procedures that are specific for the lower Missouri River Basin to prescribe and assess population control measures (Agencies involved: NGPC, MDC, USFWS).

a. Evaluate a suite of gears and sampling logistics to determine an effective and efficient method to sample all sizes of Silver and Bighead Carp in a variety of aquatic systems.

b. Determine the size distribution, relative abundance, and other population characteristics of the Silver and Bighead Carp populations in a variety of aquatic systems to help identify areas where population control measures can be implemented.

c. Pair fishery sampling efforts with eDNA sampling sites to validate eDNA results.

3. Characterize the historic and current fish community in the inter-reservoir reach and the Lower Missouri River to assess the impacts to the fish community pre- and post-invasion as well as provide baseline data for comparison to prescribe and assess future management actions. (Agencies involved: NGPC, MDC).

a. Deploy fish community assessment gears in the inter-reservoir reach and, in the lower Missouri River, use the data collected from Objective 2.1 to characterize the fish community and select native fish species.

b. Determine the size distribution, relative abundance, and other population characteristics of select fish species to help identify potential differences between areas with and without established Invasive Carp populations.

c. Utilize historic fisheries data (i.e., Pallid Sturgeon Population Assessment or Benthic Fishes) to determine changes in the associated fish community diversity, richness, size distribution, relative abundance, relative condition, and other population dynamics parameters.

4. Develop a computer-based application based on previous Bighead Carp and Silver Carp research and monitoring in the Upper Mississippi River for expeditious laboratory identification of fish eggs collected during ichthyoplankton sampling as part of an early detection protocol for Bighead, Silver, Grass or Black Carp. (Agency involved: IA DNR).

Project Highlights:

Objective #1:

- Neither Silver nor Bighead Carp eDNA has been detected by qPCR in samples taken above the major barriers in the Vermillion (Lake Vermillion Spillway) and Big Sioux (the falls at Falls Park) Rivers. This suggests the barriers of those two rivers have been successful in preventing upstream Invasive Carp movement.
- No Invasive Carp were collected above Gavins Point Dam, the lowest dam on the mainstem Missouri River.
- The Dakotas eDNA pilot project was initiated with two sampling events collecting 500 water samples from three Missouri River tributaries in June and late August/early September.
- The Bozeman Fish Health Center has been able to successfully set up their eDNA processing capabilities as a result of this work.

Objective #2:

- In the mainstem Missouri River below Gavins Point Dam, boat electrofishing was the most effective sampling method and Silver Carp were the most frequently sampled Invasive Carp species
- In the lower Missouri River tributaries, Silver Carp was overwhelmingly the dominant Invasive Carp sampled with only a handful of Grass Carp and Bighead Carp sampled. No Black Carp were sampled.
    - Catches of Invasive Carp using the two electrofishing settings varied but seemed to produce similar catch per unit effort.
    - Size classes of Silver Carp were similar for all tributaries with low numbers of fish in the 450mm to 550mm range. This range of lengths corresponds to 3-5 years of age based on otoliths from earlier pilot work in 2020.
    - The Missouri River at the mouth of the Platte River was the only location that did not have a bimodal length-frequency distribution for Silver Carp.
    - Sex ratios of Silver Carp indicate a slightly higher proportion of females in tributaries than in the mainstem river.
- Throughout all Missouri River tributaries, the electrified dozer trawl proved to be an efficient gear for capturing Silver Carp, providing demographic information similar to collections in Nebraska and Missouri using conventional boat electrofishing.
    - Since 2020, 302 electrified dozer trawl transects across 17 Missouri River tributary confluences collected over 10,500 Silver Carp ranging in size from 40 mm to 857 mm and in age from age-0 to 13 years old as well as over 50 Bighead Carp ranging in size from 215 mm to 960 mm and in age from age-0 to 11 years old.
    - In 2021, sampling included 138 electrified dozer trawl transects across 14 tributaries in four states, with variable effort per tributary. Silver Carp catch rates were variable across the basin and there was no pattern of CPUEs longitudinally or by sizes of fish

collected by tributary. The target RSE of CPUE ≤ 25 was established to increase confidence in relative abundance estimates and was met for just over half of the tributaries and the overall data compilation. These data are utilized to describe Silver Carp population dynamics useful for population management and control.

Objective #3:

- In the mainstem Missouri River below Gavins Point Dam, 6,109 fish were captured with Shorthead Redhorse being the most numerous. Fish communities below Gavins Point Dam (Invasive Carp present) and Fort Randall Dam (Invasive Carp absent) will be analyzed for potential impacts to their composition and structure in response to the establishment of the carp species.
- In the lower Missouri River tributaries, sampling in all tributaries was hindered by low water for the duration of the sampling season but overall, 4,521 fish comprising 55 species were sampled.
    - Sampling resulted in more species of fish in tributaries compared to their associated Missouri River bends except for the Nodaway River.
    - The most abundant species sampled in each tributary varied. Gizzard shad (*Dorosoma cepedianum*) was the most abundant fish sampled in the Lamine River, Shortnose gar (*Lepisosteus platostomus*) in the Grand River, and Green sunfish (*Lepomis Cyanellus*) in both the Platte River and Nodaway River.

Objective #4:

- WhoseEgg is a powerful tool for classifying fish eggs without the need to obtain costly genetic identifications but needs to be expanded to include eggs from other rivers throughout the US to become a more robust identification tool.

State Report: South Dakota

Methods

Water samples were collected three times in both the Big Sioux and Vermillion rivers. Extensive precautions were taken to prevent eDNA contamination between samples and sampling sites. All samples were collected while standing on the shore to reduce the risk of boots or waders contaminating the water with eDNA. Disposable nitrile gloves were worn during sample collection and were replaced after each sampling site. Two water samples were collected at each location using 2-Liter HDPE bottles sterilized with 20% bleach solution for ≥ 10 seconds and rinsed with distilled water (Coulter et al. 2019). The most downstream site in each river was sampled first, and each successive sample was collected upstream of the previous sample to avoid DNA cross-contamination. Two negative control samples (two, 2-L bottles of distilled water) were collected below and above each barrier during each sampling event. After sampling, bottles were placed on ice until filtration was carried out in the lab or the field. Sample filtration was split evenly between field-filtered and lab-filtered methods to determine if the time spent driving back to USD caused a significant amount of DNA degradation in the samples. Samples were filtered through 1.5 µm glass microfiber filters (Eichmiller et al. 2014) using a magnetic polyphenylsulfone filter funnel (Eichmiller et al. 2014; Nukazawa et al. 2018; Coulter et al. 2019). All supplies (filter funnels, forceps, coolers, etc.) were sterilized with 20% bleach for ≥ 10 seconds and rinsed with distilled water before and after each use and between samples. Each filter was stored in 95% ethanol in a 15 mL microcentrifuge in the lab freezers at -20°C until DNA extraction occurred.

Vermillion River samples were taken on August 5 and September 22, 2021 at two below-barrier sites and seven above-barrier sites. A substantial fish kill took place below the spillway in the Vermillion River prior to the first sampling event of 2021. Fish carcasses of various species, including Invasive Carps, could be observed floating in the water, and it was evident that a large blue-green algae bloom had developed. eDNA results conclude that only Bighead Carp were present below the barrier in the Vermillion river. A rain event washed most of the carcasses downstream prior to the second sampling event, but some bones and other carcass remnants were still present in and around the water at the time of sampling. No live fish were seen at the below-spillway sampling sites during either sample. Due to low water levels below the spillway, the third round of water samples were collected on October 28, 2021 only at sites upstream of the spillway (Figure 1).

Big Sioux River samples were taken on August 17 and September 24, 2021 at three below-barrier sites and 6 above-barrier sites. Silver or Bighead Carp were seen swimming immediately below the lowest chain of waterfalls during the first sampling event, and this is confirmed with qPCR results, but fish were not visible during the second sampling event. Due to low water levels downstream of the Falls, the third round of water samples on October 29, 2021 were collected only at sites upstream of the Falls (Figure 3).

In total, 98 water samples and 20 field blanks were collected from both rivers. DNA extraction was carried out in a designated DNA sequencing room at the University of South Dakota and according to the Qiagen DNeasy Blood and Tissue Extraction protocol (Qiagen, inc.). Extracted samples were stored at -20 degrees C. After extraction, all samples were loaded into agarose gels to test for the presence of DNA in the extracted samples and extracted blanks. Four µL of SYBR-green dye and 2 µL of sample were loaded into each lane of the gel and allowed to run at 75 V for ~ 60 minutes.

Previously published sequences for the Bighead and Silver Carp forward-primer, reverse-primer, G-block megamer, and probes were used for eDNA qPCR analysis. The forward and reverse primers, as well as the G-block sequence, contained DNA sequences conserved between the two species. One probe per species was used to detect the presence of either species' DNA in the extracted samples in the qPCR thermocycler. A 10^6 dilution series of the G-Block megamer was used in all qPCR analysis, which formed the baseline standard series of known DNA present in each sample. 96-well plates were loaded with nine field samples, one blank, and a positive control in quadruplicate, as well as a triplicate of spiked samples to ensure no inhibition was occurring during qPCR. A row of no-template controls and two columns of the standard dilution series were included on each plate. Amplification curves were generated during each run for each sample and each probe (Figure 3a). Standard curves were calculated for each plate (Figure 3b), from which the quantity of DNA present in each sample could be calculated. Only presence (positive quantification) or absence (zero quantification) was recorded.

Results & Discussion

The agarose gel tests did not identify species-specific DNA, however, they were used to determine whether field samples contained any amount of DNA, and whether DNA contamination had occurred in the field blank samples. This was a preliminary step to qPCR analysis. Contamination in field blanks would be evident if the lanes containing a field blank sample showed any DNA bands. However, none of the field blank extractions exhibited a DNA band, indicating no DNA contamination occurred in the blanks between sampling, filtering, storage, or extraction (Figure 2).

At this time 60 of the extracted samples have been analyzed using qPCR, with 50 yet to be processed from the first round of water sampling. Average standard curve R^2 values were 0.96 for both Silver and Bighead Carp markers. Average percent efficiency was 86.08% and 85.45% for Silver and Bighead Carp markers, respectively. qPCR results indicate that during the first sampling session, both Silver and Bighead Carp were detected below the Falls, but only Bighead Carp were detected below the Vermillion Spillway. During the second sampling session, only Bighead Carp were detected below both barriers. Most importantly—to date, there have been zero positive detections above either the Vermillion Spillway or the Falls, suggesting that either Invasive Carp populations do not exist in the sample areas above the barriers, or any populations that may exist above the barriers are in such low densities that their DNA went undetected during the sampling process. A second water sampling season is planned for Summer 2022.

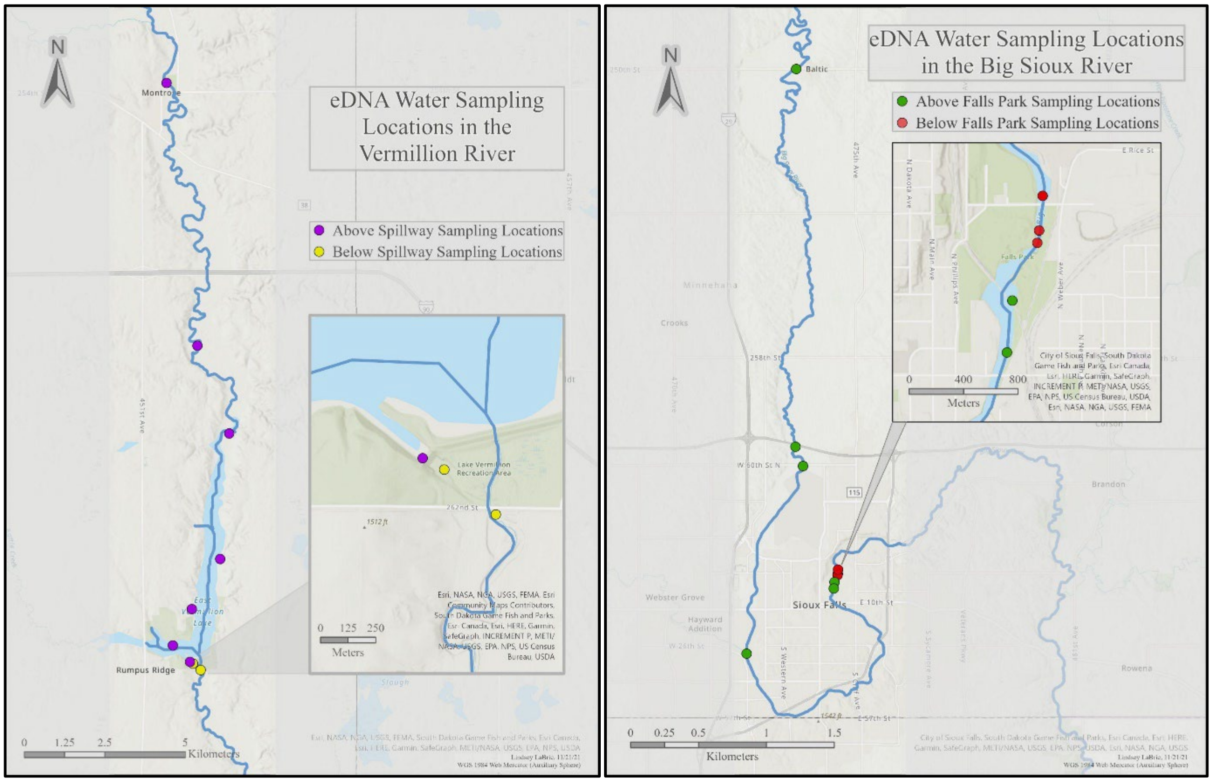


Figure 1: Maps of eDNA water sampling locations in the Vermillion River with an inset map detailing below-spillway sampling locations (left pane) and the Big Sioux River with an inset map detailing below-barrier sampling locations (right pane). Map panes and inset maps are in different scales, noted by scale bars in each map frame.

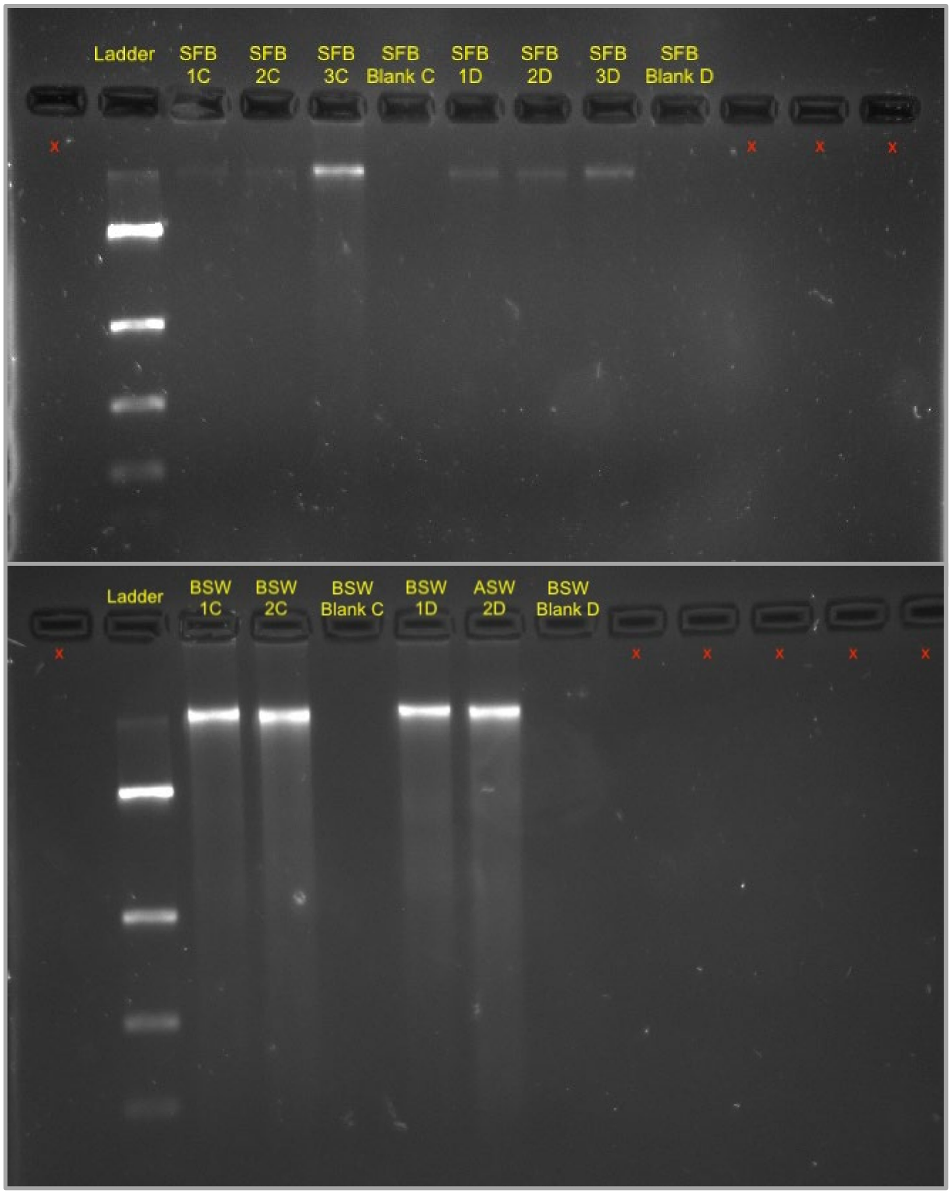
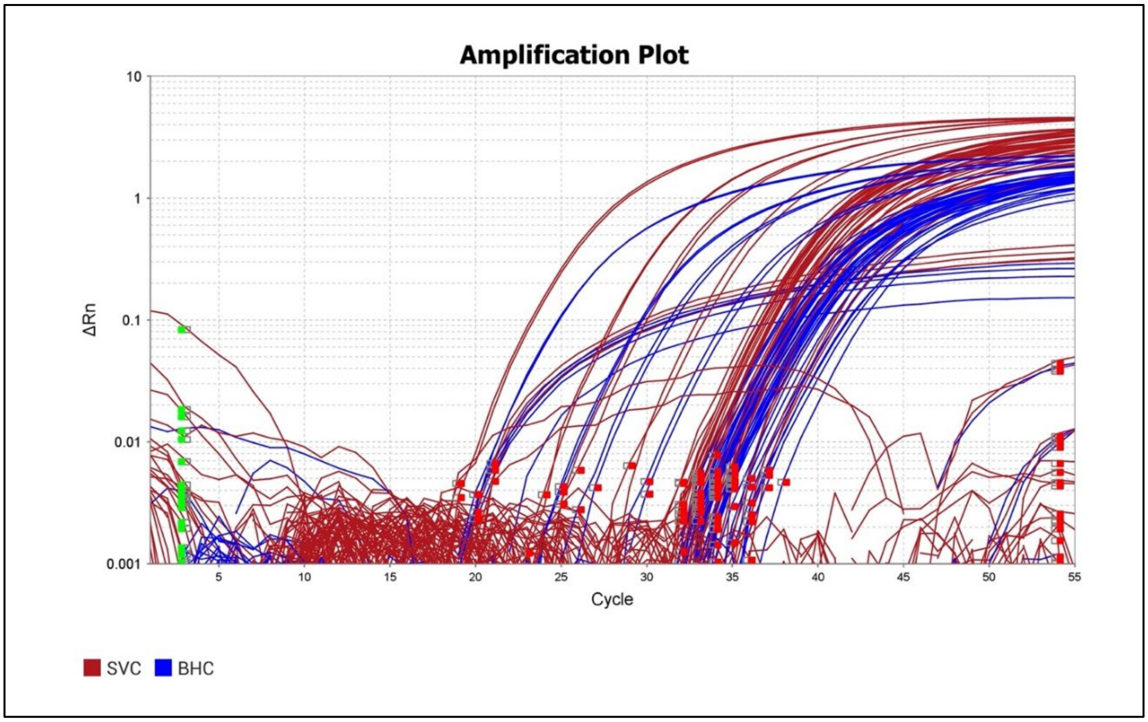


Figure 2: Gel electrophoresis results for samples taken below the Falls in the Big Sioux River (top pane) and below the spillway in the Vermillion River (bottom pane). Lanes containing blank samples do not show evidence of DNA contamination.



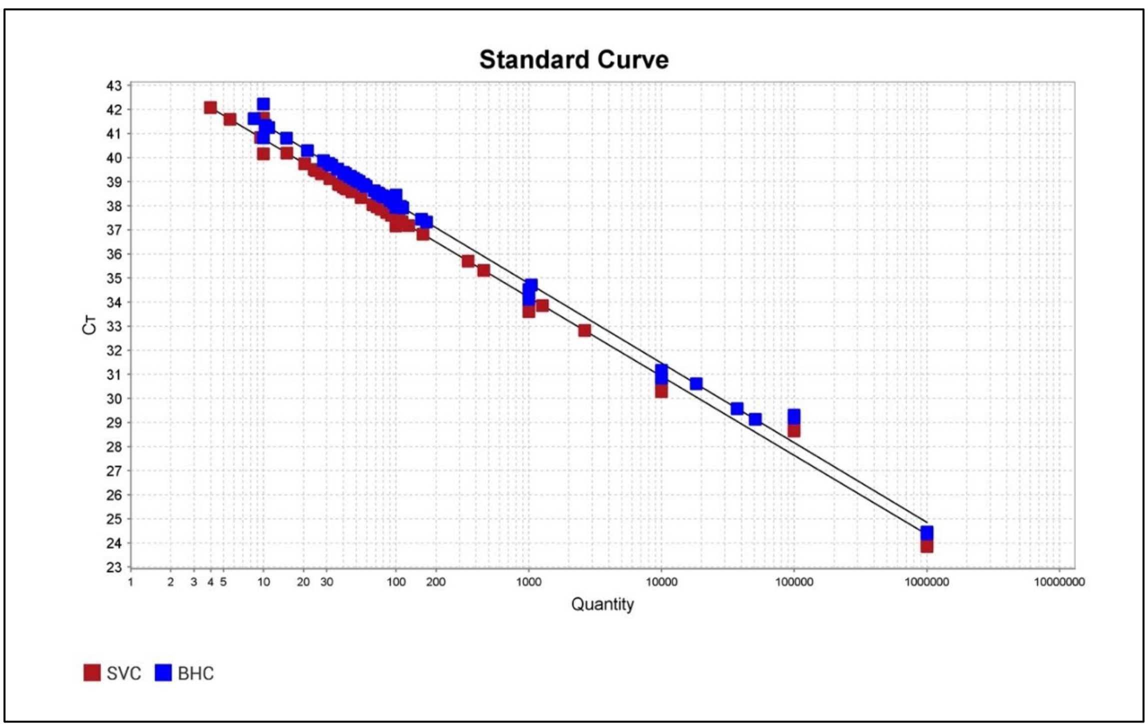


Figure 3a: An amplification curve graph generated from one plate during the qPCR process (top).

Figure 3b: A standard curve regression generated from one plate during the qPCR process (bottom).

Recommendations

Altering the water sampling regime to encompass more replicates per site and/or more sites upstream of the barriers in the Vermillion and Big Sioux would be beneficial. Taking more samples per site and generally at more sites may increase eDNA detection probability. Water sampling sessions should commence in early summer (as opposed to fall as in the first sampling season) to increase detection probability as well. Additionally, samples should be taken in locations where populations of Silver and Bighead Carp are known to exist, for example in locations where telemetered individuals are found in the James River, so that a baseline eDNA detection probability can be established for the methods described in this study. Thus, confidence would increase for any negative or positive detections found using qPCR.

References

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State Report: Nebraska

Methods

Sampling occurred below Fort Randall and Gavin's Point dams, two mainstem Missouri River hydroelectric dams. Gavin's Point Dam is the northern extent of the range of Invasive Carp species (Hypophthalmichthys spp.). In the summer of 2021, sampling was initiated below each dam. Sampling sites were split into 5 rkm (river kilometer) reaches directly below each dam. During one week per month both banks, any islands, and accessible inlets in each reach were sampled using boat electrofishing. Concurrently, mini-fyke nets and two sizes of hoop nets were utilized within and beyond each reach.

Captured fish were identified, measured for total length to nearest millimeter, and weighed to nearest gram. In addition, sagittal otoliths were removed from Invasive Carp species (Bighead Carp, Silver Carp, Grass Carp). Once in the lab, otolith preparation followed procedures laid out in Beamish (1979). Otoliths were mounted in epoxy and two dorsal-ventral cuts were made next to the core using a low-speed diamond saw. Otoliths were photographed underneath a microscope and consensus aging was performed among readers to reduce reader bias.

Results and Discussion

Between May 24, 2021 and November 13, 2021, a total of 6,109 fish were captured. Due to damage or equipment restraints, 217 fish were unable to be weighed and measured, leaving 5,892 measured fish. Of these, 2,111 were captured below Fort Randall Dam and 3,781 were captured below Gavin's Point Dam.

Shorthead redhorse were the most numerous fish sampled, with 1,415 sampled (Table 1), accounting for just over 23% of our total catch. Other species of note captured were one American eel (946mm, 1,880g), one lake sturgeon (741mm, 2,520g), and 85 paddlefish (weights from 880-35,020g). One hundred four Invasive Carp were captured below Gavin's Point Dam: 69 Silver Carp, 30 Grass Carp, and 5 Bighead Carp. Figure 1 shows their length weight distribution by species. No Black Carp were sampled below either dam, and no Invasive Carp were sampled within the reach below Fort Randall Dam. Invasive Carp were sampled at a rate of 7.05 fish per electrofishing hour below Gavins Point Dam. A further breakdown of the species sampled in both locations can be found in Table 1. Invasive Carp otolith processing is ongoing, and no aging data are ready to report at this time.

A total of over 32 hours were spent boat electrofishing this past season, 27 large hoop nets and 29 small hoop nets were deployed for a total of 644 and 692 net hours, respectively. Additionally, 30 mini-fyke nets were deployed for a total of 686 net hours. A breakdown of the sampling effort split among gear types can be found in Table 2.

Carp sampled below Gavin's Point Dam are being aged using otoliths extracted during sampling. Using these data will provide valuable information on age, growth rate, and size structure of the Invasive

Carp species present. Information extrapolated from back-calculating length at age will be especially important in gaining information for younger age classes as we have not sampled any age 0 Invasive Carp to date. Due to limited success sampling Invasive Carp species using current methods (boat electrofishing, mini-fyke nets, and hoop nets) we are planning to utilize bow fishing tournaments in the coming season to help increase our otolith count.

The locations where Invasive Carp were sampled was consistent with the current body of knowledge. Silver, Grass, and Bighead Carp were sampled downstream of Gavin's Point Dam. Within all reaches sampled, no Black Carp were sampled. Concurrent environmental DNA (eDNA) sampling at sites downstream of Gavins Point Dam have also given no indication of Black Carp being present in the Nebraska reach of the Missouri River.

Fish communities below both Gavin's Point Dam (Invasive Carp present) and Fort Randall Dam (Invasive Carp absent) are being analyzed for potential impacts to their composition and structure in response to the establishment of the carp species. This work is ongoing. To help account for differences in extraneous factors that differentiate Gavin's Point and Fort Randall Dam we are exploring grouping species by ecological function. We will continue to assess the biological data in response to the presence/absence of Invasive Carps in addition to incorporating environmental data (e.g., temperature, depth, discharge, etc.) as potential interacting parameters that influence changes in overall structure and function of the two fish communities we are studying.

Recommendations

To improve future results of this project we will need to solidify more effective methods of sampling Invasive Carp. We are hoping to utilize bow fishing tournaments, but when utilizing bow fishing tournaments, the full age class distribution is unlikely to be sampled. This mirrors some of our own issues with sampling smaller age classes. Finding younger fish and identifying potential habitat use may allow for more efficient "spot checking" to help identify the edge of invasion as early as possible.

References

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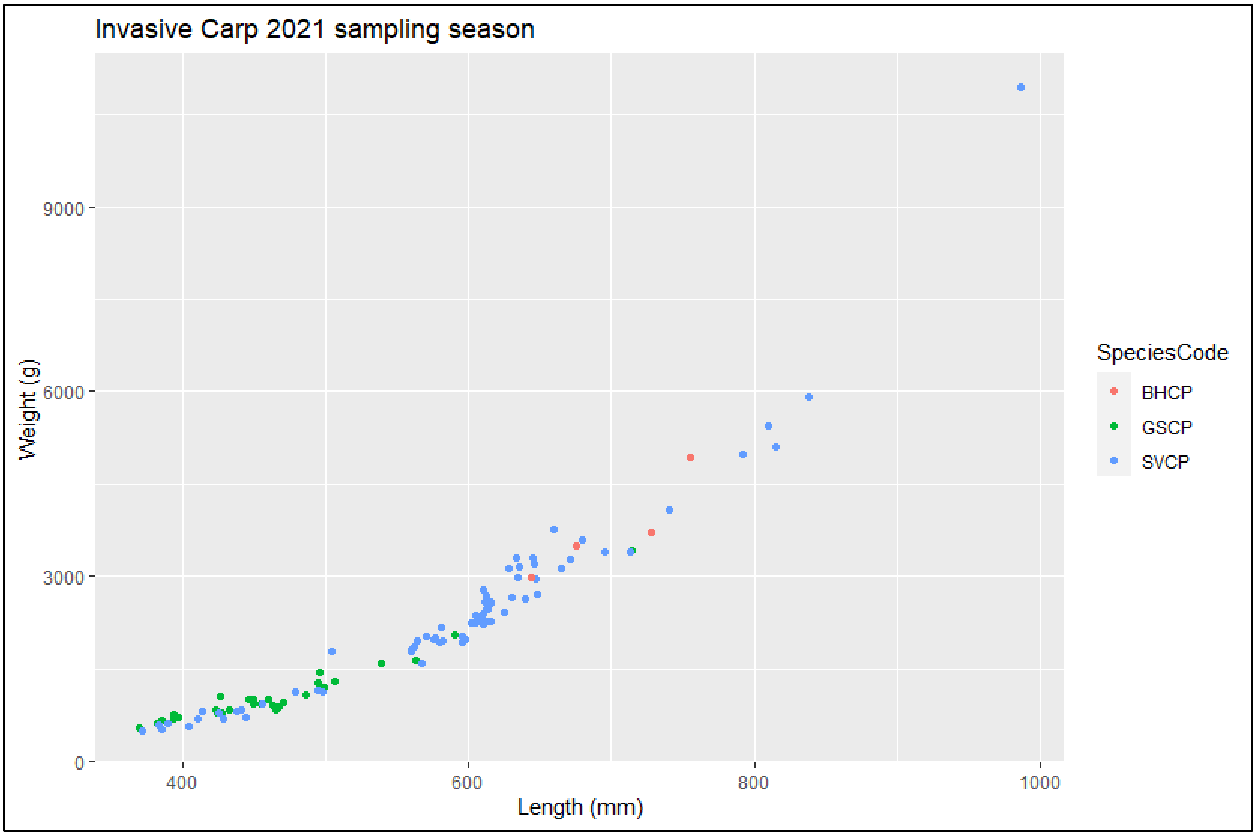

Figure 1. Weight versus length relationship for Bighead Carp (BHCP), Grass Carp (GSCP), and Silver Carp (SVCP) sampled below Gavin's Point Dam, Yankton, SD, summer 2021.

Table 1. Species counts of fish sampled (boat electrofishing, mini-fykes, hoop nets) below each dam during 2021.

Species	Fort Randall	Gavin's Point
American Eel	0	1
Black Crappie	0	3
Bighead Carp	**0**	**5**
Bluegill	9	50
Bigmouth Buffalo	20	79
Brown Trout	1	0
Blue Sucker	2	378
Common Carp	178	281
Channel Catfish	149	156
Emerald Shiner	2	15
Flathead Catfish	2	6
Freshwater Drum	25	194
Goldeye	13	282
Green Sunfish	0	1
Grass Carp	**0**	**30**
Gizzard Shad	2	435
Highfin Carpsucker	3	1
Johnny Darter	2	2
Lake Sturgeon	0	1
Largemouth Bass	0	6
Longnose Gar	0	42
Mooneye	0	6
Northern Pike	23	104
Paddlefish	53	33
Quillback	95	87
Red Shiner	9	13
Rock Bass	0	3
Rainbow Trout	2	0
River Carpsucker	261	142
River Shiner	77	31
Spotfin Shiner	87	91
Saugeye	0	1
Shorthead Redhorse	930	485
Skipjack Herring	0	4
Smallmouth Buffalo	42	373
Smallmouth Bass	80	63
Suckermouth Minnow	7	0
Shortnose Gar	123	317
Shovelnose Sturgeon	0	4
Sand Shiner	1	1
Sauger	1	3
Silver Carp	**0**	**69**
Unidentified Fish	4	0
Unidentified Micropterus	0	1
Walleye	41	7
Wiper	7	3
White Bass	16	11
White Sucker	3	0
Yellow Perch	1	0
Unidentified Cyprinid	18	0
Totals	2289	3820

Table 2. Effort allocated by gear type and site. Electrofishing is in hours of actual pedal (on) time and net hours indicate the total hours each net type was fished during 2021.

Gear Type	Gavins Point	Fort Randall	Total
Electrofishing Hours			
Boat Electrofishing	14.61	18.02	32.63
Net Hours			
Mini-Fyke Net	371.17	315.57	686.73
Large Hoop Net	317.92	327.07	644.98
Small Hoop Net	342.48	349.57	692.05
Net Hour Total	1031.57	992.20	2023.77

State Report: Iowa

Methods

App Access and Architecture

WhoseEgg is free and available online at https://whoseegg.stat.iastate.edu/. The app is accessible from any device with a browser but was developed to perform best when used on a computer. The app was built using R code (R Core Team 2021) and the R package Shiny (Chang et al. 2021). WhoseEgg is hosted on an R server that allows the app to connect to R to perform the necessary computations. Data uploaded to WhoseEgg will not be saved or redistributed in any manner to protect the privacy of user's data. The code, random forests, and training data associated with WhoseEgg are available in the supplemental material, on GitHub (https://github.com/goodekat/WhoseEgg).

WhoseEgg is divided into six pages listed in the top panel of the WhoseEgg 'Home' page (Figure 1). The structure of WhoseEgg allows users to easily flow through the app. Users begin at the 'Home' page and progress left to right through the other pages. The 'Home' page contains information to familiarize users with WhoseEgg, including the purpose of WhoseEgg and instructions for how to use the app. The 'Home' page also includes the locations where the training data were collected and the species included in the training data to help users determine whether the models in WhoseEgg are appropriate for their data.

The 'Data Input', 'Predictions', and 'Downloads' pages contain interactive tools that allow users to provide their own data and acquire predictions. The flowchart included on the WhoseEgg 'Home' page describes the steps to obtain predictions (Figure 1).

1. First, the user uploads a spreadsheet with the necessary egg characteristics via the 'Data Input' page. The spreadsheet must be an Excel or csv file and formatted appropriately. WhoseEgg provides a downloadable Excel template (included in the supplemental material) with data validation helpers to assist users format their data correctly (Figures 2a and 2b). Additionally, errors and warnings appear in WhoseEgg if the uploaded data are not formatted correctly (Figure 3).
2. Second, the user obtains predictions from the WhoseEgg random forests for the uploaded egg data on the 'Predictions' page.
3. Third, the user downloads a spreadsheet from the 'Downloads' page containing the uploaded data, some additional egg characteristics provided by WhoseEgg, and the random forest predictions.

Each page contains more detailed instructions and additional interactive features to assist users.

The 'Help' and 'References' pages are designed to be accessed at any time. The 'Help' page contains details on the egg characteristics and random forests. Answers to frequently asked questions are also included on the 'Help' page. The 'References' page lists the details of the references mentioned throughout the app.

Training Data and Random Forests

WhoseEgg uses three random forests to separately predict the family, genus, and species of a fish egg based on characteristics of the egg. We trained the random forests using a compilation of the training data from Camacho et al. (2019; 734 and 541 fish eggs from 2014 and 2015, respectively) and the validation data from Goode et al. (in press; 703 fish eggs from 2016). The eggs in both studies were sampled from locations in the Upper Mississippi River basin (Figure 5). The data sets contained genetic identifications and egg characteristics. See Camacho et al. (2019) and Goode et al. (in press) for additional details about the egg sampling, subsampling, genetic identification, and egg characteristic measurement procedures. We identified 29 eggs from 2016 with incorrect data entries. We were able to correct 23 of the observations and removed 6 of the eggs. Thus, the WhoseEgg random forests were trained on a total of 1,972 eggs but provide comparable estimates to the Goode et al. (in press) combined validation model (analysis and results included in the supplemental material).

We used 17 egg and environmental characteristics as predictor variables for the WhoseEgg random forests developed by Camacho et al. (2019) and Goode et al. (in press; Table 1). WhoseEgg requires users to provide a slightly different set of variables when uploading their data. Since the two coefficient of variation variables and the ratio of embryo to membrane are functions of other predictor variables, users do not provide them. WhoseEgg will compute these metrics based on the other variables. Additionally, WhoseEgg does not require users to provide Julian day. Instead, users provide the year and day of the month when the egg was collected and WhoseEgg computes Julian day. The remaining predictor variables must be provided by users. WhoseEgg permits additional variables to be included in the uploaded spreadsheet. However, these variables will not be used by the random forest when making predictions.

We used the genetically identified family, genus, and species levels as the response variables. For all three taxonomic levels, Grass Carp, Silver Carp, and Bighead Carp were grouped into the category of "Invasive Carp" due to similar egg characteristics among these species. The training data contained other species in the same family and genus as Invasive Carp, so the family and genus of Invasive Carp (excluding Invasive Carp) were also categories in the response variables of family and genus. The distribution of eggs per species was imbalanced with Invasive Carp, Freshwater Drum (*Aplodinotus grunniens)*, and Emerald Shiner (*Notropis atherinoides)* composing most of the eggs in the training data (Table 2).

We trained the WhoseEgg random forests using the *randomForest* R package (Liaw and Wiener 2002). Each model was trained with 1,000 trees. The other tuning parameters were set to the default values in *randomForest*. Parameters were specified to be consistent with Camacho et al. (2019) and Goode et al. (in press). WhoseEgg returns several values from the random forests for each egg observation in the uploaded data:

- *Random forest probabilities*: Since random forests are ensembles of many trees (1,000 in the case of WhoseEgg), each tree returns a prediction. The proportion of trees that return a prediction of a particular class (on out-of-bag observations) is interpreted as the probability that a randomly generated tree (under the conditions used by a random forest) will predict an observation to be in a specific class (Cutler et al. 2007). WhoseEgg returns the random forest probability for each class within the family, genus, and species levels contained in the training data.
- *Random forest prediction*: With random forests, the response variable level with the highest random forest probability is considered the random forest prediction. WhoseEgg returns the prediction for the family, genus, and species levels.

Limitations and User Responsibility

WhoseEgg is a powerful tool for classifying fish eggs without the need to obtain costly genetic identifications, but as with all models, there are assumptions and limitations users must be aware of. First, random forests used by WhoseEgg at the time of writing this article are trained and validated on data from the Upper Mississippi River basin. Due to possible variation in fish egg characteristics across regions, additional validation is required to know how the models will perform in other regions. One option for users in other regions is to perform their own validation similar to the one in Goode et al. (in press) by first applying WhoseEgg to genetically identified eggs from the region of interest. If the models perform well on Invasive Carp, it provides evidence that WhoseEgg will return trustworthy predictions on future eggs from that region. If the models do not perform well, new random forest models could be developed using similar approaches as Camacho et al. (2019) and Goode et al. (in press) to identify fish eggs in different regions or across multiple regions.

A second limitation of WhoseEgg is that models are only able to return predictions that are in the training data taxonomic levels (Table 1). If a new collection of eggs contains a different family, genus, or species, WhoseEgg will not be able to correctly predict the egg. If other species are likely be present in egg collections, WhoseEgg should be used with caution. If other species have different characteristics from Invasive Carp and the goal of using WhoseEgg is to identify Invasive Carp, WhoseEgg could still be a useful tool for identifying Invasive Carp. However, if other species have similar characteristics to Invasive Carp, WhoseEgg may incorrectly predict these eggs as Invasive Carp.

A third limitation is that the validation of the random forest in WhoseEgg was focused on the classification of Invasive Carp and not other species present in the training data. Random forests generally were successful at predicting the identity of other fish eggs, but because the success of identifying other species was not specifically assessed, we urge users to be cautious if there is an interest in focusing on the identification of different species. As with the regional limitation, users who are interested in applying WhoseEgg to identify other species could perform a validation focusing on the other species of interest. If the WhoseEgg training data contain a large amount of the species of interest, the validation could be performed on the WhoseEgg training data. Otherwise, a new dataset with more observations from the species of interest should be used.

The three limitations discussed indicate that a user of WhoseEgg has the responsibility to acknowledge if their data are not appropriate to use with the WhoseEgg models. In addition to considering the location of data collection and possible fish species present in the data, users should also consider whether the egg characteristics in their data fall in range of the training data egg characteristics. If egg characteristics fall outside of the training data ranges, the random forests will be forced to extrapolate, which could lead to untrustworthy predictions. WhoseEgg alerts users if issues are found with the formatting of the uploaded egg characteristics, but the final check of data correctness is the responsibility of the user.

Results and Discussion:

Example

Here, we present an example using WhoseEgg to obtain predictions on a set of fish eggs from the WhoseEgg training data collected in 2016 at the mouth of the Iowa River in Pool 18 of the Upper Mississippi River (Figure 5). This location was selected since it is an area that has been actively monitored to observe Invasive Carp reproduction along the invasion front (Camacho et al. in press). The data set contains the egg characteristics measured on 215 fish eggs.

Data Input Page

The ‘Data Input’ page is divided into two panels (Figure 6). The left panel includes instructions. The main panel contains a description of the page and interactive tables. The steps for providing data to WhoseEgg are as follows.

1. Download the spreadsheet template by clicking the “Download Template” button (solid red box in Figure 6).
2. Fill in the spreadsheet with the egg characteristics. The instruction panel contains a link to the help page where the information on how to measure the required egg characteristics is provided. The main panel contains spreadsheet format requirements under ‘Spreadsheet Specifications’. We created a csv file called ‘example-data.csv’ (available in the supplemental material) that contains the necessary egg characteristic from the 215 fish eggs of interest, an egg ID, and river and site variables.
3. Upload the spreadsheet by clicking the “Browse” button selecting the appropriate file (gold short-dashed box in Figure 7). If there are any format issues, WhoseEgg prints a warning or error message at the top of the page at this point. Since no messages appear with our example, WhoseEgg has not detected any issues with the format of our data. After a spreadsheet is uploaded with no errors, WhoseEgg prints a table with the uploaded data under ‘Egg Characteristics’ (blue long-dashed box in Figure 6).
4. Perform a manual check of the data to ensure that it has uploaded correctly using the tables under ‘Egg Characteristics’. Both tables are interactive and allow users to filter the data using the search box above the table and sort the data by clicking on a variable name. The table in the ‘Input Data’ tab contains the uploaded data (Figure 6), and the table in the

'Processed Data' tab contains a dataset created by WhoseEgg with the predictor variables (Figure 7). Note that the variables of River and Site are included in the input data table but not in the processed data table since they are not used as predictor variables by the models. The variable of Julian Day, however, has been added to the processed data.

5. At this point, we are ready to obtain predictions. The user may either click on the 'Jump to Predictions' button on the 'Data Input' page or click on the 'Predictions' tab at the top of the page (Figure 6).

Predictions Page

The 'Predictions' page is also divided into two panels (Figure 8). The left panel contains instructions. The main panel includes a description of the page and interactive tables. The steps for obtaining predictions are as follows.

1) Provide the data using the instructions for the 'Data Input' page given above.
2) Click the 'Get Predictions' (solid red box in Figure 8). The text below the button indicates that if a new spreadsheet is added after the button has been clicked once, the predictions will automatically update. After the button is clicked, a table and a plot appear in the main panel below 'Table of Predictions' (gold short-dashed box in Figure 8) and 'Visualizations of Predictions' (blue long-dashed box in Figure 8), respectively.
3) Inspect the 'Table of Predictions' and 'Visualizations of Predictions'.
    a) The 'Table of Predictions' contains seven variables. The first variable is the Egg ID provided in the uploaded spreadsheet. The remainder of the variables are the random forest predictions (variables ending with 'Pred') and corresponding random forest probabilities (variables ending with 'Prob') for the family, genus, and species levels.
    b) Under 'Visualizations of Predictions', the plots that appear in the 'Summary of Predictions' tab summarize the predictions made by the random forests on all observations in the uploaded data. A bar chart is created for each taxonomic level. The bar charts show the levels included in the random forest predictions and the number of predictions per level (blue long-dashed box in Figure 8). In our example data, most predictions fall in the family of *Sciaenidae*, the genus of *Aplodinotus*, and the species of Freshwater Drum. The second most frequent category in each taxonomic level is Invasive Carp. Note the number of Invasive Carp predictions varies from 55 at both the family and genus levels to 57 at the species level. Additional work outside of WhoseEgg could be done after the predictions are downloaded to investigate which observations are predicted differently across the taxonomies by the random forests to gain insight into why the random forests made different predictions for these observations.
    c) The 'Individual Egg Predictions' tab under 'Visualizations of Predictions' allows users to select an egg of interest. Then bar charts are provided showing the corresponding random forest probabilities for all categories within family, genus, and species. When the 'Individual Egg Predictions' tab is selected, the message "Please select a row in the table of predictions to view plots" is shown. The user clicks on a row in the 'Table of Predictions' (Figure 9A) and

the bar charts corresponding to the selected egg will appear (Figure 9B). In our example, 56 eggs are predicted to have a species of Invasive Carp and 47 (84%) of the eggs have a random forest probability greater than 80% for Invasive Carp. While the model mostly returns Invasive Carp predictions with high random forest probabilities, it may be of interest to further explore the eggs with lower random forest probabilities. Here, we consider the egg with the lowest random forest probability for Invasive Carp out of the eggs with a random forest species prediction of Invasive Carp (Figure 9A; egg 77). Only 37% of the trees voted for egg 77 to be an Invasive Carp. Since this is a low percentage, we are interested in knowing what other species received votes from the random forest trees. The bar chart of random forest species probabilities for egg 77 shows that approximately 27% of the trees in the species random forest voted for Speckled Chub (*Macrhybopsis aestivalis*) and approximately 17% of trees in the species random forest voted for Freshwater Drum.

4) Move to the 'Downloads' page by either clicking on the 'Jump to Downloads' button or the 'Downloads' tab at the top of the 'Predictions' page (Figure 8).

Downloads Page

The 'Downloads' page has the same set up as the 'Data Input' and 'Predictions' pages with an instructions panel on the left and an overview and interactive tables in the main panel (Figure 10). The steps for downloading the data are as follows.

1) Upload data and compute predictions done previously.
2) Click on the 'Preview Data' button (solid red box in Figure 10). An interactive table appears under 'Download Preview Table'. This table allows the user to preview the spreadsheet available for download from WhoseEgg. The table is too long to show all the columns at once, but a horizontal scrolling option allows the user to see all columns. The table includes all initial variables uploaded to WhoseEgg, additional variables computed by WhoseEgg to obtain the random forest predictions, random forest predictions, and random forest probabilities for all categories within the three taxonomic levels.
3) Select a file type for download (xlxs, xls, or csv) using the selector option (gold short-dashed box in Figure 11).
4) Download the data table by clicking on the 'Download Predictions' button (blue long-dashed box in Figure 11).

After Download

The user may use the downloaded results for further investigation. For example, we explore the relationship between the Invasive Carp random forest probabilities and predictions for the species model. We create a violin plot of the Invasive Carp probabilities versus predictions (Figure 11). Most of the eggs predicted to be Invasive Carp have high random forest probabilities (above 0.8) and most of the eggs predicted to be a species other than Invasive Carp have low random forest probabilities for Invasive Carp (below 0.25). We could elect to genetically identify the handful of eggs predicted to be Invasive Carp but with low probabilities. This would enable us to gain confidence in those eggs. The results from

WhoseEgg suggest there are Invasive Carp eggs present at the mouth of the Iowa River and help us to identify a possible subset of eggs for genetic identification if deemed necessary.

Future Work

Currently, WhoseEgg has been developed and validated only for fish eggs captured in the Upper Mississippi River. It is unknown how successfully the model could work in other locations. In the future, the models in WhoseEgg could be updated to include data from other regions and additional fish species. Data may also be added to increase the number of observations for species other than Invasive Carp and validation could be done to make WhoseEgg a tool for predicting species other than Invasive Carp. Furthermore, the functionality of WhoseEgg could be extended by including options such as a manual input of data for one egg of interest and visualizations comparing relationships between the input data and the WhoseEgg training data. WhoseEgg also provides a step in the direction of the production of a phone app that would allow users to take a picture of a fish egg on a phone and return a random forest prediction. Beyond the identification of fish eggs, this web-based application demonstrates the potential for fisheries scientists to make their work more accessible to other professions in our field. We encourage others to develop similar web-based tools for other complicated models that will make them more accessible and help to facilitate their use and application.

Table 1. WhoseEgg random forest predictor variables with definitions and training data means (and standard deviations) or levels (and proportion of eggs per level).

Variable	Definition	Mean (standard deviation) or levels (proportion)
Compact or diffuse	Whether the embryo is compact or diffuse	Compact (0.87); Diffuse (0.13)
Conductivity (μ/cm)	Conductivity of the water at the time of collection	462.21 (103.03)
Deflated membrane	Whether the membrane is deflated or not	Yes (0.59); No (0.41)
Egg stage	Stage of the egg when collected (based on Kelso and Rutherford (1996))	1 (0.15); 2 (0.01); 3 (0.09); 4 (0.22); 5 (0.10); 6 (0.12); 7 (0.10); 8 (0.08); Broken (<0.01); Diffuse (0.13)
Embryo diameter average (mm)	Average of four measurements of the embryo diameter	1.36 (0.42)
Embryo diameter coefficient of variation	Coefficient of variation of four measurements of the embryo diameter	0.1 (0.08)
Embryo diameter standard deviation (mm)	Standard deviation of four measurements of the embryo diameter	0.14 (0.14)
Embryo to membrane ratio	Ratio of the embryo diameter average to the membrane diameter average	0.67 (0.2)
Julian day	Julian day when the egg was collected	167.99 (27.21)
Larval length (mm)	Length along the midline for all eggs in stages 6-8 (otherwise set to 0)	0.49 (1.12)
Membrane diameter average (mm)	Average of four measurements of the membrane diameter	2.27 (1.04)
Membrane diameter coefficient of variation	Coefficient of variation of four measurements of the membrane diameter	0.07 (0.07)
Membrane diameter standard deviation (mm)	Standard deviation of four measurements of the membrane diameter	0.17 (0.17)
Month	Month when the egg was collected	5.85 (0.98)
Pigment presence	Whether there is pigment present on the egg	Yes (0.29); No (0.71)
Sticky debris	Whether there is debris on the egg	Yes (0.23); No (0.77)
Temperature (°C)	Temperature of the water when the egg was collected	23.39 (2.95)

Table 2. Taxonomic levels and number of eggs per species in the training data collected from pools 14-20 of the Upper Mississippi River during 2014-2016.

Family	Genus	Common name	Number of eggs in training data
Catostomidae	Carpiodes	Carpsuckers species (other)	1
		Quillback	1
		River Carpsucker	8
	Ictiobus	Bigmouth Buffalo	7
		Black Buffalo	1
		Buffalo species (other)	10
		Smallmouth Buffalo	2
Clupeidae	Alosa	Skipjack Shad	1
	Dorosoma	Gizzard Shad	2
Cyprinidae	Cyprinella	Spotfin Shiner	6
	Luxilus	Common Shiner	1
	Macrhybopsis	Silver Chub	36
		Speckled Chub	28
	Notropis	Channel Shiner	32
		Emerald Shiner	201
		River Shiner	16
		Sand Shiner	1
		Shiner species (other)	69
	Pimephales	Fathead Minnow	5
Hiodontidae	Hiodon	Goldeye	7
Invasive Carp	Invasive Carp	Invasive Carp	782
Moronidae	Morone	Striped Bass	17
		White Bass	1
Percidae	Etheostoma	Banded Darter	1
	Percina	Common Logperch	1
	Sander	Walleye	2
Sciaenidae	Aplodinotus	Freshwater Drum	733

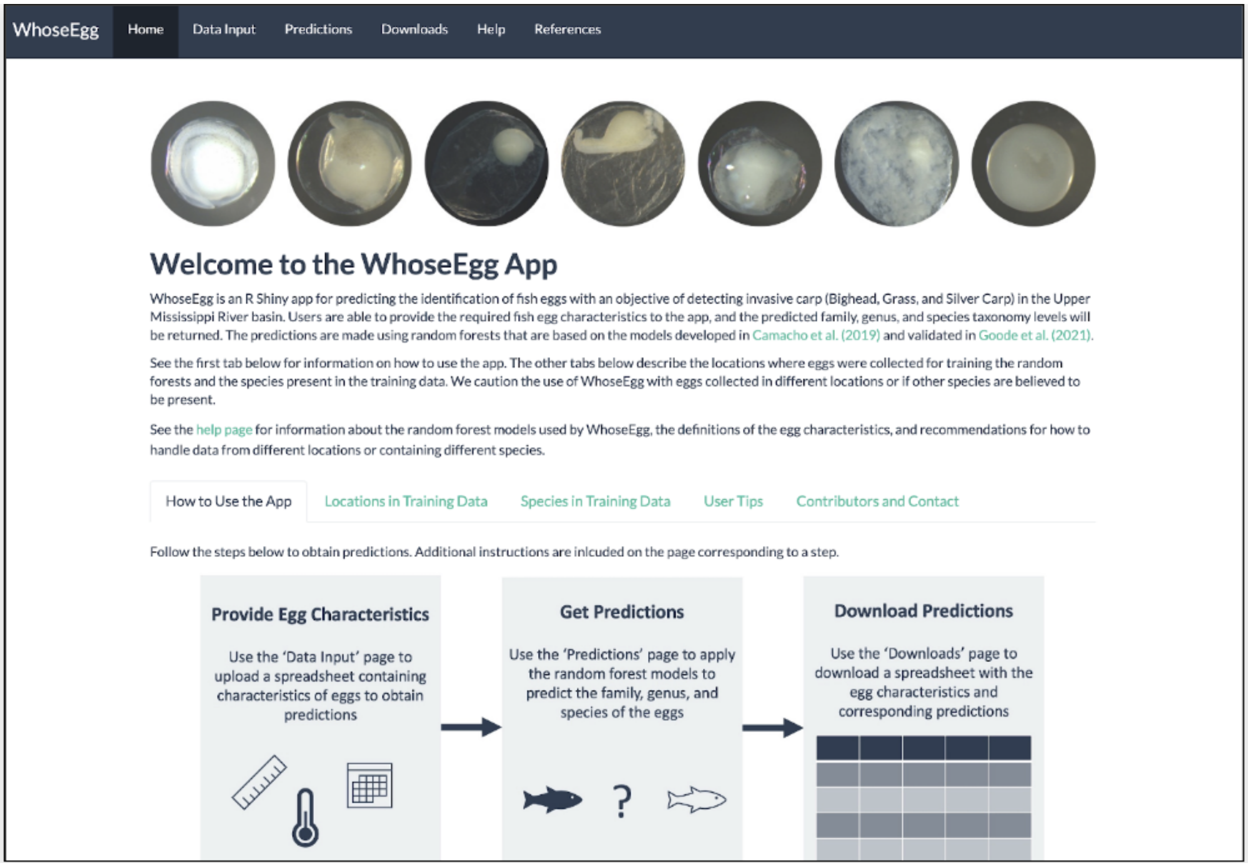

Figure 1. Homepage of WhoseEgg (Whoseegg.stat.iastate.edu) with a description of the app and a flowchart with instructions of how to use the app to obtain fish egg identification predictions.

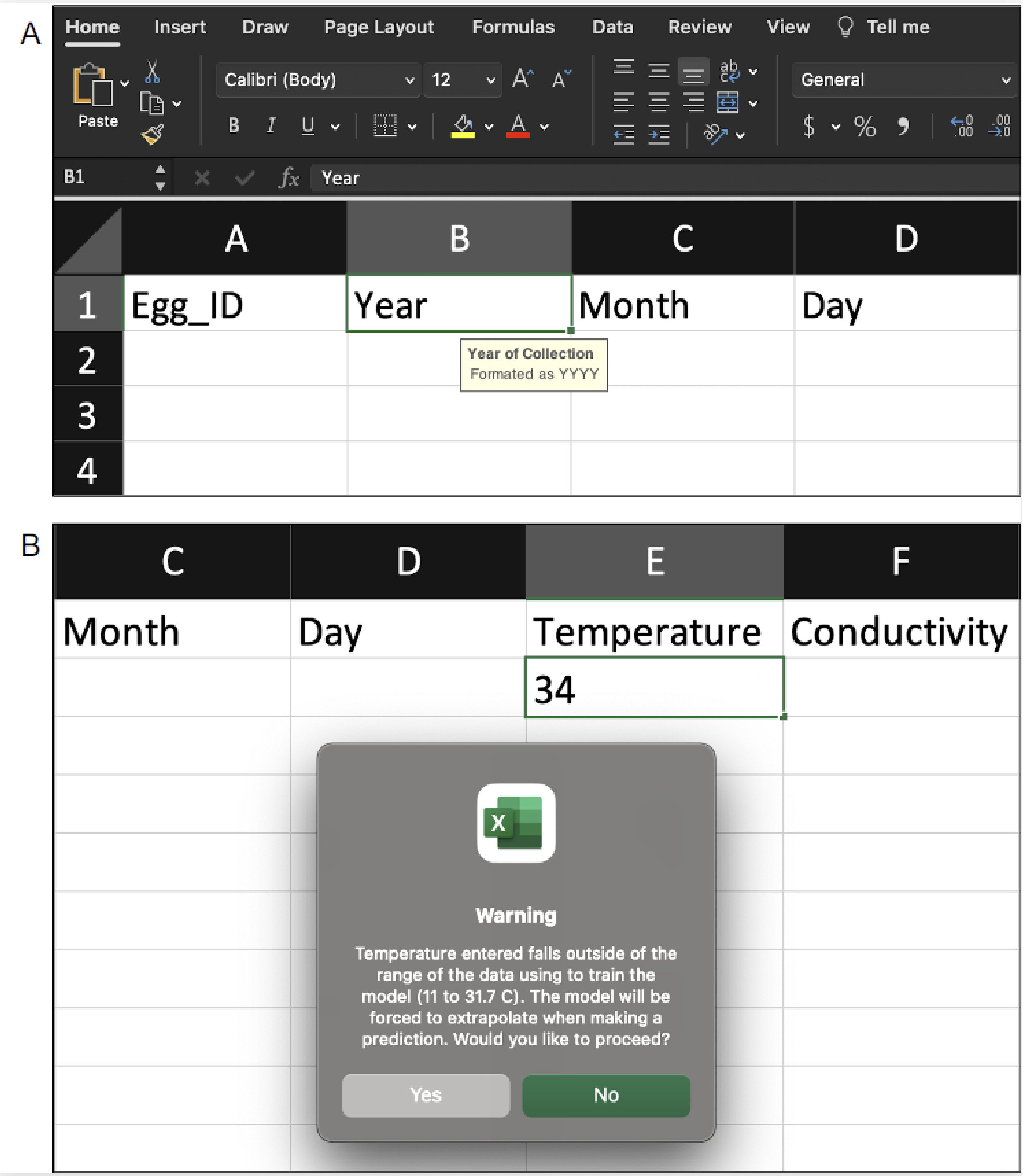

Figure 2. Examples of the helpers in the spreadsheet template to assist users correctly format egg characteristic data to uploaded to WhoseEgg. (A) When a column is selected, a description of the variable and necessary format appears. (B) If an observation is entered incorrectly/falls outside of the range of the WhoseEgg training data, an error/warning appears.

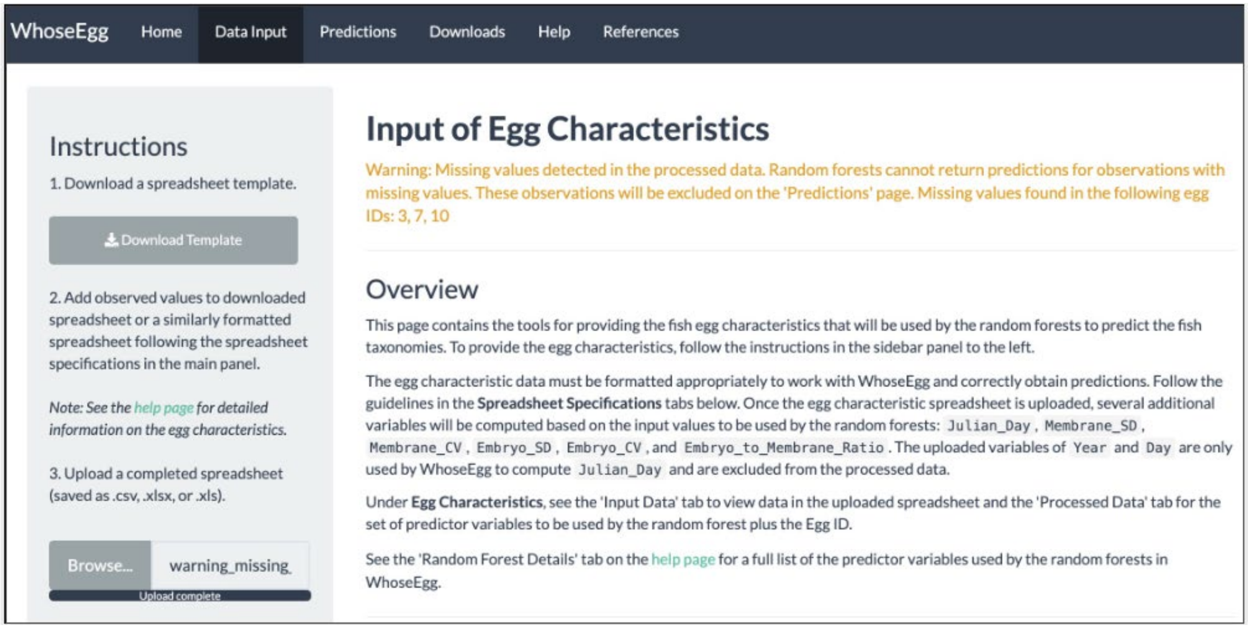

Figure 3. Example of a warning message in WhoseEgg that appears if there is an issue with the uploaded data. In this case, some observations are missing values.

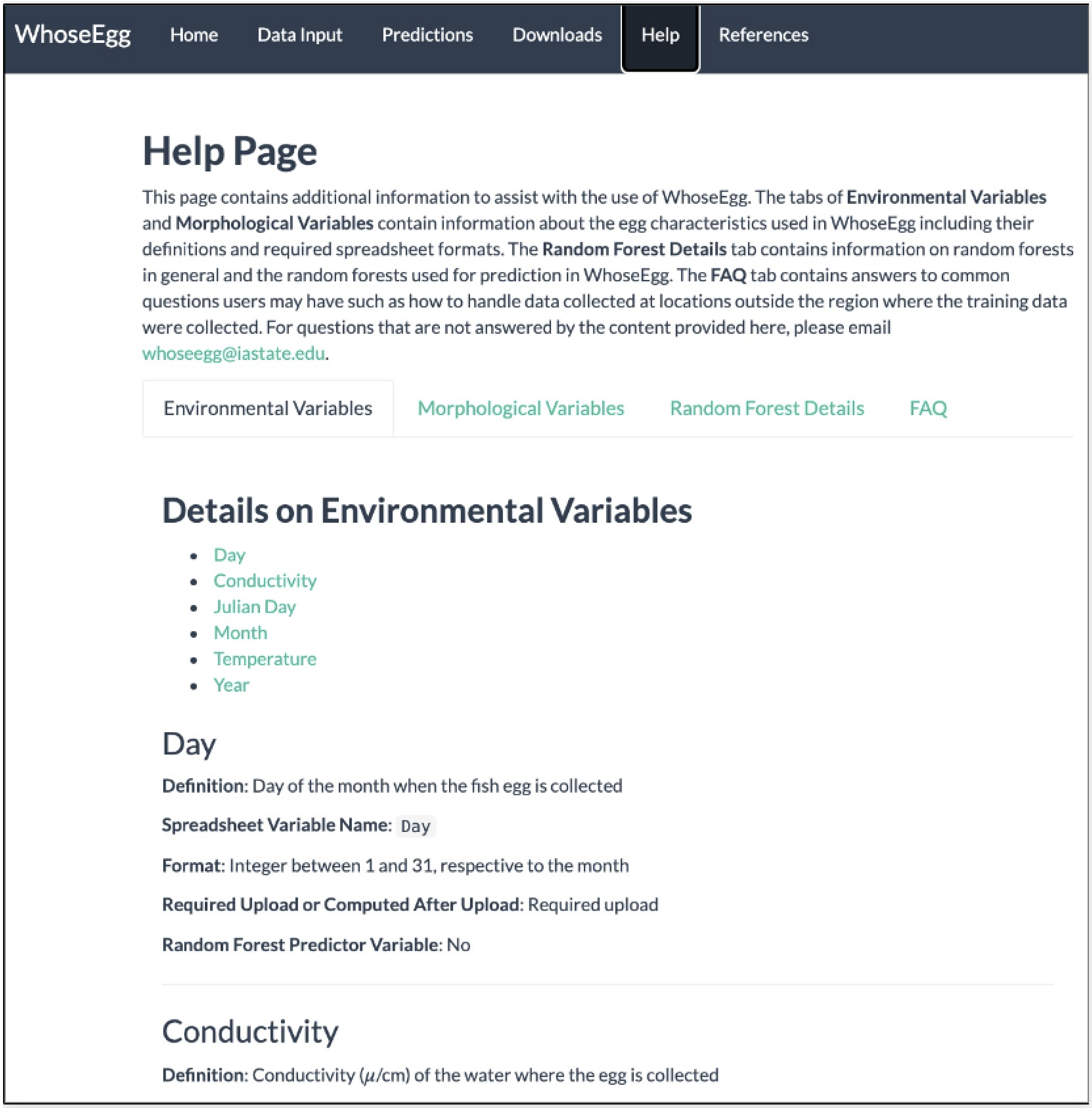

Figure 4. A portion of the WhoseEgg 'Help' page. The information for the environmental variable of 'Day' is visible and shows an example of the information available for the egg characteristics.

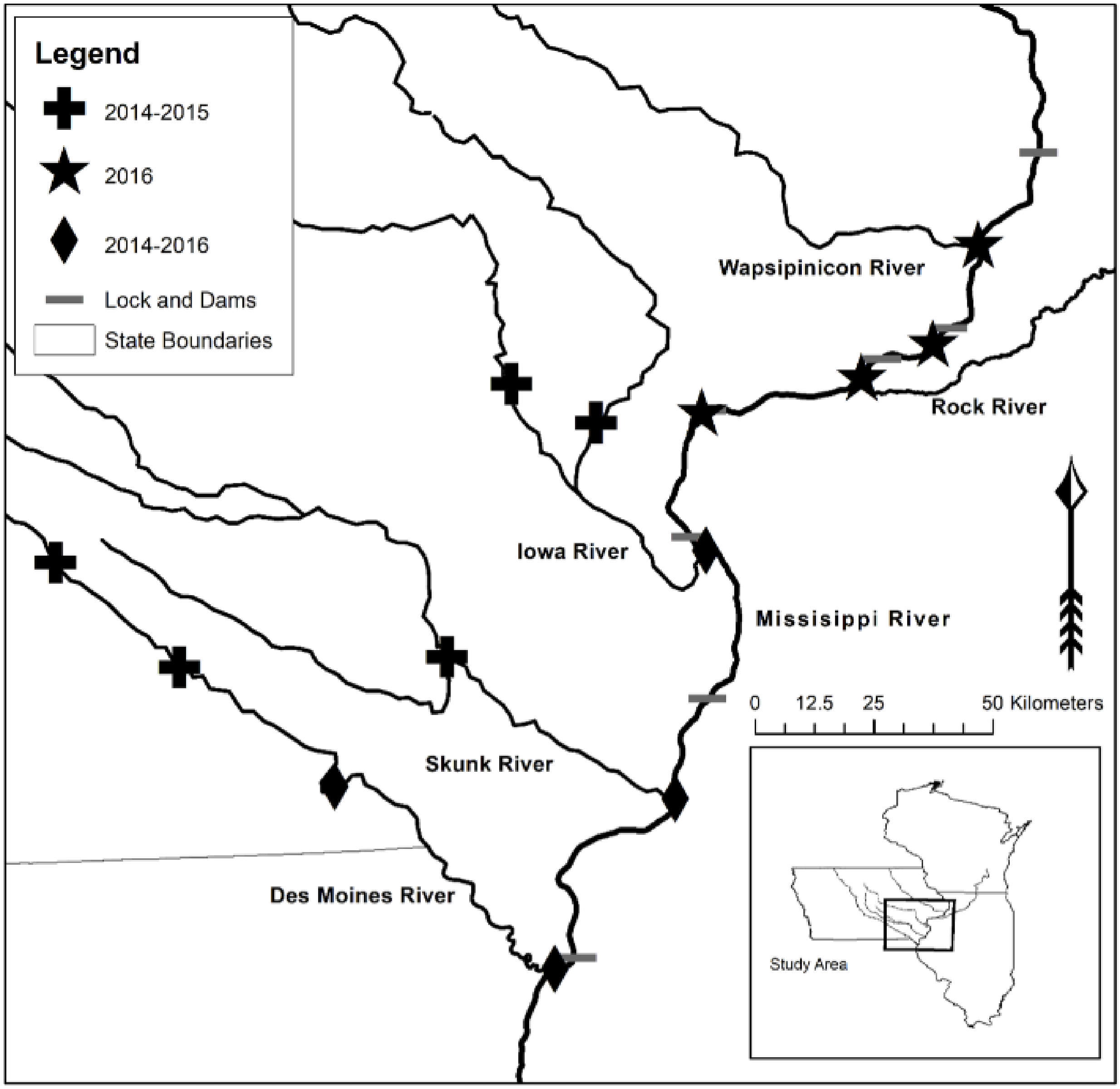

Figure 5. Upper Mississippi River and tributary rivers in Iowa and Illinois, USA where eggs were collected during 2014-2015 (plus), 2016 (star), or 2014-2016 (diamond). Map of sampling locations acquired from Goode et al. (in press).

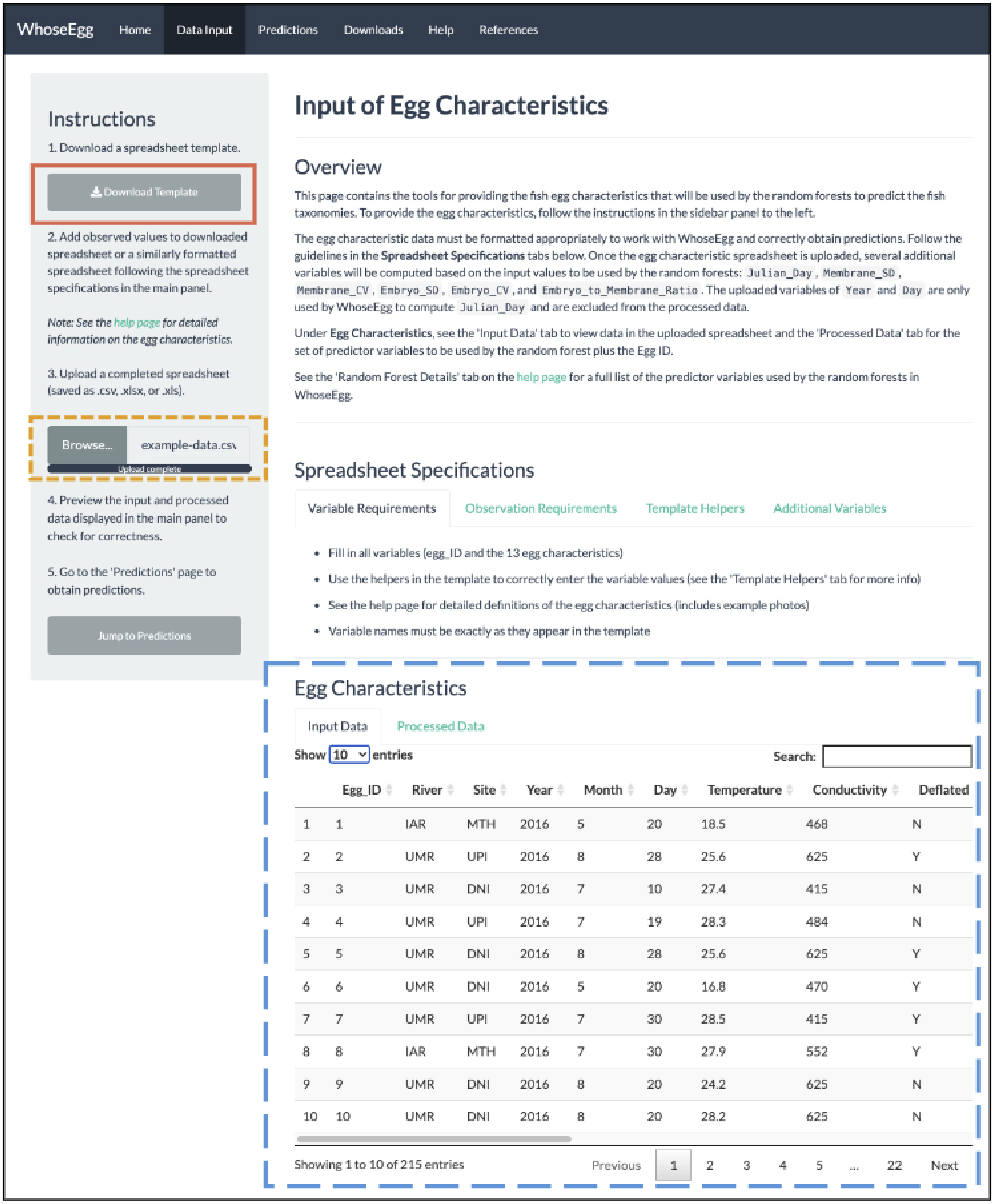

Figure 6. Screenshot of the 'Data Input' page showing the content in WhoseEgg after the example spreadsheet of fish egg characteristics were uploaded.

Egg Characteristics

Input Data | Processed Data

Show 10 entries Search:

	Egg_ID	Month	Julian_Day	Temperature	Conductivity	Deflated	Pigment	Egg_
1	1	5	141	18.5	468	N	N	D
2	2	8	241	25.6	625	Y	N	8
3	3	7	192	27.4	415	N	Y	3
4	4	7	201	28.3	484	N	N	1
5	5	8	241	25.6	625	Y	N	1
6	6	5	141	16.8	470	Y	N	D
7	7	7	212	28.5	415	Y	N	7
8	8	7	212	27.9	552	Y	N	8
9	9	8	233	24.2	625	N	N	1
10	10	8	233	28.2	625	N	N	1

Showing 1 to 10 of 215 entries Previous 1 2 3 4 5 … 22 Next

Figure 7. Processed data corresponding to the example in Figure 6.

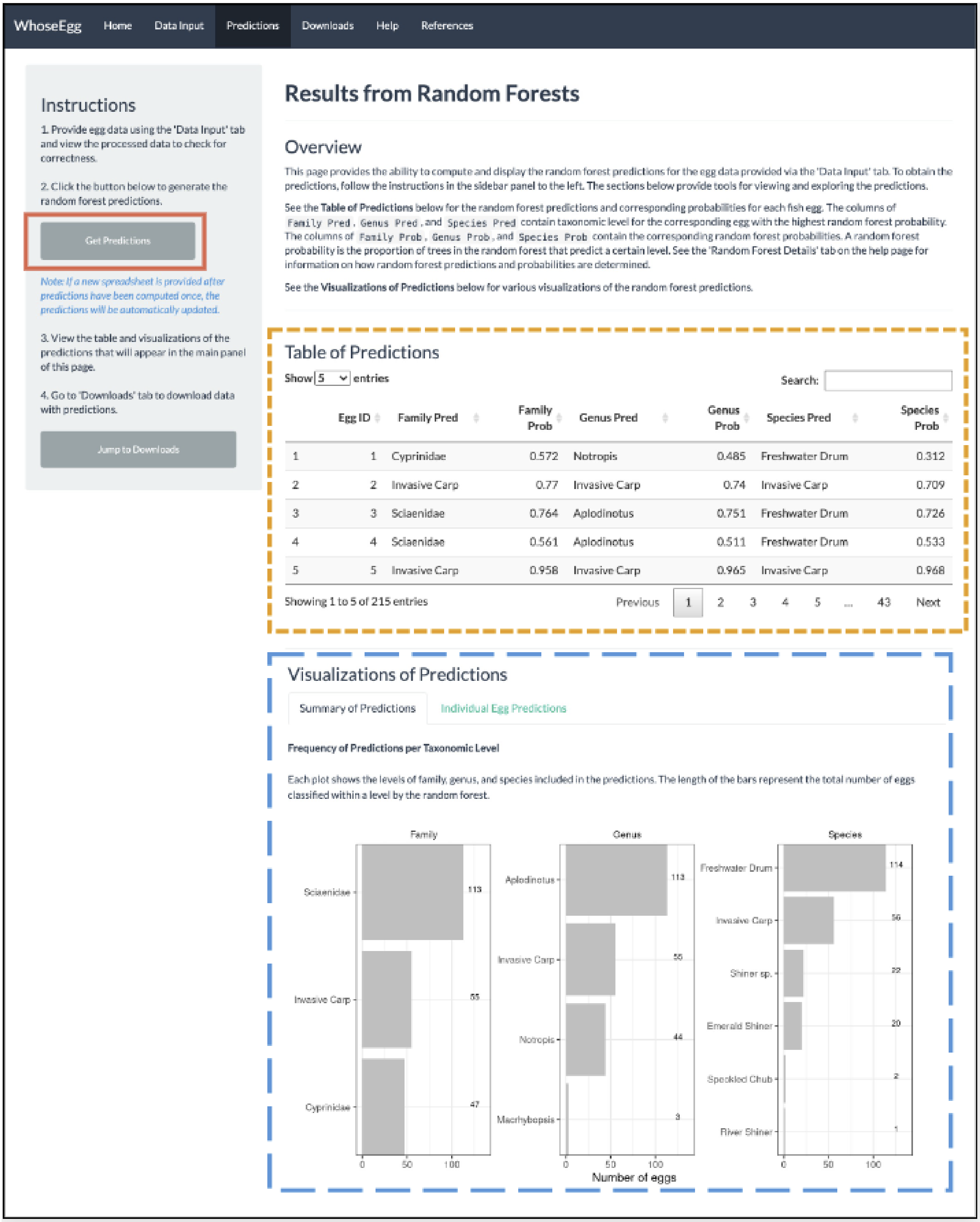

Figure 8. 'Predictions' page corresponding to the example after the 'Get Predictions' button was clicked.

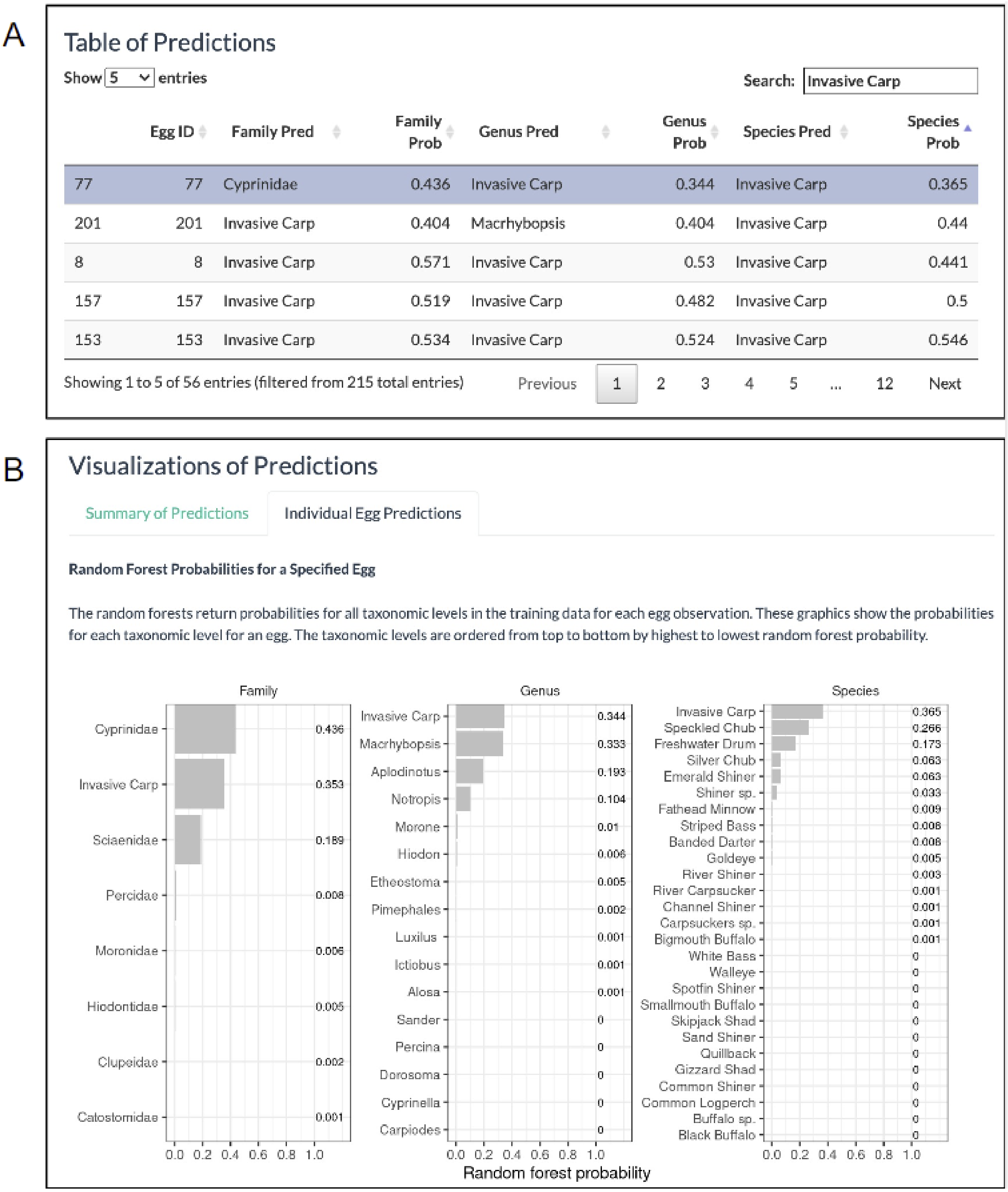

Figure 9. ‘Individual Egg Predictions’ visualization corresponding to the example data. (A) The ‘Table of Predictions’ filtered to only include rows with at least one prediction of Invasive Carp and sorted from lowest to highest species probability. The egg with the lowest random forest probability of Invasive Carp (the first row) has been selected. (B) Bar charts of random forest probabilities for all categories within a taxonomic level corresponding to the egg selected in A.

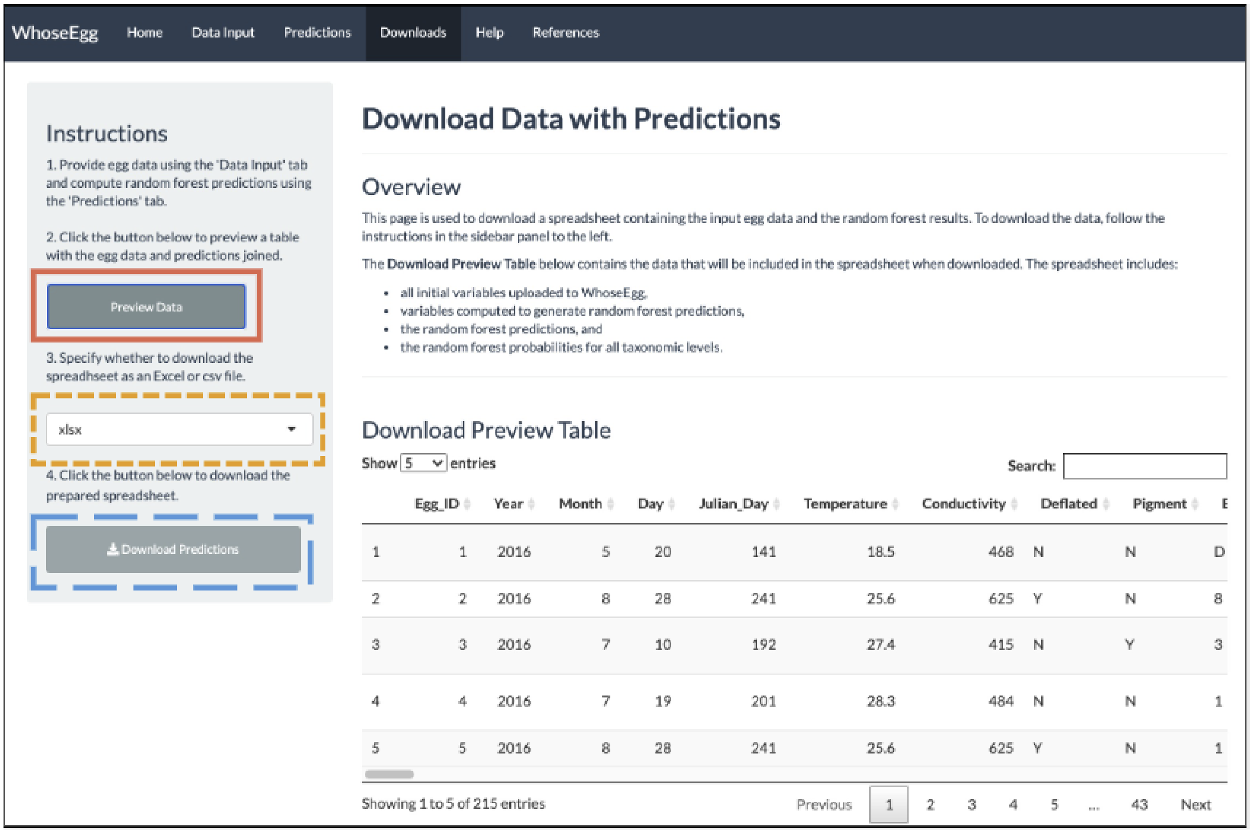

Figure 10. ‘Downloads’ page corresponding to the example after the ‘Preview Data’ button was clicked.

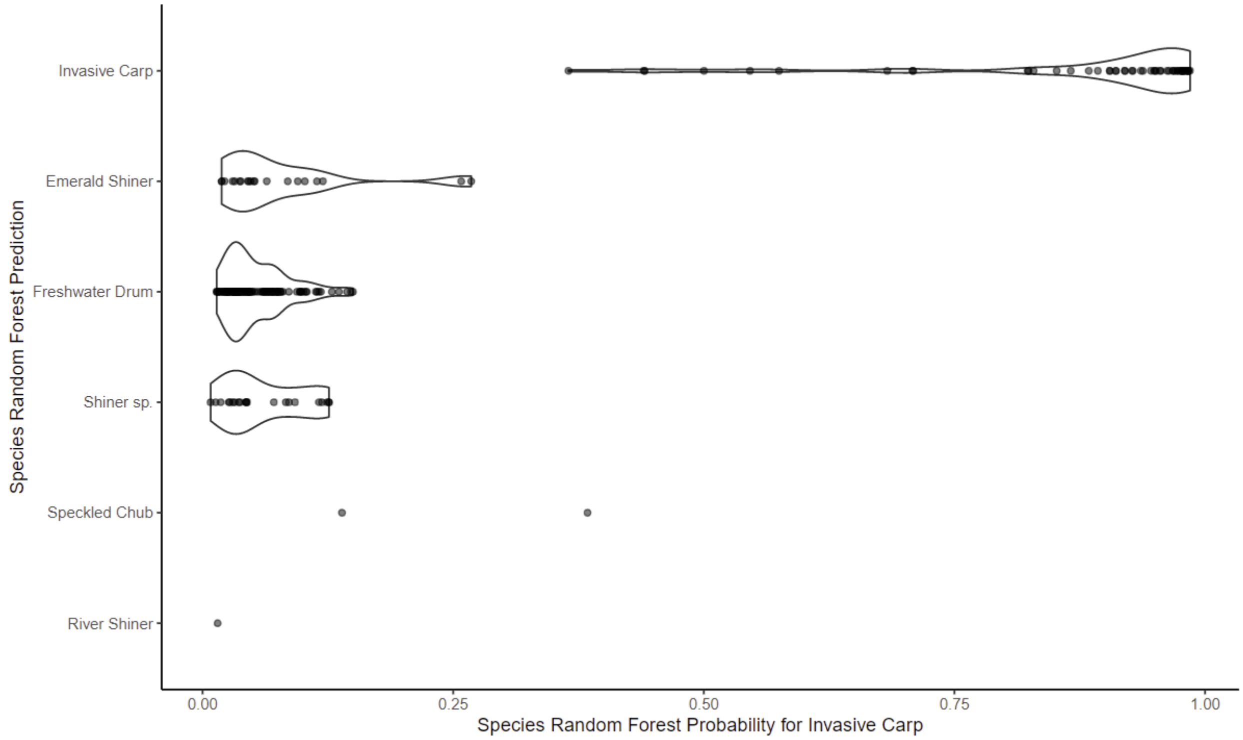

Figure 11. Violin plot of species random forest probabilities for Invasive Carp separated by the species random forest predictions.

State Report: Missouri

Methods

Four tributaries of the Missouri River that have known detections of Silver Carp (*Hypophthalmychthys molitrix*) were selected for sampling: Lamine River (MO River kilometer 325), Grand River (MO River kilometer 338), Platte River (MO River kilometer 629), and Nodaway River (MO River kilometer 745). The tributaries had varying drainage areas with the Grand River (20,429 km^2) having the largest and the Nodaway the smallest (4,647 km^2). Sampling for Invasive Carp took place from August to November. The lower 40 rkm of the tributaries were separated into 10 rkm sampling units. For sampling in the Missouri River, the bend at the mouth of each tributary was used as the sampling unit.

Two frequency and duty cycle settings (60 Hz and 40% duty cycle, 40 Hz and 20% duty cycle) for pulsed DC electrofishing runs were tested. For both settings the goal was to run 20 Amps. These settings were selected based on pilot work done in August 2020, literature review (i.e., Roth 2018, and Hammen et. al. 2019) and expert solicitation of electrofishing for Invasive Carp. A target of 10 runs per setting per site was used for sampling. Each run was 5 minutes in length and involved an aggressive serpentine pattern going down stream. Drivers would swing out to the deeper side of the segment and then quickly push any fish into the shallow side. This pattern would be repeated until the time limit was reached. Each boat consisted of an MLES Infinity box with 7000w generator, double hoops, and 2 dip netters. For Invasive Carp samples, catch per unit effort was calculated as number of fish per hour for electrofishing runs.

All species were measured in millimeters and weighed to the nearest ten grams. For Invasive Carp, sex was recorded if determination was possible. Habitat descriptors (i.e., pool, run, thalweg) were recorded for each run along with water temperature, and depth. Aging structures were collected from 5 fish per 50mm length group <600mm and then 10 fish per 25mm length group ≥600mm for each tributary and associated Missouri River bend. Whole lapilli otoliths were taken, mounted on a slide with epoxy, sanded until middle of otolith was reached, and polished until the focus and all annuli were visible. Otoliths were read by a minimum of two independent readers with aging experience and then ages compared, and agreement reached. If agreement could not be reach or the otolith was not readable, then it was discarded from the analysis.

Fish community sampling was conducted from June through August. Sampling units delineated for Objective 1 were used for fish community sampling. Tributary sampling gears and regime was based on Dunn and Paukert 2020. The same gears used in tributaries were used in the associated Missouri River bends. Fifteen electrofishing runs were targeted for each tributary site. Settings reflected typical fish community settings (60 Hz 30% duty cycle) and used a MLES Infinity box with a 7000w generator, double hoop boat, and 2 dip netters. Three mini-fyke nets (3 mm mesh, 4.5 m lead, 1.2 m wide x 0.6 m tall frame) were set per tributary site. Six otter trawls per tributary site were performed. Otter trawls consisted of a 2.4m envelope style trawl composed of 4mm mesh attached to 0.76m x 0.38m doors. Trawls were fished off the bow of the boat and pulled downstream with a sample target of 50 m distance. Missouri River bend sampling was based on Welker and Drobish 2016. Eight mini-fyke nets were set in each Missouri River bend along with 8 otter trawls and 10 electrofishing runs.

Results and Discussion

Silver Carp Population Demographics

Sampling in all tributaries was hindered by low water during the sampling season (Figures 1-4). This made almost all but the lower 10 rkm (i.e., Site 1) of each tributary inaccessible. Silver Carp was the overwhelmingly dominant Invasive Carp species (n = 2,482) in all tributaries and their associated Missouri River bends. Only a handful of Bighead Carp (*Hypophthalmichthys nobilis*, n = 3) and Grass Carp (*Ctenopharyngodon idella*, n = 14) were sampled. No Black Carp (*Mylopharyngodon piceus*) were sampled. Silver Carp sampled in tributaries that had a sex determined had a lower ratio of males to females while the carp sampled in the mainstem sites were opposite (Table 1). However, most male: female ratios were very close to 1:1.

Size distributions in all tributaries exhibited bimodal peaks with very few fish in the 500mm range or below 350mm (Figures 6 - 13). The largest sized Silver Carp (>900mm) generally were found in the Platte and Nodaway rivers. The Missouri river at the mouth of the Platte River was the only site sampled where length frequencies were not bimodal (Figure 10). Both electrofishing settings produced similar length distributions for each site (Figures 6-13).

Otoliths for the 2021 sampling year are still being processed for aging. Pilot work from August 2020 showed that mean lengths at age for Silver Carp are similar between tributary complexes (Figure 14). However, in 2021, an ANOVA on Ranks indicated mean lengths did vary between sites with the Lamine River sites being significantly different ($P<0.001$) with smaller size individuals than all other sites except for the upper Platte River site (PR2) (Figure 15). Also, Silver Carp mean lengths at the Missouri River site of the Platte River were significantly different ($P<0.05$) with larger individuals than other sites with the exception of three sites at the upper Grand River site (GR2) and Missouri River sites at the Nodaway and Grand River (Figure 15). Combining age data with the length frequencies indicate a potential lack of 3 - 5-year-old fish (2015-2017 year classes). Another potential hypothesis to the missing lengths groups is that Silver Carp have rapid growth up to 550 mm. Silver Carp length to weight was similar at all sites indicating condition was similar between the Missouri River sites and tributaries in 2021 (Figure 16). The forthcoming aging data will shed more light on potential absent year classes. The 2020 age data also showed that fish ≥10 years old were sampled only in the Nodaway and Platte River complexes. The oldest fish aged was 12 years old in the Platte River.

Electrofishing Comparison

Catches of Silver Carp were variable for each group of electrofishing settings being evaluated. In the tributaries mean catch per unit effort for the 40 Hz 20% duty cycle setting ranged from 122 fish/hr to 300 fish/hr, while for the 60 Hz 40% duty cycle setting it was 108 fish/hr to 234.9 (Table 1) (Figure 5). Catch rates for the mainstem river bends sampled were lower ranging 67.2 fish/hr to 83.7 fish/hr for the

40 Hz 20% duty cycle and 63.6 fish/hr to 144 fish/hr for the 60 Hz 40% duty cycle settings (Figure 5). Power output in tributaries tended to be higher (3600W to 7000W) than the mainstem Missouri River sites (1680W to 5250W). Lower mean catch per unit effort in the mainstem bends may be a result of lower densities of carp, or lesser efficiency of the gear on the larger river. Initial results do not suggest that there is a difference in catch per unit effort between the two electrofishing settings regardless of site or waterbody, although more sampling is needed.

Having a better understanding of the efficiencies that can be accomplished with a common fisheries gear used by state agencies will ultimately help managers in their Invasive Carp efforts. This information can then be used to better plan removal and management efforts.

Fish Community

Sampling in all tributaries was hindered by low water for the duration of the sampling season (Figures 1-4). Boat motor issues also hindered crews' ability to complete sampling using all gears in both tributaries and the mainstem river bends (Table 2). A total of 4,521 fish comprising 55 species were sampled. Sampling resulted in more species of fish in tributaries compared to their associated Missouri River bends except for the Nodaway River (Table 2). The most abundant species sampled in each tributary varied. Gizzard shad (*Dorosoma cepedianum*) was the most abundant fish sampled in the Lamine River, Shortnose gar (*Lepisosteus platostomus*) in the Grand River, and Green sunfish (*Lepomis Cyanellus*) in both the Platte River and Nodaway River.

Additional species sampled during targeted Silver Carp work but not during fish community included Paddlefish (*Polyodon spathula*) and Bigmouth buffalo (*Ictiobus cyprinellus*). The lack of these important species in fish community sampling is likely the result of not being able to complete the entire suite of gears. The mean relative weight of Paddlefish captured in the Grand River was higher than those sampled in mainstem sites (Table 3). Bigmouth buffalo mean relative weights were similar for all sites where there was enough data collected to calculate the metric. However, sample sizes were low for both species and future data needs to be collected. Bigheaded Carp have been reported to directly compete with these native fish (Schrank et al. 2003, and Sampson 2005) so their presence in the fish community will be important to monitor as management efforts take place in these localized areas.

Recommendation

Finish the last two years of gear evaluation and use data to compare to other novel gears (i.e., dozer trawl). Use most efficient gear as a standard for obtaining population demographic data so that strategic management and removal efforts can be planned. Continue to collect fish community data to evaluate any potential changes due to management and removal efforts.

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Table 1. Sampling effort and catch results for each site.

Site[1]	# of Samples	40Hz 20% Mean CPUE (± S.D.)[2]	60Hz 40% Mean CPUE (± S.D.)[2]	Total # Silver Carp	Total Weight (kg)	Male : Female
MRLR	20	83.7 (70.2)	77.9 (56.9)	817	962	--
LR1	20	128.4 (32.5)	207.5 (59.9)			--
LR2	20	225.4 (89.6)	234.9 (174.1)			--
MRGR	20	78 (47.4)	144 (121.6)	624	1,013	1.1 : 1
GR1	15	122 (40.2)	108 (79.6)			0.8: 1
GR2	9	300 (128.1)	213 (119.8			
MRPR	20	38.6 (20.9)	92.2 (59.5)	602	1,047	1.5 : 1
PR1	24	179.6 (66.7)	218.38 (104.8)			0.87 : 1
PR2	6	NA	136.8 (55.7)			
MRNR	20	67.2 (58.6)	63.6 (64.3)	439	647*	1.3 : 1
NR1	20	139.2 (132.9)	174 (132.8			0.9 : 1

*Not all fish were weighed due to equipment issues.

[1]MRLR = Missouri River at mouth of Lamine River, LR1 = Lamine River site 1, LR2 = Lamine River site 2, MRGR = Missouri River at mouth of Grand River, GR1 = Grand River site 1, GR2 = Grand River site 2, MRPR = Missouri River at mouth of Platte River, PR1 = Platte River site 1, PR2 = Platte River site 2, MRNR = Missouri River at the mouth of the Nodaway River, NR1 = Nodaway River site 1.

[2]Catch per unit effort = # Silver Carp/hr.

Table 2. Sampling effort and number of species and fish at each tributary and associated Missouri River bend.

Site[1]	Mini Fyke	Electrofishing	Otter Trawl	Number of Fish	Number of Species
MRLR	8	--	--	345	22
LR	6	29	12	1504	33
MRGR	8	--	--	836	26
GR	6	30	12	727	39
MRPR	8	--	--	214	22
PR	6	15	12	349	36
MRNR	8	--	--	337	27
NR	3	--	3	209	22

[1]MRLR = Missouri River at mouth of Lamine River, LR = Lamine River, MRGR = Missouri River at mouth of Grand River, GR = Grand River, MRPR = Missouri River at mouth of Platte River, PR = Platte River, MRNR = Missouri River at the mouth of the Nodaway River, NR = Nodaway River.

Table 3. Relative weight of Paddlefish and Bigmouth Buffalo.

Site[1]	Number of Paddlefish	Mean W_r (± S.D.)	Number of Bigmouth Buffalo	Mean W_r (± S.D.)
MRLR	9	65.7 (±6.88)	2	94.49 (±7.62)
MRGR	--	--	9	95.95 (±13.71)
GR	2	91.84 (±25.25)	5	91.96 (±8.13)
MRPR	6	69.78 (±11.17)	--	--

[1]MRLR = Missouri River at mouth of Lamine River, MRGR = Missouri River at mouth of Grand River, GR = Grand River, MRPR = Missouri River at mouth of Platte River.

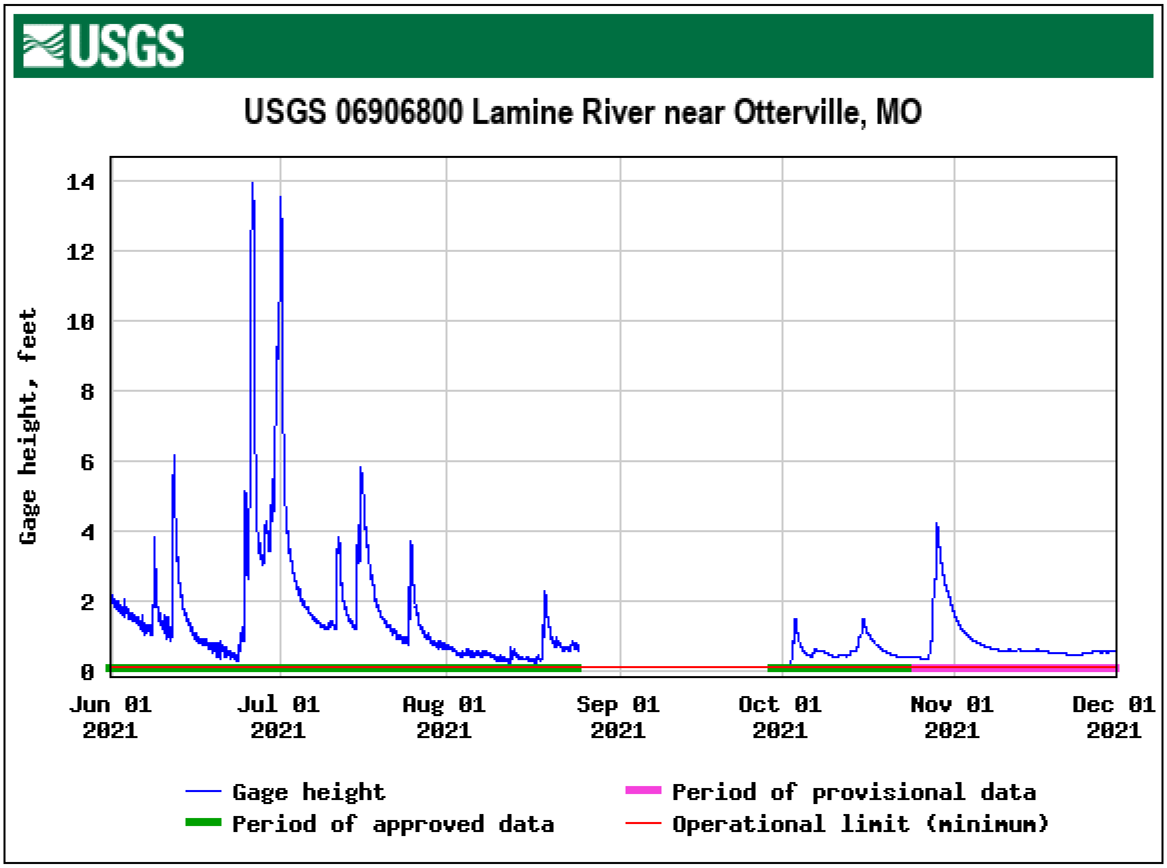

Figure 1. Gage height at USGS gage station for the Lamine River near Ottersville, MO for August 1, 2021 to November 30, 2021.

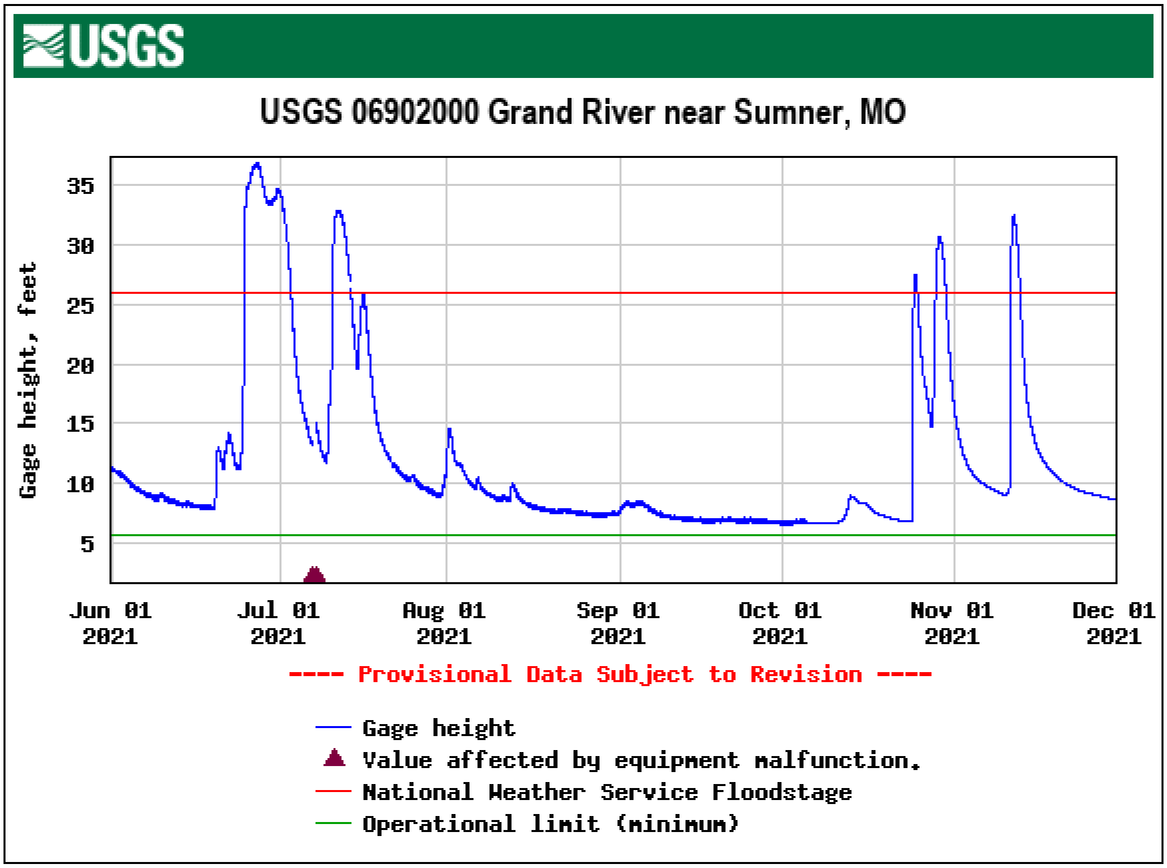

Figure 2. Gage height at USGS gage station for the Grand River near Sumner, MO for August 1, 2021, to November 30, 2021.

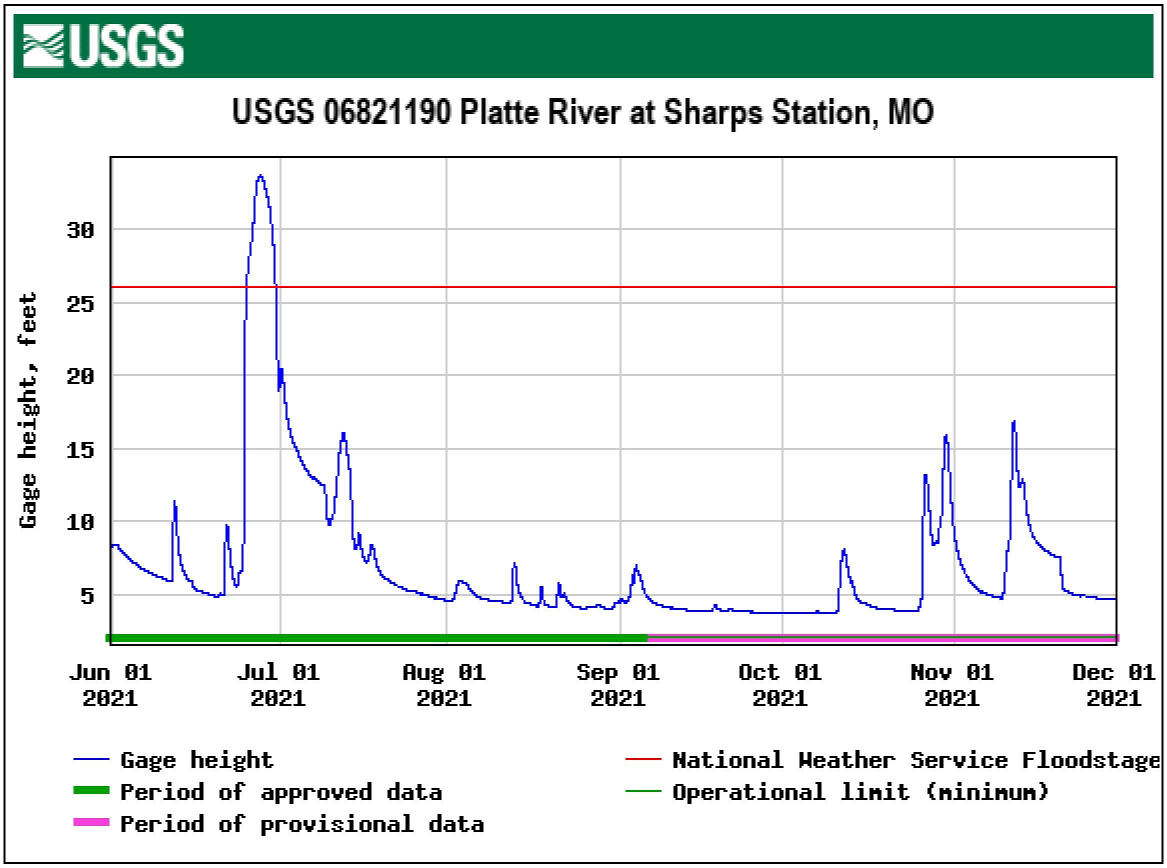

Figure 3. Gage height at USGS gage station for the Platte River near Sharps Station, MO for August 1, 2021, to November 30, 2021.

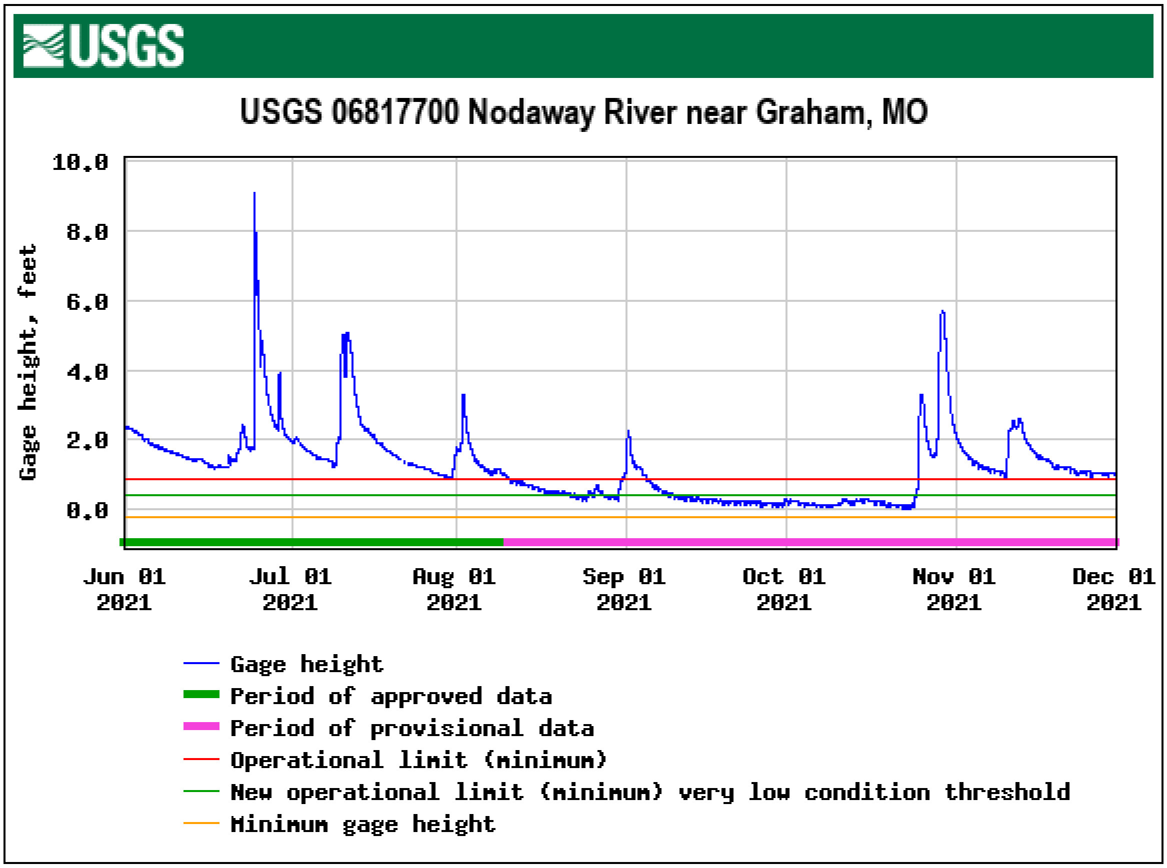

Figure 4. Gage height at USGS gage station for the Nodaway River near Graham, Mo for August 1, 2021, to November 30, 2021.

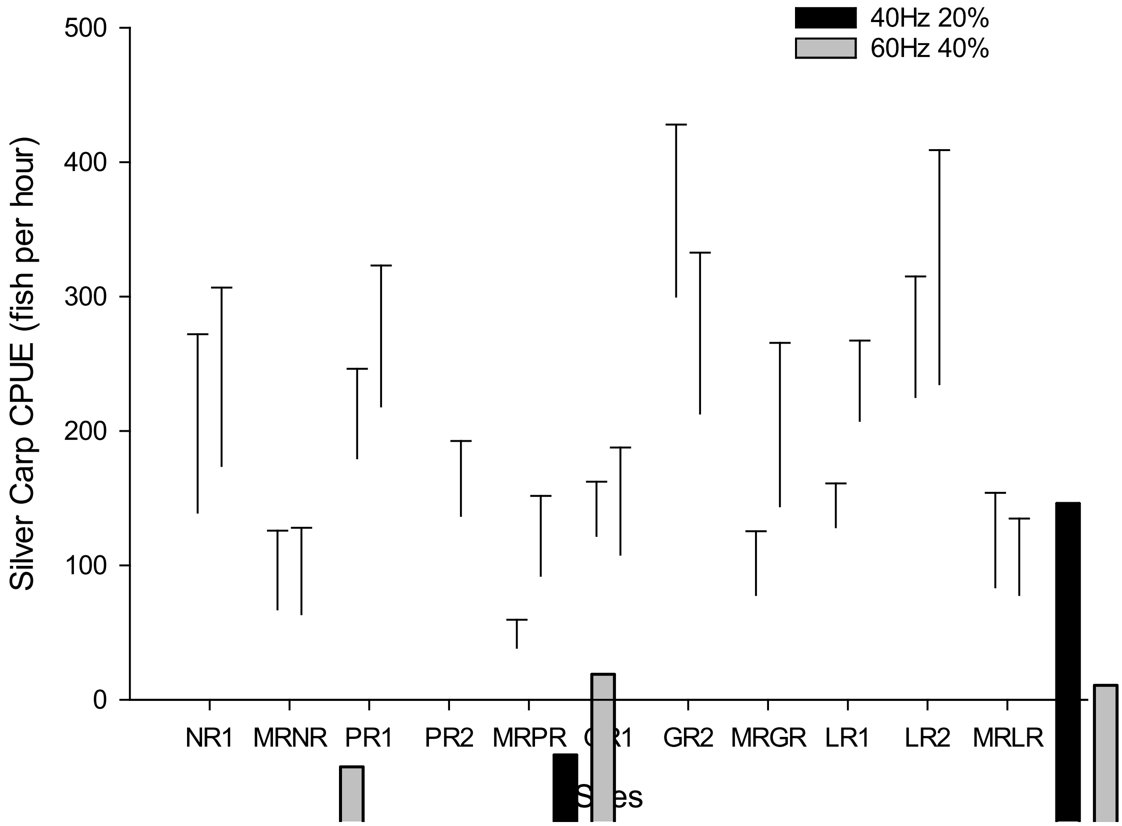

Figure 5. Catch per unit effort of Silver Carp by site in 2021. Black bars represent samples with 40Hz and 20% duty cycle and gray bars represent samples from 60Hz and 40% duty cycle.

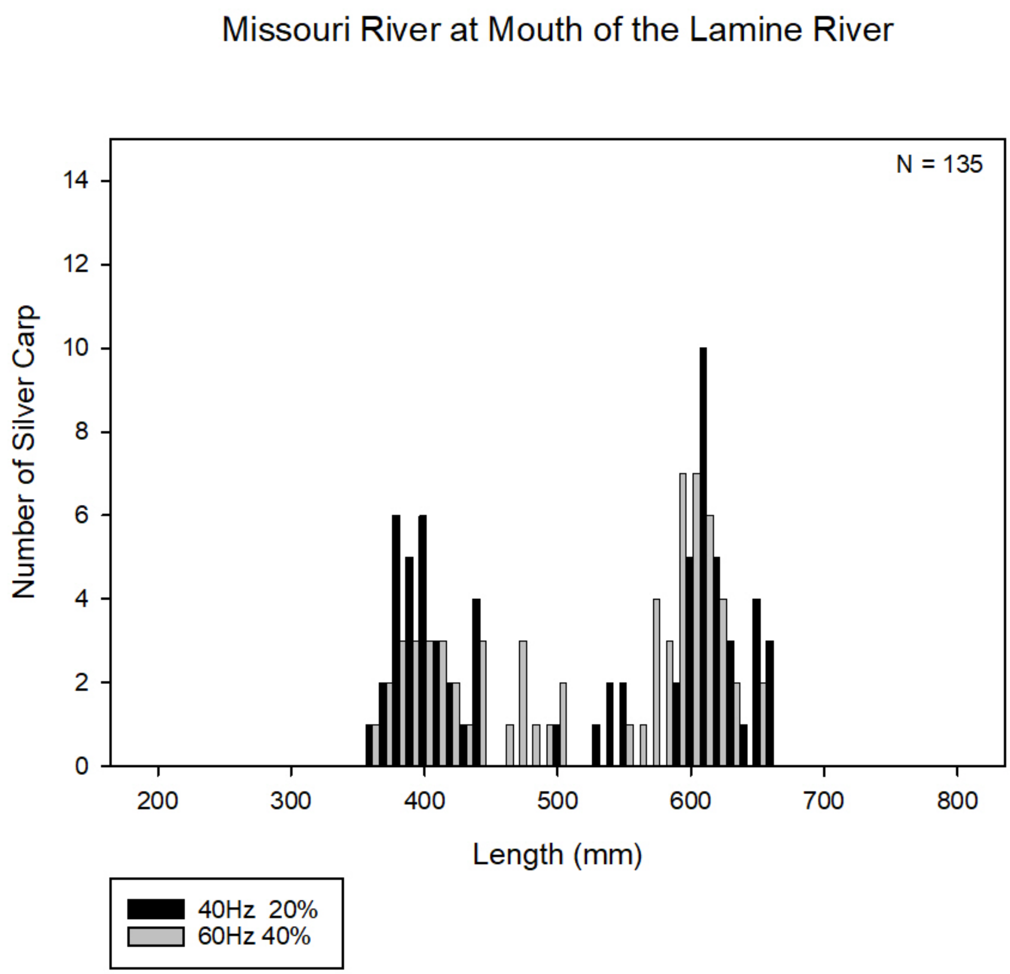

Figure 6. Silver Carp Length-Frequency distribution for the Missouri River at the Mouth of the Lamine River. Bins are 10mm length groups.

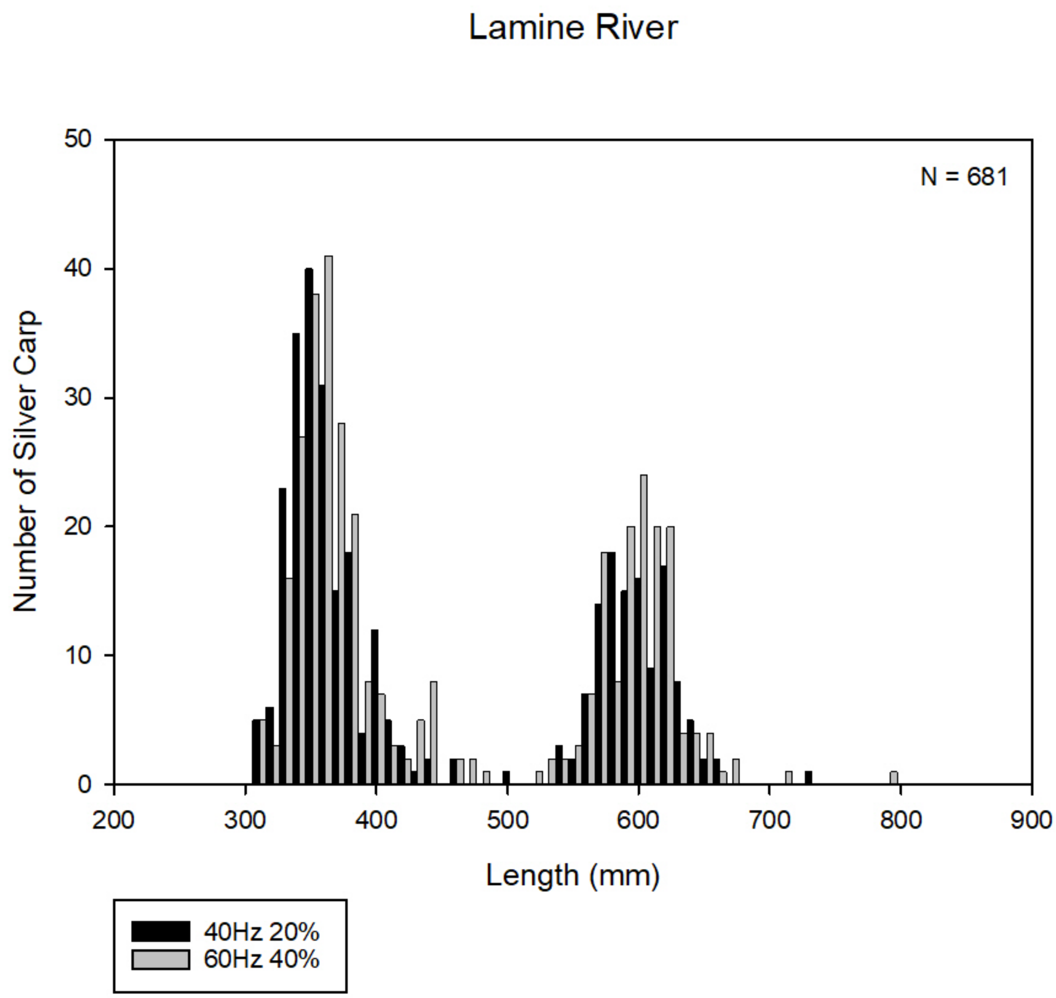

Figure 7. Silver Carp Length-Frequency distribution for Lamine River. Bins are 10mm length groups.

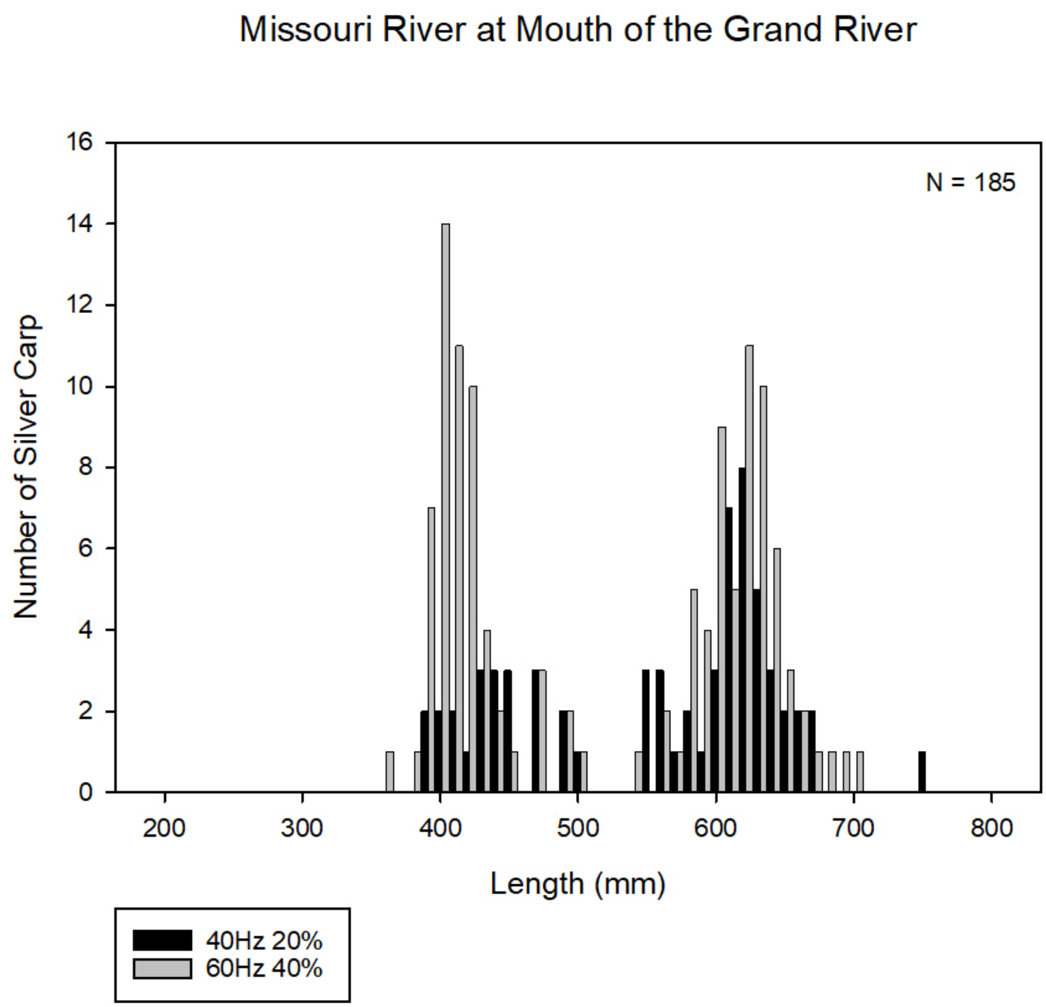

Figure 8. Silver Carp Length-Frequency distribution for the Missouri River at Mouth of the Grand River. Bins are 10mm length groups.

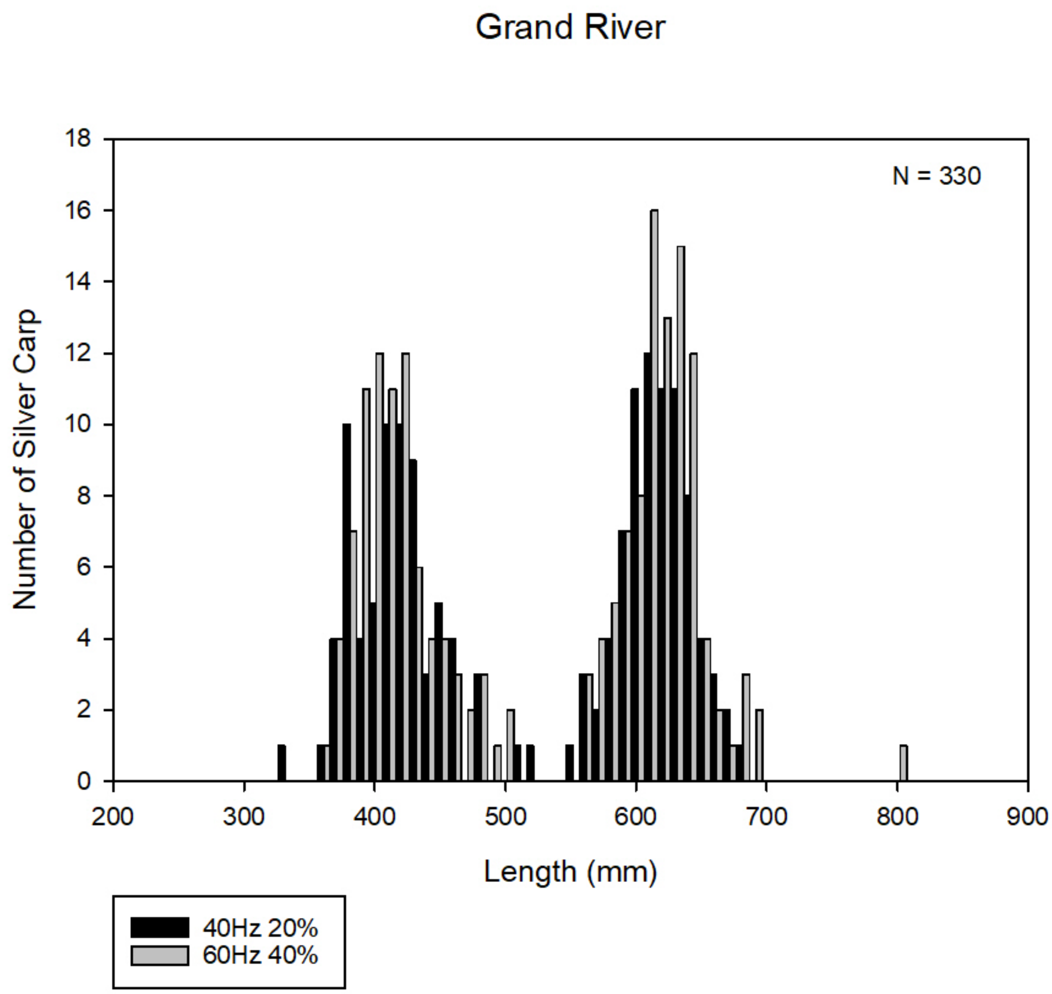

Figure 9. Silver Carp Length-Frequency distribution for Grand River. Bins are 10mm length groups.

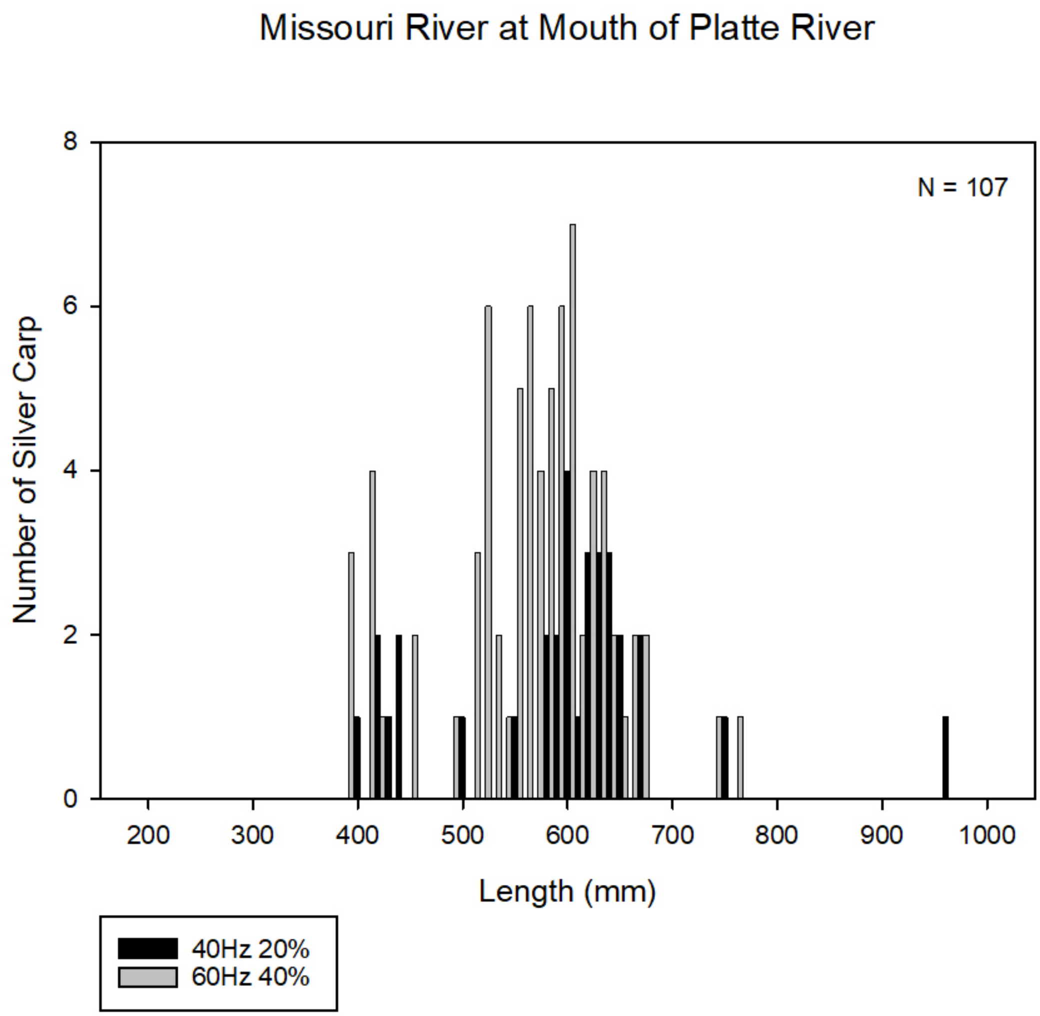

Figure 10. Silver Carp Length-Frequency distribution for Missouri River at Mouth of Platte River. Bins are 10mm length groups.

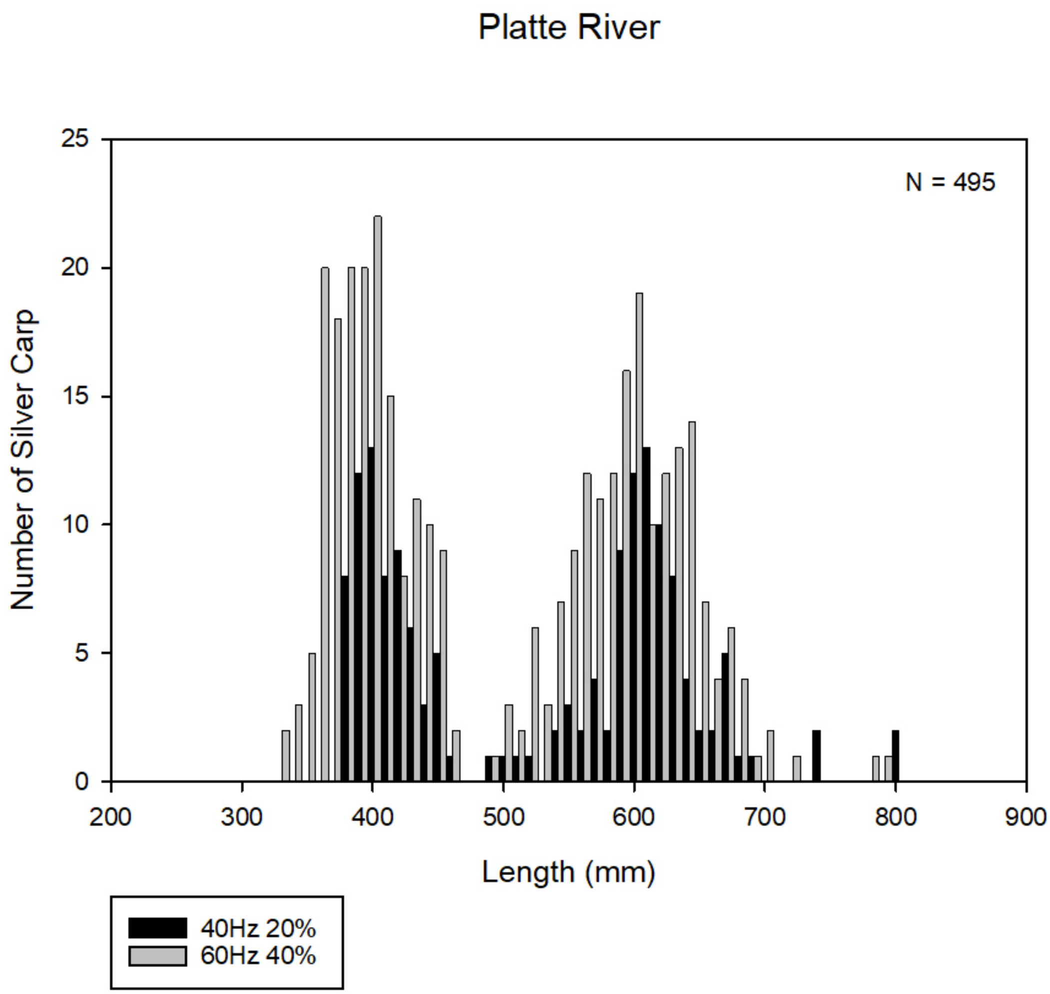

Figure 11. Silver Carp Length-Frequency distribution for Platte River. Bins are 10mm length groups.

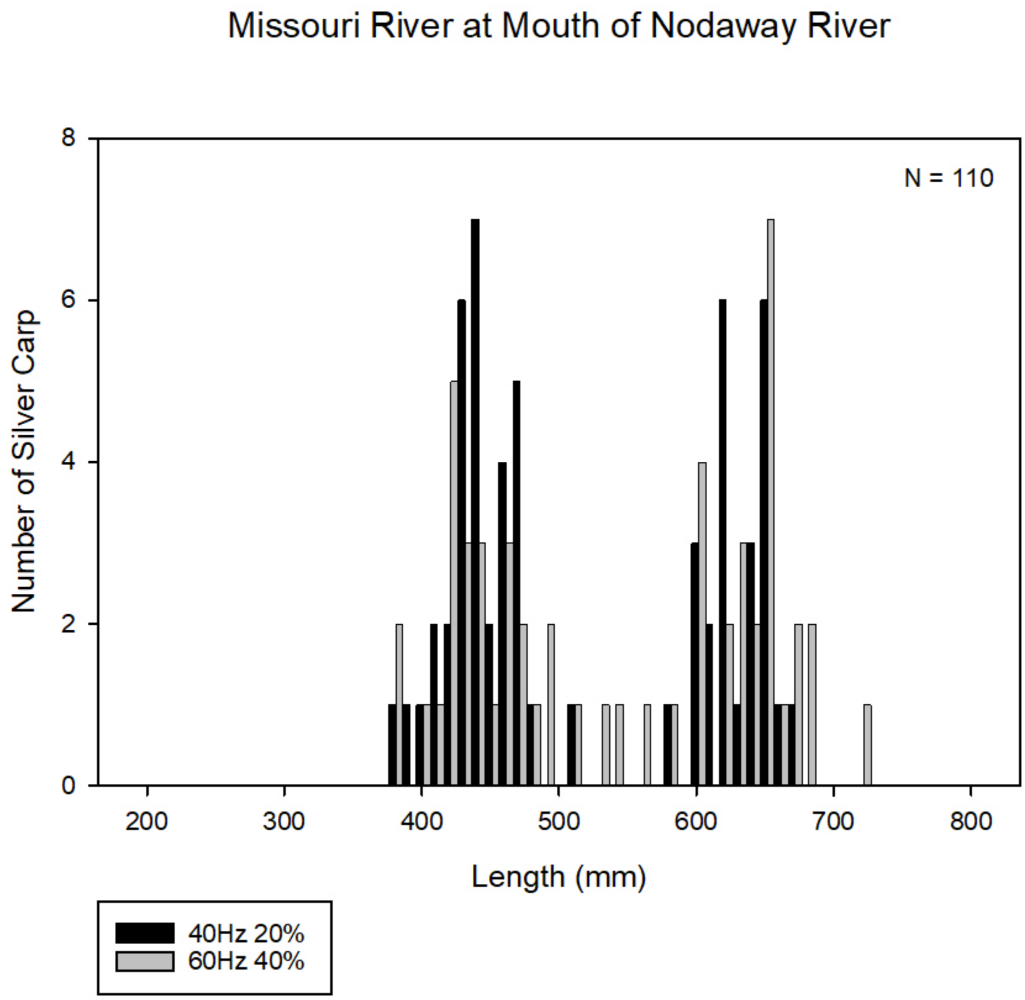

Figure 12. Silver Carp Length-Frequency distribution for Missouri River at Mouth of Nodaway River. Bins are 10mm length groups.

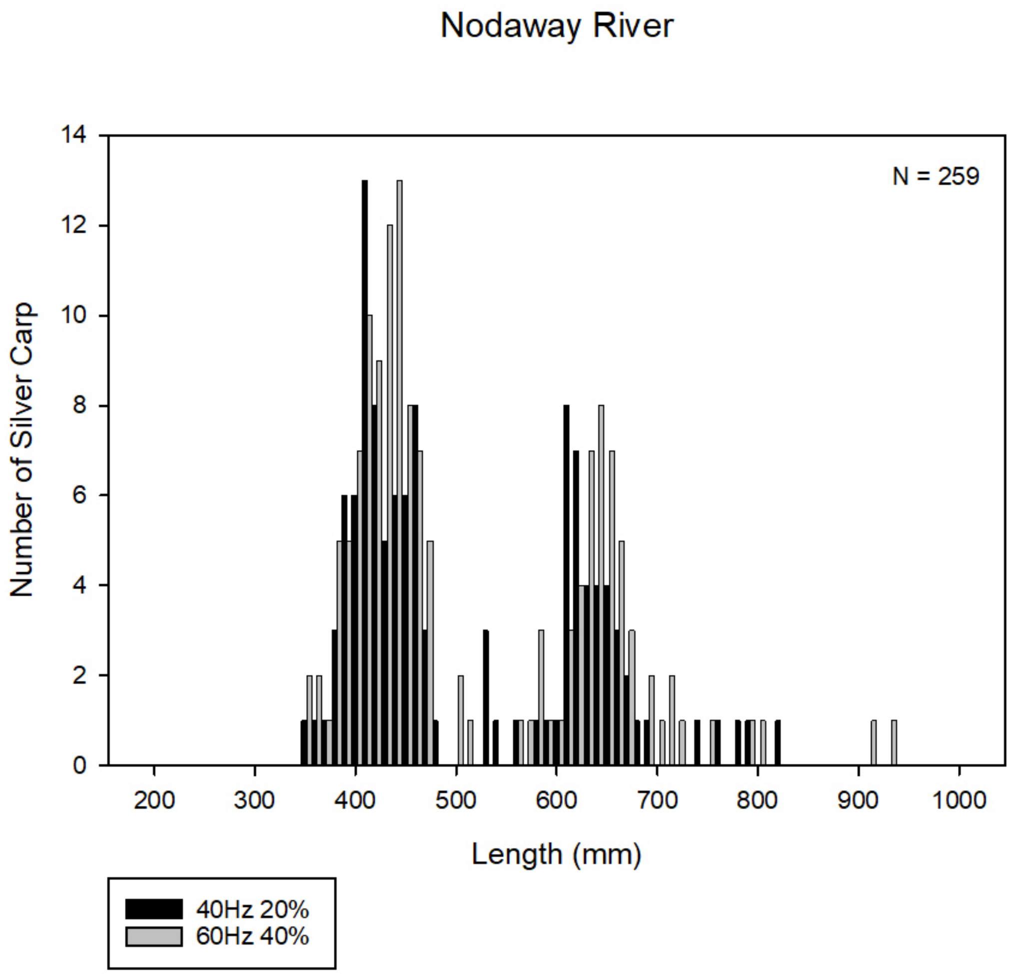

Figure 13. Silver Carp Length-Frequency distribution for Nodaway River Site 1. Bins are 10mm length groups.

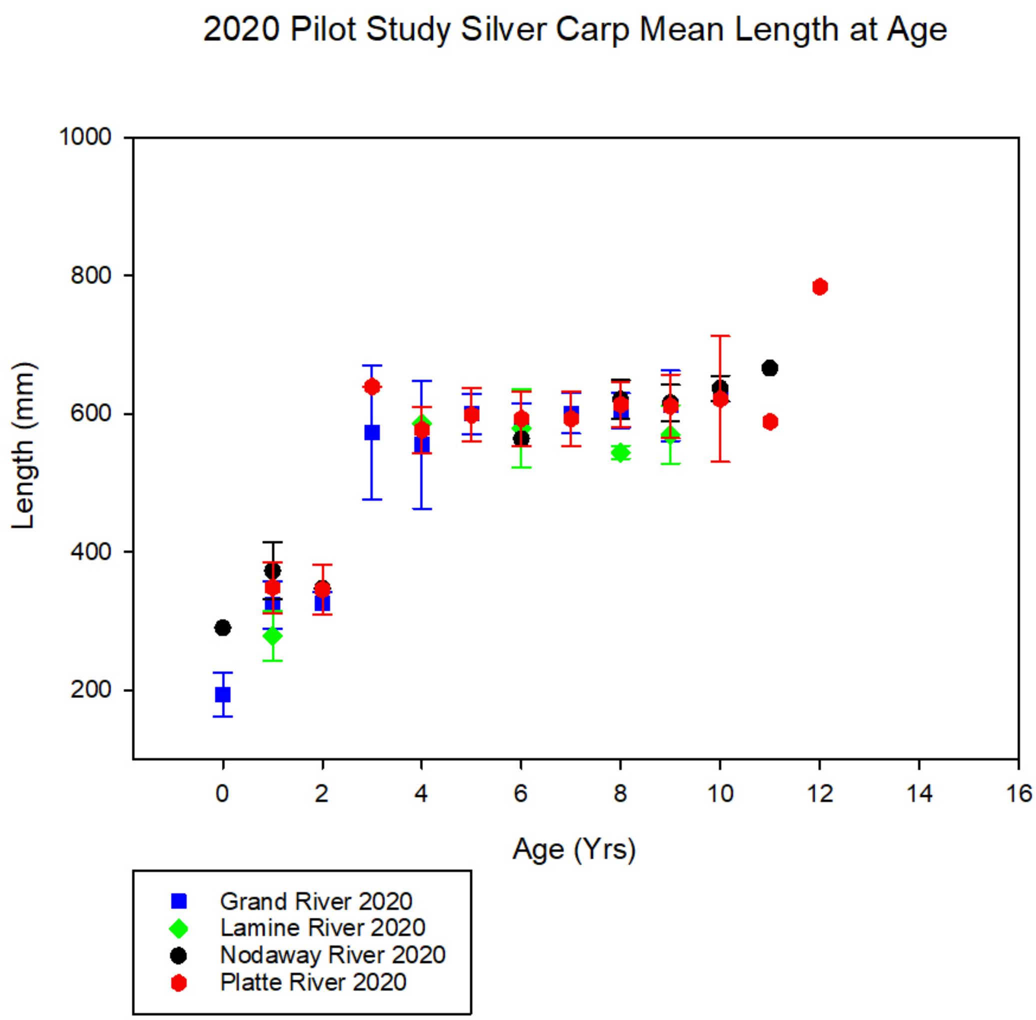

Figure 14. Mean lengths by age of Silver Carp sampled during 2020 pilot work for both tributaries and their associated Missouri River bends combined. Bars indicate 1 standard deviation.

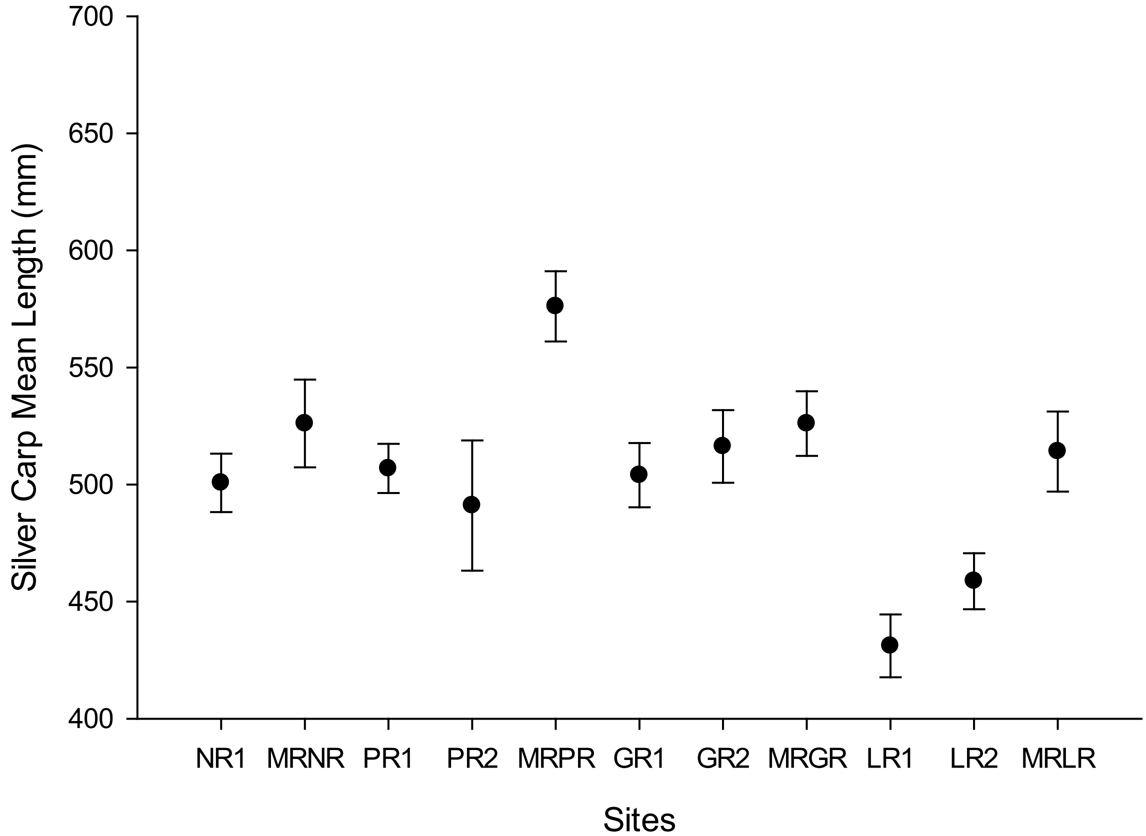

Figure 15. Mean lengths ±2E of Silver Carp by Site in 2021. NR1 = Nodaway River site 1, MRNR = Missouri River at the mouth of the Nodaway River, PR1 = Platte River site 1, PR2 = Platte River site 2, MRPR = Missouri River at mouth of Platte River, GR1 = Grand River site 1, GR2 = Grand River site 2, MRGR = Missouri River at mouth of Grand River, LR1 = Lamine River site 1, LR2 = Lamine River site 2, MRLR = Missouri River at mouth of Lamine River.

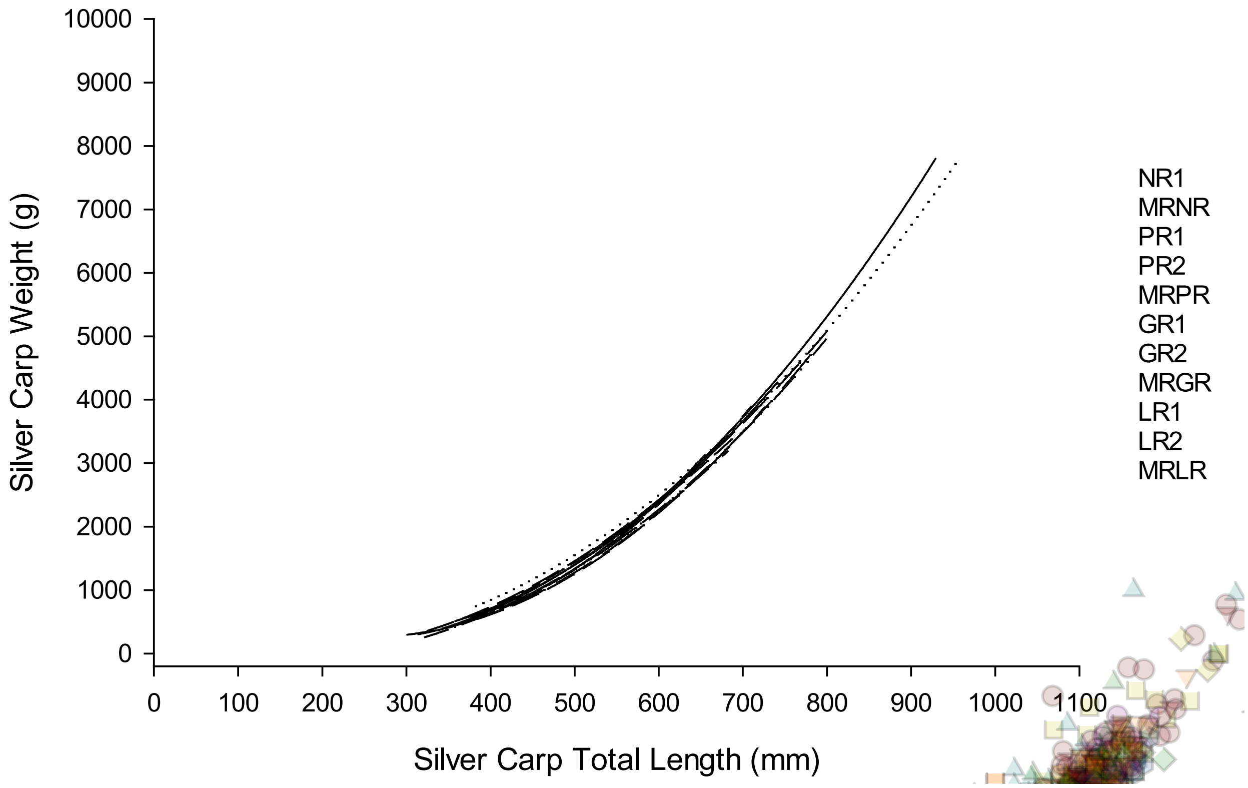

Figure 16. Silver Carp length and weight by site fitted with regression lines from 2021 sampling. NR1 = Nodaway River site 1, MRNR = Missouri River at the mouth of the Nodaway River, PR1 = Platte River site 1, PR2 = Platte River site 2, MRPR = Missouri River at mouth of Platte River, GR1 = Grand River site 1, GR2 = Grand River site 2, MRGR = Missouri River at mouth of Grand River, LR1 = Lamine River site 1, LR2 = Lamine River site 2, MRLR = Missouri River at mouth of Lamine River.

Agency Report: Agency: U.S. Fish and Wildlife Service (USFWS) Columbia Fish and Wildlife Conservation Office

Methods:

The objective of this activity was to quantify relative abundance, recruitment, growth, and mortality of Bighead and Silver Carp in tributary confluences to inform management actions. Thirty-one Missouri River tributary confluences were targeted for sampling using an electrified dozer trawl (Hammen et al. 2019) in the fall based on navigability, which was assumed for tributaries with watersheds of at least 1,000 km^2 (Flotemersch et al. 2006). Confluences were defined as the lower 20 river km to focus on confluence fish assemblages (Thornbrugh and Gido 2010). Target effort was 20 five-minute electrified dozer trawl transects at 4.8 kph (3.0 mph) conducted along the contour of the shoreline from the downstream-most site in an upstream fashion per tributary, with one 400 m transect per river km. September and October were selected to meet recommended water temperatures of 16-22°C, more consistent catch rates of Silver Carp (Sullivan et al. 2017), and more stable water levels, which reduced the impact of fecund females on length-weight relationships and coincided with annulus formation in otoliths (Thompson and Beckman 1995). When conditions allowed, left or right bank was randomly assigned. In all other cases, samples were collected on whichever bank allowed for contouring the shore while achieving depths of approximately 1.5 to 2.5 m required to fully submerge an electrified dozer trawl frame.

Despite evidence indicating higher catch rates can be achieved at night with electrified trawl gears (Ridgway et al. 2020), daytime sampling was conducted based on dangers presented by river sampling. At each tributary, total length (mm) and weight (g) was recorded for all fish as scale sensitivity allowed. Sex of Silver Carp was determined by roughness of the pectoral fin as outlined in Wolf et al. (2018) and verified through visual observation of gonads. Lapilli otoliths were extracted from the first 10 Bighead and Silver Carp per 50 mm length bin for stock length (250 mm; Phelps and Willis 2013) and larger fish and from the first 5 sub-stock length fish per 50 mm length bin.

Otoliths were stored in coin envelopes during collection then transferred to the Columbia Fish and Wildlife Conservation Office laboratory. They were allowed to dry before being sanded on a transverse plane until the nuclei were visible. Prepared otoliths were mounted in putty, submerged in glycerol and analyzed using a Nikon SMZ25 dissecting scope by three readers to record a final age through 2/3 or consensus (Maceina and Sammons 2006; Seibert and Phelps 2013). If no agreement was reached, the otolith was not used for analysis.

Analyses were conducted on a tributary basis, for the overall basin, and compared to the previous year of data as available. Relative abundance was calculated as CPUE in fish/hour for stock and larger fish, allowing standardization to additional methods (i.e., standard electrofishing; Hammen et al. 2019), though measures of distance traveled and estimates of volume of water sampled were also recorded to allow comparisons across other gears if requested (Guy et al. 2009). Relative standard error (RSE) of CPUE was calculated to estimate precision of catches, with a target RSE of ≤ 25 (Dumont and Schlechte 2004). Length-frequency histograms were generated to provide visual observations of recruitment, growth, and mortality as well as to evaluate potential gear biases. A von Bertalanffy (1938) model was used to estimate growth parameters. Given high variability in size class strength across years, it was determined that the catch-curve method was not appropriate to assess mortality and recruitment. Therefore, recruitment stability was described using a recruitment variability index (RVI) with values that range from -1 to 1, where values closer to one indicate more stable recruitment (Guy and Willis 1995; Guy and Brown 2007). An age-length key was used to generate age estimates for unaged fish using Fisheries Analysis and Modeling Simulator (FAMS 1.64; Slipke and Maceina 2014) to generate RVI estimates. Mortality was estimated using median lengths (Hoenig et al. 1983 as described in Guy and Brown 2007) using the total dataset because there were insufficient aged fish per tributary for reliable individual estimates of L_{inf} and K, parameter estimates in von Bertalanffy growth curves that describe asymptotic length and growth coefficient, respectively.

Results:

Sampling overview

Sampling was conducted 31 August 2021 through 30 September 2021 and included a total of 138 electrified dozer trawl transects across 14 tributaries in four states with variable effort per tributary (Table 1). The target of 20 transects per tributary was only met in the Kansas River, with new record low discharges in 2021 partially responsible for limited navigability throughout the Missouri River Basin tributaries (Appendix A). A total of 5,066 fish representing 42 species were collected in 2021, of which 28 were Bighead Carp, 1 was a morphologically identified Bighead and Silver Carp hybrid, and 2,615 were Silver Carp (Table 1; see Appendix B for details on all fish collected). All Bighead Carp, the hybrid, and a subset of Silver Carp (N = 577) were aged from across Missouri River tributaries, ranging from age-1 to age-13. Of note, no Silver Carp were collected in the Osage River, despite high effort of 15 electrified dozer trawl transects (Table 1) and collection of other species (Appendix B).

Silver Carp demographics

Silver Carp catch rates were variable across the basin and there was no pattern of CPUEs (Figure 1) longitudinally or by sizes of fish collected by tributary (Figures 2-4). The target RSE of CPUE ≤ 25 was established to increase confidence in relative abundance estimates and was met for just over half of the tributaries and for the overall data compilation (Table 1). Correlation analysis yielded a negligible correlation coefficient of -0.17 between RSE of CPUE and the number of electrified dozer trawl transects.

Age data allowed for RVI and von Bertalanffy estimates. Of note, no fish smaller than 200 mm were collected in 2021, and no fish were aged as age-0. Strong age classes included 2019, followed by 2018 and 2020, whereas 2016 and 2017 were weak year classes (Figure 5). When calculated by tributary, RVI values varied considerably from 0.18 to 0.78 (Table 1) and had no readily evident drivers. When data were combined for the basin, the RVI was 0.75, indicating that recruitment throughout the basin is moderately stable. Despite high variability in length-at-age, a von Bertalanffy growth curve provided a good fit for mean length-at-age (R^2 = 0.79; L_{inf} = 683.3; K= 0.273, t_o = -1.02; Figure 5).

Length data was compiled and used to estimate instantaneous annual mortality. Using Hoenig and colleagues' (1983) method, the instantaneous annual mortality estimate was 0.26. For use in the equation, it was assumed that consistent catches of Silver Carp in the electrified dozer trawl began at 150 mm TL.

Given the amount of data collected on Silver Carp, it was possible to provide additional information about the population that may be useful to managers, including length-weight relationships, relative stock density (RSD) estimates, relative weights as a surrogate for body condition, and percent male. Length-weight regressions (Weight = a×Lengthb) were performed for Silver Carp by tributary (Figure 6) in SigmaPlot (Version 12.5) and were similar across tributaries. Relative weights across tributaries were generally below the average value of 100 (Figure 7). The percent of Silver Carp which were male was generally within 10% of a random probability of 50% (Figure 7). RSDs were generally higher in tributaries farther upstream in the basin (Figure 8).

Comparisons to 2020

2021 sampling timeframe, geographical scope, and effort was similar to the 16 tributaries and 164 total transects sampled in September and October 2020, and water discharges were low in both years (Appendix A). Greater abundance but lower richness was observed in 2020 sampling (9,867 fish representing 36 species), of which a higher percentage were Silver Carp (80% in 2020 compared to 52% in 2021). In both years, Bighead Carp represented less than 1% of the catch, and no hybrids were collected in 2020. The lack of Silver Carp in the Osage River despite high effort was consistent between years (Figure 1).

Catch rates decreased in tributaries lower in the Missouri River Basin in 2021 compared to 2020 and increased in tributaries farther upstream, except for the Big Sioux River (Figure 1). High catch rates in 2020 were generally associated with high numbers of Silver Carp between stock (250 mm) and quality (450 mm) sizes (Figures 2-4), however there is no discernable pattern for CPUEs in 2021. In 2020, six of 16 tributaries met the ≤ 25 RSE for CPUE target, with a range of 16-47 overall. In both years, correlation analyses yielded a negligible correlation coefficient of -0.22 between RSE of CPUE and the number of electrified dozer trawl transects.

In 2021, catches included larger fish than in 2020 (Figure 2), a trend which appeared across tributaries (Figures 3-4). Strong and weak year classes were the same between years, and growth within the population was observed from 2020 to 2021, as was evident in the shift to larger lengths in the length-frequency histogram (Figure 2). The estimated von Bertalanffy growth curve was similar in 2021 to the growth curve in 2020 except for the initial size at age (Figure 5), as 2020 data was highly influenced by high, variable catches of age-0 and age-1 fish and age-0 fish were not present in 2021.

Relative weights and percent male generally decreased in 2021 across tributaries compared to 2020 values (Figure 7). RSDs show a general decrease in 2021 farther upstream in the basin (Figure 8), with higher CPUE (Figure 1) and a wider length range, including smaller fish, in the length-frequency distribution (Figure 4) when compared to 2020.

Bighead Carp collections

One hybrid Bighead x Silver Carp and 28 Bighead Carp were captured across the sampling season in 2021 (Table 2). Comparatively, 23 Bighead Carp and no hybrids were collected in 2020. Not enough data was collected to estimate population parameters for this species in either year.

Discussion

The relationship between watershed size and navigability appeared weak given the hydrological data and effort per tributary. Instead, channel morphology, human made barriers, boat access, and discharge collectively influenced the ability to sample a tributary confluence and the upstream extent to which the electrified dozer trawl remained effective. However, the lack of correlation between RSE and the number of electrified dozer trawl transects suggests that sampling farther upstream in tributaries may not increase confidence in parameter estimates. Instead, slightly lower water levels in 2021 may have reduced overall available habitat and created more consistent catches within tributary confluences than existed in 2020. Repeated sampling events in the same habitats (river km) within the sample period (fall) might reduce RSE of CPUE and increase confidence of CPUE and other estimates and could be explored in future years.

Recruitment seemed to be strongly tied to successful spawning events, as strong and weak year classes were represented similarly between years, including the relatively strong 2020 year class. Additional years of information can further inform whether successful spawning events, rather than other factors such as predation, starvation, or winterkill, drive recruitment and inform if disrupting spawning events could be a viable management approach.

Growth was highly variable among individuals in the Missouri River Basin. However, variability in length-at-age within and between populations is common in riverine fish (Britton 2007; Paukert and Makinster 2009). Silver Carp are also protracted spawners (Kolar et al. 2007), allowing for additional variance in size at age, particularly at young ages. Additional aging structures could be collected in future years if managing agencies need tributary-specific growth information.

Hoenig and colleagues (1983) developed their method of estimating instantaneous annual mortality using median length to be a robust alternative to other length-based mortality estimates, indicating that their method would be less sensitive to variability in year class strength. This method seems most appropriate for Silver Carp, as strong and weak year classes are very evident in the 2020 and 2021 data collected, precluding catch-curves as a reliable method to estimate mortality. For Silver Carp in the Missouri River Basin, natural mortality estimates likely reflect the true natural mortality, as there is very little management-oriented activity focused on increasing mortality at this time. However, as concerted efforts to conduct mass removals of Silver Carp occur, harvest mortality should be calculated separately to assess impacts and to determine whether removal efforts result in additive or compensatory mortality.

Relative weight is a measure of body condition indicated as a percent of a standard weight that can provide insights into fecundity, food availability, and overall health of the population (Murphy and Willis 1996). The standard weight equation for Silver Carp developed by Lamer (2015) is currently used, though it was developed based on a 50th percentile of available data instead of the 75th, as is standard practice (Murphy and Willis 1996), meaning that a relative weight of 100 indicates average body condition instead of above average condition. Trends through time and comparisons across basins are still possible, though interpretations of individual values should be informed by the development process for the equation. High abundances of smaller fish (<350 mm) generally influenced lower relative weights in both years of this population assessment, likely due to higher sustained growth prior to sexual maturity (50% maturity occurs around 310 mm; Erickson et al. 2021).

As Silver Carp demographics information is collected across basins, comparisons can be made between highly managed systems (such as the Illinois River with ongoing removal efforts) to the Missouri River Basin, where removal and other management efforts are in their infancy. Of note, Silver Carp K and L_{inf} values of the Missouri River Basin von Bertalanffy growth curve were comparable to those from the Mississippi and Ohio river basins, while the mortality estimate was near the high end of variable mortality estimates in those basins (Erickson et al. 2021). The Illinois River, however, displayed lower and less variable L_{inf}, K, and natural mortality estimates on average for Silver Carp than the Mississippi, Ohio (Erickson et al. 2021), and Missouri river basins. As management efforts such as removals or perhaps disrupted spawning occurs, we should expect to see shifts in relative abundance, recruitment, growth, and mortality estimates as observed in the Illinois River Basin.

Recommendations:

- Standardized population assessments should be conducted to facilitate comparisons through time and across basins to assess the overall impact of management efforts on Invasive Carp populations and inform future management actions. Additional years of data through this project could provide valuable insight into recruitment, growth, and mortality drivers and patterns; relative abundance natural fluctuations; population delineation; and multi-scale effects of management efforts.

- This assessment can be strengthened if combined with other Missouri River Basin Invasive Carp Partnership or Missouri River Recovery Program data sources to create a basin-wide picture of Invasive Carp populations that would enable managers to strategically place barriers, target removal efforts, or disrupt key spawning events and have confidence that those management actions are having an impact on the local or regional Invasive Carp population.

- Current protocols could be adjusted to increase consistency of data collections and inclusion of other habitats. This could include focusing the number of tributaries to those that can be consistently sampled in a variety of discharge conditions, adding mainstem Missouri River habitats (particularly in low discharge years), and conducting repeat sampling events to increase confidence in demographic estimates.

- A project assessing the efficiency of Bighead Carp sampling approaches is needed.

- Where appropriate, managers can utilize these data to inform plausible management and control approaches.

Table 1. Summary of 2021 Missouri River tributary confluence sampling for Silver Carp (SVCP) using an electrified dozer trawl. Tributaries are ordered by the Missouri River rivermile (RM) of their confluences. Total number of electrified dozer trawl transects (N), fish collected, and SVCP are listed. Catch per unit effort (CPUE) of stock (250 mm) and larger SVCP is reported with standard error in parentheses, as well as the relative standard error (RSE) of SVCP CPUE. Minimum total length (TL; in mm) and maximum TL as well as recruitment variability index (RVI) is listed for SVCP. Maximum age used to calculate RVI is listed in parentheses. The summary column lists total for transects, fish, and SVCP and averages for CPUE, CPUE RSE, and RVI.

Waterbody	**RM**	**Watershed (km^2)**	**Transects (N)**	**All fish (N)**	**SVCP (N)**	**CPUE (#/hr)**	**CPUE RSE**	**Min TL (mm)**	**Max TL (mm)**	**RVI**
Gasconade River	104	9,262	10	145	59	76.1 (45.0)	59.0	330	630	0.46 (13)
Osage River	130	38,943	15	76	0	0.0 (0.0)	--	--	--	--
Moreau River	138	1,511	2	95	82	492.0 (36.0)	7.3	265	326	0.74 (3)
Perche Creek	170	1,038	7	517	212	418.0 (116.8)	27.9	200	660	0.62 (9)
Lamine River	202	6,880	18	1,335	515	359.0 (34.7)	9.7	311	675	0.71 (13)
Little Chariton River	227	7,943	6	155	88	168.4 (63.8)	37.9	207	342	0.72 (3)
Grand River	250	20,429	13	250	149	137.5 (34.9)	25.4	300	690	0.55 (11)
Crooked River	314	921	7	603	366	631.8 (83.8)	13.3	240	595	0.46 (10)
Kansas River	367	288,343	20	235	120	72.1 (11.3)	15.7	410	780	0.18 (12)
Platte River (MO)	391	6,322	8	228	130	208.1 (45.3)	21.8	345	720	0.20 (11)
Nishnabotna River	542	7,730	6	263	162	364.3 (107.0)	29.4	395	805	0.78 (11)
Big Sioux River	734	19,257	8	281	77	115.0 (16.9)	14.7	469	830	0.23 (11)
Vermillion River	772	6,784	9	527	375	552.1 (63.4)	11.5	442	712	0.27 (9)
James River	800	48,092	9	356	280	451.6 (123.4)	27.3	375	713	0.37 (13)
Summary			**138**	**5,066**	**2,615**	**237.9 (22.1)**	**9.3**	**200**	**830**	**0.75 (13)**

Table 2. Bighead Carp and hybrid Bighead x Silver Carp collection information for fall 2021 using an electrified dozer trawl, wherein each line represents a single fish.

Date	Waterbody	Species	Length (mm)	Weight (g)	Age	Sex
9/2/2021	Big Sioux River	Bighead Carp	688	3400	11	Female
9/2/2021	Big Sioux River	Silver/Bighead hybrid	626	2070	5	Male
9/16/2021	Crooked River	Bighead Carp	400	670	3	Male
9/16/2021	Crooked River	Bighead Carp	340	400	2	Unknown
9/16/2021	Crooked River	Bighead Carp	360	550	2	Unknown
9/16/2021	Crooked River	Bighead Carp	330	530	2	Unknown
9/16/2021	Crooked River	Bighead Carp	380	500	2	Unknown
9/16/2021	Crooked River	Bighead Carp	310	320	2	Unknown
9/16/2021	Crooked River	Bighead Carp	315	340	2	Unknown
9/16/2021	Crooked River	Bighead Carp	410	760	3	Female
9/16/2021	Crooked River	Bighead Carp	335	400	2	Unknown
9/16/2021	Crooked River	Bighead Carp	310	290	2	Unknown
9/16/2021	Crooked River	Bighead Carp	325	370	3	Unknown
9/16/2021	Crooked River	Bighead Carp	380	570	2	Unknown
9/16/2021	Crooked River	Bighead Carp	320	310	2	Unknown
9/16/2021	Crooked River	Bighead Carp	345	460	2	Male
9/16/2021	Crooked River	Bighead Carp	340	400	2	Unknown
9/22/2021	Grand River	Bighead Carp	390	640	4	Male
9/15/2021	Kansas River	Bighead Carp	760	4330	9	Male
9/15/2021	Kansas River	Bighead Carp	745	3920	10	Female
9/10/2021	Lamine River	Bighead Carp	885	7000	11	Female
9/9/2021	Lamine River	Bighead Carp	520	1590	2	Male
9/9/2021	Lamine River	Bighead Carp	960	8620	9	Female
9/14/2021	Nishnabotna	Bighead Carp	500	1550	4	Male
9/7/2021	Perche Creek	Bighead Carp	345	480	2	Unknown
8/31/2021	Vermillion River	Bighead Carp	605	2400	10	Male
8/31/2021	Vermillion River	Bighead Carp	620	2420	8	Male
8/31/2021	Vermillion River	Bighead Carp	710	3560	9	Male
8/31/2021	Vermillion River	Bighead Carp	633	2230	9	Unknown

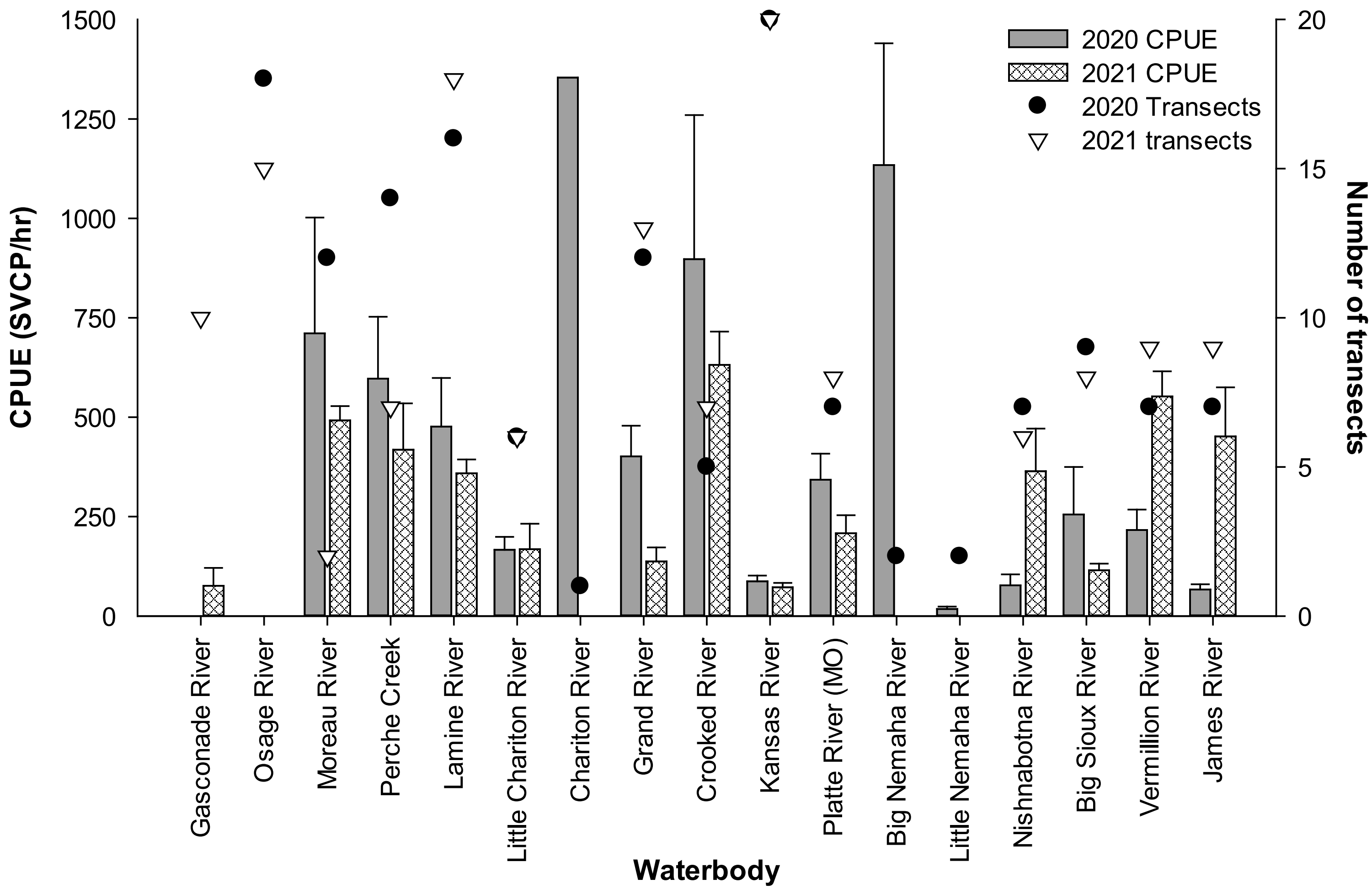

Figure 1. Catch per unit effort (CPUE; Silver Carp [SVCP] per hour) with standard error bars of Silver Carp captured in Missouri River tributaries with an electrified dozer trawl in 2020 (grey bars) and 2021 (hatched bars). The number of electrified dozer trawl transects for each tributary are indicated by a second y-axis on the right for 2020 (black circle) and 2021 (open triangle).

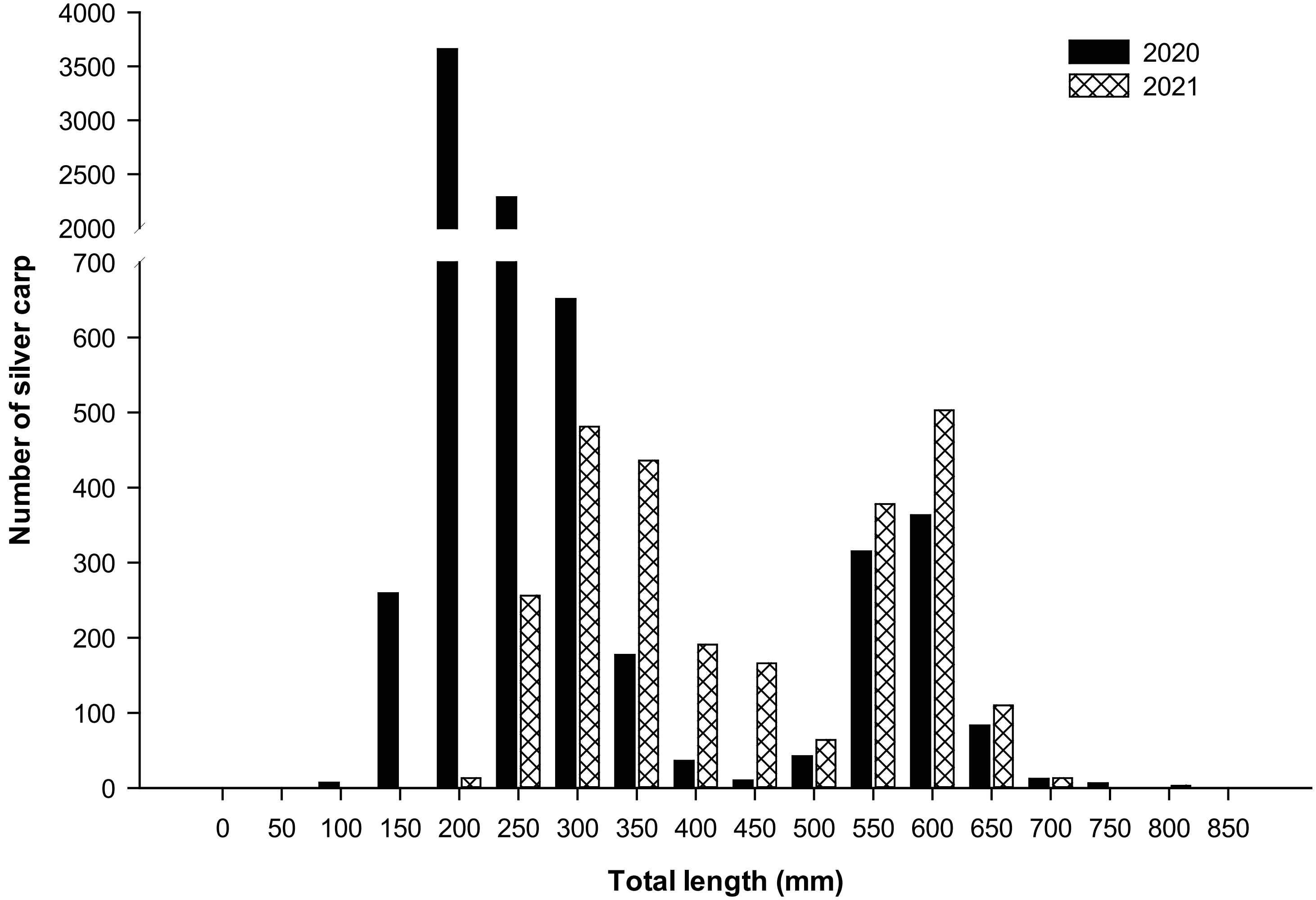

Figure 2. Length-frequency distribution of all Silver Carp captured with an electrified dozer trawl in 2020 and 2021 in 16 and 14 Missouri River tributaries, respectively. Fish were categorized by 50 mm TL bins. Please note the break in the y-axis.

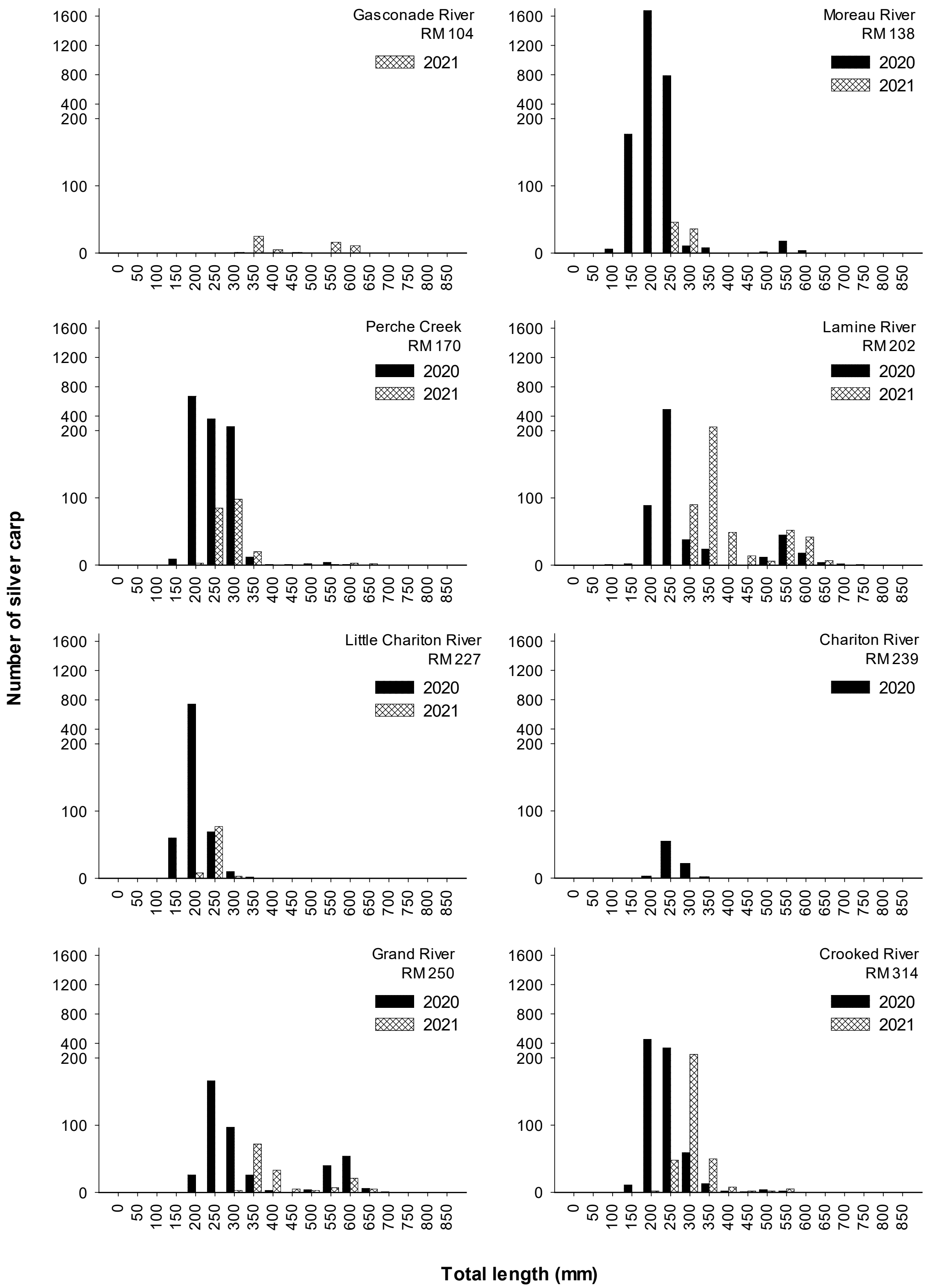

Figure 3. Length-frequency distribution of Silver Carp captured with an electrified dozer trawl in 2020 and 2021 in Missouri River tributaries. Fish were categorized by 50 mm TL bins. Please note the skewed y-axis that allows for smaller sample sizes to be seen.

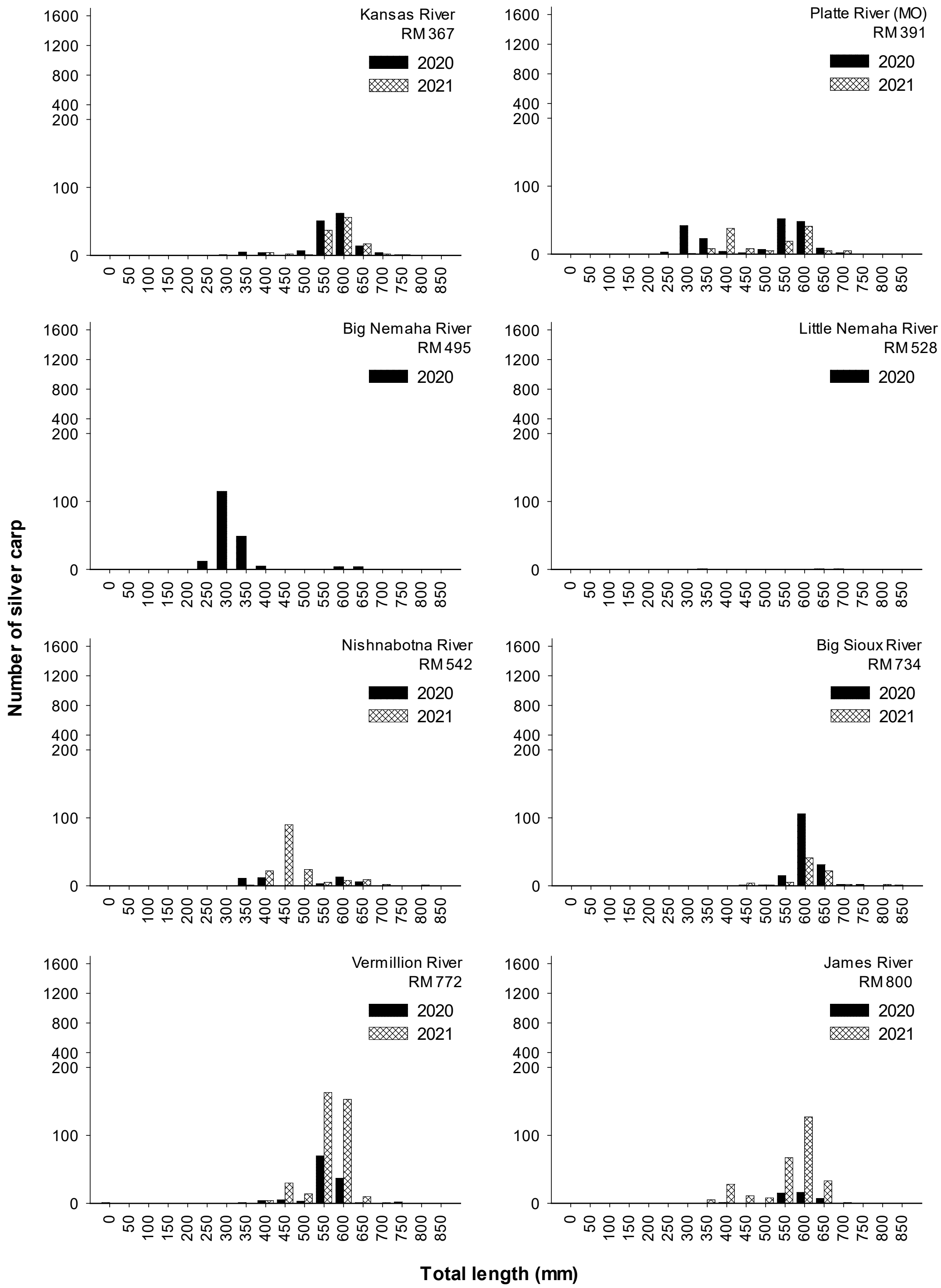

Figure 4. Length-frequency distribution of Silver Carp captured with an electrified dozer trawl in 2020 and 2021 in Missouri River tributaries. Fish were categorized by 50 mm TL bins. Please note the skewed y-axis that allows for smaller sample sizes to be seen. Little Nemaha had 3 Silver Carp caught in the 350, 650 and 700 length bins in 2020.

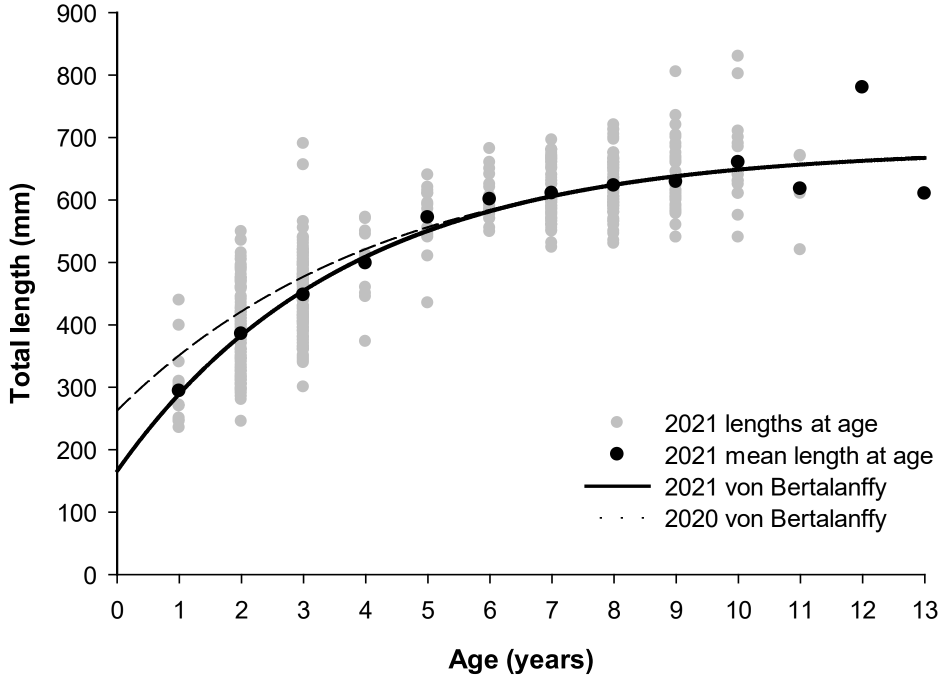

Figure 5. Length at age, with all Silver Carp (grey dots) in 2021, mean length at age (black dots) in 2021, and the von Bertalanffy growth curves for all Silver Carp aged in 2021 (N = 577, solid line) and 2020 (N = 528; dotted line).

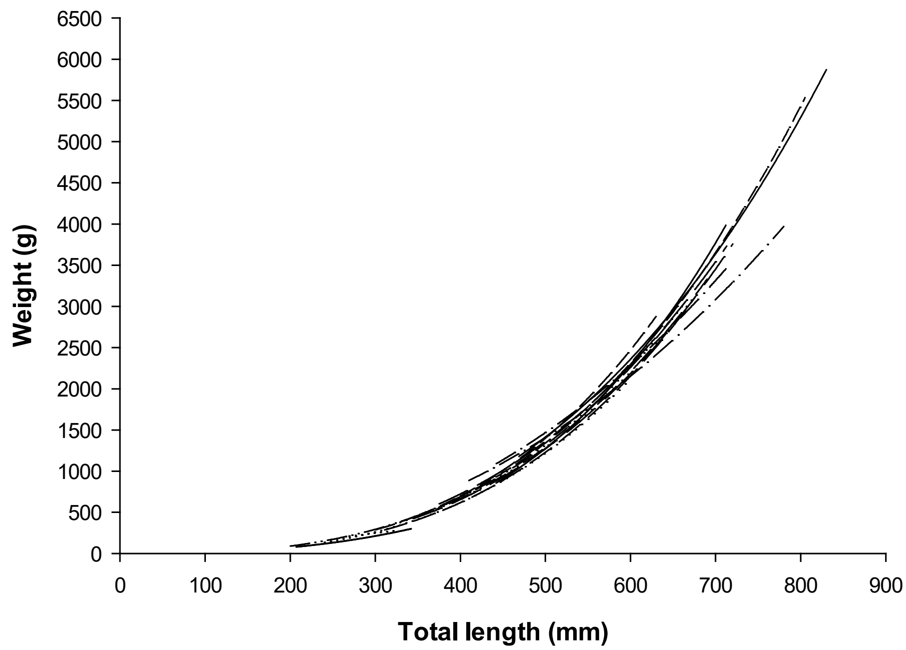

Figure 6. Length-weight regressions for Silver Carp collected in Missouri River tributaries in Fall 2021, with a regression for each tributary. Regressions are not labeled due to the closeness of data and associated regressions.

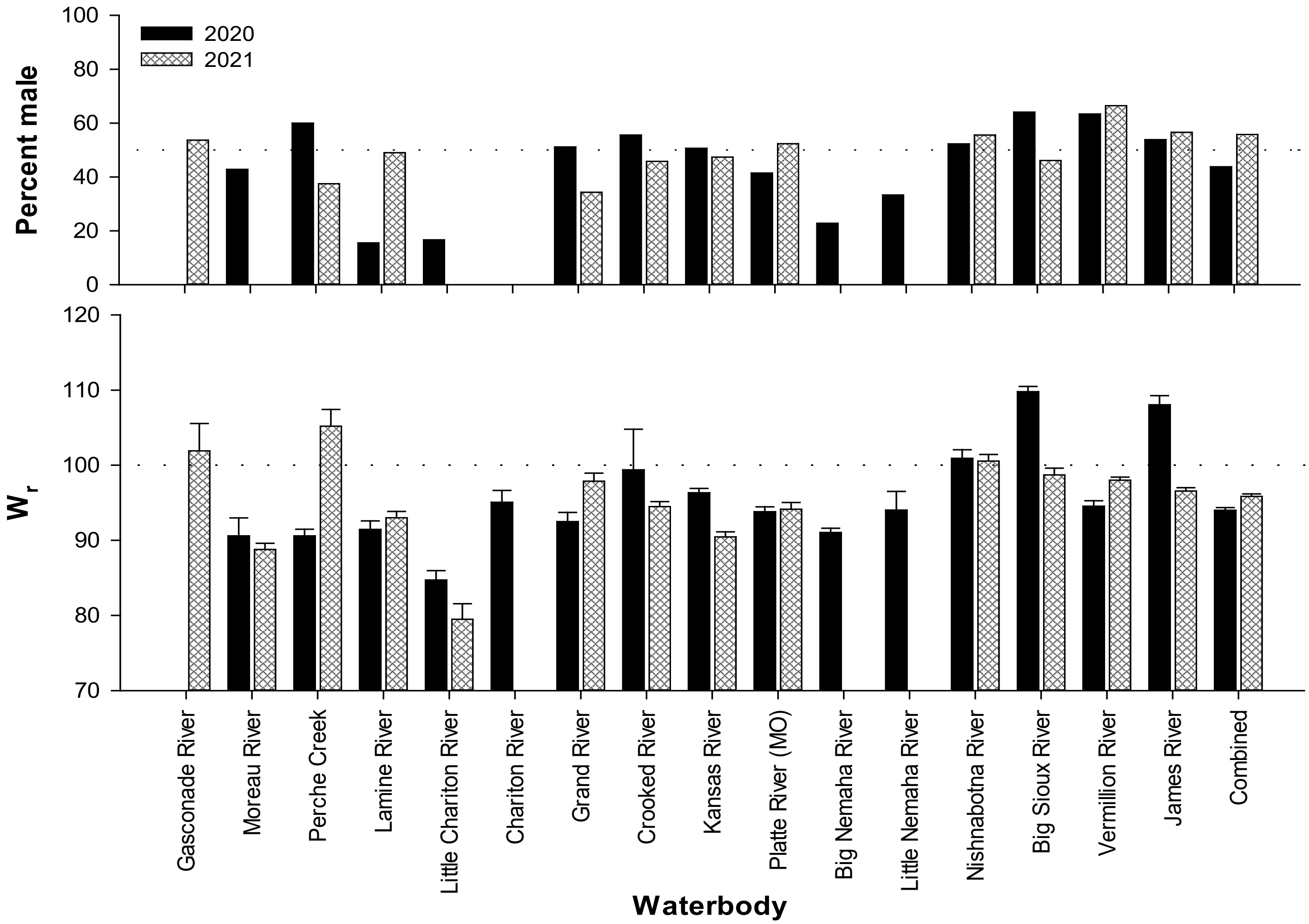

Figure 7. Percent male and relative weight (W_r) for Silver Carp collected by an electrified dozer trawl in Missouri River tributary confluences. Reference lines indicate 50% male, and W_r of 100 for ease of interpretation. Missouri River tributaries are listed in ascending order according to Missouri River river mile, with values for all combine data at the far right.

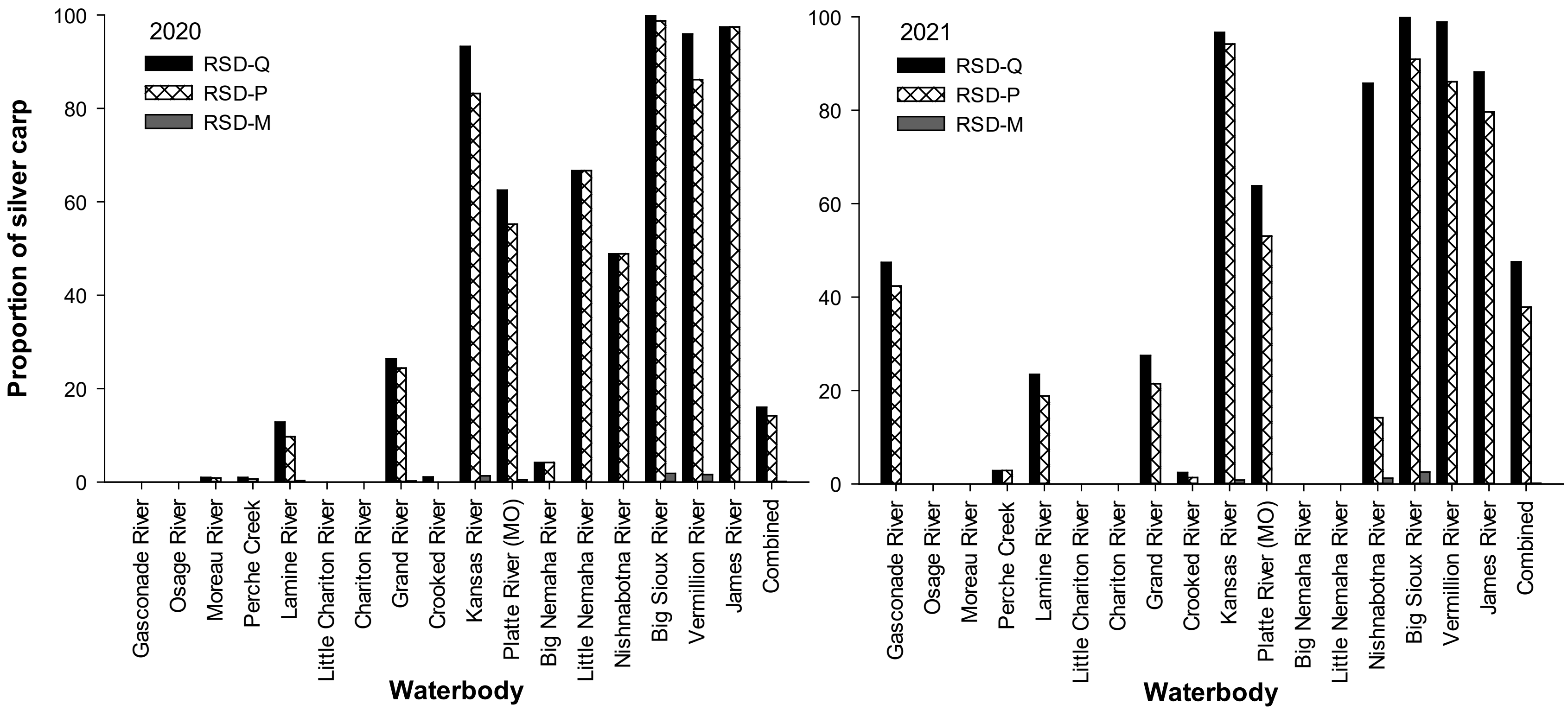

Figure 8. Relative stock densities (RSD) of Silver Carp collected by an electrified dozer trawl in Missouri River tributary confluences as well as all data for three categories: quality (Q; 450 mm), preferred (P; 540 mm), and memorable (M; 640 mm). Size categories correspond to those outlined in Phelps and Willis (2013). Missouri River tributaries are listed in ascending order according to Missouri River river mile, with values for all combined data at the far right.

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Appendix A. Hydrological data

The following series of figures depict percent daily discharge in each of the targeted Missouri River tributaries selected for Silver Carp population assessment sampling. Percent daily discharge was calculated using historical discharge values as well as daily discharge values. For each day, percent daily discharge was calculated as:

$$\frac{Q_{obs} - Q_{min}}{Q_{max} - Q_{min}} \times 100$$

Where Q_{obs} is the observed discharge on the selected date in the analyzed year, Q_{min} is the minimum discharge value in historical data, and Q_{max} is the maximum discharge value in historical data. A zero means that a new historical low was set and a 100 means a new historical high was set. The 7-day rolling average was then calculated (black and grey lines) and plotted to reduce the flashy appearance of short-lived events on smaller systems. All discharge data were obtained through the U.S. Geological Survey National Water Information System (Web Interface; https://waterdata.usgs.gov/nwis/rt). Nearest gage stations were used to the mouth of the tributary of interest (Table A.1).

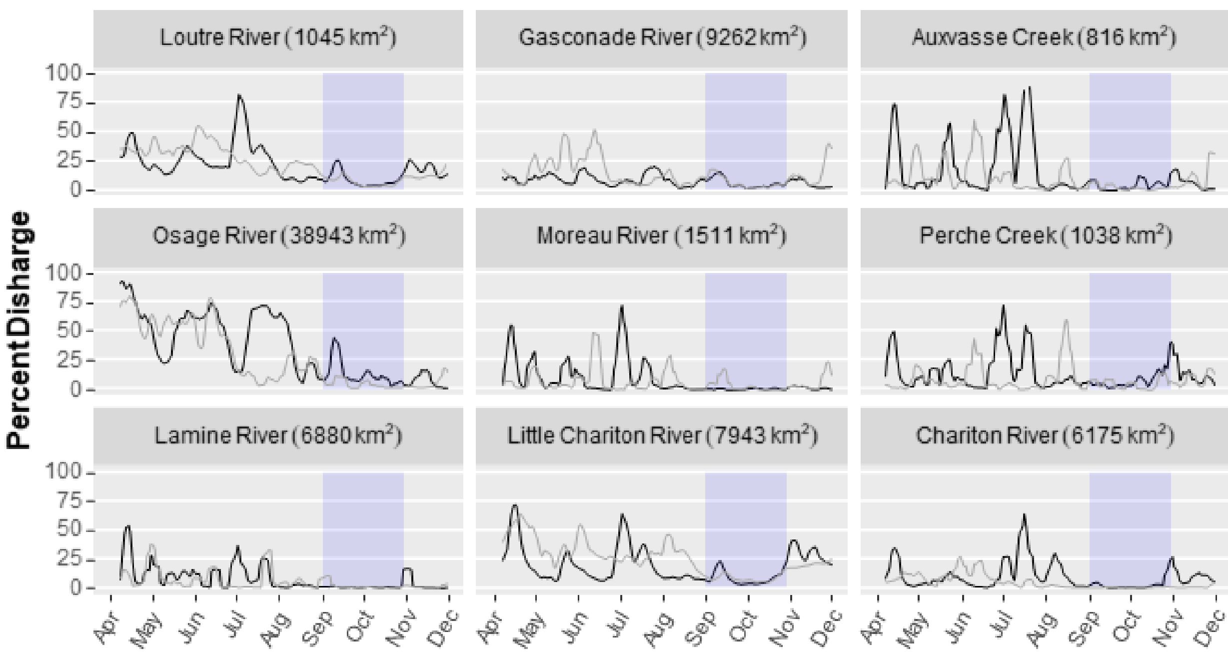

Figure A.1. Seven-day rolling averages of percent daily discharges for 2020 (grey line) and 2021 (black line), where a purple bar highlights the fall sampling period for Silver Carp.

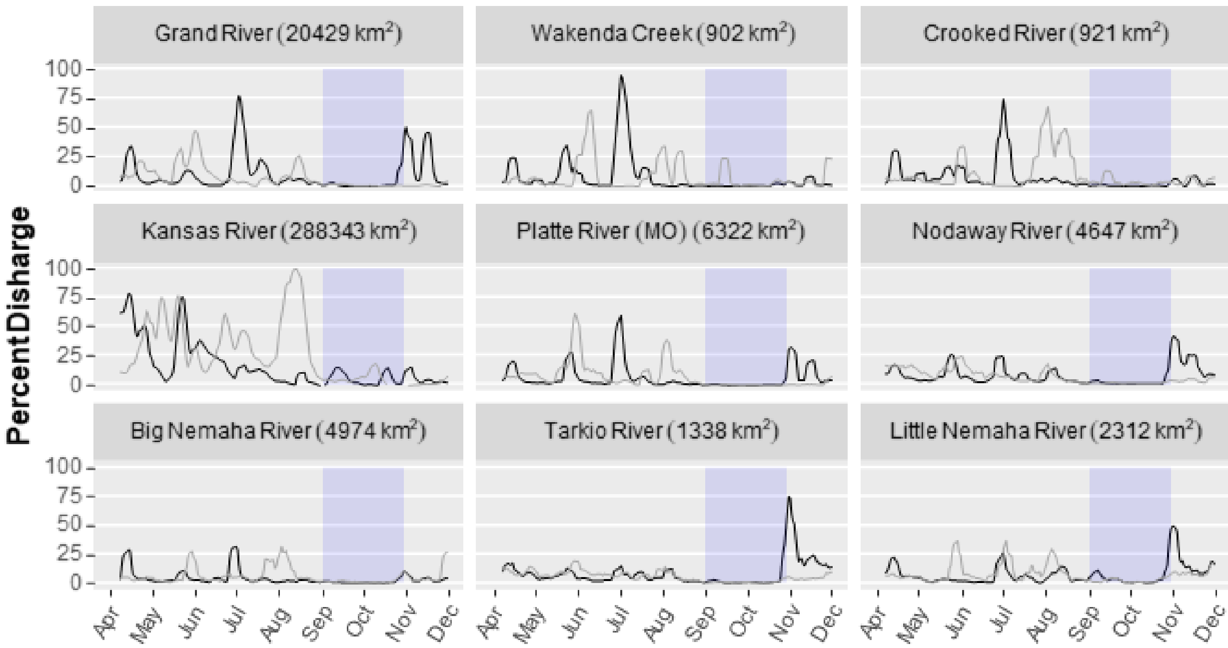

Figure A.2. Seven-day rolling averages of percent daily discharges for 2020 (grey line) and 2021 (black line), where a purple bar highlights the fall sampling period for Silver Carp.

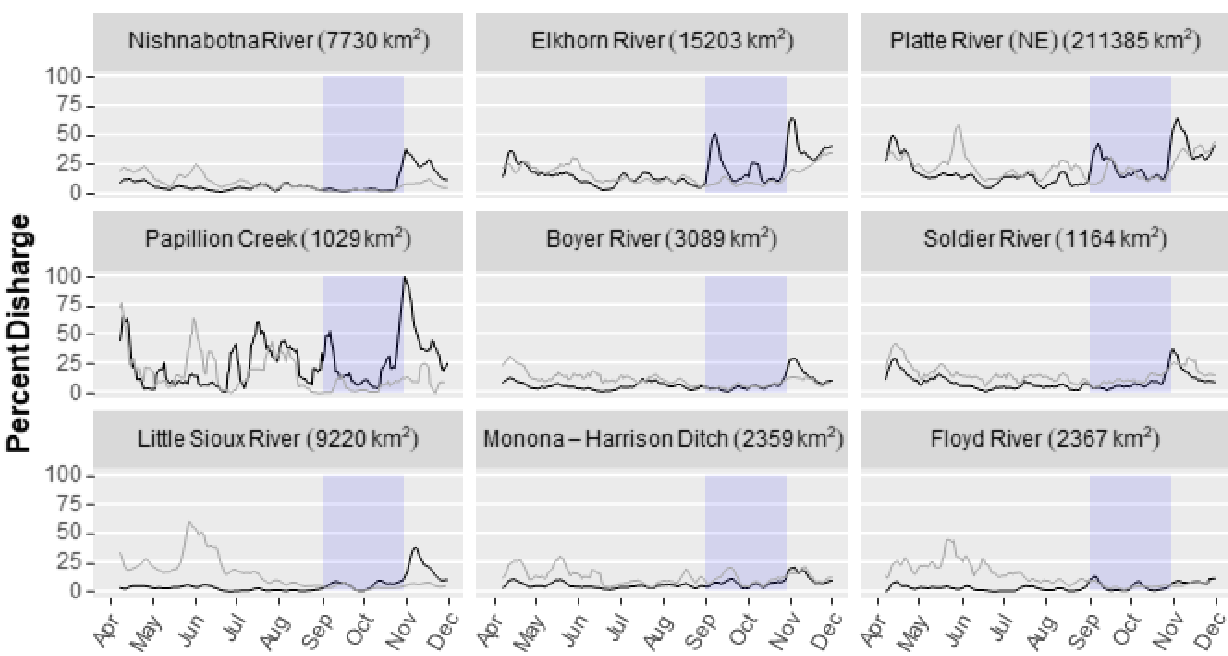

Figure A.3. Seven-day rolling averages of percent daily discharges for 2020 (grey line) and 2021 (black line), where a purple bar highlights the fall sampling period for Silver Carp.

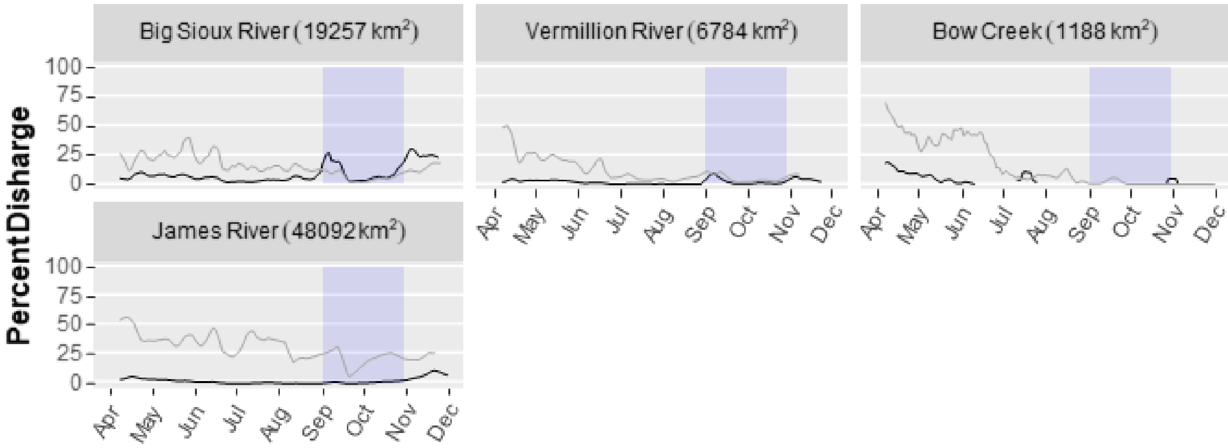

Figure A.4. Seven-day rolling averages of percent daily discharges for 2020 (grey line) and 2021 (black line), where a purple bar highlights the fall sampling period for Silver Carp.

Table A.1. Gaging stations used for each tributary, listed in order by Missouri River rivermile (RM). The Elkhorn River was added per partner input as a potential surrogate and is a tributary to the Platte River in Nebraska.

Waterbody	Gage station ID	Gage station water	State	RM	Drainage area (km^2)
Loutre River	6934500	Missouri River	MO	97	1,045
Gasconade River	6934000	Gasconade River	MO	104	9,262
Auxvasse Creek	6927240	Auxvasse Creek	MO	121	816
Osage River	6926510	Osage River	MO	130	38,943
Moreau River	6910750	Moreau River	MO	138	1,511
Perche Creek	6910230	Hinkson Creek	MO	170	1,038
Lamine River	6906800	Lamine River	MO	202	6,880
Little Chariton River	6906500	Missouri River	MO	227	7,943
Chariton River	6905500	Chariton River	MO	239	6,175
Grand River	6902000	Grand River	MO	250	20,429
Wakenda Creek	6896000	Wakenda Creek	MO	263	902
Crooked River	6895000	Crooked River	MO	314	921
Kansas River	6892518	Kansas River	KS	367	288,343
Platte River (MO)	6820500	Platte River	MO	391	6,322
Nodaway River	6817700	Nodaway River	MO	463	4,647
Big Nemaha River	6815000	Big Nemaha River	NE	495	4,974
Tarkio River	6813000	Tarkio River	MO	508	1,338
Little Nemaha River	6811500	Little Nemaha River	NE	528	2,312
Nishnabotna River	6810000	Nishnabotna River	MO	542	7,730
Platte River (NE)	6805500	Platte River	NE	595	211,385
Elkhorn River	6800500	Elkhorn River	NE		
Papillion Creek	6610795	Papillion Creek	NE	596	1,029
Boyer River	6609500	Boyer River	IA	635	3,089
Soldier River	6608500	Soldier River	IA	664	1,164
Little Sioux River	6607500	Little Sioux River	IA	669	9,220
Monona-Harrison Ditch	6602400	Monona-Harrison Ditch	IA	670	2,359
Floy River	6600500	Floyd River	IA	731	2,367
Big Sioux River	6485500	Big Sioux River	IA	734	19,257
Vermillion River	6479010	Vermillion River	SD	772	6,784
Bow Creek	6478522	Bow Creek	NE	787	1,188
James River	6478513	James River	SD	800	48,092

Appendix B. All fish collections

Table B.1. All fish collected by electrified dozer trawl in fall 2021 by species and tributary, where Missouri River rivermile (RM) and number of transects (N) are listed for each tributary.

Species	Gasconade River RM 104 N = 10	Osage River RM 130 N = 15	Moreau River RM 138 N = 2	Perche Creek RM 170 N = 7	Lamine River RM 202 N = 18
Bighead carp	-	-	-	1	3
Bigmouth buffalo	-	-	-	-	3
Blue catfish	-	-	-	-	-
Blue sucker	-	-	-	-	-
Bluegill	-	-	-	2	2
Brook silverside	1	-	1	-	2
Bullhead minnow	-	-	-	-	-
Channel catfish	-	-	-	-	-
Common carp	-	3	1	3	2
Emerald shiner	19	1	-	5	58
Freshwater drum	1	-	-	-	-
Gizzard shad	47	15	1	267	702
Golden redhorse	-	5	-	-	-
Goldeye	3	31	1	5	16
Grass carp	1	-	1	4	-
Green sunfish	-	-	-	-	-
Highfin carpsucker	1	-	-	-	-
Largemouth bass	-	-	-	-	-
Longnose gar	7	1	2	3	8
Mooneye	-	7	-	-	-
Paddlefish	-	-	1	-	1
Quillback	-	1	-	-	-
Red shiner	-	-	-	-	5
Redfin shiner	-	-	-	-	-
River carpsucker	2	1	-	3	2
Sand shiner	-	-	-	-	-
Sauger	-	-	-	-	-
Shorthead redhorse	-	-	-	-	1
Shortnose gar	1	-	1	3	9
Silver carp	59	-	82	212	515
Silver/Bighead carp hybrid	-	-	-	-	-
Silver chub	-	-	-	-	-
Silver redhorse	-	1	-	-	-
Skipjack herring	-	4	-	-	-
Smallmouth buffalo	3	5	3	8	6
Spotfin shiner	-	-	-	-	-
Spottail shiner	-	-	-	-	-
Unidentified buffalo	-	-	-	-	-
Unidentified carpsucker	-	-	-	-	-
Unidentified shiner	-	-	-	-	-
Walleye	-	-	-	-	-
Western mosquitofish	-	-	-	-	-
White bass	-	1	1	1	-
Total	**145**	**76**	**95**	**517**	**1,335**

Table B.1 con't

Species	Little Chariton River RM 227 N = 6	Grand River RM 250 N = 13	Crooked River RM 314 N = 7	Kansas River RM 367 N = 20	Platte River (MO) RM 391 N = 8
Bighead carp	-	1	15	2	-
Bigmouth buffalo	12	4	16	-	-
Blue catfish	-	1	-	2	3
Blue sucker	-	-	-	8	-
Bluegill	-	-	-	-	-
Brook silverside	-	-	-	-	-
Bullhead minnow	2	-	-	-	-
Channel catfish	3	-	7	-	-
Common carp	10	-	10	2	2
Emerald shiner	6	12	57	49	53
Freshwater drum	4	-	2	2	-
Gizzard shad	5	49	66	4	9
Golden redhorse	-	-	-	-	-
Goldeye	1	20	7	12	21
Grass carp	2	1	-	1	1
Green sunfish	-	-	-	-	1
Highfin carpsucker	3	-	-	-	-
Largemouth bass	-	-	-	-	-
Longnose gar	2	5	11	12	-
Mooneye	-	2	-	-	-
Paddlefish	1	-	4	-	-
Quillback	-	-	-	-	-
Red shiner	-	-	-	2	-
Redfin shiner	-	-	-	-	-
River carpsucker	-	-	3	1	1
Sand shiner	-	-	-	-	-
Sauger	-	-	-	-	-
Shorthead redhorse	-	-	1	-	-
Shortnose gar	7	3	25	6	2
Silver carp	88	149	366	120	130
Silver/Bighead carp hybrid	-	-	-	-	-
Silver chub	-	-	-	1	-
Silver redhorse	-	-	-	-	-
Skipjack herring	-	-	-	-	1
Smallmouth buffalo	9	3	12	7	-
Spotfin shiner	-	-	-	-	-
Spottail shiner	-	-	-	-	-
Unidentified buffalo	-	-	-	4	-
Unidentified carpsucker	-	-	-	-	-
Unidentified shiner	-	-	-	-	-
Walleye	-	-	-	-	-
Western mosquitofish	-	-	-	-	4
White bass	-	-	1	-	-
Total	**155**	**250**	**603**	**235**	**228**

Table B.1 con't

Species	Nishnabotna River RM 542 N = 6	Big Sioux River RM 734 N = 8	Vermillion River RM 772 N = 9	James River RM 800 N = 9	Total
Bighead carp	1	1	4	-	28
Bigmouth buffalo	-	7	12	3	57
Blue catfish	-	-	-	-	6
Blue sucker	-	1	-	-	9
Bluegill	-	2	1	-	7
Brook silverside	-	-	-	-	4
Bullhead minnow	-	-	-	-	2
Channel catfish	-	-	-	-	10
Common carp	7	4	10	1	55
Emerald shiner	14	7	7	6	294
Freshwater drum	1	1	3	-	14
Gizzard shad	17	113	64	3	1,362
Golden redhorse	-	-	-	-	5
Goldeye	3	14	6	15	155
Grass carp	4	1	1	-	17
Green sunfish	-	-	-	-	1
Highfin carpsucker	-	-	-	-	4
Largemouth bass	-	3	-	-	3
Longnose gar	6	1	8	8	74
Mooneye	-	-	-	-	9
Paddlefish	-	-	-	8	15
Quillback	-	-	-	-	1
Red shiner	2	17	-	-	26
Redfin shiner	-	1	-	-	1
River carpsucker	17	3	15	2	50
Sand shiner	-	-	2	2	4
Sauger	2	-	-	-	2
Shorthead redhorse	-	-	-	-	2
Shortnose gar	22	4	5	12	100
Silver carp	162	77	375	280	2,615
Silver/Bighead carp hybrid	-	1	-	-	1
Silver chub	-	-	-	-	1
Silver redhorse	-	-	-	-	1
Skipjack herring	-	-	-	-	5
Smallmouth buffalo	5	8	13	13	95
Spotfin shiner	-	3	-	-	3
Spottail shiner	-	5	1	-	6
Unidentified buffalo	-	-	-	-	4
Unidentified carpsucker	-	1	-	-	1
Unidentified shiner	-	5	-	-	5
Walleye	-	1	-	-	1
Western mosquitofish	-	-	-	-	4
White bass	-	-	-	3	7
Total	**263**	**281**	**527**	**356**	**5,066**

Agency Report: Agency: US Fish and Wildlife Service: Bozeman Fish Health Lab, Missouri River Fish and Wildlife Conservation Office, and Great Plains Fish and Wildlife Conservation Office

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

Work Summary

Missouri River Basin Water Samples Collected

USFWS staff from Whitney Genetics Lab, 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 with the Missouri River. 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 Invasive 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 for Region 6. With the guidance of staff from the Whitney Genetics Lab, 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 Whitney Genetics Lab and others to enhance quality assurance/quality control standards. Staff from the Whitney Lab reviewed all BFHC Invasive Carp eDNA SOPs and provided feedback on lab layout and workflow plans. Whitney Lab 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, following a day behind 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 Fish Health Centers in Wisconsin and 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 project being coordinated through the University of South Dakota in cooperation with South Dakota Game, Fish & Parks; is working in the same habitats areas to determine distribution and passage of in channel structures of Invasive Carp. The pilot work is supplementing data that will be used to help guide this research.

Figure 1. June 2021 sampling was conducted in the east fork of the Vermillion River near Canistota, South Dakota and in the Big Sioux River below the Falls in Sioux Falls, South Dakota. Yellow dots indicate eDNA sample collection sites (100 per river). The center picture depicts eDNA sampling at the Vermillion River site. Maps and photo are courtesy of Jenna Bloomfield (USFWS, La Crosse FWCO).

Figure 2. September 2021 sampling was conducted in the confluences of the Vermillion (near Vermillion, South Dakota), James (near Yankton, South Dakota), and Big Sioux (near Sioux City, Iowa) rivers. Yellow dots indicate eDNA sample collection sites (100 per river), maps courtesy of Jenna Bloomfield (USFWS, La Crosse FWCO). The top center picture depicts eDNA sampling at the James River site (photo courtesy of Sam Stukel, USFWS, Gavins Point NFH). Bottom right photo is of a Silver Carp that jumped into the eDNA sample boat on the Big Sioux River (photo courtesy of Kyle Von Ruden, USFWS, Midwest Fisheries Center).