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), South Dakota State University (SDSU)

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

Statement of Need:

In collaboration with multiple stakeholders, the USFWS and the Aquatic Nuisance Species Task Force 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 ga reat 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.

Objectives:

Objective 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).

- Sub-Objective 1: 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 communication the results.

- Sub-Objective 2: 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.

- Sub-Objective 3: 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.

- Sub-Objective 4: 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.

Objective 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).

- Sub-Objective 1: 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.

- Sub-Objective 2: 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.

- Sub-Objective 3: Pair fishery sampling efforts with and eDNA sampling sites to validate eDNA results.

Objective 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).

- Sub-Objective 1: 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.

- Sub-Objective 2: 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.

- Sub-Objective 3: 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.

Project Highlights:

Objective #1:

- In the Big Sioux River, neither Silver nor Bighead Carp eDNA has been detected by qPCR in samples taken above the major barrier to fish movement (a natural chain of waterfalls located at Falls Park in Sioux Falls.

- However, in the Vermillion River, both Silver and Bighead Carp eDNA has been detected above the barrier (a spillway at the downstream end of Lake Vermillion. This may suggest the presence of carp above the Spillway and in Lake Vermillion.

- No Invasive Carp were collected above Gavins Point Dam, the lowest dam on the mainstem Missouri River and no positive detections for Black Carp in Nebraska's waters

- Statewide water sample assessment using eDNA from interior rivers and streams has suggested a slightly broader range of distribution than is currently confirmed within Nebraska.

Objective #2:

- Approximately 350 Silver Carp otoliths have been collected for otolith microchemistry analysis
    - Water chemistry appears to show potential for distinguishing between eastern South Dakota's rivers and the mainstem Missouri River
    - Strontium isotopes did not differentiate enough in the eastern SD tributaries to assign differences in otolith chemistries

- 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

- Observed spatial differences in Silver Carp GSI and batch fecundity in the Platte River and its tributaries.

- Verified the Wolf-Lederman method is an accurate sex-assignment method for Silver and Bighead Carp.

- We collected 420 ichthyoplankton samples across 13 sites on the Big Sioux, Little Sioux, Floyd, and Boyer rivers between April 27th – August 5th, 2022.
    - Detected a large spawning event of invasive carp in the Big Sioux River on the Iowa/South Dakota border
        - Captured approximately 4,500 invasive carp larvae and 300 eggs on June 15th, 2022 near Akron, Iowa.

        - Larval invasive carp densities (~6,300 larvae/100 m^3) are substantially higher than larval densities reported in other areas, including the Illinois River.
    - We also captured larvae upstream on the Little Sioux River and downstream on the Boyer River

- We sampled 36 2nd – 5th order stream sites using backpack electrofishing and seining in 12 HUC8 watersheds in the Missouri River basin between May-October 2022.
    - We captured 36,403 individuals of 43 species but did not collect juvenile invasive carp.
    - We did capture seven adult Silver Carp, two adult Silver x Bighead Carp hybrids, and eight adult Grass Carp.

- We also sampled eight off-channel habitats along the Missouri River proper using backpack electrofishing, seine nets, clover-leaf minnow traps, and mini-fyke nets.
    - We collected 33 species in off-channel habitats, including seven juvenile Silver Carp (mean TL = 70 ± 12 mm SE) at three off-channel sites (a side-channel within Lower Hamburg Bend Conservation Area in Atkinson County, MO, a backwater in Big Bear Park in Thurston County, NE, and a backwater in Schilling Lake Wildlife Management Area in Cass County, NE) during October.
    - We collected juvenile Silver Carp in water <0.5 m with silty-sand substrates, little to no velocity, and little macrophytes, woody debris, and canopy cover. We will repeat these sampling efforts during 2023 to be better able to elucidate the drivers of juvenile invasive carp recruitment and identify key habitats for management.

- In the lower Missouri River tributaries, Silver Carp was the most abundant invasive carp with 1,687 sampled with a total weight of 2,600kg (>5,900lbs) of fish. Ten Grass Carp and three Bighead Carp were also collected. No Black Carp were sampled.
    - Silver Carp catch per unit effort varied between sites using two electrofishing settings (60Hz/40% duty cycle and 40Hz/20% duty cycle) but usually produced similar catch rates at the same sample sites. Both settings are probably suitable options for sampling Silver Carp.
    - Silver Carp catch rates were higher in the Platte River than other tributaries. Catch rates of Silver Carp at Platter River site 1 were significantly higher utilizing both electrofishing settings than Grand River site 1 and the Missouri River bend at the Nodaway River with both settings and Nodaway River site 1 and Grand River site 2 utilizing 40Hz and 20% duty cycle.
    - Size classes of Silver Carp were similar between the Lamine and Grand River with both tributaries dominated by fish 400mm to 480mm. Sampling in all tributaries collected few fish below 400mm and low numbers of fish in the 500mm to 550mm range. However, individuals >600mm were more frequently sampled in the Platte and Nodaway River than in the Lamine and Grand River. Also, Silver Carp >600mm were more frequently collected in the Missouri River bends associated with the Lamine and Grand River than both tributaries.

- Size distributions and length at age data of Silver Carp in 2022 indicate limited reproduction over the last two years in the four tributaries and associated Missouri River bends. This may be due to persistent low river levels in the lower Missouri River basin.
- Silver Carp sex ratios of males to females tended to be close to a 1:1 ratio at most sites except for the Grand River and Missouri River Bend at the Platte River, where a higher ratio of males was sampled than females.

- To provide demographic data to help define invasive carp populations in tributaries of the Missouri River the electrified dozer trawl completed 405 transects across 17 tributaries between 2020 – 2022. Almost 20,000 fish were collected, of which nearly 12,000 were Silver Carp that ranged from 40 – 940 mm TL.

- Hydroacoustic sampling was introduced in 2021 in select tributaries to test the feasibility of estimating Silver Carp densities with the goal of providing more stable, reliable estimates between years. This technique yielded Silver Carp density estimates that were used for assessing the success of a targeted invasive carp removal in a Missouri River tributary.

Objective #3:

- In the mainstem Missouri River below Gavins Point Dam, Emerald Shiners and Shorthead Redhorse are 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.

- Documented spatiotemporal trends in naïve occupancy with the lower Platte River and it’s tributaries.

State Report: South Dakota

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

Project Title: Define the spatial distribution and population demographics of invasive carp populations and the associated fish community in the Missouri River Basin

Methods:
In 2022, water samples were collected three times in both the Big Sioux and Vermillion Rivers. Standard 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. One water sample was collected at each location using one, 2-Liter HDPE bottle 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. One negative control sample (one, 2-L bottle of distilled water) was "collected" by opening the lid thereby exposing the blank sample to field conditions, tightly closing the lid, and dipping the bottle into the water below and above each barrier during each sampling event. After sampling, bottles were placed on ice until filtration was carried out in the field. 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, rinsed with distilled water, and dried before and after each use and between samples. Each filter was stored in 95% ethanol in a 15 mL polypropylene Falcon tubes in the lab freezers at -20°C until DNA extraction occurred.

Vermillion River samples were taken on May 24, July 12, and August 18, 2022 at four below-barrier sites and fourteen above-barrier sites (Figure 1). Big Sioux River samples were taken on May 26, July 11, and August 17 and September 24, 2022 at five below-barrier sites and eleven above-barrier sites (Figure 2). We observed live carp of undetermined species at all water sampling sessions below the barriers. We also collected six water samples (five samples and one field blank) from Silver Lake near Freeman, SD (43.448763, -97.410816) upon request of South Dakota Game Fish and Parks in response to a fish kill that had happened earlier in the summer. We observed dead Silver and/or Bighead Carp scattered along the shoreline of Silver Lake and live Common Carp swimming in the lake, but we observed no live Silver or Bighead Carp.

In total, 115 water samples and 18 field blanks from both rivers were collected. eDNA extraction was carried out in a designated DNA sequencing room at the University of South Dakota following the Qiagen DNeasy Blood and Tissue Extraction protocol (Qiagen, inc.). Extracted samples were stored at -20 degrees C in a lab freezer until qPCR analysis. Previously published sequences for the Bighead and Silver Carp forward-primer, reverse- primer, G-block megamer, and probes were used for eDNA qPCR analysis

(Erickson et al. 2017). 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' eDNA in the extracted samples in the qPCR thermocycler. A dilution series of the G-Block megamer was used during qPCR analysis, which formed the baseline standard series of known DNA present in each sample. 96-well plates were loaded with eight field samples, a field blank, an extraction blank and a positive control, each in quadruplicate. Each plate also contained a triplicate of spiked samples to ensure no inhibition was occurring during qPCR. Ten, no-template control wells (nano-pure water) and two replicates of the standard dilution series were included on each plate. Amplification curves were generated during each run for each sample and each probe. Standard curves were calculated for each plate, from which the quantity of DNA present in each sample could be calculated.

Results & Discussion

At this time 86 of the 133 samples collected in 2022 have been analyzed using qPCR. qPCR results indicate that both Silver and Bighead Carp were present below both barriers (Figure 3). To date, there have been zero positive detections above the barrier in the Big Sioux River, suggesting that either Invasive Carp populations do not exist in the sample areas above the Falls, or any populations that may exist above the barriers are in such low densities that their eDNA went undetected during the sampling process. In the Vermillion River, both Silver and Bighead Carp eDNA was detected in 3 separate samples in Lake Vermillion after two separate qPCR runs (Figure 4). These samples are likely true positives because there is no evidence of DNA contamination in the field blanks, extraction blanks, or negative qPCR controls. However, as an extra step to increase certainty in the positive detections, these three samples will be re-extracted from their original filters and re-run with qPCR. Additionally, if this step yields eDNA detections, another round of water samples may be collected from those sites in Lake Vermillion which will be processed following the sampling protocol described above.

We are currently working on a Bayesian model to determine the likelihood of positive detections in our above- and below-barrier samples. The model will estimate the probability that any positive samples are true positives, which is especially important to providing a greater level of confidence in positive detections in above-barrier samples. This will, in turn, increase confidence of South Dakota Game Fish and Parks in carrying out any management actions in response to a positive detection above the barriers.

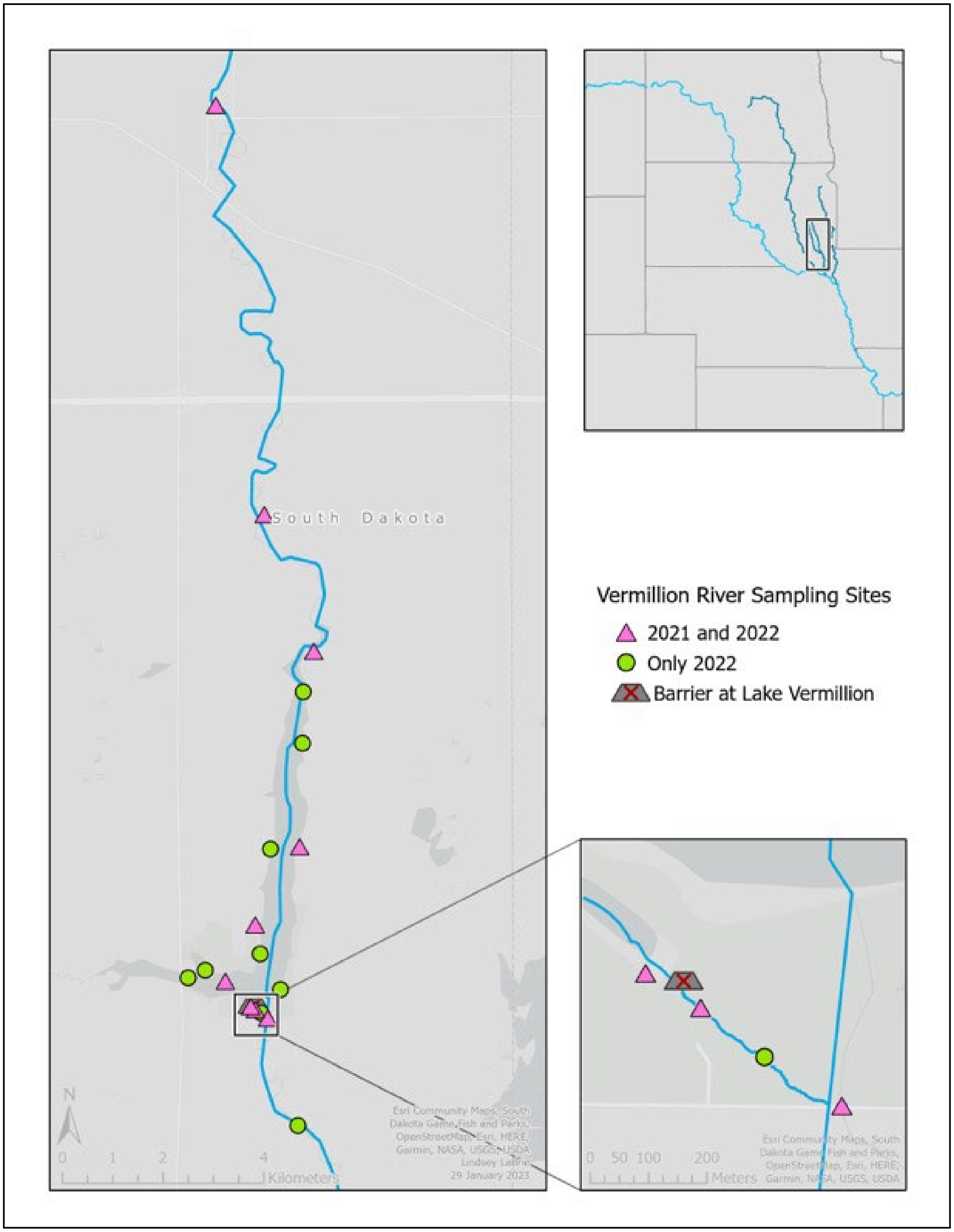

Figure 1. Water sampling locations in the Vermillion River. Sites sampled in both years are represented as pink triangles and sites sampled only in 2022 are represented as green circles.

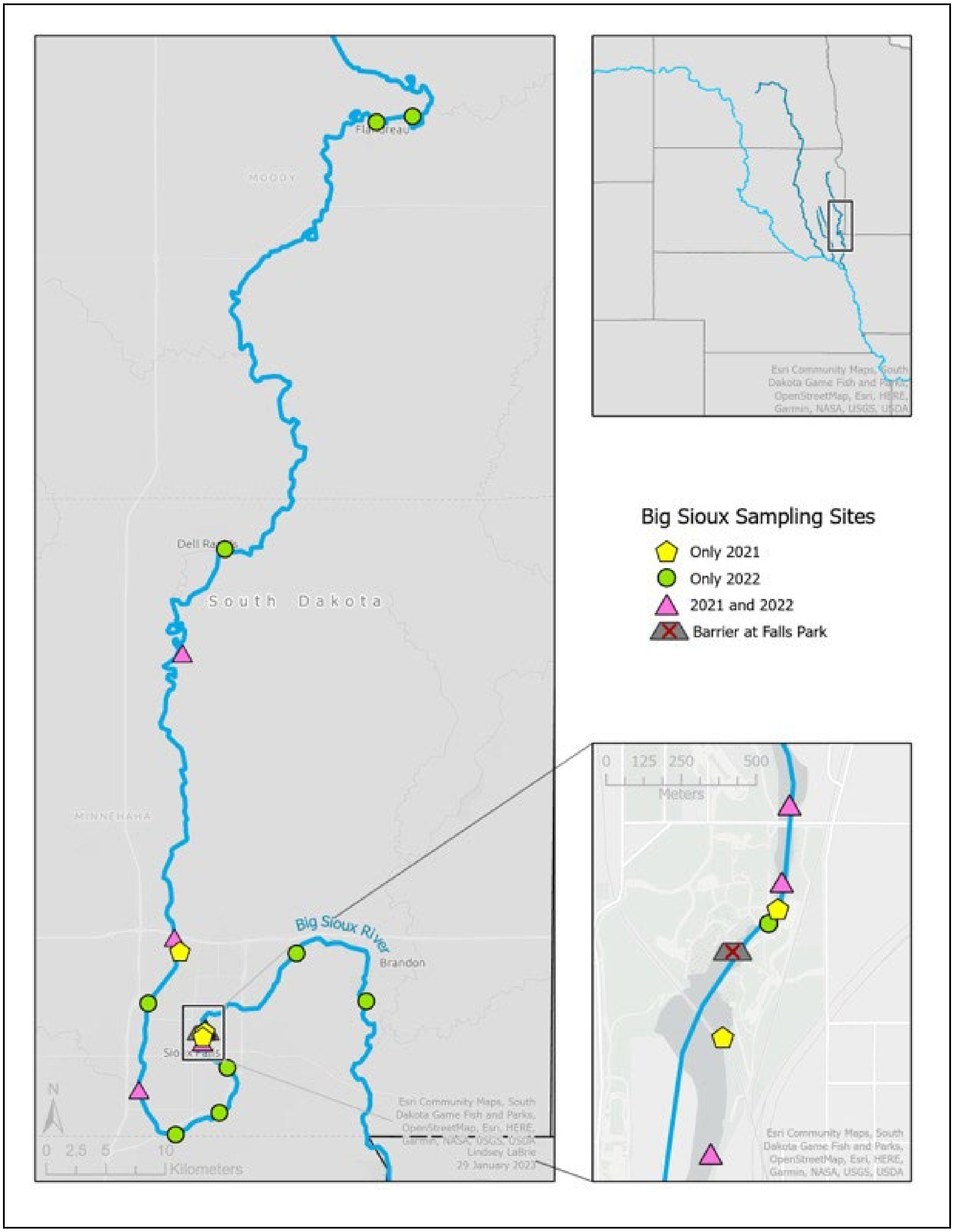

Figure 2. eDNA water sampling locations in the Big Sioux River. Sites sampled in both years are represented as pink triangles, sites sampled only in 2021 are represented as yellow hexagons, and sites sampled only in 2022 are represented as green circles.

Average eDNA quantity (copies/µL)		
Vermillion River		
	Below barrier	Above barrier
Silver carp	0.205	0.00022
Bighead carp	3.221	0.01051
Big Sioux River		
	Below barrier	Above barrier
Silver Carp	0.092	0.0
Bighead Carp	0.283	0.0

Figure 3. A table of average quantities of Silver and Bighead Carp eDNA above and below each barrier in the Vermillion and Big Sioux Rivers.

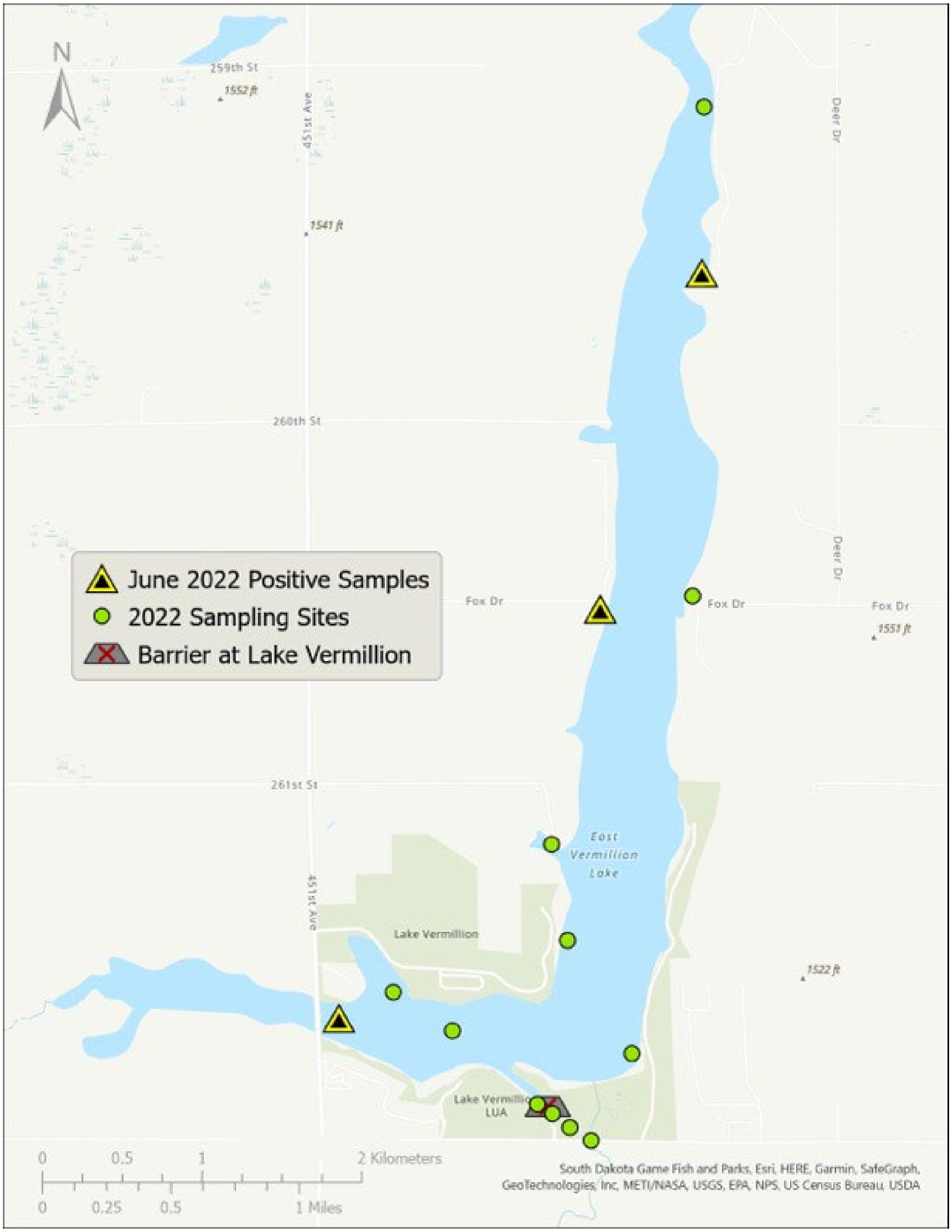

Figure 4. Locations of positive eDNA samples in the Vermillion River detected with qPCR. Samples are from June 2022 and are currently under reanalysis to ensure they are true positives.

Recommendation:

Expanded and continued eDNA water sampling above both barriers is recommended to provide a clearer picture of invasive carp populations above the two barriers, especially in the Vermillion River. This is important for early detection of Silver and Bighead Carp populations to inform early management strategies.

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State Report: South Dakota

Agency: South Dakota Department of Game, Fish, and Parks (SDGFP), South Dakota State University (SDSU), and University of Idaho, Kennedy LIFE Lab

Project Title: Determining natal origins and movement patterns of Silver Carp in eastern South Dakota tributaries through otolith microchemistry

Methods: Water samples for trace elements were collected from two locations on each of the James, Vermillion, and Big Sioux rivers and from three locations on the Missouri River in June and November, 2021. Additional water sampling data was collected as part of a larger Mississippi River Basin Panel study in 2021, and one additional sample was collected from each of the three tributaries in June and August 2021. In 2022, two samples per tributary, three from the Missouri River, and one each from the West Fork Vermillion River and Split Rock Creek were collected in June, August, and November.

For trace element and oxygen isotope analysis, water was collected in 250-mL polypropylene bottles of from each site. Water samples were transferred to an acid-washed polypropylene syringe, filtered through a Whatman Puradisc 0.45 µm filter, and stored in scintillation vials wrapped with Parafilm to prevent evaporation and fractionation (Kendall and Caldwell 1998; Shiller 2003). Filtered samples were then shipped to the Center for Trace Analysis at the University of Mississippi for processing of Ca, Ba, and Sr trace elements and $\delta^{18}O$.

In June 2021, a second water sample was collected at each site in a 200-mL acid-washed, polyethylene bottle and stored unfiltered. These samples were sent to the Kennedy Laboratory for Integrative Fish Ecology Lab for processing of ^{87}Sr and ^{86}Sr isotopes.

Otolith collection

Silver Carp were collected from 2020-2022 using a combination of targeted sampling and as by-catch during other field sampling events on the three eastern South Dakota tributaries to the Missouri River. We used a cast net to target fish on the Big Sioux River and sampled both rivers using boat electrofishing. Each fish was measured for total length (mm), and lapillus otoliths were extracted using a plastic forceps and stored in polypropylene centrifuge vials for future otolith microchemistry analysis.

Results and Discussion

Trace element and $\delta^{18}O$ water sampling data demonstrate spatial variability in water chemistry signatures. Mean Sr:Ca ratios decrease in the three eastern SD tributary rivers from west to east, but the mean mainstem Missouri River signature is higher than all three tributary rivers (Figure 1). Further, there appears to be a substantial difference in mean Ba:Ca ratios between the Big Sioux River and the James and Vermillion rivers (Figure 1). Mean $\delta^{18}O$ ratios had substantial overlap among the three eastern SD tributaries, but the Missouri River signatures were substantially lower (Figure 2).

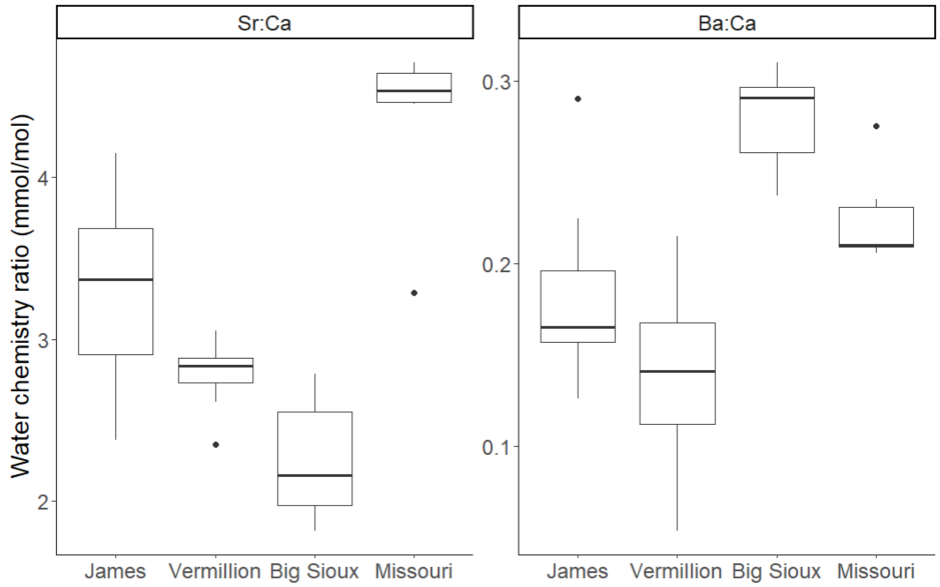

Figure 1. Boxplots of water Sr:Ca and Ba:Ca ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021 and June 2022.

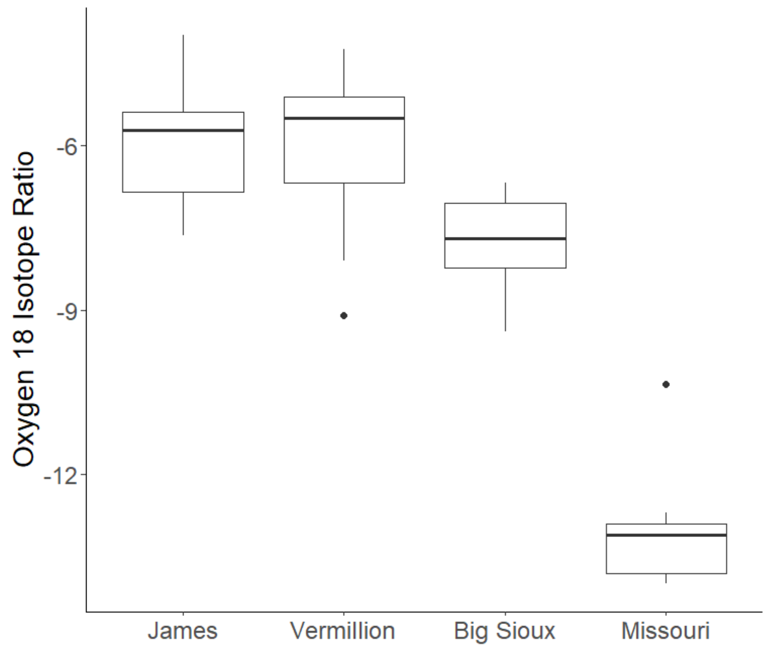

Figure 2. Water oxygen-18 ($\delta^{18}O$) isotope ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021 and June 2022.

Results from strontium isotope analysis show that water samples differ to the fifth decimal among all of the rivers, with the Big Sioux River having different signatures at the second decimal (Table 1). While differences in water chemistry are likely reproduceable, otolith chemistry signatures obtained during laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) would not be sensitive enough to identify differences among the James, Vermillion, or Missouri Rivers. As a result, we did not conduct any additional strontium isotope water sampling and will not pursue this as a technique for LA-ICMS with Silver Carp otoliths.

Table 1. Strontium isotope water chemistry results for each site on the three eastern SD tributaries and the Missouri River sampled in June 2021.

River	Site	***87Sr/86Sr***	***±2SE (ratios included)***
Missouri	Sioux City	0.7089097	0.000004, 149 of 160
	Clay County	0.7088912	0.000003, 148 of 160
	James River Island	0.7088996	0.000003, 146 of 160
James	HWY 81 - Shramm boat access	0.7088220	0.000004, 151 of 160
	Mitchell, SD	0.7088414	0.000005, 149 of 160
Vermillion	Highway 19 bridge crossing	0.7086468	0.000003, 151 of 160
	Highway 42 bridge crossing	0.7088947	0.000003, 150 of 160
Big Sioux	Akron, IA	0.7098262	0.000003, 146 of 160
	Ninemile boat access	0.7100143	0.000004, 151 of 160

Otolith collection

A total of 47, 37, and 38 Silver Carp otoliths were collected from the Big Sioux, James, and Vermillion rivers in 2020 and 2021. Total lengths ranged from 269-761 mm. In 2022, low water prevented sampling on the Vermillion River, but 94 and 112 Silver Carp from the James and Big Sioux rivers were sacrificed for otolith microchemistry. Total lengths of fish collected in 2022 ranged from 367 to 841 mm. Additional juvenile (presumed age-0) Silver Carp were collected in the upper James River in southern North Dakota in fall 2021 (n = 2) and lower James River (approximately rkm 38) in fall 2022 (n = 18), and several juvenile Silver Carp were collected by USFWS staff in 2020 in the mainstem Missouri River.

Carp otoliths from 2020 and 2021 were sent for processing to Southern Illinois University for LA-ICPMS, and results are pending. Otoliths collected in 2022 and from all juvenile Silver Carp will be sent to Southern Illinois University in March 2023 for LA-ICPMS. Water sampling and continued otolith collection will continue into spring/summer 2023.

Recommendation:

Otolith microchemistry appears to be a possible tool for identifying natal origins among the eastern South Dakota tributaries to the Missouri River. Additional time is needed to process otoliths and analyze data to understand natal origins of Silver Carp in these systems.

References:

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State Report: Nebraska

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

Project Title: Assessment of invasive carp distribution in Nebraska: Emphasis on early detection monitoring on the mainstem Missouri River and leading-edge investigations on tributaries and periphery streams.

Methods:

Invasive Carp (Bighead, Silver, and Grass Carp) have been detected in the Missouri River as far upstream as Gavin's Point Dam. Black Carp have also been detected in the lower Missouri River, but have not been detected as far upstream as Gavin's Point Dam. While management agencies have some idea of the impact these fish can have on their environment, their impact on the fish community is unknown. Conserving the Missouri River fish community will require a thorough understanding of the impact Invasive Carp have on these fish communities. It is imperative to study the population dynamics of Invasive Carp, leading to a better understanding of how Invasive Carp impact fish communities both now and in the future.

This study is comparing the fish communities in the five river kilometer (Rkm) reaches below Gavin's Point Dam (Invasive Carp detected) and Fort Randall Dam (Invasive Carp not detected). It will also provide information about the growth rates of Invasive Carp in the reach below Gavin's Point Dam.

In the summer of 2022, sampling was continued below Gavin's Point and Fort Randall dams using a suite of gears designed to capture a large segment of the diversity in the fish communities. The primary sampling method was boat electrofishing. One week per month both banks, any islands, and accessible inlets in the five Rkm reaches below the dams were sampled. Concurrently with boat electrofishing, mini-fyke nets, and two sizes of hoop nets were utilized to sample different portions of the fish community. To find appropriate sites for mini-fyke nets, some gears were deployed beyond the five Rkm reaches below each dam. Low water conditions and mechanical issues with the shocking boat limited sampling below Fort Randall Dam. As a result, no hoop nets were deployed and limited electrofishing was restricted to the first 2 Rkm below Fort Randall Dam.

Captured fish were measured for total length in millimeters and weight in grams. Sturgeon species were measured to fork length and Paddlefish were measured from eye to fork. Additionally, sagittal otoliths were removed from Invasive Carp for future aging and back calculations.

We also collected water samples monthly during the summer months from the mainstem river at Nebraska City, Blair, and below Gavin's Point Dam for eDNA analyses in search of possible Black Carp detections. Additional water samples for eDNA were also collected from Lewis and Clark Lake for detection of Bighead Carp and Silver Carp. Furthermore, monthly water samples are being collected across the state by partners involved in concurrent research that are providing an additional layer of potential Carp detection across the interior Nebraska rivers and streams.

Results and Discussion:

Between May 16, 2022 and October 1, 2022, a total of 3738 fish were captured. Due to damage or sampling constraints (e.g., only the first 25 fish per species per sample were measured with the remaining counted), 650 fish were not weighed and measured, leaving 3088 measured fish. Of these, 1268 were captured below Fort Randall Dam and 2470 were captured below Gavin's Point Dam. In 2021 6109 fish were captured with 5892 measurable fish with 2111 below Fort Randall Dam and 3781 below Gavin's Point Dam. The decreased catch in 2022 is not overly surprising given reduced sampling effort due to lower water conditions that restricted accessibility.

In 2022 Emerald Shiner were the most numerous fish sampled, with 647 sampled totaling 17% of our total catch by count (Table 1). However, 501 of these fish were caught in a single mini-fyke net. Shorthead redhorse, the most abundant species from last year accounted for 11% of the total catch. Other species of note included one Muskellunge (1105mm, 15100g), 3 Shovelnose Sturgeon, and 48 Paddlefish (weights from 6400-25200g). One hundred and forty-three Invasive Carp were captured below Gavin's Point Dam: 115 Silver Carp, 18 Grass Carp, and 10 Bighead Carp. Forty of the Silver Carp and 6 of the Bighead Carp were obtained from anglers during the opening weekend of the Paddlefish snagging season. The total number of Carp caught during the 2021-2022 field seasons was 15 Bighead Carp, 184 Silver Carp, and 48 Grass Carp (*Figure 1*). No Black Carp were sampled below either dam, and no Invasive Carp were sampled within the reach below Fort Randall Dam.

Water samples collected for eDNA analyses continued to suggest no additional range expansion upstream in the Missouri River during 2022. Specifically, we have had no positive detections for Black Carp in Nebraska's waters. Bighead Carp and Silver Carp have not been detected above Gavin's Point Dam using eDNA techniques.

Statewide water sample assessment using eDNA from interior rivers and streams has suggested a slightly broader range of distribution than is currently confirmed within Nebraska. We are currently coordinating with concurrent projects to assess population dynamics of Invasive Carps at the University of Nebraska-Lincoln and Nebraska Game and Parks personnel to implement on the ground sampling to confirm these expansions.

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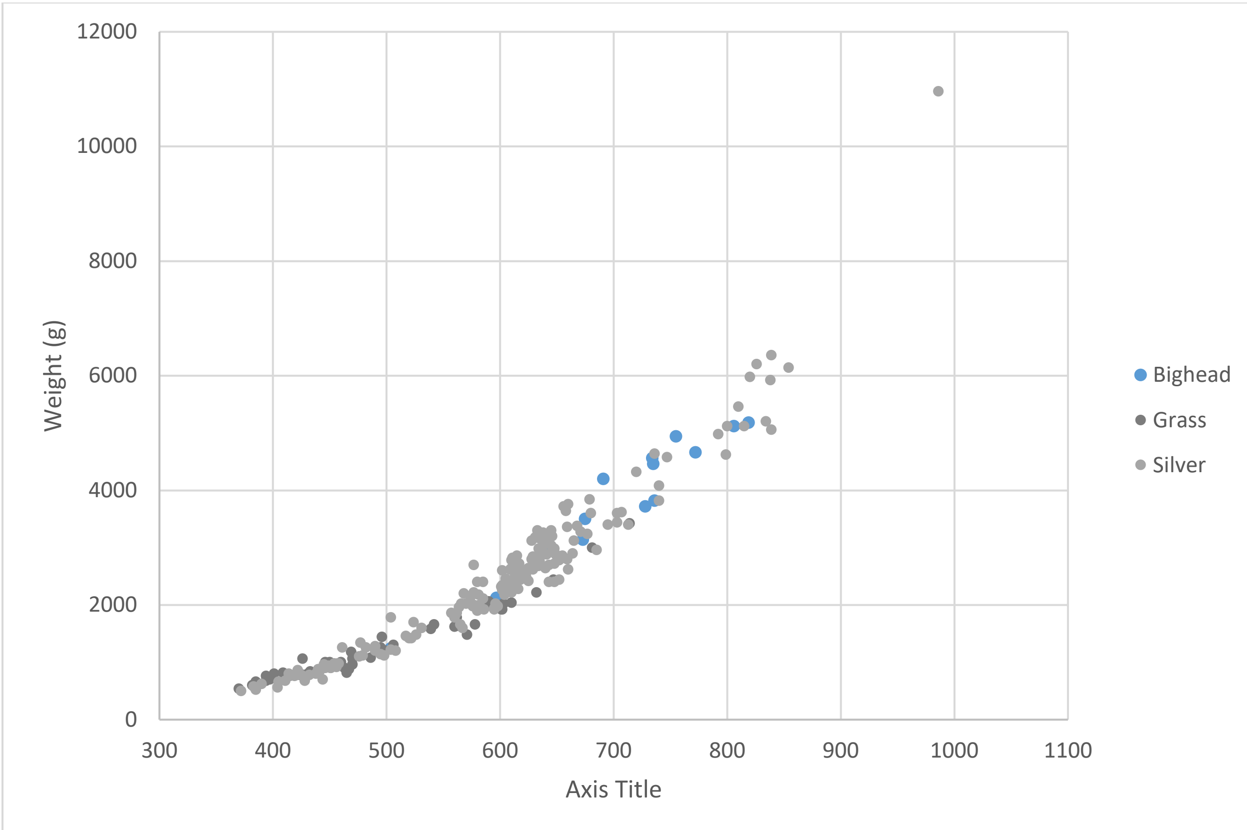

Figure 1. Weight versus length relationship for Bighead Carp, Grass Carp, and Silver Carp sampled below Gavin's Point Dam, Yankton, SD during 2021 and 2022 sampling seasons.

	2021		2022	
Species	Fort Randall	Gavin's Point	Fort Randall	Gavin's Point
American Eel	0	1	0	0
Bighead Carp	0	5	0	10
Bigmouth Buffalo	20	79	7	30
Bigmouth Shiner	0	0	1	0
Black Bullhead	0	0	0	1
Black Crappie	0	3	0	1
Blue Sucker	2	378	0	249
Bluegill	9	50	2	74
Brassy Minnow	0	0	55	0
Brown Trout	1	0	0	0
Channel Catfish	149	156	1	158
Common Carp	178	281	47	88
Creek Chub	0	0	23	0
Emerald Shiner	2	15	637	10
Fathead Minnow	0	0	2	0
Flathead Catfish	2	6	0	24
Freshwater Drum	25	194	5	110
Gizzard Shad	2	435	6	376
Goldeye	13	282	5	176
Grass Carp	0	30	0	18
Green Sunfish	0	1	0	2
Highfin Carpsucker	3	1	0	0
Johnny Darter	2	2	0	1
Lake Sturgeon	0	1	0	0
Largemouth Bass	0	6	0	3
Longnose Gar	0	42	0	17
Mooneye	0	6	0	18
Muskellunge	0	0	0	1
Northern Pike	23	104	7	7
Orangespotted Sunfish	0	0	0	3
Paddlefish	53	33	22	24
Quillback	95	87	34	71
Rainbow Trout	2	0	0	0
Red Shiner	9	13	3	2
River Carpsucker	261	142	34	86
River Shiner	77	31	0	1
Rock Bass	0	3	0	11
Sand Shiner	1	1	0	0
Sauger	1	3	1	2
Saugeye	0	1	0	1
Shorthead Redhorse	930	485	146	269
Shortnose Gar	123	317	38	112
Shovelnose Sturgeon	0	4	0	3
Silver Carp	0	69	0	115
Skipjack Herring	0	4	0	1
Smallmouth Bass	80	63	25	53
Smallmouth Buffalo	42	373	15	181
Spotfin Shiner	87	91	104	132
Spottail Shiner	0	0	2	0
Suckermouth Minnow	7	0	0	0
Unidentifid Notropis	0	0	10	0
Unidentified Centrarchid	0	0	0	3
Unidentified Cyprinid	18	0	5	0
Unidentified Fish	4	0	12	1
Unidentified Micropterus	0	1	0	0
Unidentified Sanders	0	0	1	0
Walleye	41	7	9	10
White Bass	16	11	8	15
White Sucker	3	0	1	0
Wiper	7	3	0	0
Yellow Perch	1	0	0	0
Totals	2289	3820	1268	2470

Table 1. Species Counts of fish sampled (boat electrofishing, mini-fykes, and hoop nets) below each dam during the Summer of 2021 and 2022.

State Report: Nebraska

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

Project Title: Assessment of Silver Carp and Bighead Carp in the Platte River, Nebraska: Emphasis on population distribution, population demographics, and reproduction.

Methods:

Larval Sampling:

Ichthyoplankton sampling was conducted from May to August in 2022 across 5 sites: Waterloo (Elkhorn River), Leshara (Platte River), Louisville (Platte River), Plattsmouth (confluence of the Platte to the Missouri), and Omaha (Missouri River upstream of the Platte River confluence; Figure 1). Ten-minute stationary ichthyoplankton tows were set using conical ichthyoplankton nets (0.5-m mouth diameter, 500-µm mesh) at eight randomly-selected locations across a 5km river reach at each site. Water temperature, pH, dissolved oxygen and conductivity were measured using a YSI. Water depth to the nearest cm was measured with a depth stick. Net contents were washed into the cod end bucket then transferred to whirlpaks. Eggs and larvae were then sorted from debris and preserved in 95% ethanol for identification.

Young-of-year (YOY) sampling was conducted in the fall (October-November) of 2022. Seine nets were used at adult sampling site locations (see below; Figure 2). Four, 15m long seine-hauls were conducted at each site. Total length measurements of captured fish were collected for the first 25 individuals of each species. Each species with more than 25 individuals had individuals counted. Silver Carp and Bighead Carp young-of-year were preserved in 95% ethanol for further processing.

Adult Sampling:

Sampling took place during the spring (May), summer (July), and fall (October) of 2022.
Sampling was conducted at eleven sites in the lower Platte River basin and one site in the Missouri River. Sample sites were distributed across seven river reaches which are defined as follows:

1. Missouri (approx. MO River kilometer 630)
2. Lower Platte (confluence of Platte/Missouri to the confluence of Platte/Elkhorn)
3. Middle Platte (confluence of Platte/Elkhorn to the confluence of Platte/Loup)
4. Upper Platte (confluence of Platte/Loup and upstream on the Platte River)
5. Elkhorn (confluence of Platte/Elkhorn and upstream on the Elkhorn River)
6. Loup (confluence of Platte/Loup and upstream on the Loup River)
7. Salt Creek (confluence of Platte/Salt Creek and upstream on Salt Creek).

Adult fish were collected using high frequency, boat-mounted electrofishing (60 hz, 25% duty cycle, targeting 20 amps). We used an ETS (Wisconsin) Electrofishing Systems control box mounted on a 4.9-meter, (16 foot) aluminum Jon boat. Sampling runs were 900 seconds (15 minutes) and were conducted from upstream to downstream. Low flows severely restricted boat maneuverability, but runs were conducted in a serpentine manner when water level allowed. When low water levels prohibited the use of an electrofishing boat, we used an M.L.E.S. electrofishing tote barge. We attempted to maintain consistent electrofishing effort and operated the tote barge with settings similar to the Jon boat electrofishing unit.

We sampled under a “space for time” robust design, occupancy model framework similar to what is described in Sullivan (2019). Each sample site was separated into four, independent sub-samples that each received one 900 second sampling run. Each subsample was approximately one kilometer apart from the next to establish independence. Each subsample was considered a repeated sample for its respective site.

Bigheaded carp were identified down to species by checking for the presence of a ventral keel and examining gill raker morphology; Silver carp possess a ventral keel that extends from the anal opening to the gill membrane and gill rakers that are fused and sponge-like (Schofield 2005). Total length (mm), weight (g), and sex were recorded for each individual. Sex was determined using the rapid sex assignment method described in Lederman (2022) and Wolf (2018) and was verified by opening the stomach cavity and inspecting gonads. This method was tested for accuracy during our fall sampling period on 154 individuals. We collected fin clips (for an additional aging structure) by cutting the primary pectoral fin ray with diagonal cutting pliers near the base of the pectoral fin. We collected tissue samples for genetic analysis by cutting a clip of tissue near the base of the anal fin. These samples were also retrieved using diagonal cutting pliers. Genetic tissue samples were placed in buffer solution and placed in a deep freezer for storage. Lapilli otoliths were collected from each fish (Seibert 2013). A reciprocating saw was used to decapitate the fish and expose the brain cavity; the otoliths were then retrieved using plastic forceps and were stored in paper coin envelopes.

Female gonads were removed, weighed to the nearest gram, and preserved in 10% buffered formalin for batch fecundity estimates and histological analyses. Gonadosomatic index (GSI) was calculated as 100 multiplied by the ratio of wet gonad weight to wet body weight. Batch fecundity was estimated as weight of both ovaries multiplied by the enumeration of 1g of ovary sampled from 3 locations (i.e., front, middle, back of ovary). Histological analyses will be conducted at the Nebraska Veterinary Diagnostic Center at UNL to categorize oocyte stage for spawning phenology assessments. The presence or absence of oocyte stages from each individual will be analyzed to determine reproductive phase according to methods by Brown-Peterson et al. (2011).

Results and Discussion:

Larval Sampling:
Eggs, larvae, and age-0 fish were collected from 336 ichthyoplankton samples (Table 1). From fall seining, a total of 6468 fish were collected across 28 species (Table 2). Three YOY Silver Carp were captured at the Platte River confluence with the Missouri River.

Adult Sampling:

We collected 564 silver carp and 20 bighead carp during all sample seasons in 2022. 69 silver carp were captured during the spring, 340 were captured during the summer, and 155 were captured during the fall. All bighead carp were collected during the summer sampling season; none were captured during the spring and fall. Because numbers of bighead carp were so few, they have been excluded from further analysis. We collected a total of 2,879 fish across 43 species (Table 3).

A total of 153 female bigheaded carp gonads were collected across all sites and all seasons. Mean Silver Carp GSI ranged from 18.3 - 6.2 (Elkhorn River – Upper Platte). Mean Silver Carp batch fecundity ranged from 163532.9 - 571993.3 eggs (Platte Confluence – Elkhorn River). Silver Carp GSI and batch fecundity were analyzed between hydrological units using a one-way ANOVA with Tukey post-hoc pairwise comparison. GSI and batch fecundity was highest in the Elkhorn River (GSI: $p = 0.007$; batch fecundity: $p > 0.05$; Figures 3-4).

Silver Carp were found at seven out of twelve sampling locations during the spring sampling period (Figure 5). No Silver Carp were found in the Loup River or in the Platte River west of the confluence of the Loup River (Figure 5). During the summer sampling period, Silver Carp appeared to expand their distribution (Figure 6). Silver Carp maintained their distribution from the spring but were also captured in the Loup River and further up Salt Creek (Figure 6). During the fall sampling period, Silver Carp distribution shifted again; presences were maintained in the Loup River and Salt Creek, but presences were lost at both sites on the Elkhorn River and at one site on the Platte River (Figure 7).

Silver Carp catch per unit effort (hereafter referred to as "CPUE") during the spring sampling period was highest in the Missouri River, followed by our most-downstream site in the Platte River at Plattsmouth, Nebraska (Figure 8). Silver Carp CPUE was comparatively higher during the summer than in the spring (Figure 9). CPUE was highest at the "Plattsmouth" Platte River site, and catch rates appeared to increase at sites further into the study area as well (Figure 9). During the fall sampling period, CPUE decreased for most sites in the Platte River basin (most noticeably at "Plattsmouth") (Figure 10). Sampling locations in the Missouri River and Salt Creek maintained similar CPUE to the previous sampling period (Figure 10).

Length frequency histograms of Silver Carp in the Platte River basin for each season appear to display similar distributions. Mean total lengths were typically near 650 mm; the smallest fish were generally around 500 mm and the largest were approximately 800 mm (Figures 11-13). Statistical tests revealed no seasonal difference between mean total lengths of Silver Carp in the Platte River Basin. However, there was a significant difference in mean total lengths at different river reaches (Figure 14). Specifically, we found that Silver Carp in the Loup River were longer on average when compared to Silver Carp in the Missouri River ($p = 0.002$). Additionally, Silver Carp mean total length in the Elkhorn River was greater than the Missouri River, respectively ($p = 0.003$). Mean total lengths did not differ significantly by sex (female: 645 mm, male: 642 mm)

Sex ratios of Silver Carp across the study area were male-skewed. The female-male ratio at our sample location in the Missouri River was 1:1.2. The female-male ratio of all sites in the Platte River Basin combined was 1:2.2. Differences in sex ratio became more apparent when calculated for each tributary. Sites on the main-stem Platte River had a female-male ratio of 1:1.9. Female-male ratios in the Elkhorn River were even more skewed at 1:2.4. Finally, we observed the most skewed ratios in the Loup River at 1:14. A one-way ANOVA paired with a Tukey HSD test revealed a significant difference between the Missouri River and the Loup River. See figures 15 and 16.

We tested the Wolf-Lederman rapid sex-assignment method on 154 Silver Carp during the fall sampling period. Pectoral fins were examined on each individual, an estimate (male/female) was made, and then gonads were checked for verification. We found the method to be 99% accurate; estimates were correct for 152 out of 154 individuals. One of the incorrect assignments was initially estimated to be male but was verified as a female. The other incorrect assignment was vice versa (ID'd as female but was male). It should be noted that both Wolf and Lederman found this method to be most accurate on individuals ranging from approximately 300 to 800 mm (Lederman 2022, Wolf 2018). Very few individuals outside of this range were captured during our fall sampling period; this likely contributed to our high accuracy.

Discussion:

We observed spatial and temporal trends in naïve occupancy and CPUE of Silver Carp in the Platte River Basin. Silver Carp appeared to expand their distribution in the summer, followed by a subsequent contraction in the fall (Figures 5-7). Similarly, Silver Carp CPUE in the Platte River Basin increased during the summer and decreased during the fall (Figures 8-10). CPUE of Silver Carp was generally higher in the Missouri River and in the Lower Platte reach. CPUE was generally lower upstream on the Platte River and in its tributaries (Loup River, Elkhorn River). We also observed spatial trends in population demographics. Silver Carp in the Elkhorn River and the Loup River were significantly longer on average than Silver Carp in the Missouri River (Figure 14). Sex ratios were male-skewed in the Platte River Basin and was more skewed further upstream in the basin (Figure 16).

Silver Carp and Bighead Carp have been shown to alter zooplankton communities and compete with native filter feeders, such as American Paddlefish *Polyodon spatula* and Bigmouth Buffalo *Ictiobus cyprinellus* (Schrank 2003; Sampson 2009; Sass 2014; Pendleton 2017). Further, Silver Carp population demographics are thought to be influenced by such community alterations as well as intraspecific competition (Stuck 2012; Sass 2014; Coulter 2018; Sullivan 2019; Erickson 2021). For example, spatial variations in Silver Carp body condition, size, and growth have often been attributed to density-dependent controls (Coulter 2018; Sullivan 2019; Werner 2022). Specifically, low densities of Silver Carp are often positively correlated with larger size, higher growth rates, and favorable zooplankton communities (Williamson 2005; Sass 2014: Coulter 2018). However, these variations in density are typically observed across a grander scale than our study area in the Platte River Basin and are often accompanied by physical barriers to dispersal (Erickson 2021; Werner 2022). While we observed greater mean total lengths of Silver Carp at sites with lower CPUE, it is probable that there are other factors contributing to this correlation. Larger individuals may be better suited to dispersing greater distances upstream during annual movements (Peters 1983; Minns 1995). However, previous research on Silver Carp movement distances and home ranges are not correlated with size or sex (Coulter 2016; Prechtel 2018).

Silver Carp sex ratios in their invasive range are often slightly male-skewed; female-male ratios of approximately 1:1 to 1:1.3 are commonly reported (Abdusamadov 1987; Williamson 2005; Kamilov 2014; Erickson 2021). However, there is a paucity of information explaining variation in sex ratios of Silver Carp and the impacts such variations may have. Additionally, I have found no sources reporting drastic male-skewed ratios such as the female-male ratio observed in the Loup River: 1:14. One possible hypothesis explaining this phenomenon could be that female Silver Carp did not move into the Platte River Basin due to poor spawning conditions (low flow) and a lack of spawning cues in 2022. However, it should be noted that previous Silver Carp studies have found no differences in movement between males and females (Coulter 2016; Prechtel 2018). More research and supplementary years of sampling should aid in explaining the observed variation.

Recommendation/Future Directions:

- Finding/purchasing a gear configuration that will allow for effective sampling in variable flows (instead of using tote barge to supplement boat electrofishing)
- Need repeated sampling to assess variability in sex ratios
- Currently in the process of analyzing genetic samples, otoliths, and microchemistry data

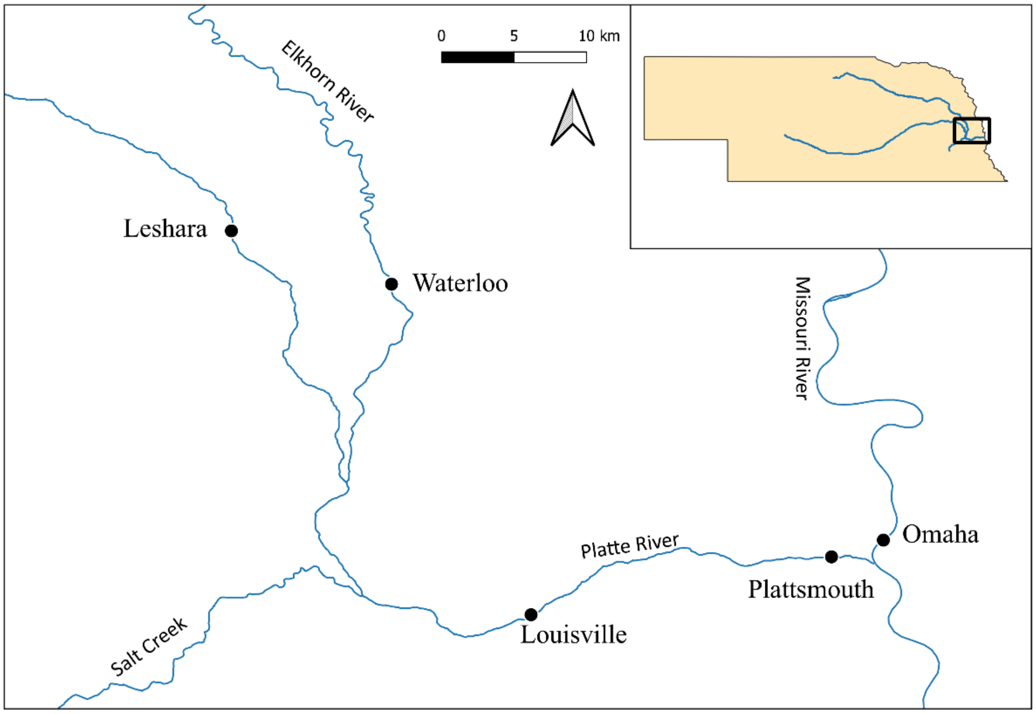

Figure 1. Ichthyoplankton sampling sites on the Platte River, the Elkhorn River, and the Missouri River in 2022.

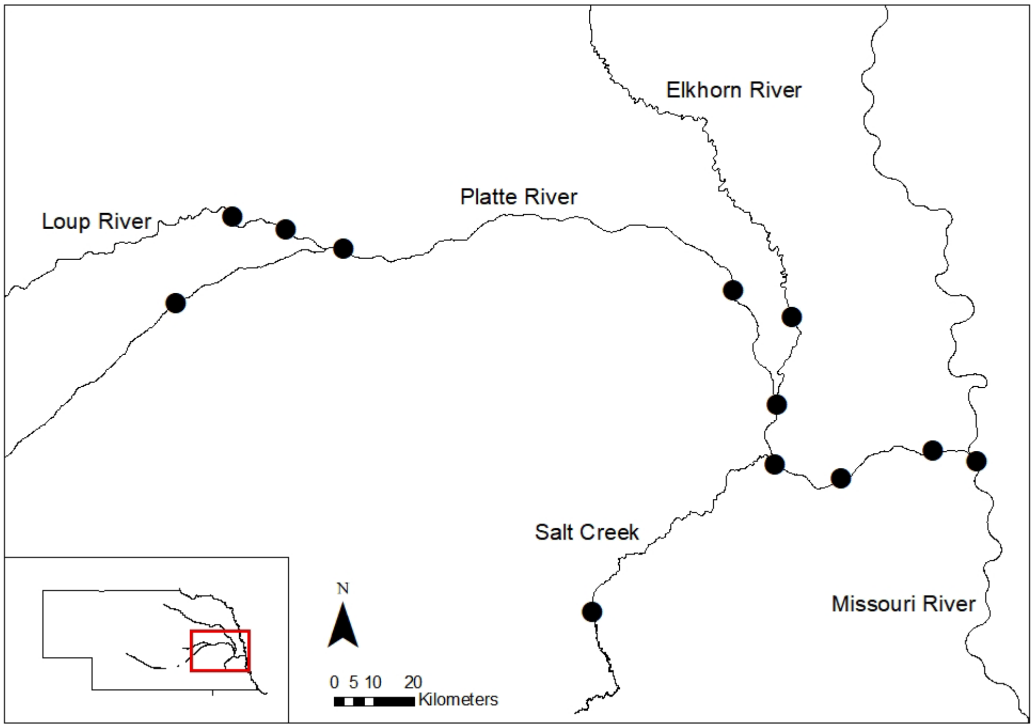

Figure 2: Study area featuring twelve sites in the lower Platte River basin and the Missouri River.

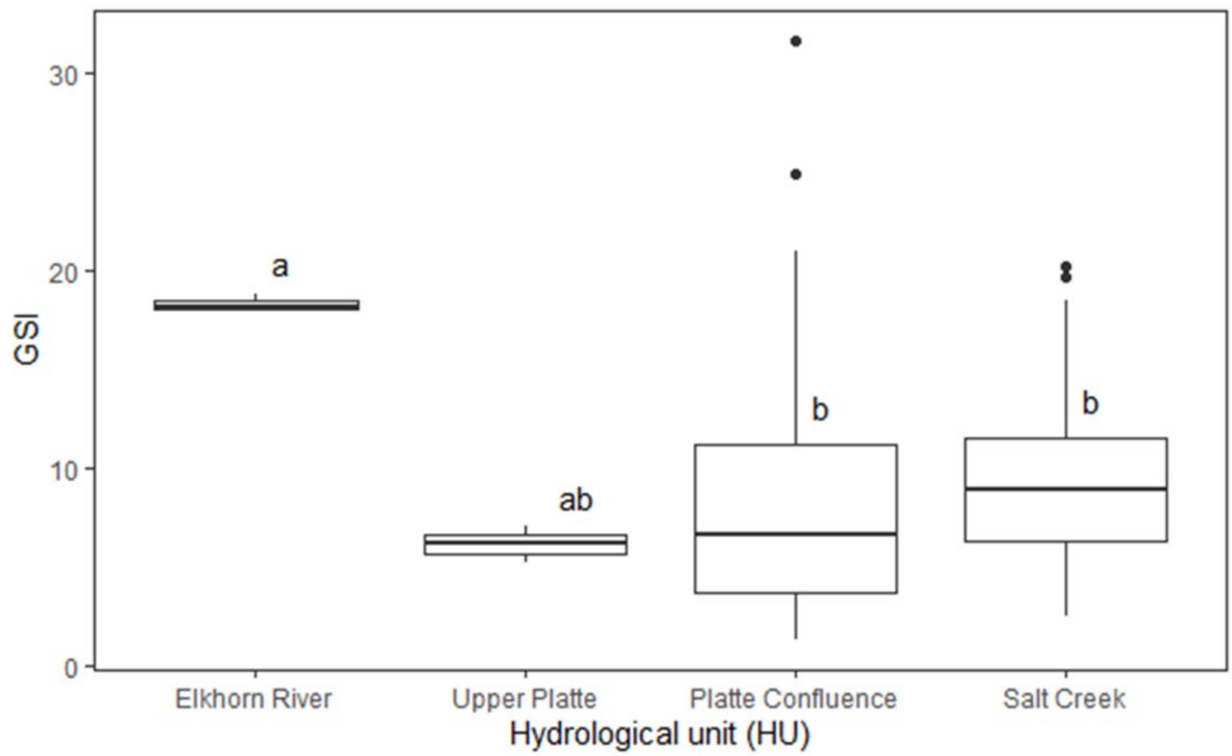

Figure 3: Female Silver Carp GSI analyzed by hydrological units (HU) across all seasons. Data was analyzed using one-way ANOVA with Tukey post-hoc pairwise comparisons.

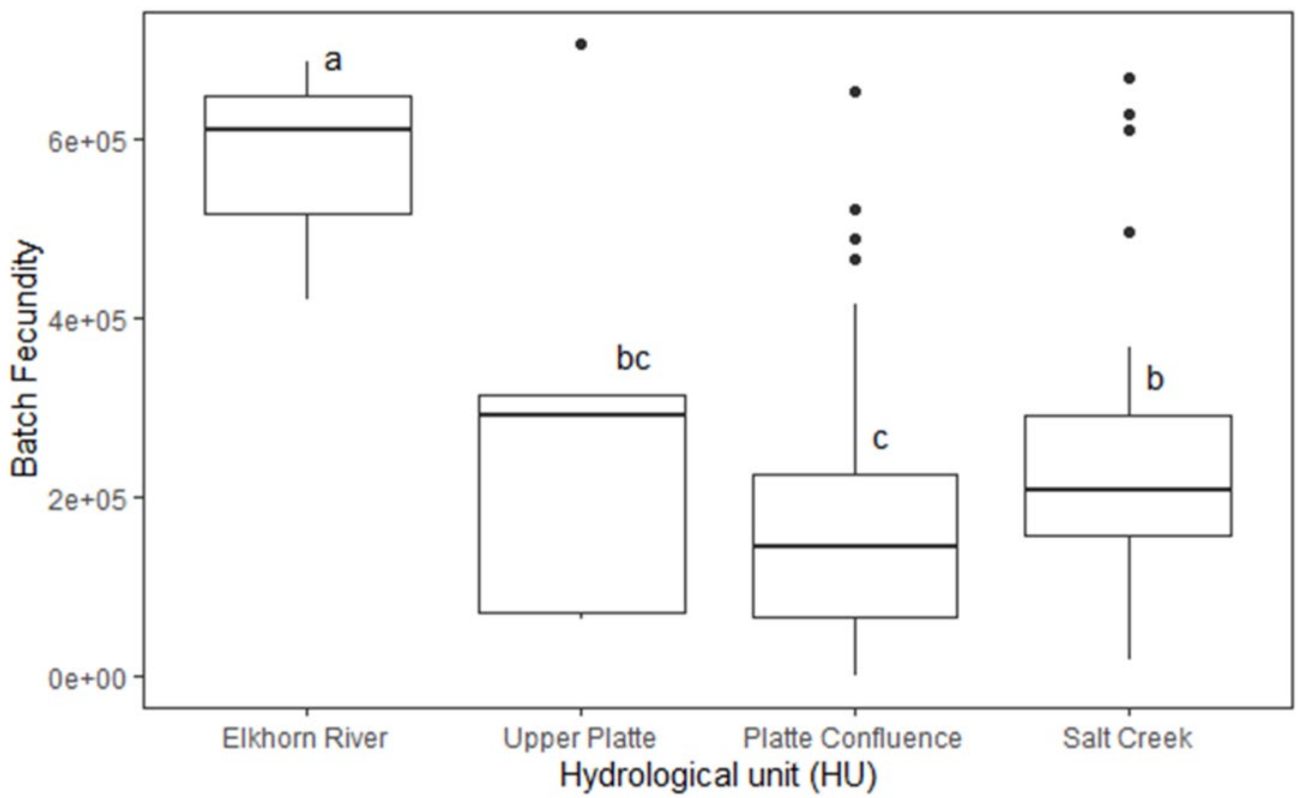

Figure 4: Female Silver Carp batch fecundity by hydrological units (HU) across all seasons. Data was analyzed using one-way ANOVA with Tukey post-hoc pairwise comparisons.

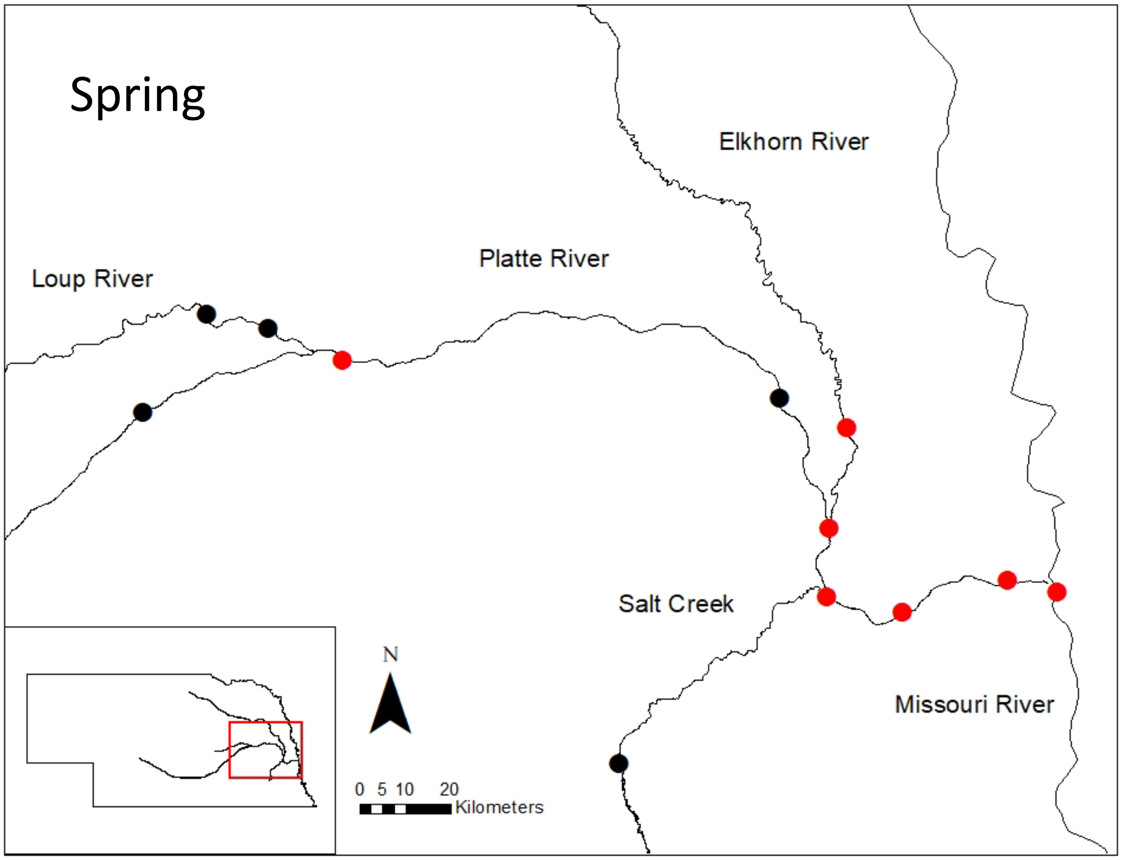

Figure 5: Map displaying naive occupancy of Silver Carp during the spring sampling period. Red circles indicate that Silver Carp were present and black circles indicate an absence.

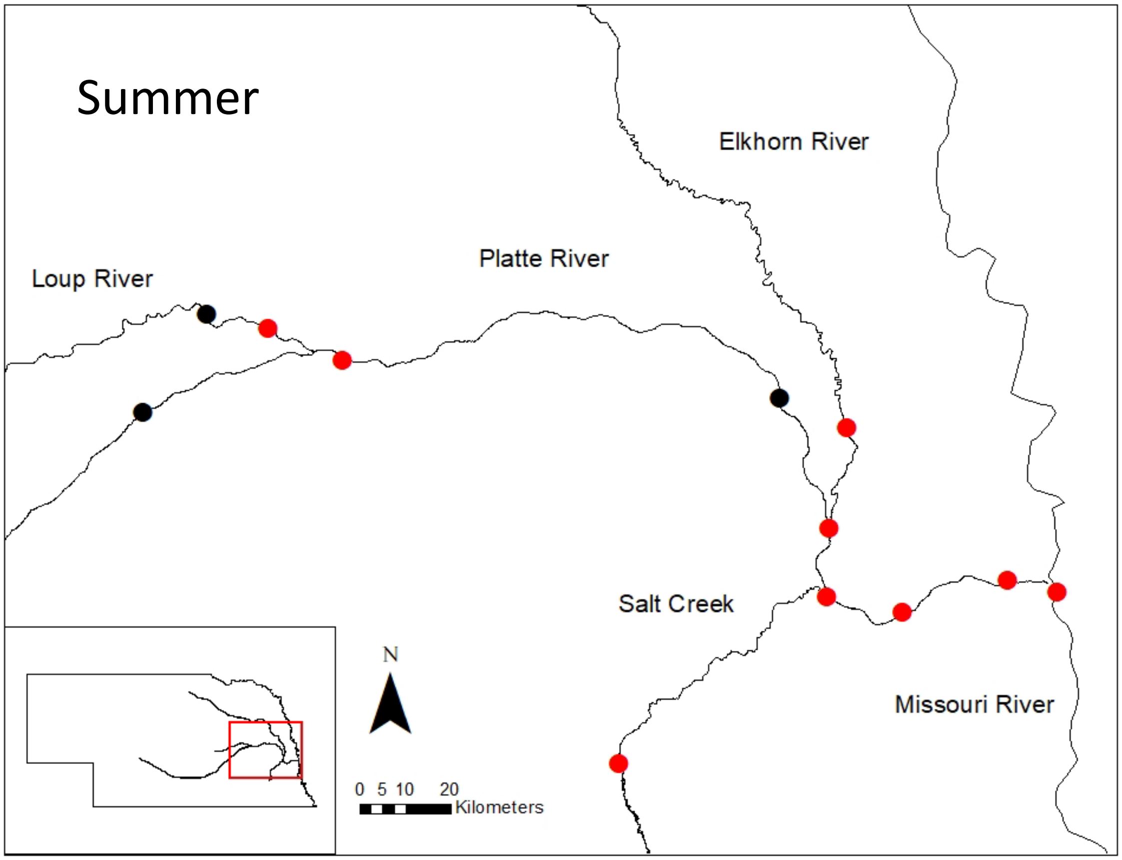

Figure 6: Map displaying naive occupancy of Silver Carp during the summer sampling period. Red circles indicate that Silver Carp were present and black circles indicate an absence.

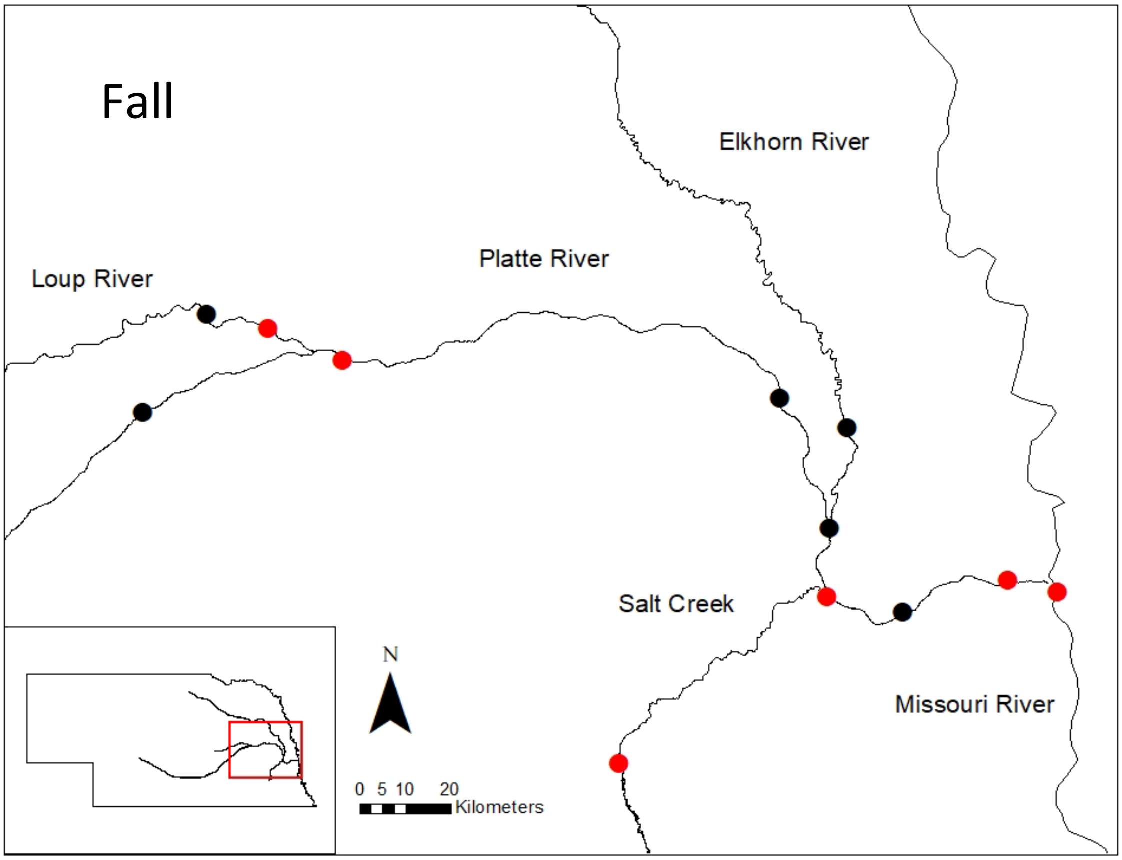

Figure 7: Map displaying naive occupancy of Silver Carp during the fall sampling period. Red circles indicate that Silver Carp were present and black circles indicate an absence.

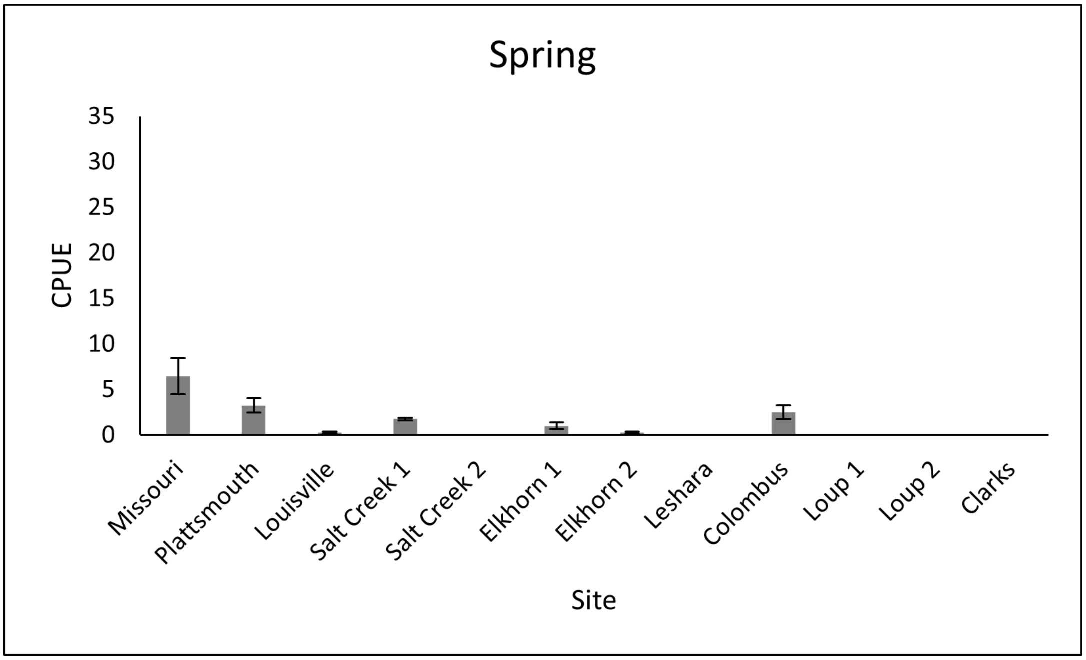

Figure 8: Catch per unit effort (CPUE) of Silver Carp during the spring sampling period.

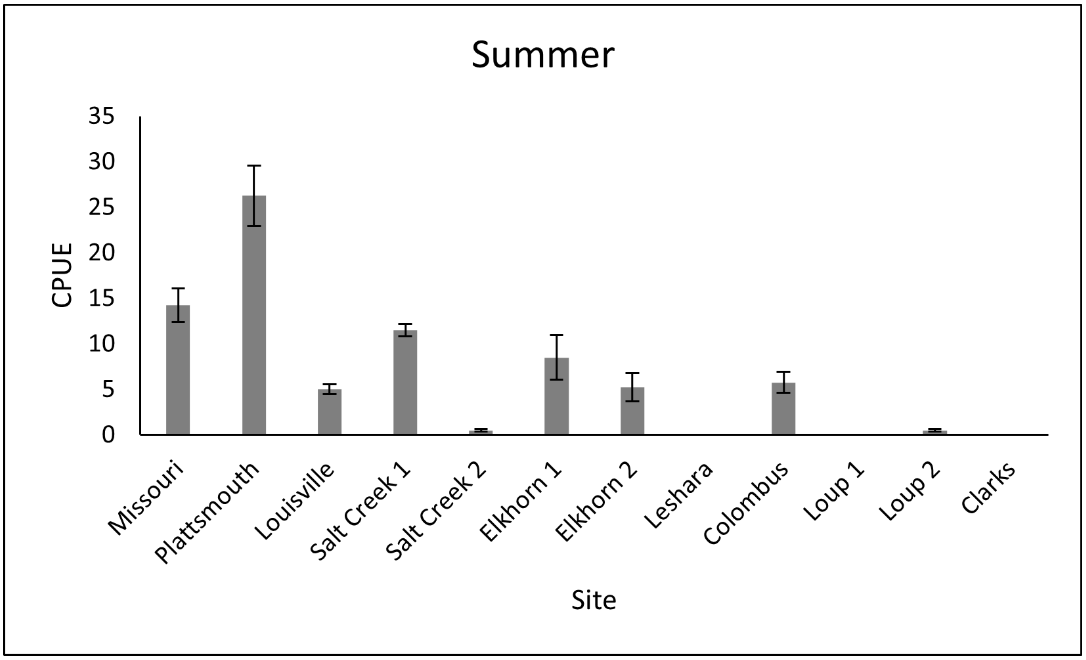

Figure 9: Catch per unit effort (CPUE) of Silver Carp during the summer sampling period.

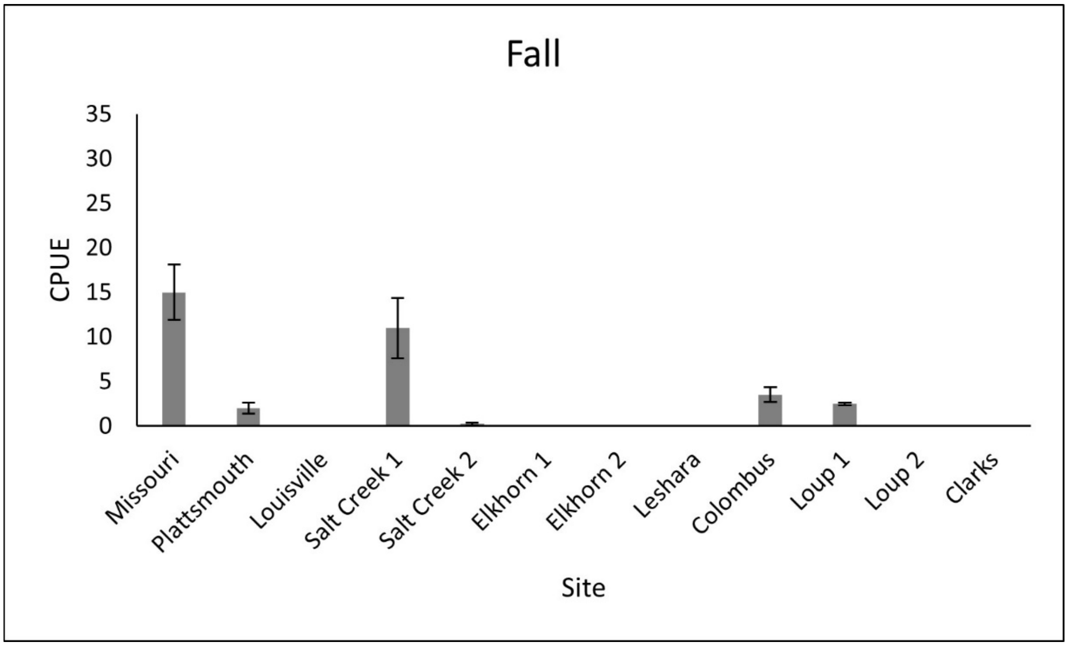

Figure 10: Catch per unit effort (CPUE) of Silver Carp during the fall sampling period.

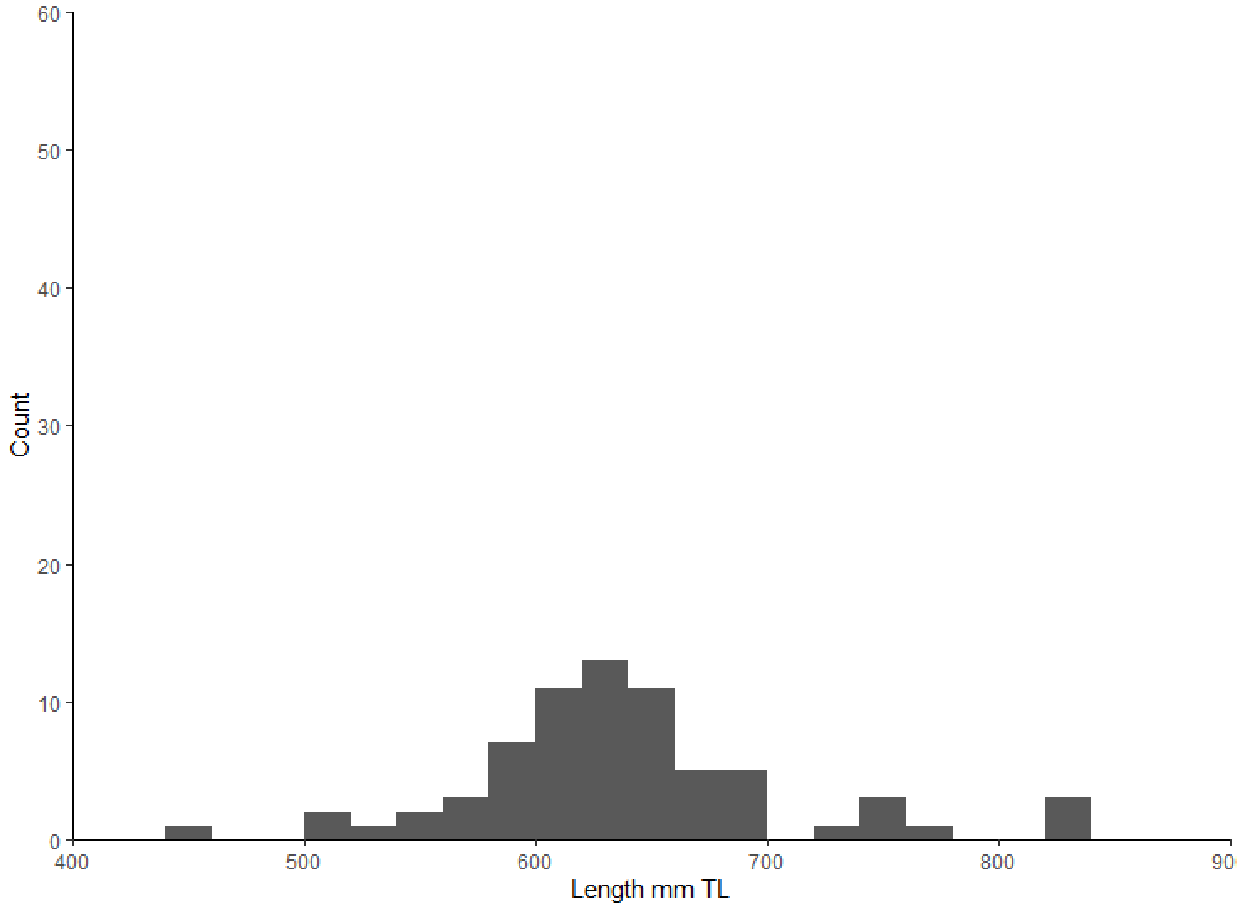

Figure 11: Length frequency histogram of Silver Carp in the Platte River during the spring sampling period (all sites combined).

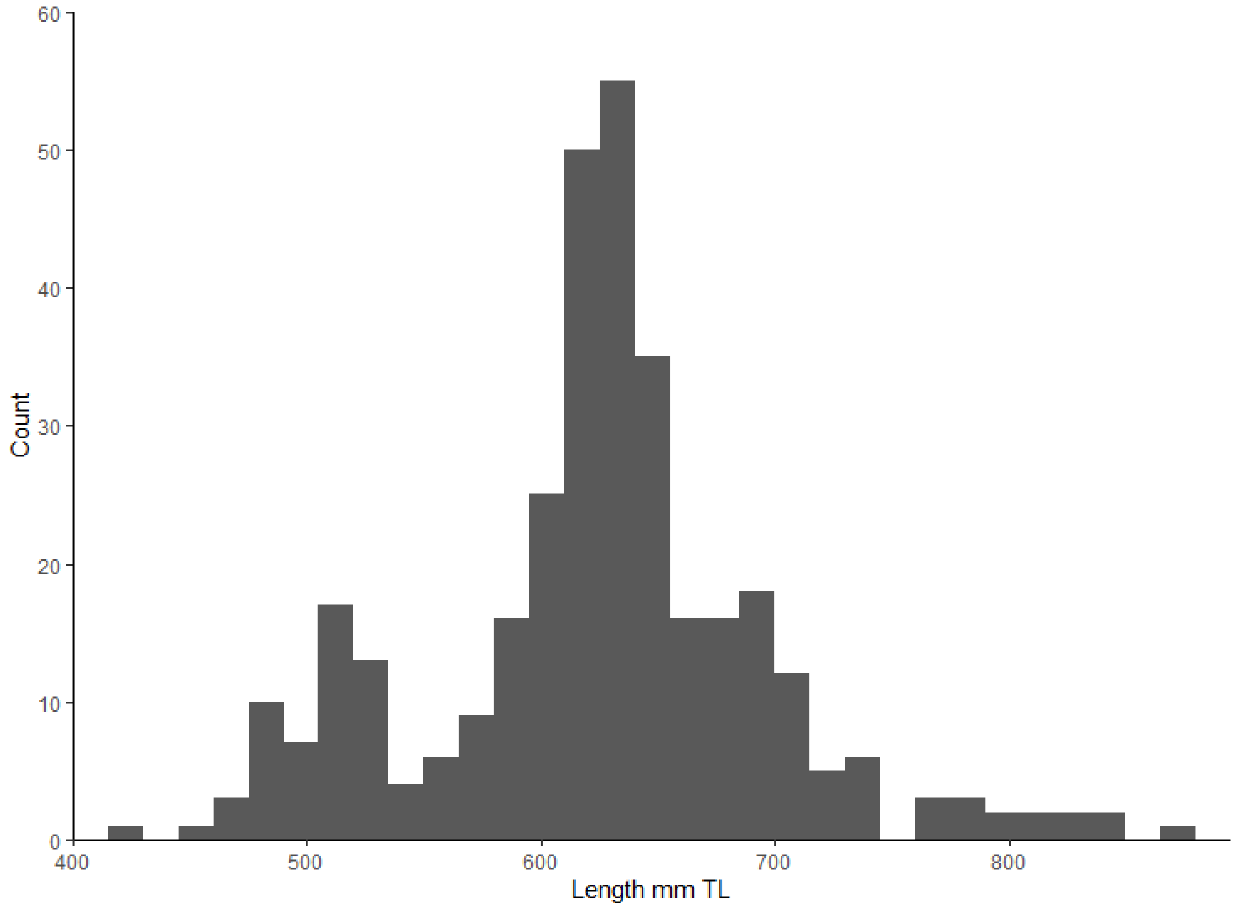

Figure 12: Length frequency histogram of Silver Carp in the Platte River during the summer sampling period (all sites combined).

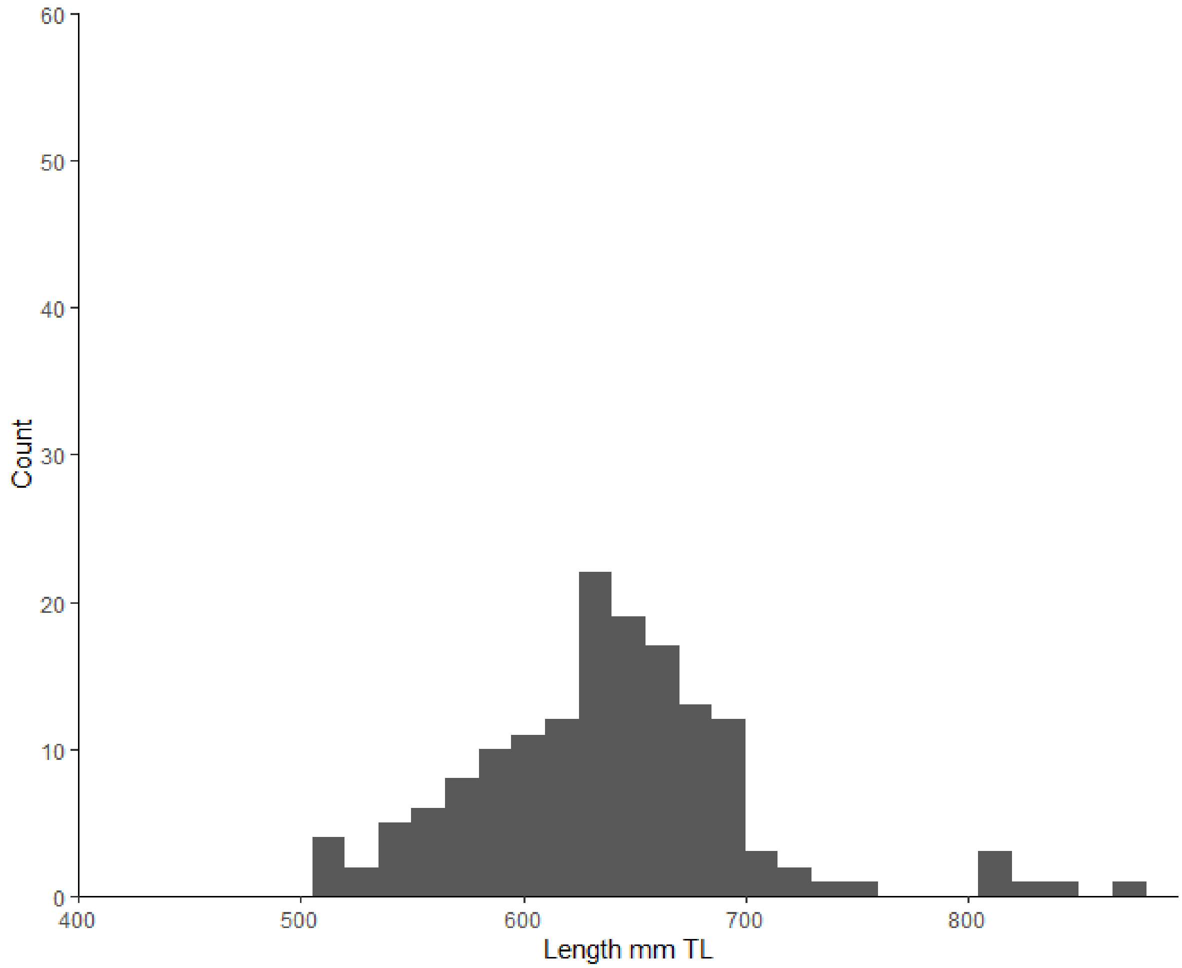

Figure 13: Length frequency histogram of Silver Carp in the Platte River during the fall sampling period (all sites combined).

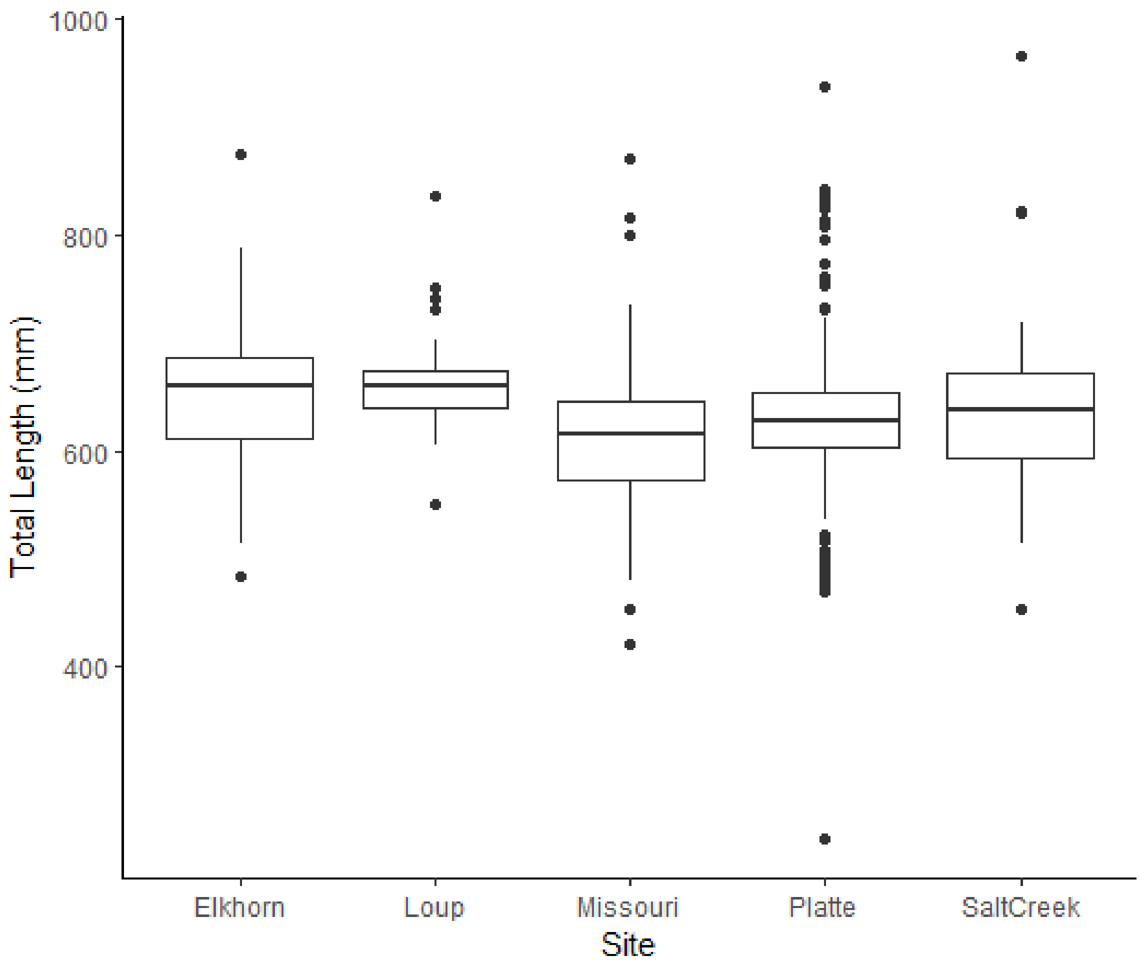

Figure 14: Box plot showing total length (mm) of silver carp captured in each stream.

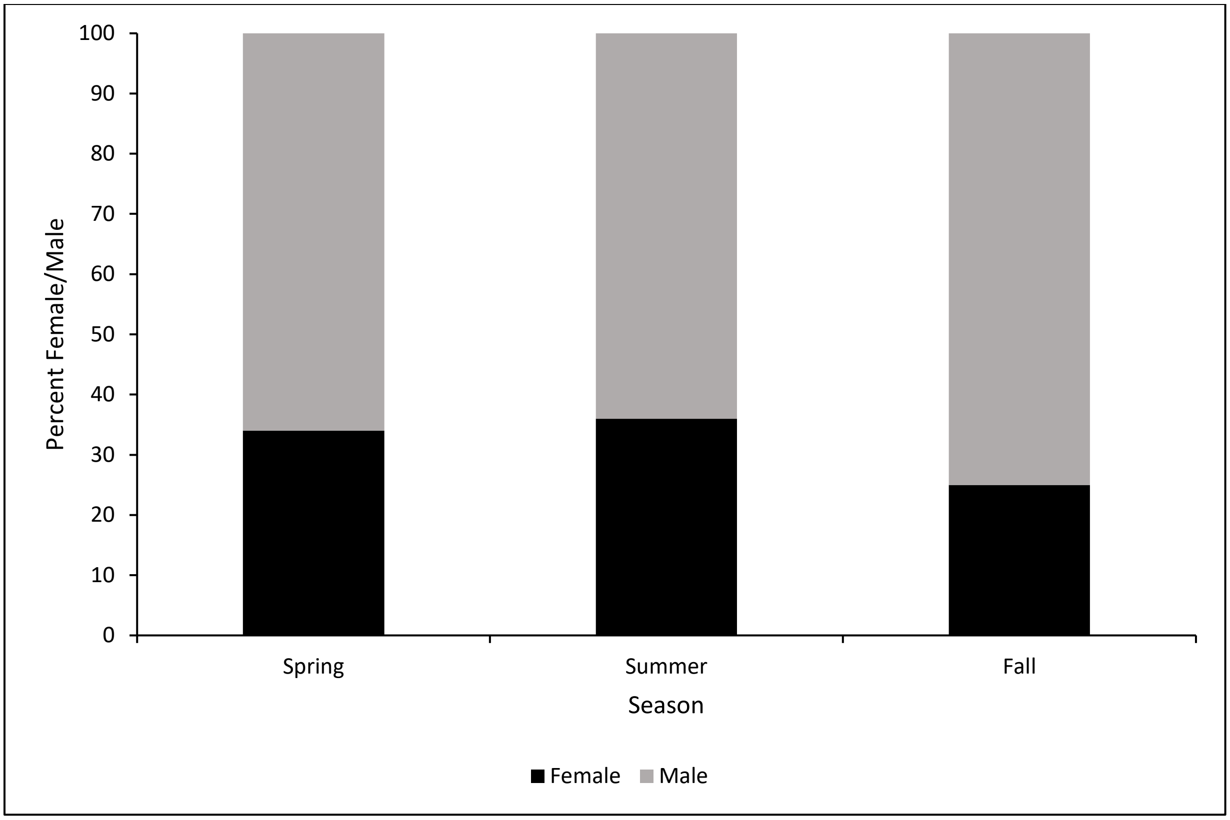

Figure 15: Percent female/male of Silver Carp in the Platte River basin. Statistical tests showed no difference between seasons.

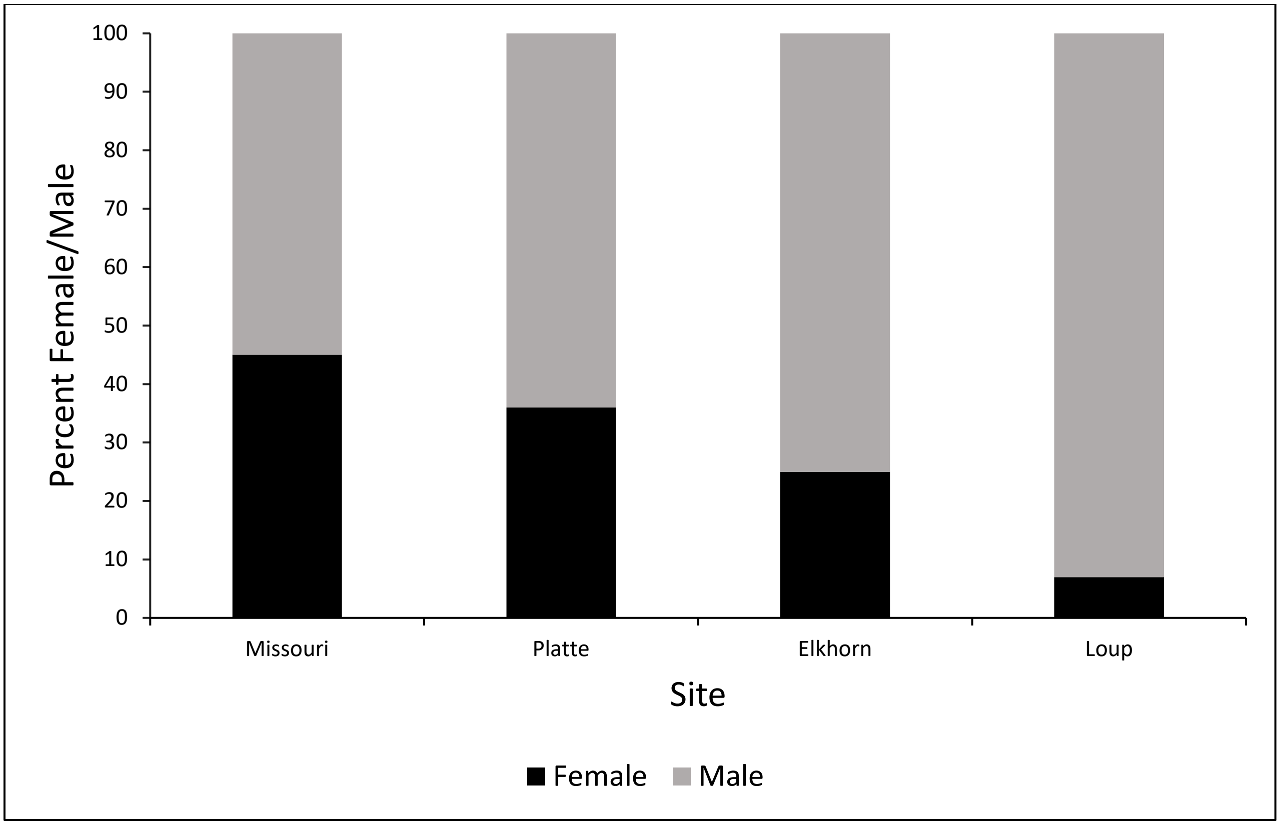

Figure 16: Percent female/male of Silver Carp in the Platte River basin. Statistical tests showed significant differences in sex ratios between the Missouri River and the Loup River.

Table 1. Larval sampling dates, sites, and river locations.

Date	Site	River
5/16	Louisville	Platte
5/17	Omaha	Missouri
5/19	Leshara	Platte
5/20	Waterloo	Elkhorn
5/23	Waterloo	Elkhorn
5/24	Omaha	Missouri
5/26	Plattsmouth	Platte
5/31	Waterloo	Elkhorn
6/1	Louisville	Platte
6/3	Leshara	Platte
6/14	Waterloo	Platte
6/15	Plattsmouth	Platte
6/16	Leshara	Platte
6/17	Omaha	Missouri
6/22	Louisville	Platte
6/24	Plattsmouth	Platte
6/27	Waterloo	Elkhorn
6/29	Leshara	Platte
7/5	Louisville	Platte
7/6	Plattsmouth	Platte
7/6	Omaha	Missouri
7/8	Waterloo	Elkhorn
7/11	Leshara	Platte
7/13	Louisville	Platte
7/14	Omaha	Missouri
7/14	Plattsmouth	Platte
7/18	Waterloo	Elkhorn
7/18	Leshara	Platte
7/19	Louisville	Plattte
7/22	Plattsmouth	Platte
7/22	Omaha	Missouri
7/27	Leshara	Platte
7/27	Waterloo	Elkhorn
8/1	Louisville	Platte
8/4	Plattsmouth	Platte
8/4	Omaha	Missouri
8/10	Louisville	Platte
8/17	Waterloo	Elkhorn

8/18	Leshara	Platte
8/19	Louisville	Platte
8/22	Omaha	Missouri
8/24	Plattsmouth	Platte

Table 2. Total fish count by species from seining.

Species	Total Count
Bigmouth Shiner	74
Bluegill	35
Brook Silverside	3
Bigmouth Buffalo	7
Channel catfish	4
Emerald Shiner	3342
Fathead minnow	5
Flathead chub	1
Freshwater Drum	8
Gizzard Shad	10
Goldeye	1
Grass Carp	2
Largemouth bass	1
Longnose Gar	3
Western Mosquitofish	27
Orange spotted sunfish	3
Quillback	2
Red Shiner	1811
River Carpsucker	39
River Shiner	330
Sand Shiner	746
Shoal Chub	11
Shortnose Gar	5
Silver Carp	3
Silver Chub	1
Spotfin Shiner	3
White Crappie	3
Total	**6468**

Table 3. Total fish counts by electrofishing.

Species	Count
Bigmouth Buffalo	8
Blue Catfish	1
Black Crappie	4
Bighead Carp	22
Bluegill	68
Bluegill X Green Sunfish	1
Bigmouth Shiner	34
Blue Sucker	69
Brook Silverside	3
Channel Catfish	387
Common Carp	221
Emerald Shiner	16
Fathead Minnow	3
Flathead Catfish	51
Flathead Chub	2
Freshwater Drum	70
Grass Carp	8
Goldeye	46
Green Sunfish	47
Gizzard Shad	109
Johnny Darter	1
Largemouth Bass	76
Longnose Gar	13
Orange Spotted Sunfish	1
Pallid Sturgeon	1
Quillback	106
River Carpsucker	448
Red Shiner	111
River Shiner	19
Smallmouth Buffalo	18
Silver Carp	600
Silver Chub	27
Suckermouth Minnow	2
Sand Shiner	26
Shortnose Gar	48
Shovelnose Sturgeon	40
Shorthead Redhorse	82
Sauger	12
Walleye	62

White Crappie	6
White Bass	5
White Perch	1
Western Killifish	1
White Sucker	3
Total	**2879**

References:

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State Report: Iowa

Agency: Iowa Department of Natural Resources (IA DNR) and Iowa State University (ISU)

Project title: Reproduction and recruitment of invasive carp in Missouri River tributaries

Methods:

Component 2.1: Assess timing and magnitude of invasive carp reproduction in the Little Sioux River using larval densities

We sampled ichthyoplankton at four sites along the Little Sioux River and nine additional sites across the Big Sioux River, Floyd River, and Boyer Rivers for a total of thirteen sites (Table 1; Figure 1). We selected sites to cover the upper, middle, and lower thirds of each river, except for the Little Sioux, which is longer and was divided into quarters. We separated all sites by at least 60 river kilometers to decrease the probability of resampling a single spawning event farther downstream. We collected three replicate ichthyoplankton samples at each site every ten days from April 27th until August 5th, 2022. Each sampling session lasted two consecutive days. We collected larval fishes with a conical ichthyoplankton net (0.5-m mouth diameter, 500-µm mesh) anchored to a bridge and held stationary in the current for approximately 4 minutes. Nets were aligned parallel to shore and facing upstream to capture drifting eggs and larvae. We only sampled the thalweg to accommodate low water levels and standardize sampling across the varied sizes of study rivers (wetted widths of sampling sites varied from 78.9 meters to 6.7 meters). A General Oceanics flowmeter was attached to the mouth of the net to record the water volume sampled (m^3) in each tow. We measured air temperature, water temperature, conductivity, and pH at each site with a dual temperature/conductivity YSI probe and a Hach HQ40D pH meter. We used the nearest upstream USGS or NOAA stream gauge to obtain daily river discharge. Twice in August we towed the net manually against the current when river discharge was too low to properly engage the flowmeter, once at the FLD-UPR site on August 5th, 2022 and once at the LSR-UPR site on August 4th, 2022. Furthermore, we did not sample the FLD-MID site on August 5th, 2022, as the thalweg depth was less than 0.5 of the net's diameter. After each tow, we rinsed net contents into the cod end, filtered it through a 500- µm sieve, and preserved the sample content in 95% ethanol.

In the lab, at least two individuals separated eggs and larval fishes from debris and stored them in glass scintillation vials for identification. After sorting, we categorized age-0 fishes as yolk-sac (presence of a yolk), larvae (yolk was completely resorbed), or juveniles (full set of developed fins). We classified embryos still encased in the egg membrane as eggs. In addition to larval stage classification, we identified all fishes to family or genus (when possible) using morphological landmarks such as yolk-sac characteristics, gut length, and myomere counts (Auer 1982; Lenat and Resh 2001). Finally, we classified invasive carp larvae as bigheaded carps (*Hypophthalmichthys molitrix* and *H. nobilis*), Grass Carp (*Ctenopharyngodon idella*), or Common Carp (*Cyprinus carpio*), following metrics provided by Chapman and George (2011) and Chapman (2006).

Component 2.2: Assess detection probabilities and spatial distribution of juvenile invasive carp throughout Missouri River tributaries in Iowa

We sampled fish assemblages at 36 stream sites across twelve HUC8 watersheds in the Missouri drainage from May – October 2022 (Figure 1). We selected sites ranging from 2nd – 5th order tributaries using a Generalized Random Tessellation Stratified (GRTS) sampling design (Stevens and Olsen 2004). Along with these randomly selected stream sites, we selected and sampled eight backwaters/side-channels along the Missouri River proper. In streams, we used both backpack electrofishing and seining to compare catches and assemblages between gears. We delineated each stream site (40 times the mean wetted width) and divided it into three equally spaced reaches separated by 50 m. We then divided reaches into two smaller sub-reaches, one for an electrofishing survey and the other for a seine haul.

Prior to fish sampling, we measured water temperature, dissolved oxygen, turbidity, pH, and conductivity using a Hach HQ40d multimeter and Hach 2100p turbidimeter. We conducted backpack electrofishing within each reach following methods similar to that of the Iowa Department of Natural Resources (2015). We used single backpack electrofishers for streams <5 m wide and two backpack electrofishers for streams >5 m. We aimed to achieve 2-5 amps of output and settings were set at 15% duty cycle at 60Hz with a voltage range of 150-250 volts, depending on conductivity and fish taxis effect (Bonar et al. 2009). We electrofished in an upstream direction ending at a block net or natural barrier. We captured stunned fishes with dip nets and placed them directly into buckets and/or live wells until the sample was completed. After the sample, we recorded effort in seconds, identified fish to species and enumerated them, and if caught, we recorded lengths and weights of invasive carp and preserved them in ethanol. After fishes were processed, we released them downstream of the electrofishing reach to avoid recapture. Following electrofishing, we conducted a seine haul (bag seine, 11 m x 1.8 m, 4.7 mm mesh) where we pulled the seine at the same speed or faster than the current in a downstream direction for 30 m to a block net or natural barrier. Once the haul was completed, we processed fishes in the same fashion as the electrofishing survey. We completed this process three times at each site for a total of three electrofishing replicates and three seine haul replicates.

In addition to sampling streams, we also sampled backwater/side channels to the Missouri River and its tributaries, as backwater areas may serve as nursery areas for juvenile invasive carp (Kolar et al. 2007). We sampled backwaters using similar methods as stream sites; however, we sampled with five replicates of each gear instead of three and we standardized backpack surveys to 30 m sections along the bank. Within each of the five reaches we also deployed one mini-fyke net (2’ x 4’ box, 21’ lead, ¼” delta mesh) and one clover leaf trap (28” x 15,” ¼” mesh) to further compare juvenile invasive carp sampling efficiency of multiple gears.

We measured physical stream habitat after fish sampling to avoid disturbance. We divided the length of each reach into four equally sized transects, for a total of twelve transects, and measured habitat parameters at the midpoint of each transect (Iowa Department of Natural Resources 2015). We measured wetted width to the nearest 0.1 m and depth, velocity, and substrate type at 10, 25, 50, 75, and 90% of the wetted width at each transect. We measured current velocity with a Marsh-McBirney velocity meter at 60% of the depth in locations less than 0.75 m and averaged between two measurements at 20 and 80% of the depths in locations greater than 0.75 m (Sindt et al. 2012). We measured instream fish cover (filamentous algae, aquatic macrophytes, woody debris/small brush, overhanging banks/vegetation, under-cut banks, boulders, artificial structure, and deep pool presence) 5 m upstream and downstream of each transect midpoint. We measured in-stream fish cover on a scale of 0 – 4 with 0 being absent, 1 being sparse (<10%), 2 being moderate (10-40%), 3 being heavy (40-75%), and 4 being very heavy (>75%) and we estimated percent bare stream bank (nearest 5%) on either side of the stream, and canopy cover in three directions using a spherical densiometer (Iowa Department of Natural Resources 2015).

Results and Discussion:

<u>Component 2.1:</u> Assess timing and magnitude of invasive carp reproduction in the Little Sioux River using larval densities

We conducted 426 larval tows across 13 sites on four rivers (Big Sioux, Little Sioux, Floyd, Boyer) during summer 2022 to assess invasive carp reproduction throughout western Iowa (Figure 1). To date, we have processed 4,988 larvae and 356 eggs from our samples with an additional 39 samples awaiting processing in the lab as of December 2022. We detected eggs and larvae at all sites, though not on all dates. Egg densities ranged from 0 to 220 eggs/100 m^3 while larval densities in individual tows ranged from 0 to 6,280 fish/100 m^3. The highest density of age-0 fishes in a single tow (6,280 fish/100 m^3) was recorded at the BSR-MID site on June 15th, 2022. In addition, this was the only sampling event where we captured age-0 invasive carp (approximately 4,500 yolk-sac and larval individuals). Of the carp identified thus far, 51.3% are genus *Hypophthalmichthys* (960 individuals) and 25.2% are *Ctenopharyngodon* (471 individuals). An additional 441 larvae could not be positively identified down to genus and are assigned broadly to "invasive carp".

All Bigheaded Carp larvae collected in the Big Sioux River ranged in age from approximately 3 days to less than 12 hours post-fertilization (Chapman and George 2011) that were likely spawned during a significant rise in flow from May 31st to June 1st (188 m^3/sec) and from June 13th to June 16th, 2022 (137 m^3/sec; Figure 3). Eggs and larvae were captured on the ascending arm of the hydrograph during this smaller, secondary peak in discharge. Water temperatures between May 31st and June 15th across the Big Sioux River sample sites increased by an average of 7.0°C to 23.6°C, 23.3°C, and 24.9°C at the upper, middle, and lower sites, respectively. We hypothesize this major temperature increase acted synergistically with elevated flows to initiate invasive carp reproduction.

The highest native fish larval density was 97.3 fish/100 m^3. Native taxa consisted overwhelmingly of *Cyprinidae* (253), followed by families *Catostomidae* (primarily genera *Ictiobus* and *Carpiodes*; 44), *Percidae* (42), *Centrarchidae* (genus *Pomoxis*; 2), and *Hiodontidae* (1). Native densities across all rivers but the Big Sioux peaked in mid to late June (Figure 4, 5). Native larval and yolk-sac fishes were captured at water temperatures between 12.7 and 31.0°C and discharges between <1 and 150 m^3/sec. Furthermore, conductivity ranged from 448 to 1,149 µS and pH varied from 7.89 to 9.84 during sessions when age-0 fishes were detected, indicating the tolerance of many native taxa to a wide range of environmental conditions. Larval fish taxonomic makeup and diversity tended to be homogenous between rivers. However, preliminary regression analyses suggest that family *Cyprinidae* is significantly associated with sites FLD-LWR and LSR-LWR, *Catostomidae* is associated with BSR-MID and BYR-MID, and *Percidae* is associated weakly with BYR-UPR and LSR-UPR, suggesting potential differences in assemblages within rivers. *Cyprinidae*, *Percidae*, and *Catostomidae* were detected in all rivers; *Hiodontidae* was only captured in the Big Sioux and *Centrarchidae* was only captured in the Floyd and Little Sioux rivers. We only detected three families in the Boyer River compared to four families in the Big Sioux, Floyd, and Little Sioux rivers (Figures 4, 5).

Late June, July, and August 2022 were characterized by high water temperatures and low water levels in the four river basins sampled. Drought conditions were likely unfavorable for invasive carp spawning and may have restricted the spawning activities of native taxa as well. The Big Sioux River is the largest (by width and volume) of the four rivers sampled and remains less channelized compared to the Little Sioux, Boyer, and Floyd Rivers. Though still impacted by the drought, the Big Sioux River consistently maintained the highest discharge of all four rivers during 2022 (Figure 6). It is therefore unsurprising we documented invasive carp reproduction in the Big Sioux River but not the other three, especially given the previous detection of young-of-the-year invasive carp in the lower Big Sioux (Hayer et al. 2014). Continued ichthyoplankton sampling in 2023 will further shed light on the extent of invasive carp reproduction in these river systems under various environmental conditions.

<u>Component 2.2:</u> Assess detection probabilities and spatial distribution of juvenile invasive carp throughout Missouri River tributaries in Iowa

We sampled fish communities at 36 river sites throughout western Iowa during 2022 to assess potential recruitment sources for invasive carp. We collected 36,403 individuals of 44 species during river sampling but did not detect juvenile invasive carp. We also sampled 8 off-channel sites along the Missouri proper where we detected juvenile invasive carp at three of these sites – a side-channel within Lower Hamburg Bend Conservation Area in Atkinson County, MO, a backwater in Big Bear Park in Thurston County, NE, and a backwater in Schilling Lake Wildlife Management Area in Cass County, NE during October. Among the three sites, we only collected seven juvenile Silver Carp (mean total length of 70 ± 12 mm SE). We collected five Silver Carp in two seine hauls while we collected one individual with backpack electrofishing and one in a mini-fyke net. We found all seven individuals in similar habitats where substrate was silty sand, depth was $\leq$ 0.5 m, there was little to no flow, little macrophytes and woody debris, and no canopy cover. We collected all juvenile Silver Carp within samples that had moderate to high abundances of Gizzard Shad (*Dorosoma cepedianum*). Our captures of juvenile Silver Carp only in backwaters and side channel habitats of the Missouri River and not in the tributaries is consistent with the hypothesis that invasive carp may preferentially use main river off-channel habitats for nursery areas (Kolar et al. 2007). However, habitat may have been limited in tributaries as water levels have been lower than average this year, especially July – October. We captured all juveniles late in the field season, suggesting that sampling late-summer through early-autumn may increase captures; however, we also only sampled off-channel habitats of the Missouri

River later in the field season. We hope with continued sampling efforts focusing on larger tributaries and off-channel habitats of the Missouri River, we will be better able to elucidate sources and drivers of invasive carp recruitment and identify key habitats for management.

In addition to juvenile invasive carp, we collected adult Silver Carp in the Little Rock River and the Tyson Bend backwater (IA). We also observed but were unable to capture adult Silver Carp at one site in the Boyer River, one site in the Big Sioux River, and the Decatur Bend backwater (IA), Louisville Bend backwater (IA), and Big Bear Park backwater (NE). We also caught adult Grass Carp in three rivers, including one site in Turkey Creek, one site in Wolf Creek Ditch, and one site in the Floyd River.

Table 1. The abbreviation of each Missouri River Basin ichthyoplankton sampling site, its associated river segment, and its latitude and longitude in Figure 1.

Site Code	**Site**	**Latitude**	**Longitude**
BSR-UPR	Big Sioux River upper	43.281397°	-96.578365°
BSR-MID	Big Sioux River middle	42.837555°	-96.561898°
BSR-LWR	Big Sioux River lower	42.626361°	-96.515384°
FLD-UPR	Floyd River upper	43.141773°	-95.887772°
FLD-MID	Floyd River middle	42.866099°	-96.109066°
FLD-LWR	Floyd River lower	42.519176°	-96.377367°
LSR-UPR	Little Sioux River upper	43.298773°	-95.176106°
LSR-UMD	Little Sioux River upper-mid	42.914653°	-95.339554°
LSR-LMD	Little Sioux River lower-mid	42.575503°	-95.726297°
LSR-LWR	Little Sioux River lower	42.081474°	-96.014105°
BYR-UPR	Boyer River upper	42.400283°	-95.156743°
BYR-MID	Boyer River middle	42.065665°	-95.344350°
BYR-LWR	Boyer River lower	41.678513°	-95.748249°

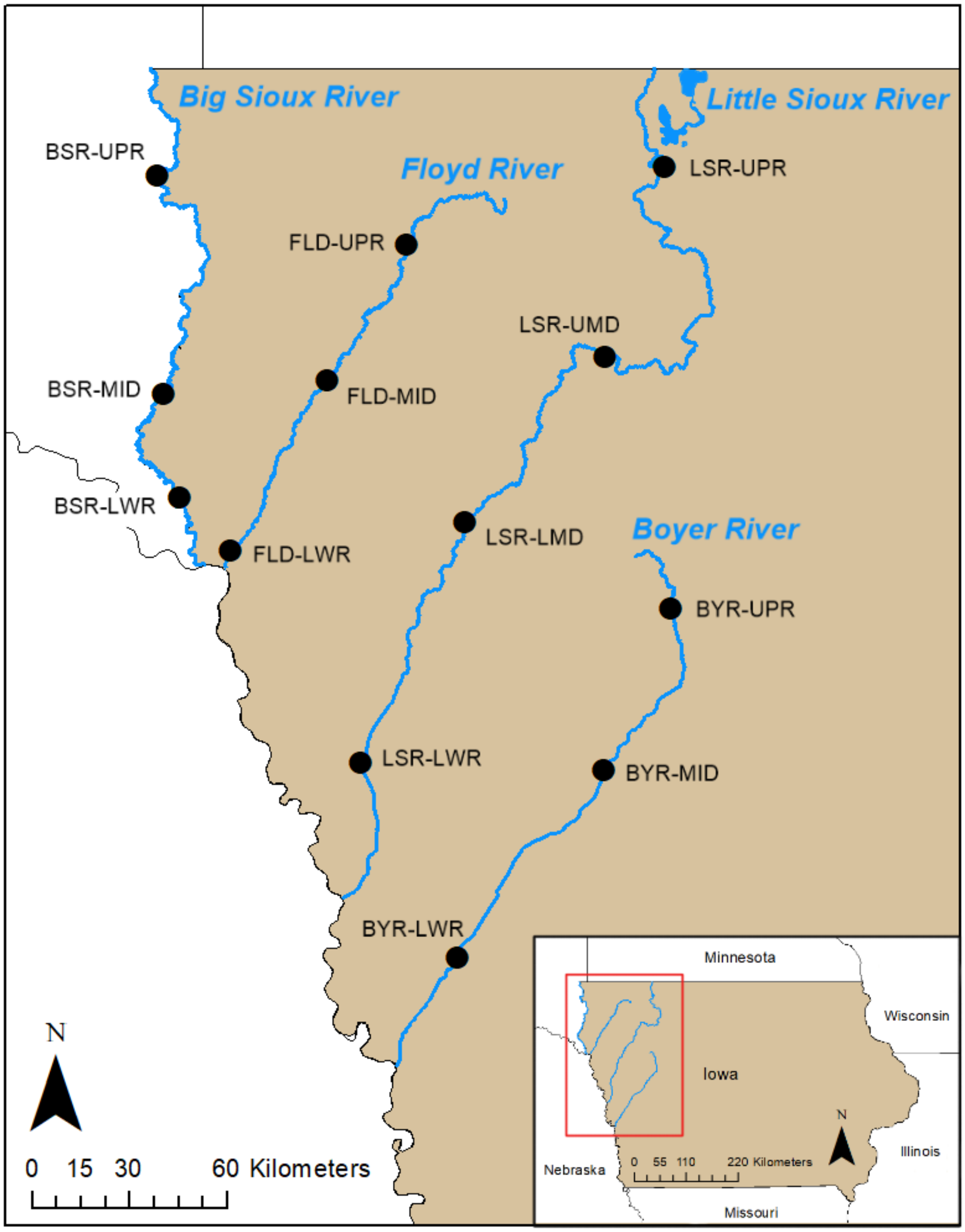

Figure 1. Locations of the thirteen sites on the Big Sioux, Floyd, Little Sioux, and Boyer Rivers in Northwestern Iowa where we sampled larval fishes during 2022. Sites were broadly selected to cover approximately equal sections of each river. See Table 1 for site abbreviation key and coordinates.

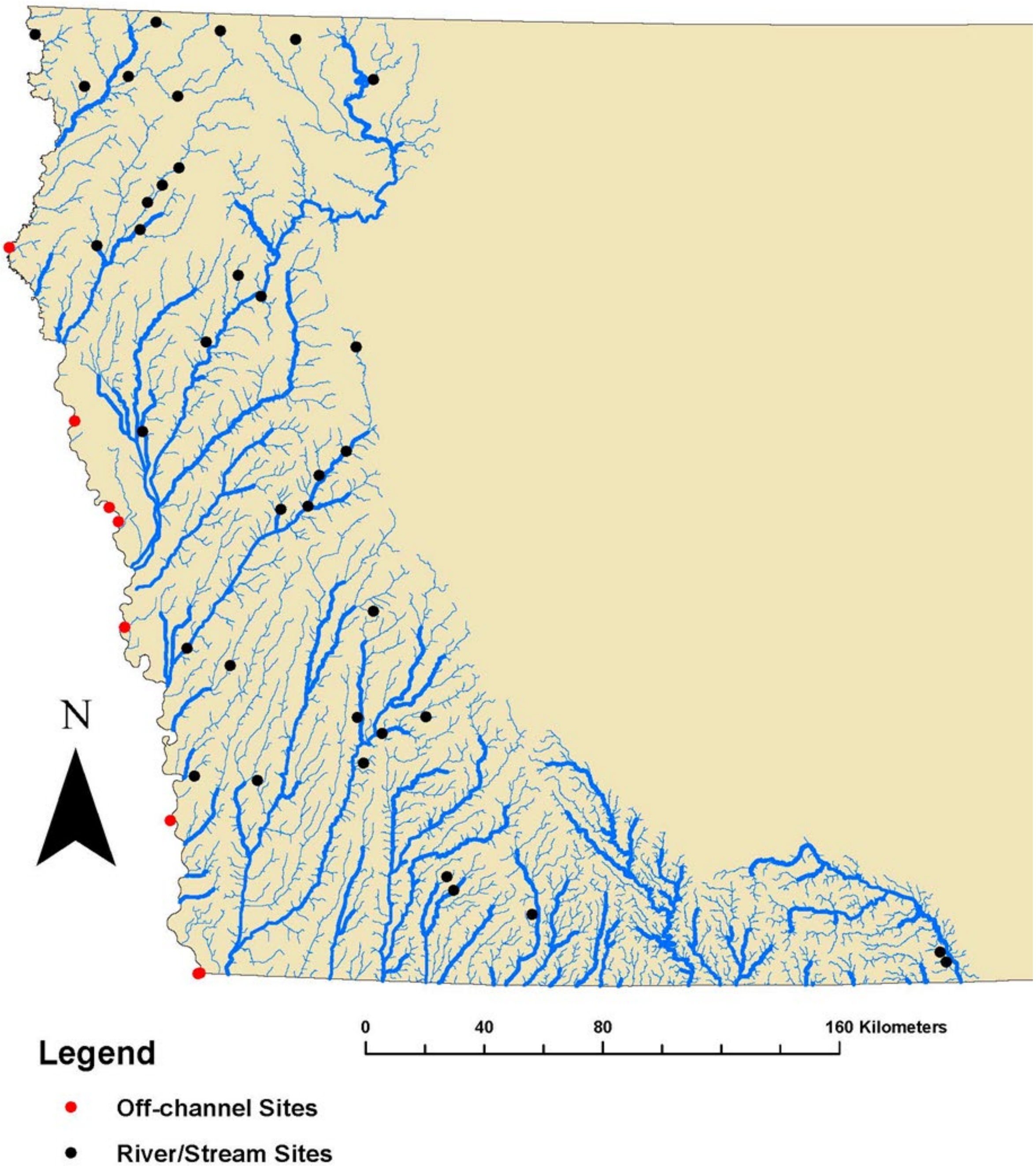

Figure 2. Locations of stream (n=36) and off-channel (n=8) sites in the Missouri River Basin where we sampled fish communities during 2022.

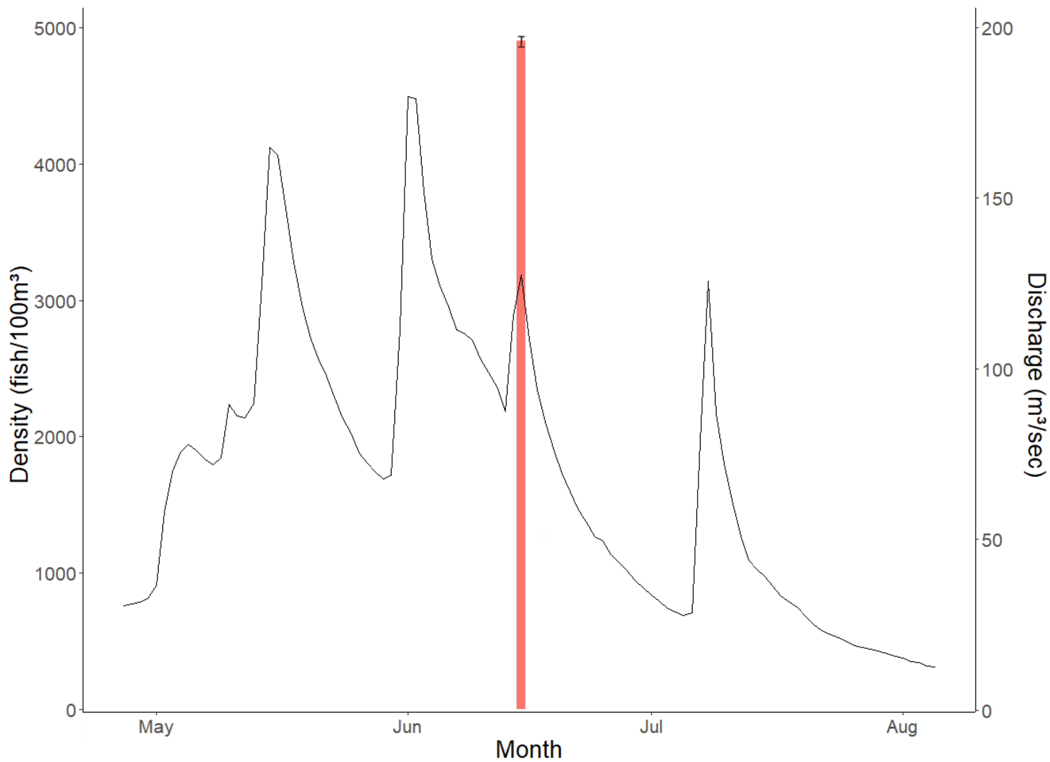

Figure 3. Larval and yolk-sac invasive carp densities (red bar; mean ± 95% CI) at the Big Sioux River middle site (BSR-MID) in relation to river discharge (m3/sec).

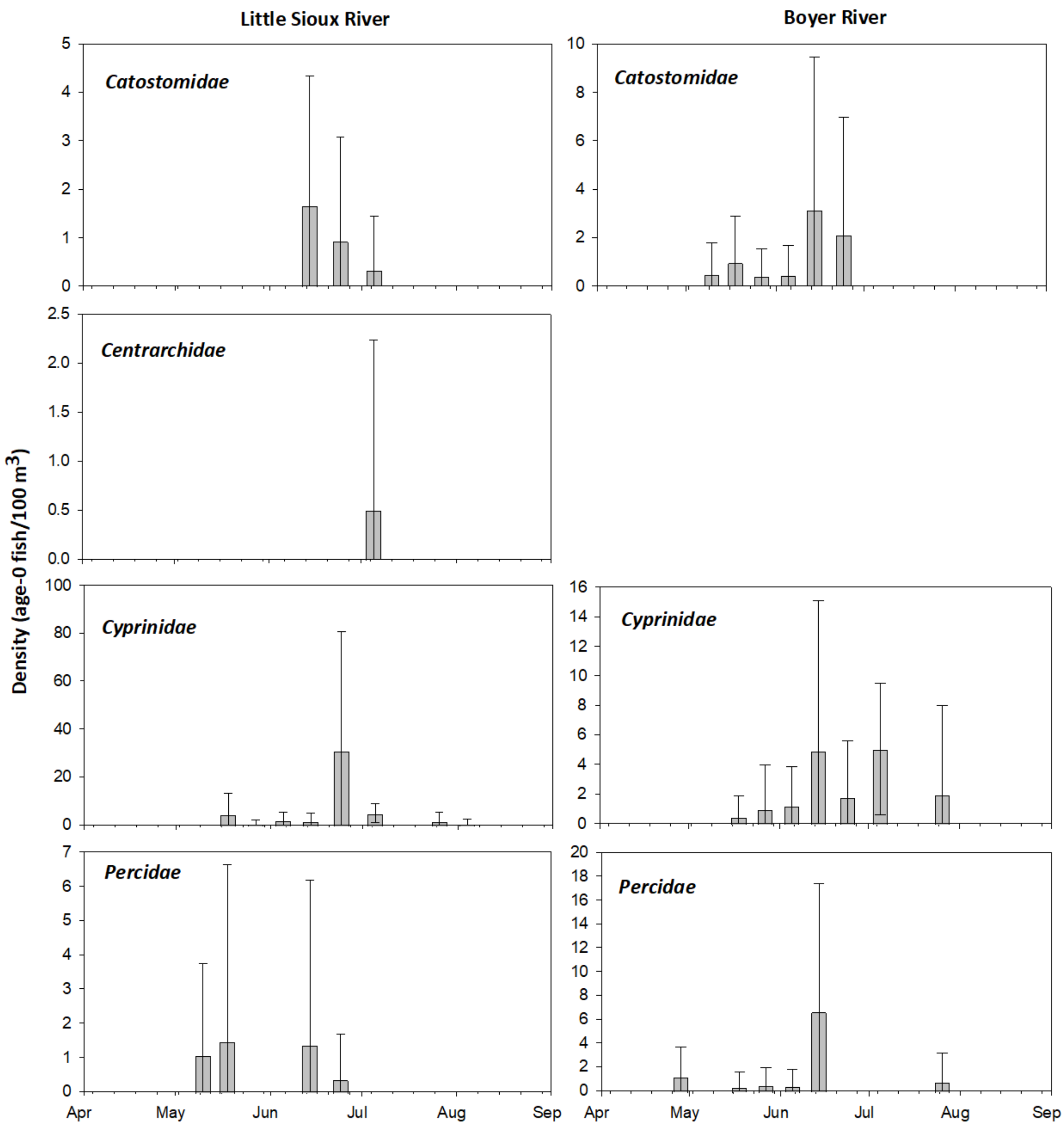

Figure 4. Density (mean ± 1 SE) of age-0 fishes from four families collected on the Little Sioux River (left) and Boyer River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

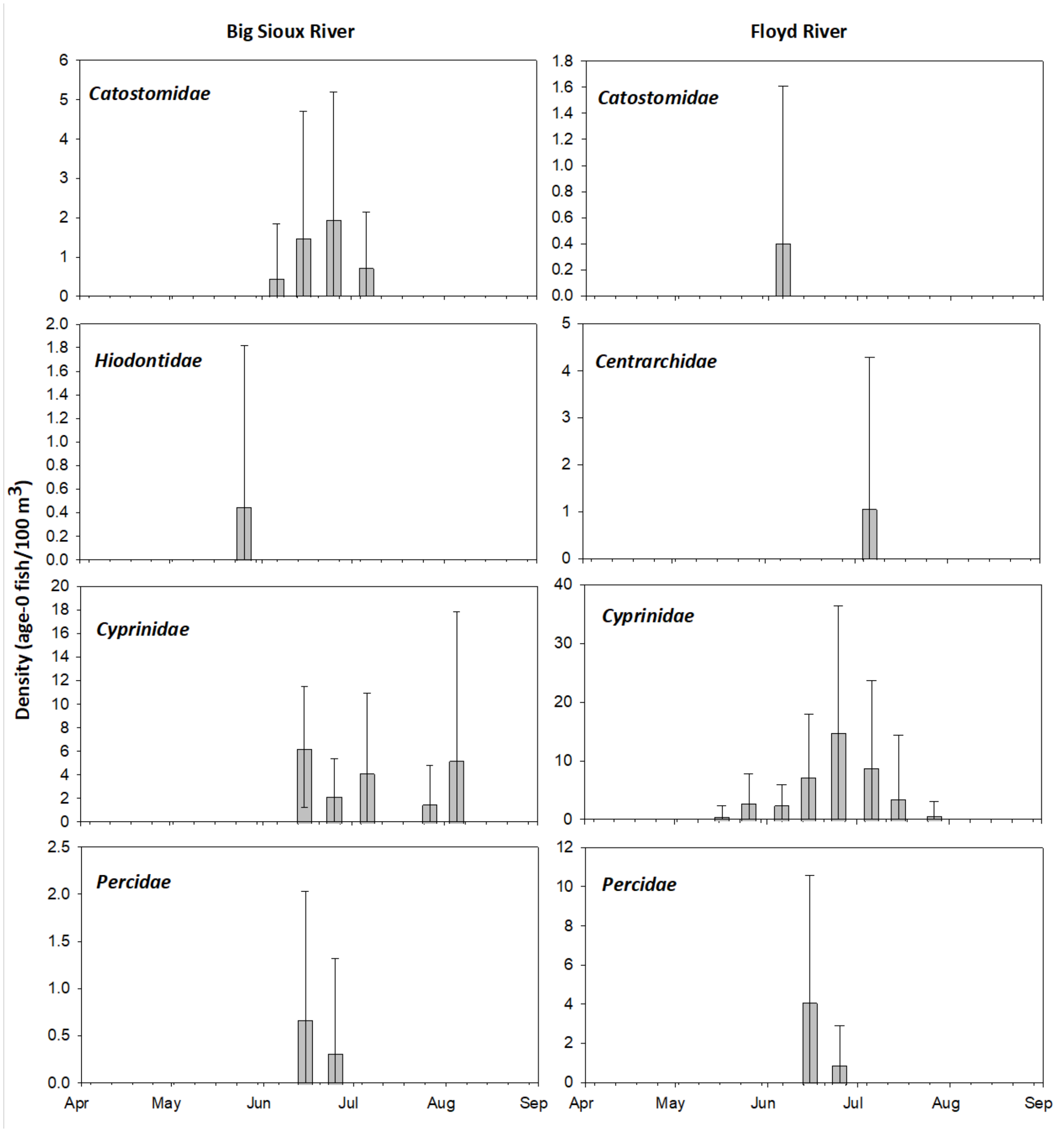

Figure 5. Density (mean ± 1 SE) of age-0 fishes from five families collected on the Big Sioux River (left) and Floyd River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

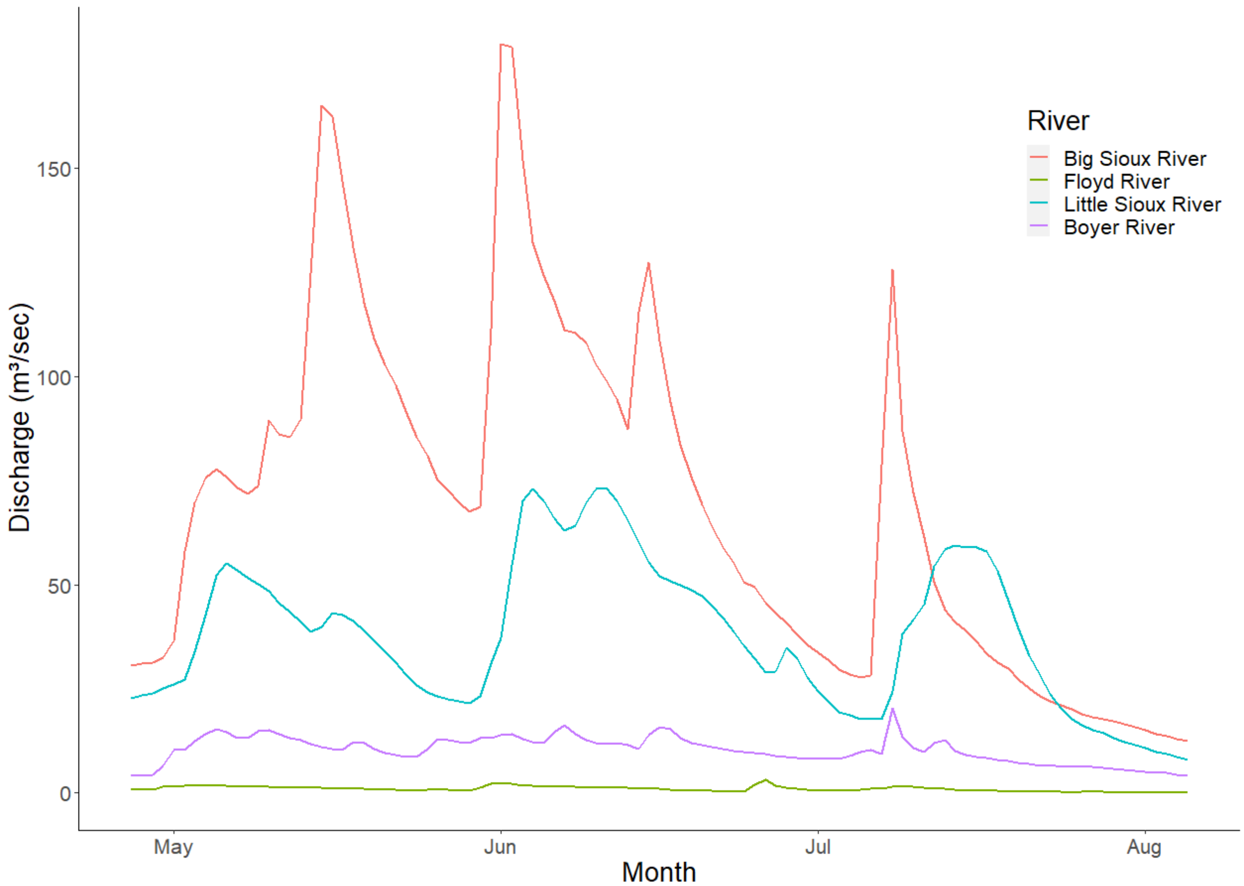

Figure 6. Discharge (m^3/sec) of the Big Sioux, Floyd, Little Sioux, and Boyer River from April 28th to August 5th, 2022.

State Report: Missouri

Agency: Missouri Department of Conservation (MDC)

Project Title: Define the population demographics of invasive carp populations and the associated fish community in the Missouri River Basin

Methods

Sampling for invasive carp took place from August to September. The lower 40 rkm of the four tributaries were separated into 10 rkm sampling units, although only the lower 20 rkm or less has been accessible during sampling the last three years. For sampling in the Missouri River at the mouth of each tributary the bend was used as the sampling unit.

Two frequency and duty cycle settings (60Hz and 40% duty cycle, 40Hz and 20% duty cycle) for electrofishing runs were tested in sample year 2022. 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., Hammen et. al. 2019, and Roth 2018), 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 a serpentine pattern going down stream. Boat drivers would swing out to the deeper side of the channel and then 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 two dip netters.

All species were measured in millimeters and weighed to the nearest gram. 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. Lapilli otoliths were collected from the first 100 individuals per tributary with no more than 20 structures collected from one run and retained for aging. Following the first 100 individuals, structures were collected on individuals unrepresented or underrepresented for each tributary and associated Missouri River bend. Whole lapilli otoliths were sanded until the otolith nucleus was reached, and polished until the focus and all annuli were visible. Otoliths were read by two independent readers with aging experience and then ages compared, and agreement reached.

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. Fifteen electrofishing runs were targeted for each tributary site. Settings reflected common fish community settings (60Hz 30% duty cycle) and used a MLES Infinity box with a 7000w generator, double hoop boat, and two 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 targeted. 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 target distance of 50m. Missouri River bend sampling was based on Welker and Drobish 2016. The same gears used in tributaries were used in the associated Missouri River bends. Eight mini-fyke nets were set in each Missouri River bend along with 10 electrofishing runs.

For invasive carp samples, catch per unit effort was calculated as number of fish per minute and extrapolated to fish per hour for electrofishing runs. Silver Carp catch rates, length groups and relative weights for native species were tested for normality ($P<0.05$). If treatment groups passed the normality test, a one way analysis of variance (ANOVA) was conducted and multiple pairwise comparisons were made using the Holm-Sidak method. When treatment groups failed normality test, analysis used a Kruskal-Wallis one way analysis of variance on ranks and when a significant difference in treatment groups was detected further analysis and multiple pairwise comparisons were made using Dunn's method. Analysis was conducted using SigmaPlot 14.5 by SYSTAT.

Relative stock densities were calculated for Silver Carp for each tributary and associated Missouri River bend. Minimum length specifications for Silver Carp were: stock = 250 mm; quality = 450 mm; preferred = 560 mm; memorable = 740 mm; trophy = 930 mm; as reported by Phelps and Willis (2013).

Sites

Four tributaries of the Missouri River with established populations of Silver Carp were selected for sampling included: 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 four tributaries had varying drainage areas with the Grand River having the largest (20,429 km^2) and the Nodaway the smallest (4,647 km^2). The lower 40 rkm were initially selected for sampling, however low water conditions in the late summer and fall in all tributaries reduced the sample area to 20 rkm or less. For sampling in the Missouri River, the bend at the mouth of each tributary was used as the sampling unit.

Results

Lamine River – Invasive Carp Sampling

Invasive carp sampling for Lamine River sites and the Missouri River bend at the Lamine was conducted the first week of August in the summer of 2022. A total of 43 electrofishing runs (13 - Missouri river bend, 15 - Lamine site 1, and 15 - Lamine site 2) and 3.76 gear hours of electrofishing resulted in 442 Silver Carp and 536 kg (1,182lbs) of fish. However, no other invasive carps were collected in sample year 2022. Power output for electrofishing in the Lamine River samples ranged from 3980w-8400w. Power output for electrofishing in the Missouri River samples ranged from 5100w-6620w. Both electrofishing settings produce variable catches. Results from a Kruskal-Wallis one way analysis of variance on ranks indicated Silver Carp catch rates were not significantly different between sites or settings ($P=0.073$) (Figure 1.)

Silver Carp lengths ranged from 387mm – 725mm across all Lamine sites, and all three sites showed a bimodal size distribution. Lamine River sites were dominated by fish from 430mm to 480mm. Silver Carp median lengths at Missouri River sites were significantly different ($P<0.001$) from Lamine River sites with larger individuals encountered at Missouri River sites utilizing both electrofishing settings (Figure 2). Stock size Silver Carp (250-449mm) comprised the highest proportion sampled over the last three years in the Lamine River (Figure 3). However, quality (450-559mm) and preferred (560-739mm) size classes accounted for most of the Silver Carp collected in the Missouri river bend at the mouth of the Lamine in 2022.

Fifty otoliths were collected from Silver Carp sampled from Lamine River sites and forty-six from the Missouri river bend at the mouth of the Lamine River. No Age-0 or Age-1 Silver carp were collected in

2022 and Silver Carp from age-2 to age-4 ranged in length from 387 to 506 mm (Figure 4). Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 0.8:1 while in the Lamine River sites it was 0.96:1.

Lamine River – Fish Community

Thirty electrofishing runs, 12 mini-fyke sets, and 4 otter trawls were conducted for all Lamine River sites. No sampling with otter trawls was conducted in Lamine River site 1 or the Missouri River bend site. Sampling resulted in a total of n = 1,259 fish (Missouri River = 429, Lamine site 1 = 235, Lamine site 2 = 595). Thirty-two species were sampled at the Lamine River sites (Lamine site 1 = 22, Lamine site 2 = 26) while 27 species were sampled at the associated Missouri river bend. Abundant species at Lamine site 1 included Gizzard Shad (29.4%), Silver Carp (15.7%), Freshwater Drum (7.6%), Silver Chub (7.2%), Green Sunfish (5.9%), Bluegill (5.5%), and Shortnose Gar (5.5%). At site 2 dominant species included Bluegill (24%), Creek Chub (16.8%), Freshwater Drum (16.6%), Gizzard Shad (11.9%), and Shortnose Gar (8.7%). Sampling in the Missouri river at the mouth of the Lamine resulted in Red Shiner (24.2%), Gizzard Shad (22.1%), Emerald Shiner (20.3%) and Silver Carp (14.9%) as the highest proportion of the fish sampled and remaining species comprising less than 5% of the catch (Table 2).

Relative weights of River Carpsucker were lower in the Lamine River compared to those sampled in the Missouri river bend at the Lamine, although not significantly different (P=0.106) (Figure 5).

Grand River – Invasive Carp Sampling

Invasive carp sampling for Grand River sites was conducted the second, third and fourth weeks of August in 2022. Forty-eight electrofishing runs (16 in the Missouri River bend, 20 Grand River site 1, 12 in Grand River site 2) and 4.53 electrofishing gear hours resulted in 245 Silver carp and 8 Grass carp and 336 kg (740lbs) of fish. Power output for electrofishing runs in the Grand River ranged from 4800w-7105w, while power output for the associated Missouri River bend ranged from 5600w-6435w. Results from a Kruskal-Wallis one way analysis of variance on ranks indicated Silver Carp catch rates were not significantly different between sites or electrofishing settings (P=0.804) (Figure 1).

Silver Carp lengths ranged from 381mm to 686mm for all sites combined. All sites displayed a bimodal length frequency distribution. However, electrofishing at the Missouri River bend associated with the Grand River yielded more Silver Carp over 600mm and Silver Carp median lengths at Missouri River sites were significantly different (P<0.001) from Grand River sites with larger individuals at Missouri River sites utilizing both electrofishing settings (Figure 2). Stock size (250-449mm) Silver Carp comprised the highest proportion collected from the Grand River in 2022 (Figure 3). However, the majority of Silver Carp sampled from the Missouri river bend were of quality (450-559mm) and preferred (560-739mm) size classes.

One hundred three otoliths were collected from Silver Carp at the Missouri river bend at the mouth of the Grand River. One hundred six otoliths were collected from Silver Carp at the Grand River sites. No age-0 or age-1 Silver carp were collected in 2022 and Silver Carp from age-2 to age-4 ranged in length from 381 to 582 mm (Figure 4).

Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 1.1:1 while in the Grand River sites it was higher at 1.3:1. Silver Carp lengths between male and females were not significantly different within sites, however, lengths of males and females were significantly different (P<0.001) between Silver Carp collected in the Missouri River bend and Grand River sites, although this is probably a factor of overall larger individuals collected in the Missouri River bend at the Grand River.

Eight Grass Carp were collected at Grand River sites in sample year 2022. Two Grass Carp measuring 492mm and 591mm were captured in the Missouri River bend at the Grand River. The remaining six Grass Carp were captured in the Grand River site 2 with sizes ranging from 475mm to 794mm with a mean length of 604mm.

Grand River – Fish Community

Thirty electrofishing runs, 6 mini-fyke net, and 12 otter trawl samples were collected in the Grand River sites. Ten electrofishing runs and 8 mini-fyke net samples were collected in the associated Missouri River bend. This resulted in a total of 3,164 fish (Missouri River bend= 816, Grand River site 1 = 2,049, and Grand River site 2 = 299). Twenty-seven species were sampled in the Grand River (Site 1 = 19 and Site 2 = 22). The Missouri River bend sampling resulted in 22 species. Missouri River samples were dominated by Freshwater Drum (81.7%). Similarly, Grand River site 1 fish community was also dominated by Freshwater Drum (89.3%). Grand River site 2 abundant species included Channel Catfish (46.2%), Blue Catfish (11.4%), Shortnose Gar (7.4%), and Green Sunfish (6%) with remaining species comprising less than 5% of the catch.

Bigmouth Buffalo mean relative weights from the Grand River were significantly lower (P<0.05) than those collected from the Nodaway River (Figure 5). River Carpsucker relative weights in the Grand River were also lower than other sites but not significantly different (P=0.106). Gizzard Shad relative weights from the Grand River were not significantly different from other sites (P=0.443). Relative weight of Paddlefish was low in the Grand River, however the sample size was small and there were no other specimens weighed at other sites.

Platte River – Invasive Carp Sampling

Invasive carp sampling for Platte River sites was conducted the first three weeks of August in 2022. Fifty-eight electrofishing runs (20 in the Missouri river bend, 20 at site 1, and 18 at site 2) and 2.92 electrofishing gear hours resulted in 770 Silver Carp, and 2 Grass Carp amounting to 1,435kg (3,164lbs) of fish. No Bighead or Black Carp were sampled. Power for Platte River runs ranged 3990w – 5600w while power for the associate Missouri river bend was 2640w-3640w. Silver Carp mean catch per unit effort at the Platte River sites (307.88 – 349.89 fish/hour) was significantly higher (P<0.001) using both electrofishing settings than the Missouri River bend site using 40pps/20Hz (44.69 fish/hour) (Figure 1). However, mean catch rates were not significantly different between both settings at the Missouri River bend site (P=0.193).

Silver Carp lengths ranged from 390mm to 827mm for all sites combined (Figure 2). All sites displayed a bimodal length frequency distribution. Median Silver Carp length (604mm) at the Missouri River bend using 60pps/ 40Hz was significantly different (P<0.05) from median lengths at Platte River sites (502-580mm) with both settings. However, lengths were not significantly different between settings at the Missouri River bend site (P=1.00). Quality (450-559mm) and preferred (560-739mm) size class Silver

Carp accounted for the majority collected from the Platte in 2022 (Figure 3). Preferred (560-739mm) size class Silver Carp accounted for the highest proportion sampled at the Missouri river bend associated with the Platte and no stock size (250-449mm) individuals were collected.

One hundred twenty otoliths were collected from Silver Carp in the Platte River sites. Four otoliths were collected from Silver Carp in the Missouri River bend at the mouth of the Platte River. No age-0 or age-1 Silver Carp were collected in 2022 and Silver Carp from age-2 to age-4 ranged in length from 390 to 609 mm (Figure 4). Silver Carp mean length at age differed between Platte River sites and Missouri River bend site, but this is probably due to the low number of otoliths collected from the Missouri River bend site. Sex ratios (Male: Female) in the Missouri river site was 1.5:1 compared to 0.8:1 in the Platte River sites combined.

Platte River – Fish Community

Thirty electrofishing runs, 6 mini-fyke net, and 12 otter trawl samples were completed in the Platte River sites. Ten electrofishing runs and 8 mini-fyke net samples were completed in the associated Missouri river bend. This resulted in 868 fish sampled (Missouri river = 281, Platte River site 1 = 214, and Platte River site 2 = 373). Twenty-two species were sampled in the Missouri river at the mouth of the Platte River. Twenty-three species were sampled at the Platte River sites (Site 1 = 20, Site 2 = 19). Abundant species in the Missouri River sample were Emerald Shiner (31%), Sand Shiner (20.3%), Freshwater Drum (16.4%), and Red Shiner (9.3%). Platte River site 1 fish community included Channel Catfish (23.8%), Freshwater Drum (18.7%), Blue Catfish (16.8%), Silver Carp (14.5%), and Gizzard Shad (6.5%). The Platte River site 2 samples included Freshwater Drum (42.1%), Blue Catfish (15.5%), Channel Catfish (9.1%), and Silver Carp (7.8%) with remaining species comprising less than 5% of the catch (Table 2).

River Carpsuckers collected in the Platte had the highest mean relative weight, although not significantly different from other sites (P=0.106) (Figure 5).

Nodaway River – Invasive Carp Sampling

Invasive carp sampling at Nodaway River sites was conducted the second and third weeks of August in 2022. Thirty-seven electrofishing runs (20 in the Missouri river and 17 at Nodaway River site 1) and 3.06 gear hours resulted in 230 Silver Carp and 3 Bighead Carp with a total weight of 415 kg (915lbs). Power output for the associated Missouri River bend samples ranged from 4200w - 5032w. Power output for the Nodaway River runs ranged 1904w - 4598w. Silver Carp catch per unit effort was not significantly different between sites or electrofishing settings (P=0.360) (Figure 1).

Silver Carp lengths ranged from 397mm to 780mm for all sites (Figure 2). All sites and electrofishing settings resulted in a bimodal distribution. Silver Carp length frequencies were not significantly different between sites or electrofishing settings (P=0.059). Preferred (560-739mm) size class Silver Carp accounted for the highest proportion from the Nodaway River in 2022 (Figure 3). However, in 2020 and 2021 stock size (250-449mm) Silver Carp comprised the highest proportion. Quality size (450-559mm) Silver Carp comprised the highest proportion at the Missouri river bend in 2022.

Ninety-five Silver Carp otoliths were collected from the Missouri river at the mouth of the Nodaway River. One hundred two otoliths were collected for aging from Silver Carp in the Nodaway River site. No age-0 or age-1 Silver Carp were collected in 2022 and Silver Carp from age-2 to age-4 ranged in length from 397 to 543 mm (Figure 4). Sex ratios (Male: Female) of Silver Carp in the Missouri River was 1.1:1 and 1:1 in the Nodaway River.

Three Bighead Carp were captured in sample year 2022 and all were captured at the Nodaway River site 1. All three were identified as females ranging in size from 537m to 618mm. Otoliths were collected for aging from two of the Bighead Carp with one individual measuring 537mm at age-4 and one at 592mm at age-5.

Nodaway River - Fish Community

Ten electrofishing runs, 3 mini-fyke net, and 3 otter trawl samples were completed in the Nodaway River site. Ten electrofishing runs and 8 mini-fyke net samples were completed in the associated Missouri river bend. Sampling resulted in 2,330 fish (Missouri river = 1,256, Nodaway River site 1 = 1,074). Twenty-six species were collected in the Missouri river samples and 33 were collected in the Nodaway River samples. Abundant species in the Missouri river samples were Emerald Shiner (32.5%), Sand Shiner (15.8%), River Shiner (13.5%), Freshwater Drum (8.1%), and Red Shiner (6.2%). Nodaway River species were dominated by Freshwater Drum (51.7%) followed by Emerald Shiner (8.8%), Silver Carp (6.9%), and Channel Catfish (5%) with remaining species comprising less than 5% of the catch (Table 2).

Nodaway River Bigmouth Buffalo mean relative weights were significantly higher ($P<0.05$) than individuals collected in the Grand River (Figure 5). River Carpsuckers relative weights were similar to other sites.

Discussion

Silver Carp was the overwhelmingly dominant invasive carp species in all tributaries and their associated Missouri River bends. Catches of Silver Carp were variable for each group of settings being evaluated. Initial results do not suggest that there is a difference in catch per unit effort between the two regardless of site or waterbody, although anecdotally 60Hz/40% duty cycle tends to elicit more of a jumping response than perhaps 40Hz/20% duty cycle which could lead to more fish jumping in the boat. Both settings are probably suitable options for sampling Silver Carp.

Ten Grass Carp and three Bighead Carp were also collected in sample year 2022. Bighead Carp and Grass Carp relative abundance may be underestimated with standard boat electrofishing. This could be due to the amount of Silver Carp jumping while electrofishing, decreasing the netter’s ability to catch Bighead or Grass Carp. In the case of Bighead Carp, they also might be occupying deeper areas not affected by standard electrofishing techniques. Additional sampling with gill nets may provide a better estimate of relative abundance for Bighead and Grass Carp while at the same time collect more robust data of native species such as Paddlefish and Bigmouth Buffalo in competition with invasive carp.

Size distributions and length at age data of Silver Carp in 2022 provide evidence to limited reproduction over the last two years in the four tributaries and associated Missouri River bends. This may be due to low river levels in the lower Missouri River basin witnessed these past two years.

Studies such as this to collect and examine invasive carp population demographic information in known areas of occurrence are paramount to better plan for control, removal, and management efforts. Also, a better understanding of the efficiencies of electrofishing settings 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.

Recommendation

Continue to collect invasive carp population demographic data in the Lamine, Grand and Platte Rivers in addition to sampling the associated Missouri River bend at the confluence of each tributary. The Nodaway River is not scheduled to be sampled next sampling season due to the fact only a small portion can be accessed. Also, consideration for future sampling may include one random or fixed mainstem Missouri River bend in proximity to each tributary but geographically distanced to gather abundance data outside the potential influence of a tributary.

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Table 1. Invasive Carp sampling effort and catch results for each site.

Site[1]	# of Samples	40Hz 20% Mean CPUE (± S.E.)[2]	60Hz 40% Mean CPUE (± S.E.)[2]	Total # Silver Carp	Total Weight kilograms(lbs)	Male : Female
MOLR	13	102.3 (50.9)	117.2 (127.1)			0.8 : 1
LR1	15	109.7 (72.5)	63.4 (37.9)	437	530 (1,168)	1 : 1
LR2	15	158.7 (36.6)	147.9 (78.5)			
MOGR	16	62.6 (27.8)	63.6 (38.6)			1.1 : 1
GR1	20	46.3 (23.1)	43.0 (23.1)	245	318 (701)	
GR2	12	46.6 (49.5)	59.1 (39.8)			1.3 : 1
MOPR	20	44.7 (36.5)	168.2 (95.5)			1.5 : 1
PR1	20	340.5 (98.1)	336.5 (79.7)	770	1,430 (3,153)	
PR2	18	307.9 (128.8)	349.9 (93.8)			0.8 : 1
MONR	20	72.0 (68.3)	56.4 (65.5)	230	408 (899)	1.1 : 1
NR1	17	44.4 (30.0)	147.5 (122.1)			1 : 1

[1]MOLR = Missouri River at mouth of Lamine River, LR1 = Lamine River site 1, LR2 = Lamine River site 2, MOGR = Missouri River at mouth of Grand River, GR1 = Grand River site 1, GR2 = Grand River site 2, MOPR = Missouri River at mouth of Platte River, PR1 = Platte River site 1, PR2 = Platte River site 2, MONR = Missouri River at the mouth of the Nodaway River, NR1 = Nodaway River site 1.
[2]Catch per unit effort = # Silver carp/hr.

Table 2. Fish Community 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
MOLR	4	10	--	429	27
LR	8	20	4	830	32
MOGR	8	10	--	816	22
GR	6	30	12	2,348	27
MOPR	8	10	--	281	22
PR	6	30	12	587	23
MONR	8	10	--	1,256	26
NR	3	10	3	1,074	33

[1]MOLR = Missouri River at mouth of Lamine River, LR = Lamine River, MOGR = Missouri River at mouth of Grand River, GR = Grand River, MOPR = Missouri River at mouth of Platte River, PR = Platte River, MONR = Missouri River at the mouth of the Nodaway River, NR = Nodaway River.

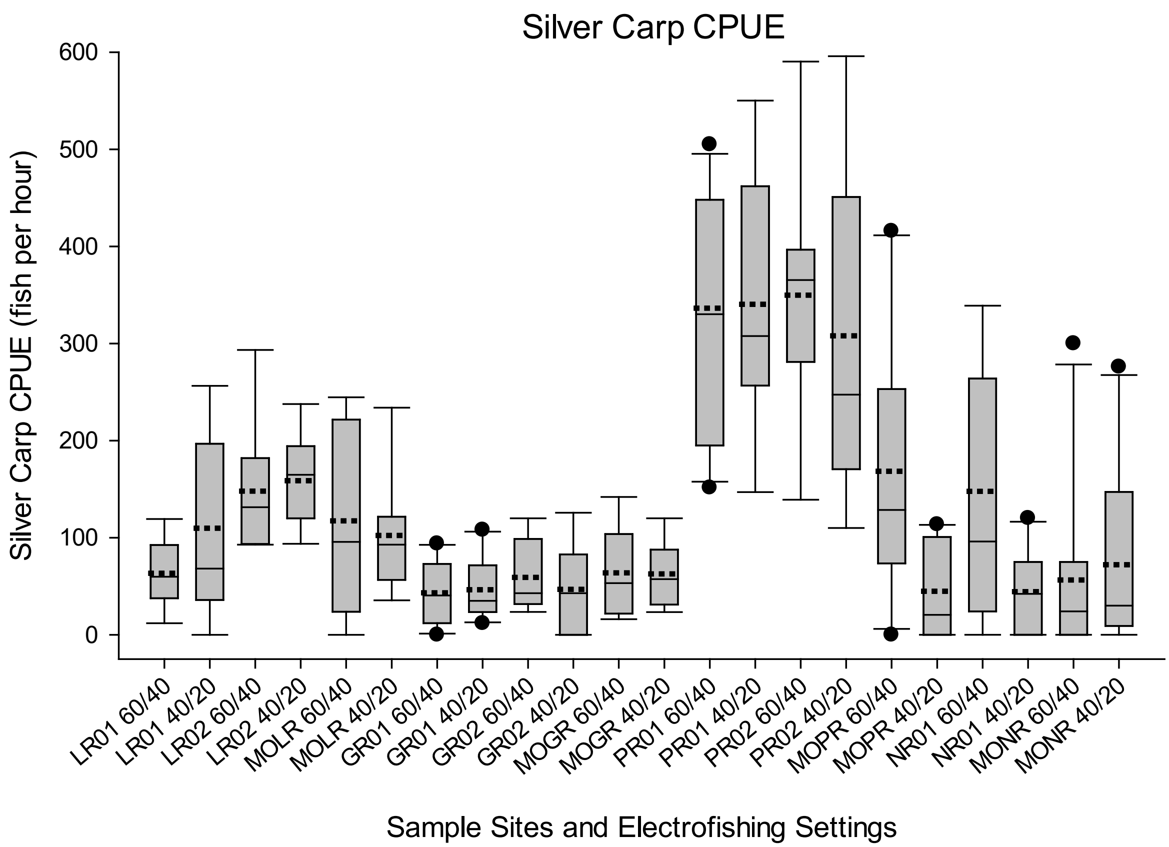

Figure 1. Box plots of Silver Carp catch per unit effort for each electrofishing setting (60Hz/40% duty cycle and 40Hz/20% duty cycle) at each site in Lamine (LR), Grand (GR), Platte (PR), Nodaway (NR) and associated Missouri River bends (MO) at each tributary. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10^{th} and 90^{th} percentiles and black circles indicate outliers.

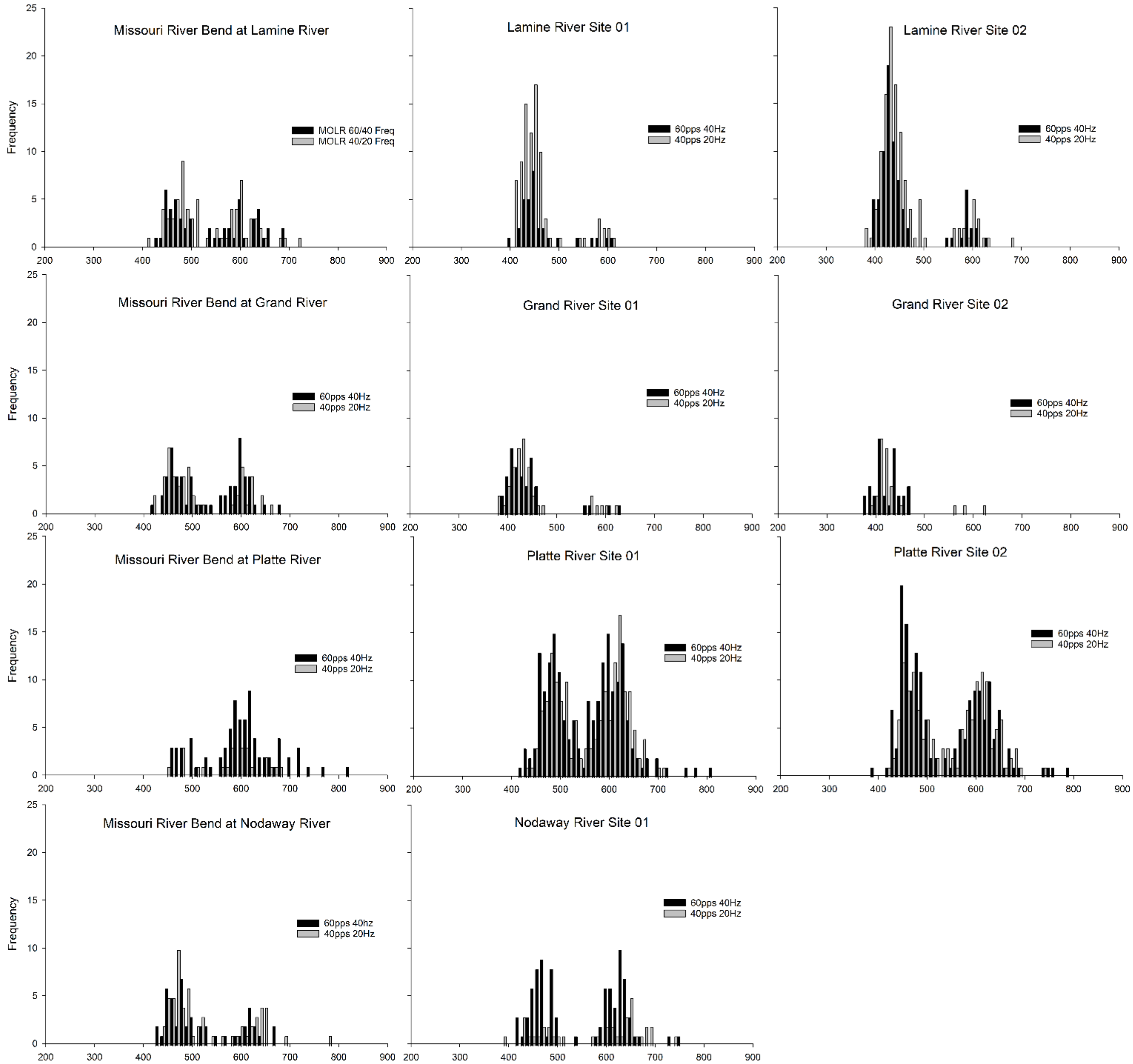

Figure 2. Length frequencies of Silver Carp in 10mm increments for all sites and electrofishing settings.

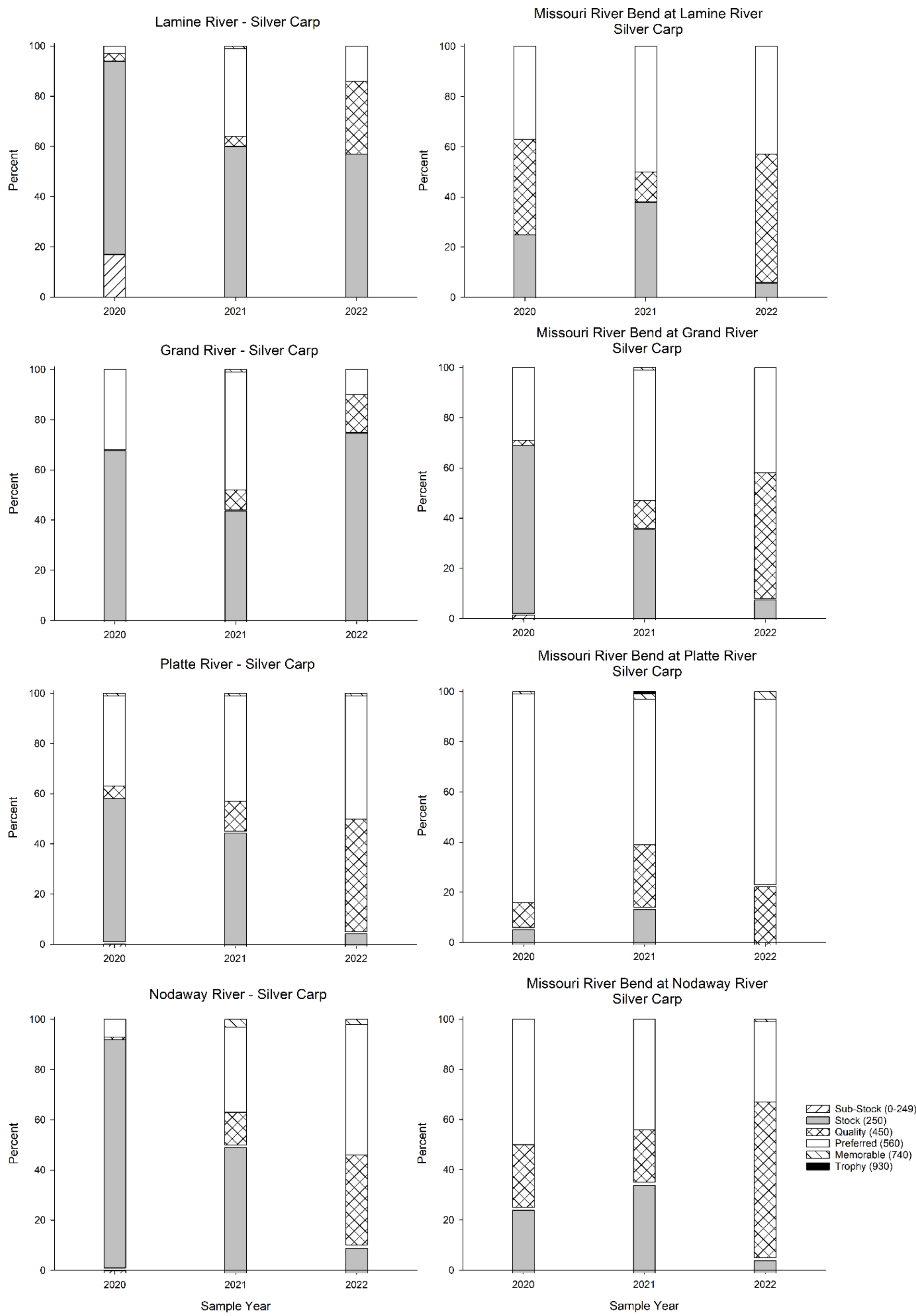

Figure 3. Incremental relative stock densities of Silver Carp based on Phelps and Willis (2013) from each tributary and associated Missouri River bend.

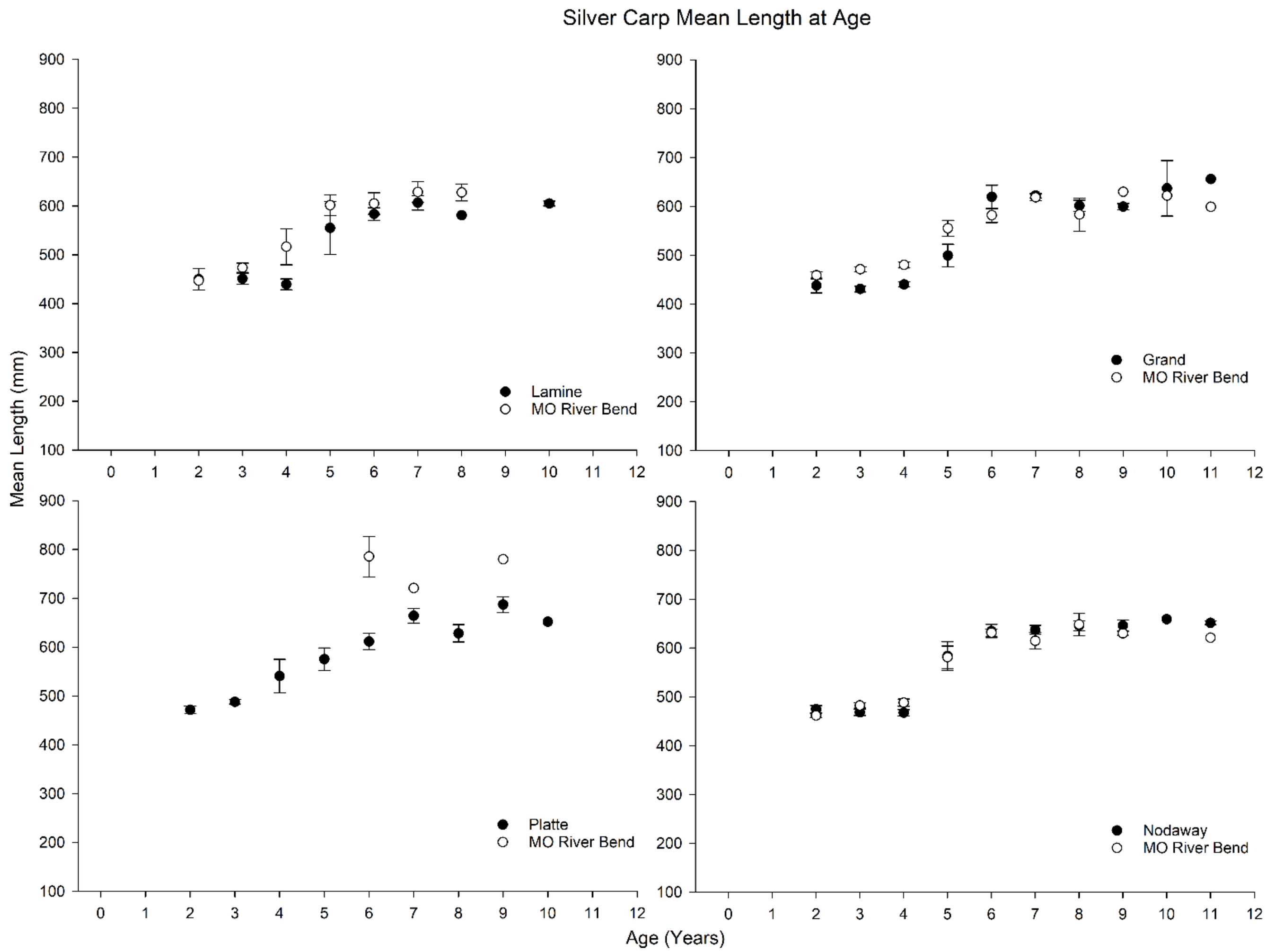

Figure 4. Silver Carp mean length at age +/- 1 standard error for each tributary and associated Missouri River bend in 2022.

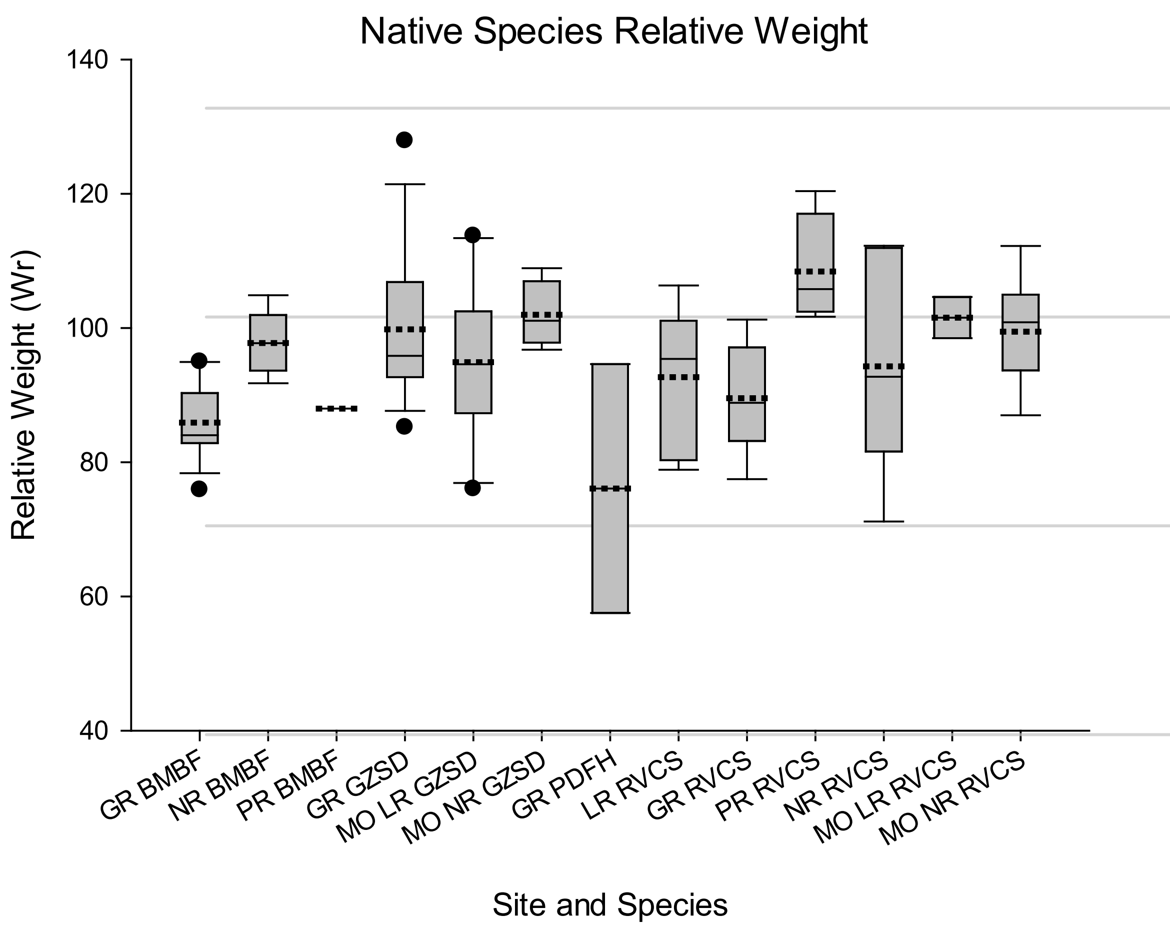

Figure 5. Box plots of relative weights for Bigmouth Buffalo (BMBF), Gizzard Shad (GZSD), Paddlefish (PDFH), and River Carpsucker (RVCS) at associated sites in Lamine (LR), Grand (GR), Platte (PR), Nodaway (NR) and associated Missouri River bends (MO) at each tributary. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

Federal Report: U.S. Fish and Wildlife Service (USFWS)

Agency: U.S. Fish and Wildlife Service (USFWS) including the Columbia Fish and Wildlife Conservation Office and the Great Plains Fish and Wildlife Conservation Office

Project Title: Population Assessment of Asian carp in Missouri River Tributary Confluences

Methods

Silver Carp demographics

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 for 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 shoreline 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 in consideration of potential 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. In 2022, lapilli otoliths were extracted from the first 100 Bighead and Silver Carp per tributary, then from additional individuals up to 20 otoliths per 50 mm length bin for stock length (250 mm; Phelps and Willis 2013) and larger fish and up to 15 sub-stock length fish per 50 mm length bin. This method differed from previous collections in 2020 and 2021, which solely targeted length bin collections. The increased number and randomization of the first 100 Silver Carp otoliths provided better age and growth estimates per tributary.

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 mutual agreement (Maceina and Sammons 2006; Seibert and Phelps 2013). If no agreement was reached, the otolith was not used for analysis.

Comparisons were conducted on a tributary basis, for the overall basin, and across years as appropriate. To analyze differences between years, a Kruskal-Wallis Test with a post–hoc Dunn's Test, applying a Bonferroni correction for all comparisons, was used. Relative abundance was calculated using catch per unit effort (CPUE; 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). A Spearman rank correlation was used to look for trends between the number of transects in relation to RSE values. Length frequency histograms were generated to provide visual observations of recruitment, growth, and mortality.

A von Bertalanffy (1938) model was used to estimate growth parameters. Given high variability in year 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). RVI estimates were only included for tributaries where there were more year classes present than missing and where there were at least three year classes present (Guy and Willis 1995; Guy and Brown 2007). Relative age frequencies were used to support and help interpret RVI values. Mortality was estimated using median lengths (Hoenig et al. 1983) for a subset of tributaries that had sufficient aged fish for estimating reliable L_{inf} and K, parameter estimates in von Bertalanffy growth curves that describe asymptotic length and growth coefficient, respectively. Body condition was calculated as relative weight using the standard weight equation found in Lamer (2015).

Hydroacoustic tributary surveys

In 2021, hydroacoustic surveys within the Missouri River Basin began to quantify relative abundance estimates. The Osage, Lamine, Grand, and Kansas rivers were prioritized due to their size, physical characteristics, and expressed interest from partner agencies. Surveys were conducted during September 28 – 30, 2021 and October 3 – 20, 2022. Split-beam hydroacoustic data was collected using a BioSonics DT-X echosounder and methods described by MacNamara et al. (2016). Surveys were conducted along the thalweg, extending 3.2 km (2 miles) upstream from the Missouri River confluence. Hydroacoustic transducers were side-facing. Thus, surveys included one upstream and one downstream transect to sample both the left and right descending banks. Using Echoview Software, transects were divided into 0.8 km (0.5 mile) intervals, yielding approximately 8 replicates per survey. Hydroacoustic and capture gear data are paired through an apportionment process as hydroacoustic data provides fish targets, estimated lengths, and water volume sampled whereas physical capture gear data is used to inform species assignment. Using capture data from previously described electrified dozer trawl sampling, proportional abundance of stock and larger Silver Carp was determined for each 50 mm length bin and applied to the corresponding hydroacoustic bins for all replicates. Replicates were used to calculate mean relative abundance and standard error of Silver Carp.

Hydroacoustic mainstem Missouri River 2022 pilot surveys

Mainstem Missouri River hydroacoustic approaches were piloted during September and October 2022. The objective of pilot collections was to determine if hydroacoustic approaches developed for large river navigation pools and mid-sized tributaries are transferrable to the conditions in the Missouri River. Considerations include abundant control structures, high flow, and turbulence, which impact the quality of hydroacoustic results. Implementation of mainstem hydroacoustic surveys will require paired capture data to inform the apportionment process, however, this was beyond the scope of the 2022 pilot collections. To address our objective, hydroacoustic surveys were conducted in main-channel and wing- and L-dike habitats. Four sections of the Missouri River were selected; these included 5.6 km (three nautical miles) upstream and downstream of the confluences with the Osage River, Lamine River, Grand River and Kansas River. Evaluation metrics including water volume sampled versus volume available, feasibility to detect fish targets in echogram data, and physical access downstream of control structures are being used to assess the feasibility of mainstem Missouri River hydroacoustic sampling efforts. Results are pending.

Electrified dozer trawl mainstem Missouri River 2022 pilot surveys

The electrified dozer trawl was deployed to assess feasibility of its use for collecting Silver Carp demographic data in mainstem habitats. The electrified dozer trawl has an expressed operating limit of river velocity less than 0.5 m/s. River velocity within the expressed limit was found behind dike complexes. At the same four tributaries sampled by hydroacoustic equipment (Osage, Lamine, Grand, and Kansas rivers), sampling occurred within the same week as hydroacoustic surveys, yet conducted to minimize disturbance to Silver Carp. For mainstem habitats, all available habitats were sampled within 5 rkm upstream and 5 rkm downstream of the tributary confluence of the four tributaries identified, where data were aggregated within 1 rkm segments to allow for replicates, up to 10 replicates per mainstem sample area.

Results

2022 Silver Carp demographics

In 2022, 103 electrified dozer trawl transects were completed across 13 tributaries (Table 1). capturing 4,621 fish representing 34 unique species (Table 1; Appendix A). Overall, 1,461 Silver Carp (32% of total catch) within the 40 – 940 mm TL range and 17 Bighead Carp (<1% of total catch) within the 342 – 780 mm TL range were captured from sampled tributaries (Tables 1 and 2), with no Bighead or Silver carp collected in the Gasconade or Osage rivers in 2022. Aging structures were collected from a subset of both Bighead (N = 13) and Silver carp (N = 900) across all tributaries. Bighead Carp ranged in age from 3 – 10 years whereas Silver Carp ranged in age from 0 – 12 years. In addition, Silver Carp under 120 mm (N = 56) were assumed to be age zero. Given the paucity of Bighead Carp collections, no further analyses were conducted for this species.

Silver Carp CPUE and CPUE RSE was variable across tributaries (Table 1; Figure 1). The target CPUE RSE (RSE ≤ 25) was only met for the Big Sioux, Grand, Kansas, and Platte rivers. A Spearman rank correlation analysis indicated that RSE was not correlated with the number of transects conducted in 2022 (coefficient = -0.54; P = 0.08). Silver Carp were collected across a broad size range (Table 1), with the highest proportion falling in the 600 – 649 mm length bin (Figure 2). No Silver Carp were collected within the 150 – 299 mm size range (Figures 3 and 4).

Silver Carp condition was similar across tributaries for 2022 with average relative weights below the expected average (W_r = 100). Only the Big Sioux and Nishnabotna rivers had relative weights over 100, with relative weights of 107 (SE = 8.2) and 103 (SE = 9.3), respectively (Figure 5). The percentage of male Silver Carp in 2022 varied from 46 – 67%. Assuming an unexploited population is 50% male, around half of the tributaries sampled were within 5% of the expected 50% male (Figure 6). Three tributaries (Big Sioux, and James and Kansas rivers) exceeded 10% higher than the 50% probability (Figure 6).

Recruitment variability indexes (RVI) were calculated for tributaries with sufficient Silver Carp age class representation (Table 3). The average RVI across all tributaries was 0.24 (SE = 0.06) with tributary values between -0.06 – 0.50, indicating recruitment is variable (Table 1). The presence of many year classes with few missing year classes across tributaries supports RVI estimates (Figures 7 and 8). Von Bertalanffy growth models were calculated for tributaries that had sufficient data to produce a good fit based off model residuals (Figure 9; Table 3).

Length data was used to estimate instantaneous annual mortality for tributaries that had well-fitted L_{inf} and K estimates from von Bertalanffy growth curves. The instantaneous annual mortality estimate of Silver Carp in the Missouri River Basin was 0.19 with variation across tributaries ranging from 0.10 – 0.31 (Table 3) for 2022 using Hoenig and colleagues' (1983) method. For use in the equation, it was assumed that consistent catches of Silver Carp in the electrified dozer trawl began at 150 mm TL, with no Silver Carp below that size included in mortality estimation.

Demographic comparisons to prior years

Sampling effort decreased in both number of tributaries sampled and number of electrified dozer trawl transects across years due to available habitat. Effort was highest in 2020 with 164 transects across 16 tributaries, followed by 2021 with 138 transects across 14 tributaries, whereas 2022 had the lowest effort with 103 transects (Table 1). Similarly, total number of fish and Silver Carp collected decreased annually, as did the percent of total catch comprised by Silver Carp. In 2020, 9,867 fish representing 36 species were collected, of which 80% were Silver Carp and less than 1% were Bighead Carp. In 2021, 5,066 fish representing 42 species were collected, of which 52% were Silver Carp and less than 1% were Bighead Carp. In all years, no Silver Carp were collected in the Osage River. In 2022, 4,621 fish representing 34 species were collected, of which 32% were Silver Carp and less than 1% were Bighead Carp. Silver Carp were only collected in the Gasconade in 2021.

Catch rates varied each year across tributaries (Figure 1) and no significant differences across tributaries existed within a given year (Kruskal–Wallis, all $P > 0.05$). A Spearman rank correlation yielded no significant finding when comparing RSE of catch rates and the number of transects completed within a year for any of the three years sampled ($P > 0.05$).

Size structure showed a strong bimodal distribution in 2020, with Silver Carp lengths increasing in 2021 and 2022 (Figure 2). The high proportion of small (200 – 300 mm) Silver Carp in 2020 declines with time, while the proportion larger (550 – 650 mm) individuals increase through time (Figure 2). Presumed age–0 Silver Carp (< 120 mm) were present in 2020 and 2022, though they represent a higher percent of the catch in 2022 (Figure 2).

Body condition of Silver Carp was reported as relative weight for each year. There was no observable pattern of relative weight across tributaries within a year (Figure 5). However, a Kruskal-Wallis showed significant differences across years for relative weight, and a Dunn's test showed significant differences across years, with a mean relative weight of 94.4 in 2020, 95.7 in 2021, and 98.4 in 2022 (adjusted P-values were all < 0.01). Silver Carp sex ratio exhibited lower variation across tributaries in 2022 than in prior years (Figure 6). Overall, the proportion of Silver Carp collected which were male was within 10% of the expected average of 0.5.

Recruitment variability for individual tributaries and for overall basin values was lower in 2022 than in 2021. In 2021, age-1 to age-13 Silver Carp yielded individual tributary RVI estimates ranged from 0.18 to 0.74, with the combined data indicating an RVI estimate of 0.75 for the overall Missouri River Basin. No RVIs were calculated for 2020 data. In addition, 2022 was the first year in which tributary-specific von Bertalanffy growth curves and instantaneous mortality estimates were available, so no comparisons were made.

Hydroacoustic tributary surveys

Silver Carp densities varied spatially and temporally. Physical capture by means of dozer trawl yielded zero Silver Carp in the Osage River, resulting in hydroacoustic-based density estimates of zero Silver Carp per 1,000 m^3. Densities among the Lamine, Grand, and Kansas rivers ranged from 1.78 to 4.49 Silver Carp per 1,000 m^3 and 2.07 to 3.6 Silver Carp per 1000 m^3 during 2021 and 2022, respectively (Figure 10). Densities for the Lamine and Kansas rivers increased from 2021 to 2022 whereas densities in the Grand River decreased (Figure 10). Results from the Lamine and Kansas rivers were attributed to natural population increases (e.g., recruitment, immigration) whereas results from the Grand River were attributed to intensive removal efforts implemented in 2022. Grand River removal efforts resulted in strong population declines in the removal area, which strongly overlapped with our 3.2 km (2 mile) survey area.

Hydroacoustic mainstem Missouri River 2022 pilot surveys

Mainstem hydroacoustic pilot data was collected during 2022. Although final results are pending, preliminary findings suggest that hydroacoustic surveys effectively samples main-channel border habitat as evidenced by clear echograms that were not adversely impacted by high water velocities, which exceeded values normally observed in navigation pools. Not surprisingly, limited fish targets were observed in main channel habitat, suggesting that this habitat type is not generally preferred by fish that utilize the upper or middle portions of the water column. In contrast to main channel efforts, sampling control structures was challenging due to access issues from low water levels, turbulence around rip rap, which creates noise in the echogram, and sediment deposition patterns such as sandbars. These challenges limit the distance that hydroacoustic equipment is able to sample. Final pilot results will be used to provide recommendations concerning the feasibility of conducting hydroacoustic surveys in mainstem Missouri River habitats during low water conditions. Sampling during normal water levels will likely be more effective, however, this prediction should be confirmed using field results.

Electrified dozer trawl mainstem Missouri River 2022 pilot surveys

Electrified dozer trawl sampling took place on the mainstem Missouri River in September and October 2022, with a total of 19 transects across 4 sites, yielding 200 Silver Carp. Suitable sampling habitats became more available as sampling moved farther downstream in the Missouri River Basin, where 2 transects were completed in the mainstem Missouri River outside the Kansas River confluence, 3 transects were completed outside the Grand River confluence, 6 transects were completed outside the Lamine River confluence, and 8 transects were completed outside the Osage River confluence. This preliminary data will be combined with other mainstem pilot data from 2020 and included in a broader spatial and temporal analysis of Silver Carp demographic data from across the Missouri River Basin to identify population differences and inform protocol refinement recommendations.

Discussion

When and where management occurs in the Missouri River Basin, information from both electrified dozer trawl demographic information and hydroacoustic relative abundance estimates could provide a baseline against which to measure success. Further analyses are being conducted to facilitate a better understanding and potential application of the results of these efforts. Nevertheless, demographic data pooled across tributaries within each year showed relatively few differences. Relative abundance had a statistical difference but not likely a meaningful difference between 2020 and 2022. Furthermore, from 2020 to 2022, RVI seemed to decrease as drought limited reproductive and recruitment opportunities, and relative weights increased with an aging population and a dominant 2019 year class approaching maturity (Illinois River Silver Carp females mature around 500 mm and males mature around 450 mm; ICRCC 2021). To ensure that demographics data are reliable for evaluating future management efforts, measures of sampling success should be examined and better defined for Silver Carp, which tend to be highly mobile and exhibit patchy distribution. A broader examination is needed for the role of precision measures such as RSE in evaluating relative abundance, as is recommendations for total sample size needed for both relative abundance and age data. Despite increasing the number of Silver Carp aged in 2022, the minimum of 100 fish per tributary was not met in every tributary, likely due to low water conditions limiting areas sampled with the current study design. Further, Coggins, Gwinn and Allen (2013) recommended a minimum sample size of 500 to 1,000 individuals with a target of at least 10 fish per 5 mm length bin to provide the most accurate and precise estimates for von Bertalanffy growth coefficients. Utilizing existing data for the Missouri River Basin as well as other Mississippi River sub-basins may provide insight into minimum sampling sizes needed as well as other, more reliable measures of success for representing Silver Carp population demographics information.

Hydroacoustic data revealed that Silver Carp densities increased from 2021 to 2022 in the Lamine and Kansas rivers, which we attributed to natural population increase (e.g., recruitment, immigration). In contrast, Silver Carp densities in the Grand River declined during the same time period, a result which was attributed to intensive invasive carp removal efforts implemented during 2022 (information on the removal effort can be found in the 2022 Missouri

River Basin Invasive Carp Partnership Annual Technical Report under Objective 3: Control and containment of invasive carp in the Missouri River Basin; MICRA, unpublished). Results from Missouri River tributaries are comparable to densities reported for other systems such as the lower navigational pools of the Illinois River, downstream of Starved Rock Lock and Dam, where peak levels ranged approximately 2 to 5 Silver Carp per 1,000 m^3 across pools in 2012 (ICRCC 2022).

Low water levels hampered sampling efforts across all years of data collection by limiting the number of navigable tributaries as well as the number of transects that could be completed within each tributary. Historically, the hydrology of the Missouri River during the data collection period, September – October, would be expected to be low compared to the previous months; however, the low water conditions the past three years have been unprecedented (USGS 2023, multiple gaging stations across Missouri River Basin). It is also unclear whether Silver Carp utilize habitat types outside of sampleable tributaries, such as the mainstem Missouri River. Silver Carp tend to select for deeper, slow-moving water with a preference for sandy substrate (Prechtel et al. 2018), which becomes more available in mainstem habitats in low water years, particularly behind dike structures. Sampling in additional habitats should be further explored, particularly for drought years when Silver Carp may be utilizing deeper, slow-moving sandy habitats found behind dikes in the mainstem Missouri River. The potential impact of changing hydrological conditions should be considered in designing future assessment projects and interpreting results from differing conditions. Natural variation across tributaries within years should be further explored to assess potential spatial patterns and temporal trends. When possible, the role of abiotic and biotic factors on relative abundance, including water levels or temperatures, habitat differences, and population density should be identified.

Recommendations

- Further explore the need and feasibility of sampling mainstem habitats to supplement tributary sampling.
- Conduct further analyses to look for spatial patterns or temporal trends within existing demographics data, identify minimum sampling criteria needed for success (sample size, RSE, age structures), and to identify areas where targeted removals may be most effective.
- Coordinate with the Missouri River partnership to determine the general need and spatial coverage of 2023 hydroacoustic surveys (e.g., tributary surveys, removal assessment surveys).
- Complete analysis of mainstem Missouri River hydroacoustic pilot data and coordinate with the Missouri River partnership to determine mainstem survey needs (e.g., locations, habitats) and research needs (e.g., effectiveness of mainstem sampling during average water levels).

Tables

Table 1. Summary of 2022 Missouri River tributary confluence sampling for Silver Carp (SVCP) using an electrified dozer trawl. Tributaries are in ascending order based on the Missouri River river mile (RM) at their respective confluences. The total number of electrified dozer trawl transects, 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 is relative standard error (RSE) of SVCP CPUE. Minimum and maximum total length (TL) and recruitment variability index (RVI) are listed for SVCP. The summary column lists total number for transects, fish, and SVCP and the averages for CPUE, and CPUE RSE.

Waterbody	RM	Watershed (km²)	Transects (N)	All Fish (N)	SVCP (N)	CPUE (#/hr)	CPUE RSE	Min TL (mm)	Max TL (mm)
Gasconade River	104	9,262	4	20	0	0 (0)	–	–	–
Osage River	130	38,943	8	16	0	0 (0)	–	–	–
Perche Creek	170	1,511	4	196	30	87 (27)	31	355	925
Lamine River	202	1,038	13	593	127	117 (35)	30	420	675
Little Chariton River	227	6,880	6	131	14	44 (15)	35	351	827
Grand River	250	7,943	12	229	71	69 (16)	23	65	725
Crooked River	314	20,429	6	344	109	421 (233)	56	55	715
Kansas River	367	921	12	573	119	135 (24)	18	430	761
Platte River (MO)	391	288,343	7	307	125	251 (43)	17	102	940
Nishnabotna River	542	6,322	6	570	147	458 (237)	52	455	820
Big Sioux River	734	7,730	8	500	274	578 (109)	19	40	829
Vermillion River	772	19,257	8	429	342	876 (228)	26	60	775
James River	800	6,784	9	713	103	135 (40)	29	46	791
Summary			**103**	**4,621**	**1,461**	**227 (35)**	**15**	**40**	**940**

Table 2. Bighead Carp collections for 2022 electrified dozer trawl sampling. Each row represents a single fish.

Date	Tributary	Species	Length (mm)	Weight (g)	Sex	Age
9/6/2022	Little Chariton River	Bighead Carp	666	2,840	Female	–
9/6/2022	Little Chariton River	Bighead Carp	500	1,380	Male	–
9/7/2022	Crooked River	Bighead Carp	474	1,070	Male	5
9/7/2022	Crooked River	Bighead Carp	377	520	Male	3
9/7/2022	Crooked River	Bighead Carp	351	460	Male	4
9/7/2022	Crooked River	Bighead Carp	376	580	Male	3
9/7/2022	Crooked River	Bighead Carp	397	580	Male	4
9/7/2022	Crooked River	Bighead Carp	352	460	Male	4
9/7/2022	Crooked River	Bighead Carp	355	470	Male	3
9/7/2022	Crooked River	Bighead Carp	375	550	Male	4
9/7/2022	Crooked River	Bighead Carp	383	560	Male	3
9/7/2022	Crooked River	Bighead Carp	355	470	Male	3
9/7/2022	Crooked River	Bighead Carp	364	530	Male	3
9/20/2022	Nishnabotna River	Bighead Carp	780	4,740	Male	–
8/30/2022	Big Sioux River	Bighead Carp	583	2,140	Male	10
8/30/2022	Big Sioux River	Bighead Carp	585	1,832	Male	–
8/31/2022	Vermillion River	Bighead Carp	685	3,080	Female	9

Table 3. Recruitment variability index (RVI), growth estimation parameters (L_{inf} and *K*) from von Bertalanffy growth models, and instantaneous annual mortality (*M*) estimates for each tributary with sufficient data. Not all tributaries had sufficient data to produce reliable results. RVI parentheses display the maximum age used for calculation.

Waterbody	Transects	SVCP	RVI	L_{inf}	*K*	*M*
Lamine River	13	127	0.44 (11)	619	0.42	0.21
Grand River	12	71	0.50 (10)	668	0.37	0.24
Crooked River	6	109	–	633	0.30	0.31
Kansas River	12	119	-0.06 (11)	652	0.40	0.10
Platte River (MO)	7	125	0.26 (10)	644	0.46	0.18
Nishnabotna River	6	147	0.07 (11)	700	0.33	0.12
Big Sioux River	8	274	0.25 (12)	655	0.44	0.21
Vermillion River	8	342	0.20 (10)	643	0.36	0.13
James River	9	103	0.25 (10)	652	0.43	0.17

Figures

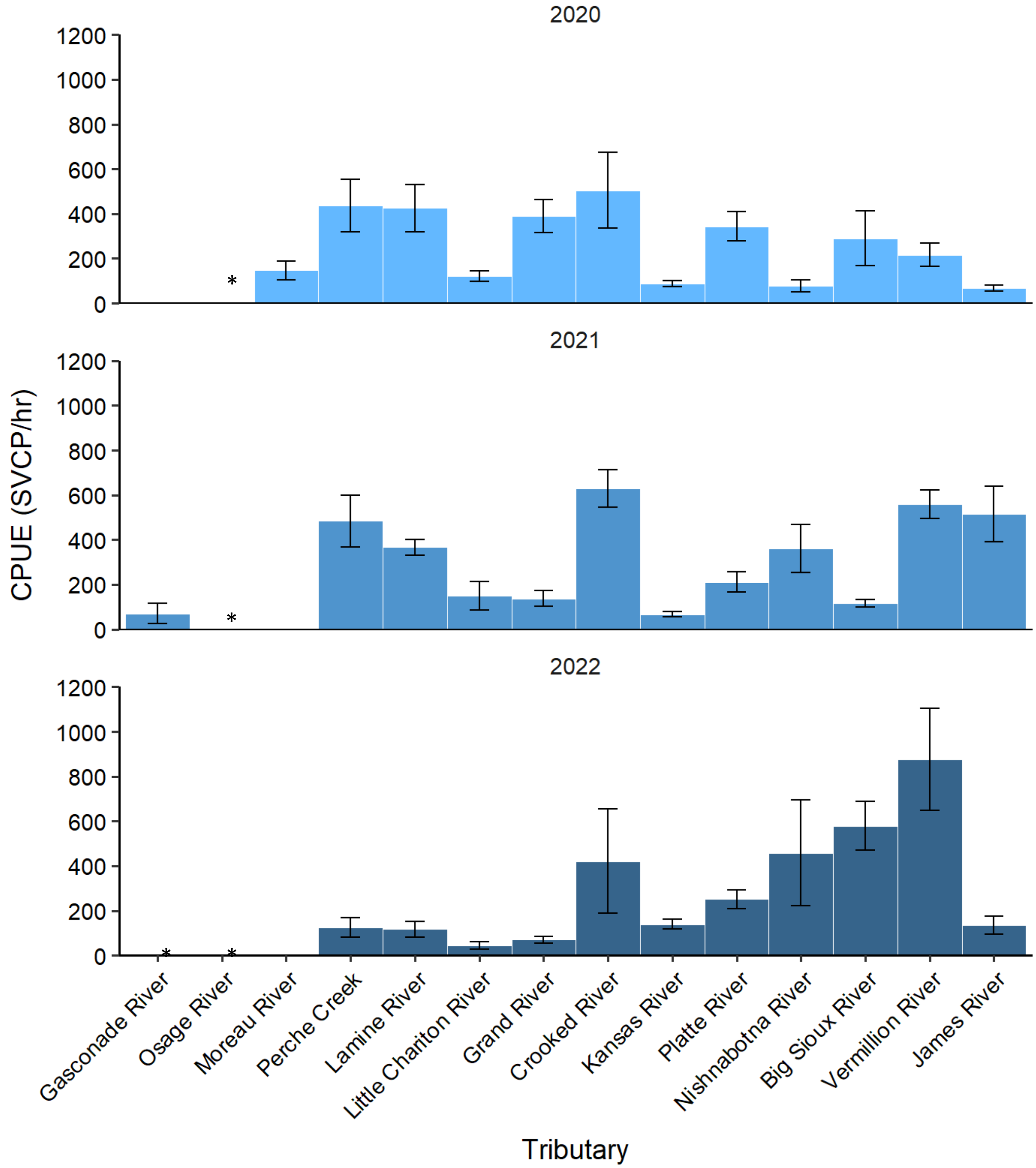

Figure 1. Mean catch per unit effort (CPUE; SVCP per hour) with standard errors of Silver Carp (SVCP) captured in Missouri River tributaries with an electrified dozer trawl in 2020 – 2022. Only tributaries with at least three transects were included. An asterisk indicates the tributary was sampled with no SVCP collected. All other missing bars indicate that no samples were collected in that tributary in the given year (Gasconade River 2020 and Moreau River 2022) or that fewer than three transects were completed (Moreau River 2021).

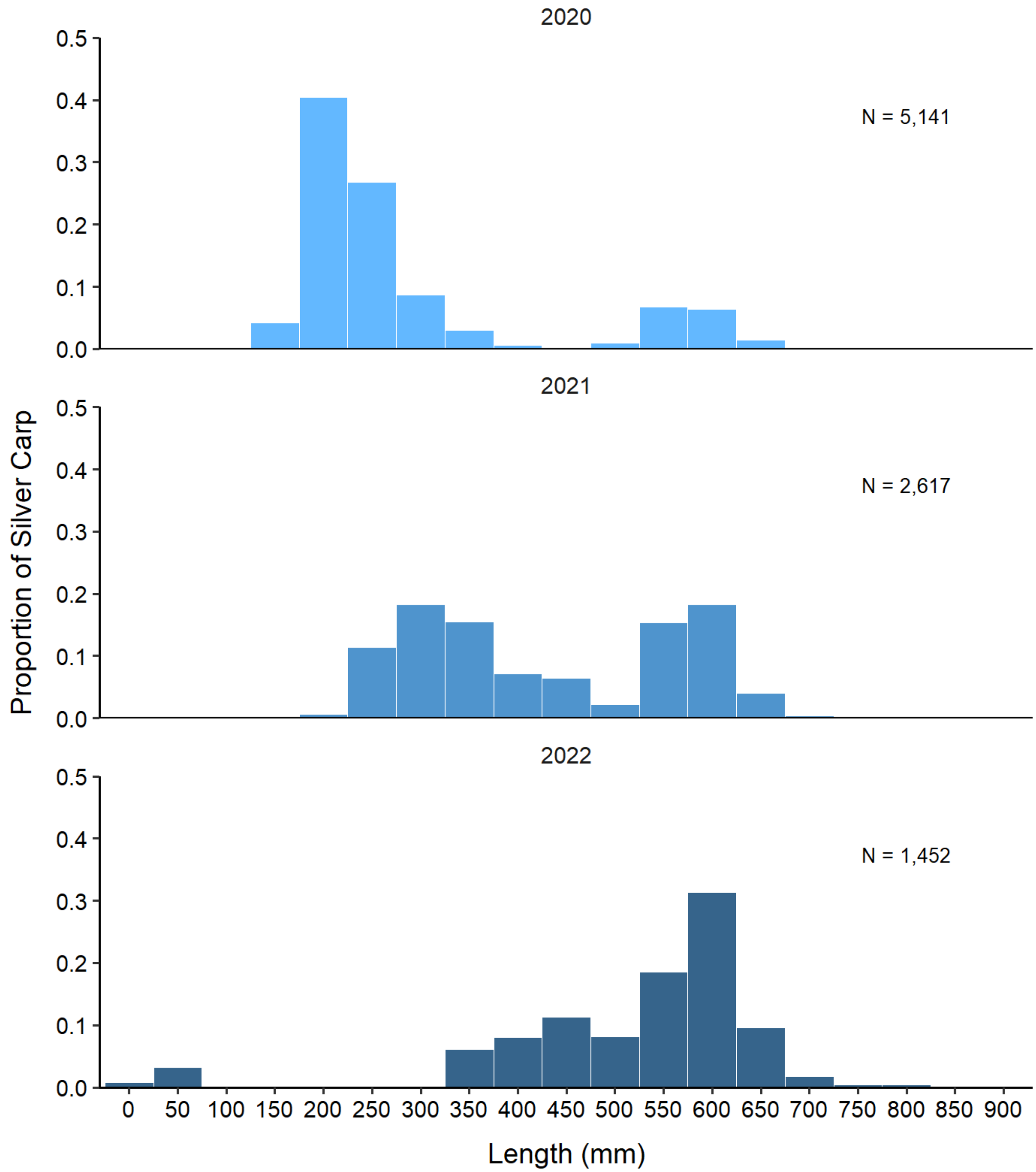

Figure 2. Relative length frequency distribution by 50 mm length bin of Silver Carp captured with an electrified dozer trawl in 2020 – 2022 in Missouri River tributaries. The number of Silver Carp with length data (N) is indicated for each year, which may not exactly match the total number of Silver Carp collected in Table 1 due to missing length data.

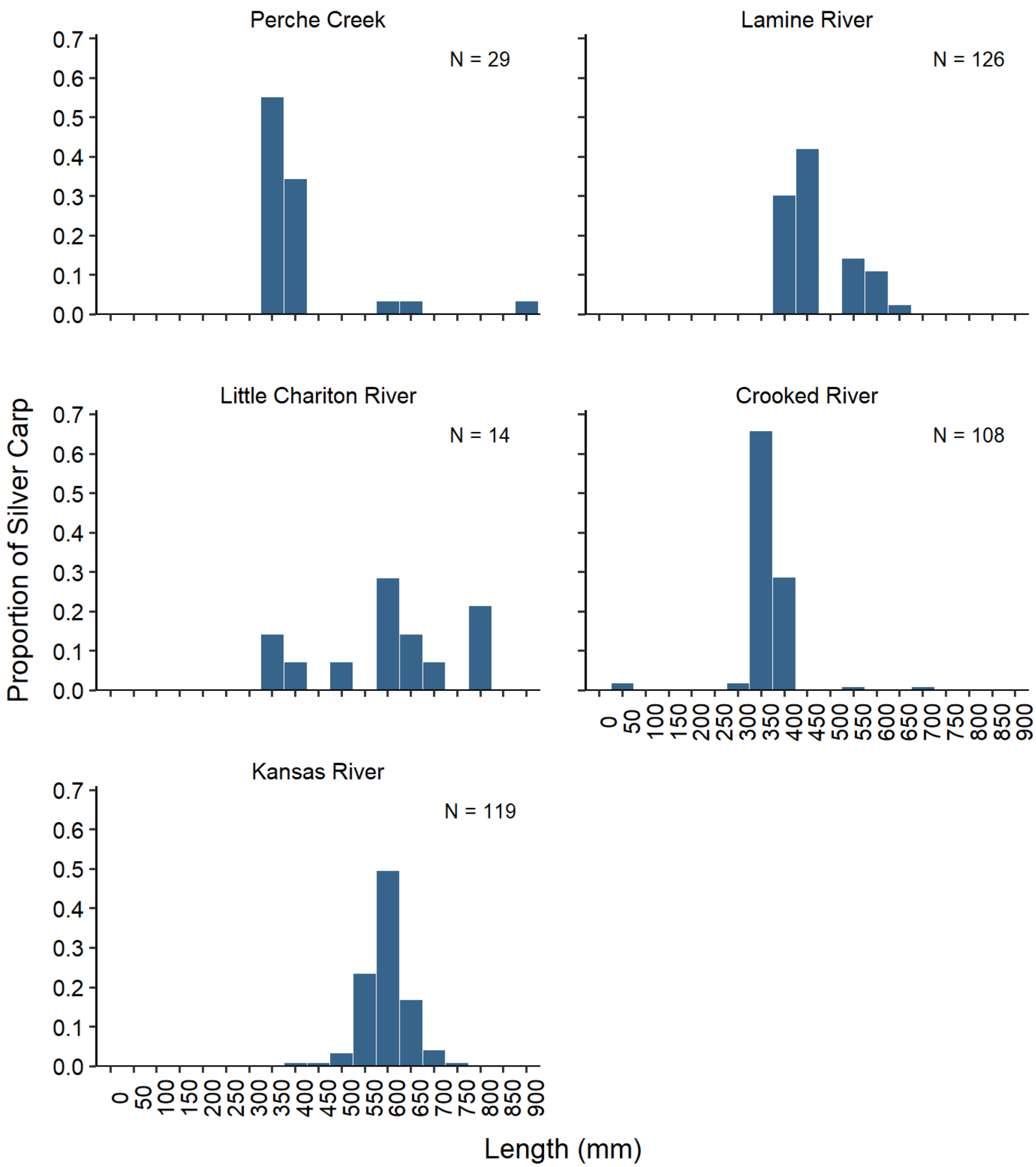

Figure 3. Relative length frequency distribution by 50 mm length bin of Silver Carp captured with an electrified dozer trawl in 2022 in each Missouri River tributary. The number of Silver Carp with length data (N) is indicated for each year, which may not exactly match the total number of Silver Carp collected in Table 1 due to missing length data.

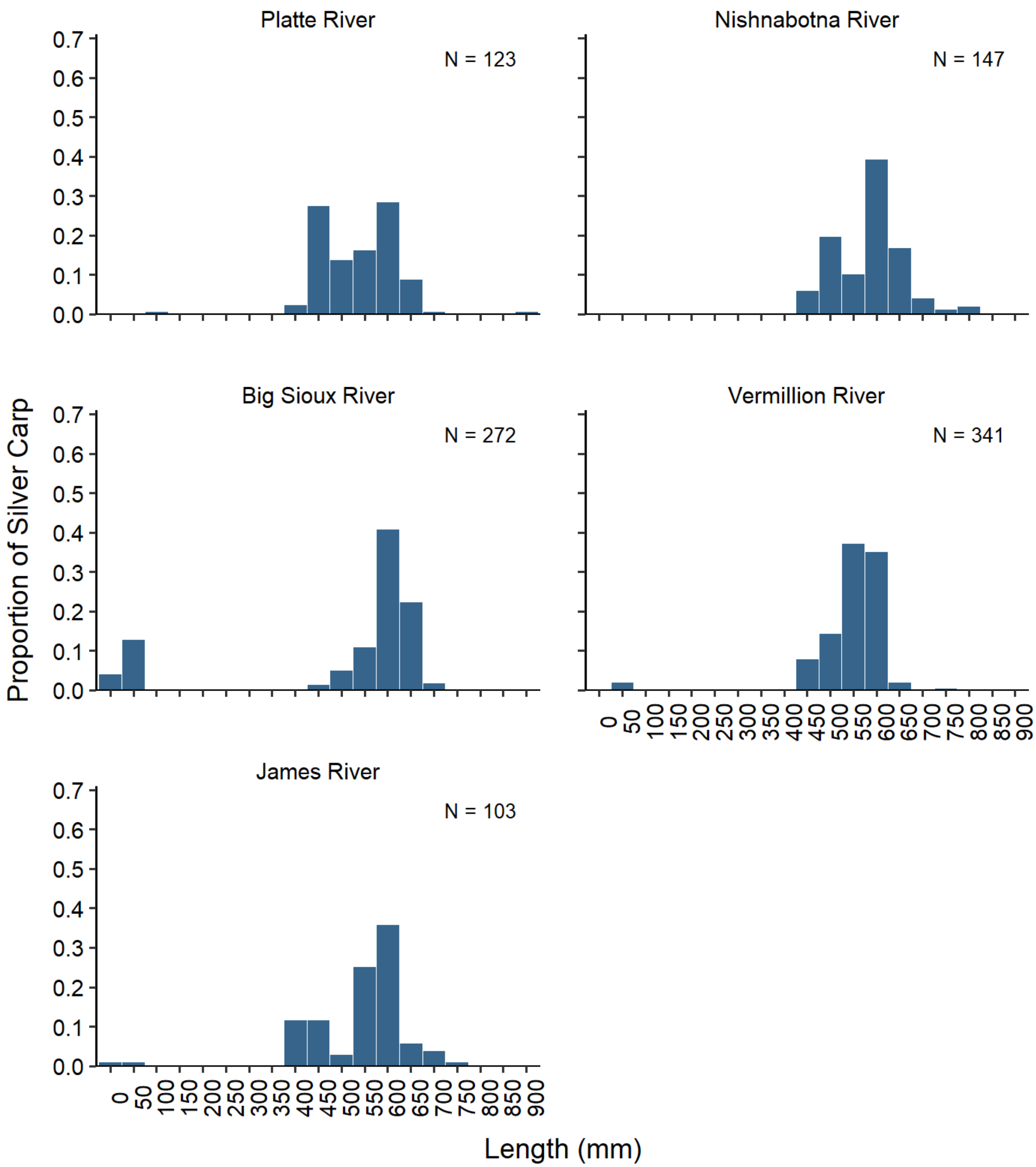

Figure 4. Relative length frequency distribution by 50 mm length bin of Silver Carp captured with an electrified dozer trawl in 2022 in each Missouri River tributary. The number of Silver Carp with length data (N) is indicated for each year, which may not exactly match the total number of Silver Carp collected in Table 1 due to missing length data.

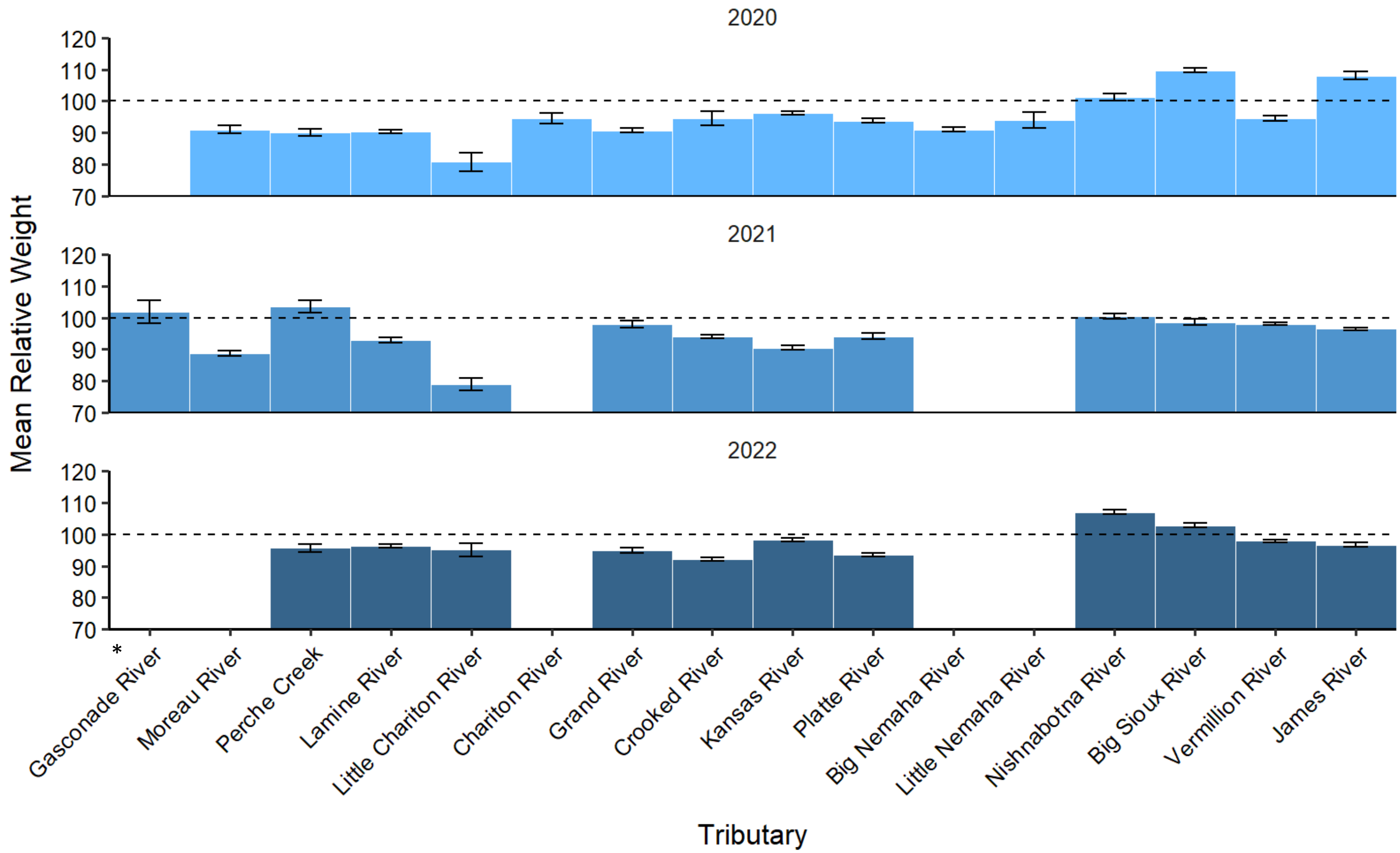

Figure 5. Mean relative weight of Silver Carp collected by tributary and year with the electrified dozer trawl. Error bars represent one standard error. A value of 100 (indicated by a dashed line) is an average body condition for Silver Carp using the equation from Lamer (2015) An asterisk indicates the tributary was sampled with no SVCP collected. All other missing bars indicate that no samples were collected in that tributary in the given year.

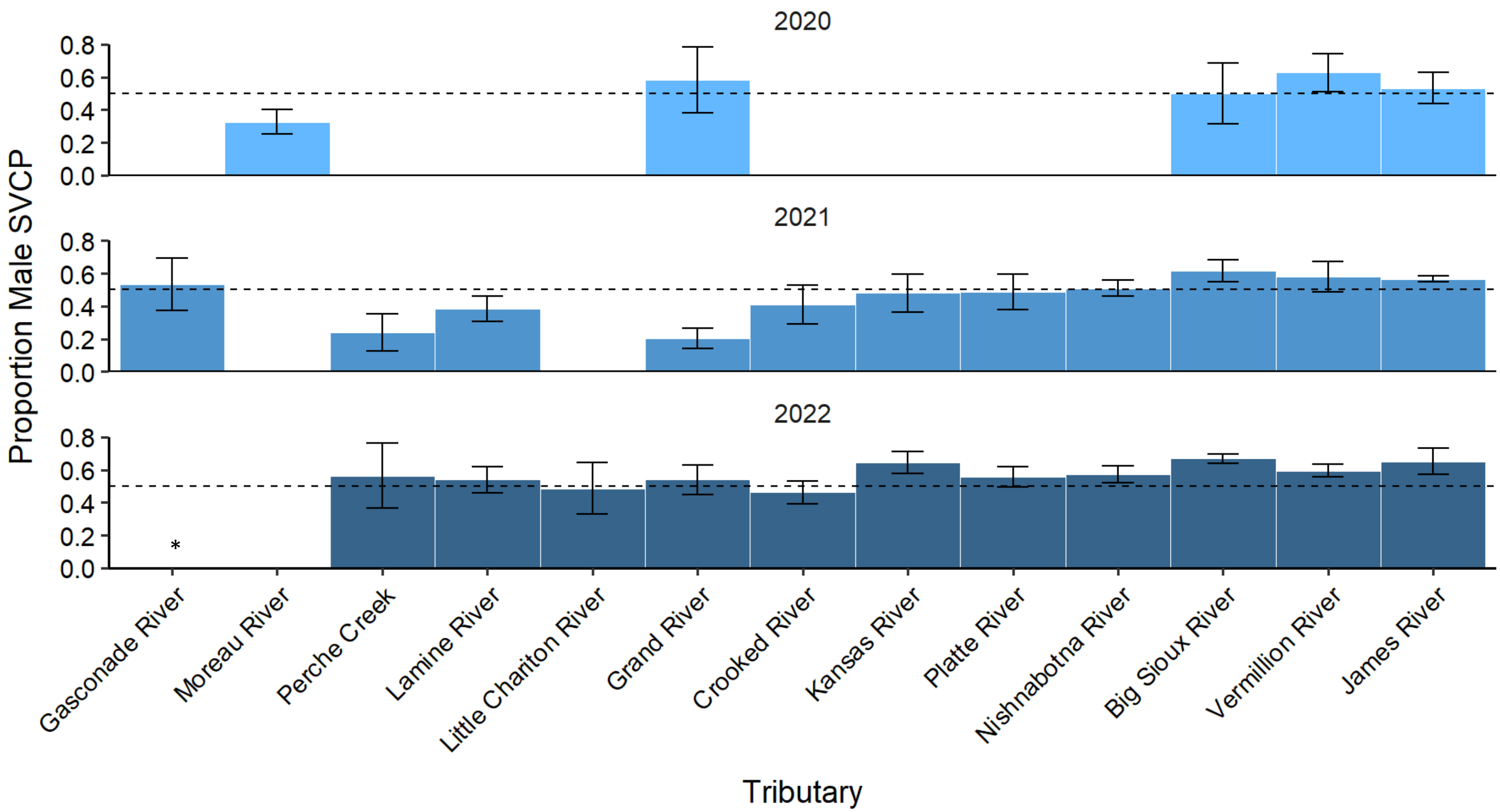

Figure 6. Mean proportion of Silver Carp that were male with standard error bars for 2020 – 2022 electrified dozer trawl sampling in Missouri River tributaries. Tributaries with less than 5 individual fish sexes were excluded or are missing bars. An asterisk indicates the tributary was sampled with no SVCP collected.

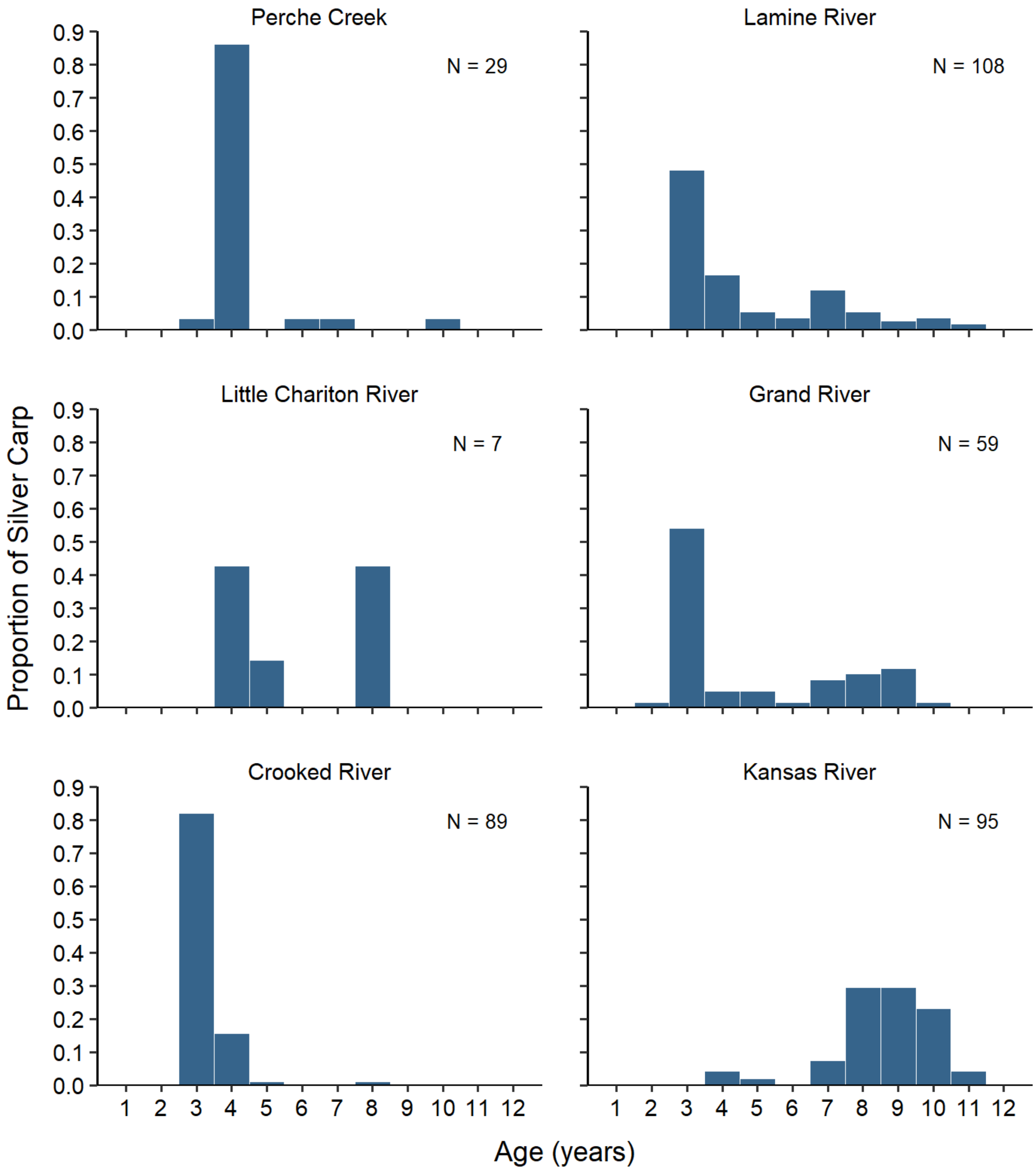

Figure 7. Relative age frequency of Silver Carp captured with an electrified dozer trawl in 2022 in each Missouri River tributary. The first 100 Silver Carp collected were aged, followed by the addition of individuals with low representation in a given 50 mm length bin. The number of Silver Carp aged is indicated for each tributary (N) and age-0 fish were excluded.

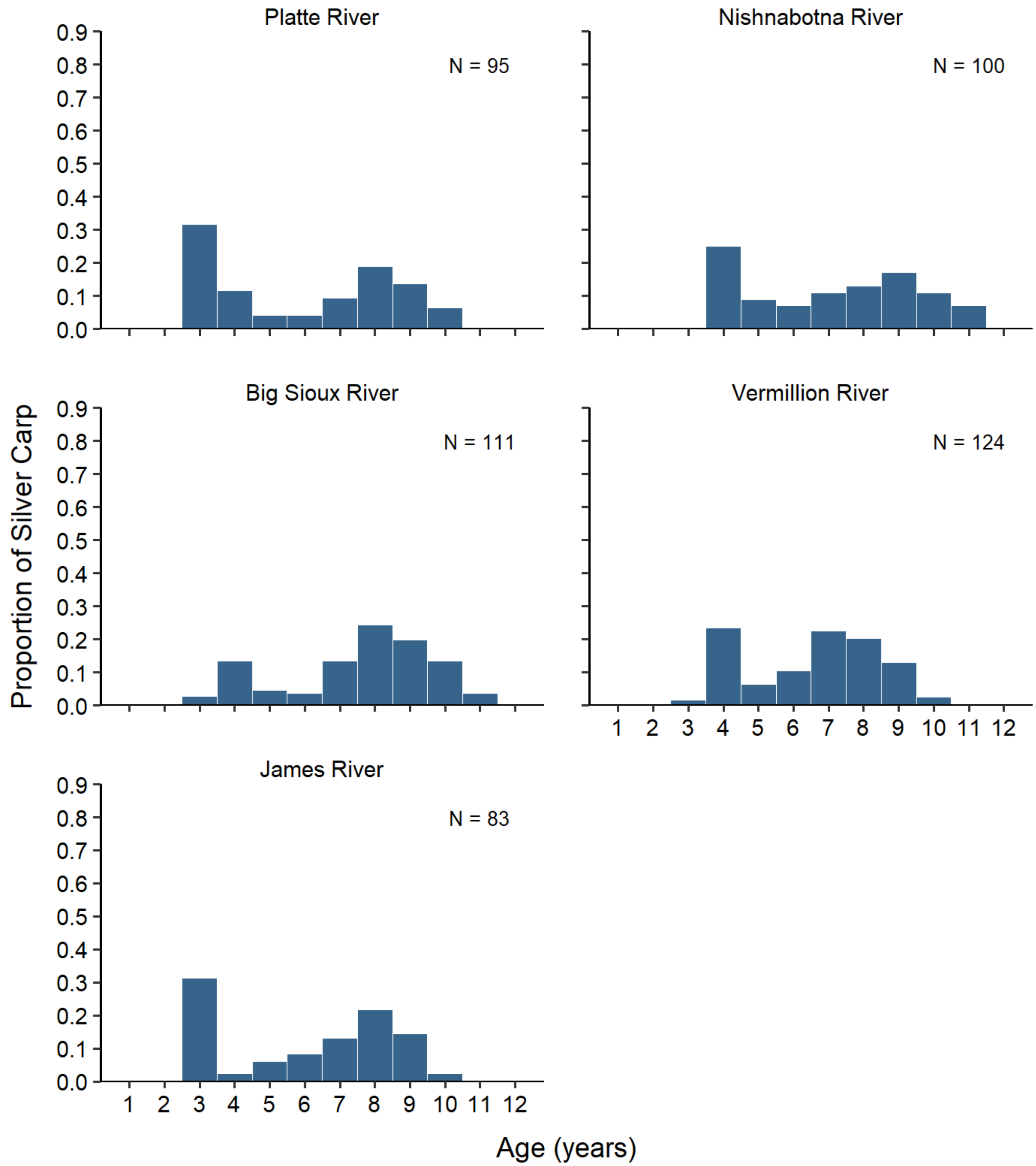

Figure 8. Relative age frequency of Silver Carp captured with an electrified dozer trawl in 2022 in each Missouri River tributary. The first 100 Silver Carp collected were aged, followed by the addition of individuals with low representation in a given 50 mm length bin. The number of Silver Carp aged is indicated for each tributary (N) and age-0 fish were excluded.

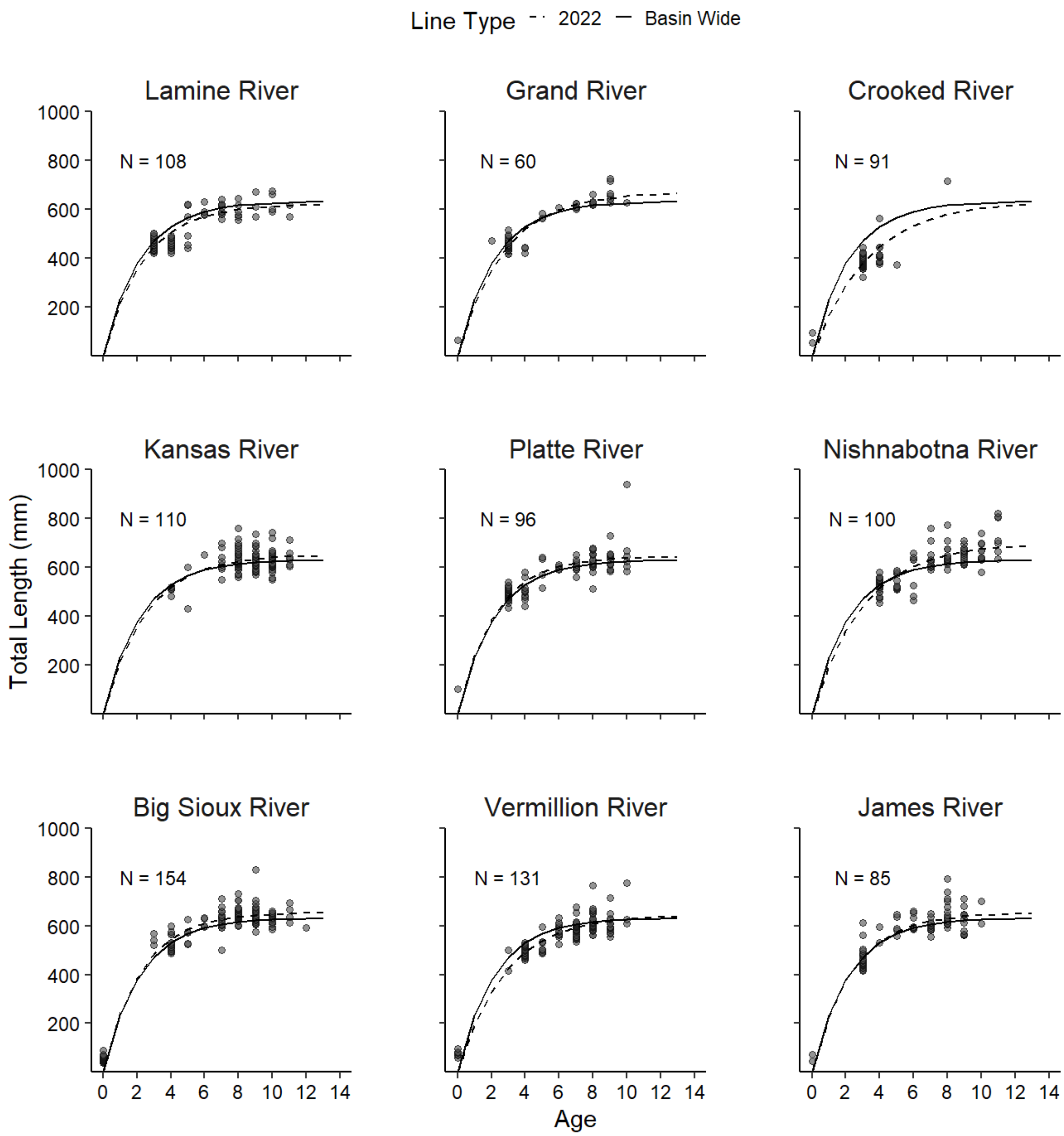

Figure 9. Length at age with for all Silver Carp collected by electrified dozer trawl in 2022, as represented by dots, where sample size (N) is indicated for each tributary. Dashed lines represent the von Bertalanffy growth curve for 2022 data for the given tributary. The solid line is the von Bertalanffy growth curve for all available Silver Carp data from Missouri River tributaries from 2020 – 2022. For all von Bertalanffy growth curves, t_0 was set to zero.

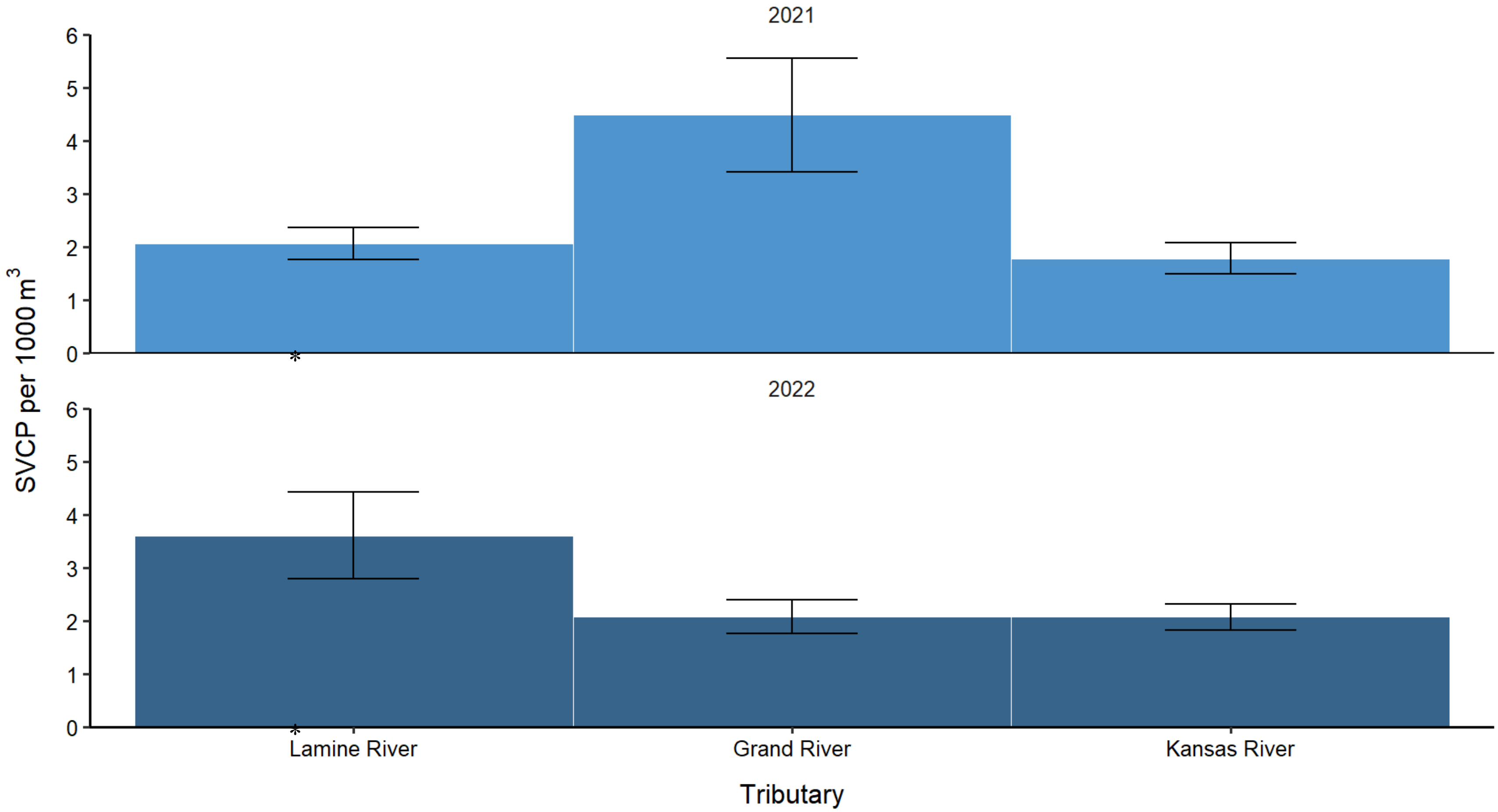

Figure 10. Hydroacoustic estimates of Silver Carp density in fish per 1,000 m^3 of water sampled by tributary and year. Although the Osage River was also sampled, no Silver Carp were collected to estimate densities.

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Appendix A

Table A.1. All fish collected by electrified dozer trawl in fall 2022 by species and tributary, where Missouri River river mile (RM) and number of transects (N) are listed for each tributary. –

Fish Species	**Gasconade River RM 104 N = 4**	**Osage River RM 130 N = 8**	**Perche Creek RM 170 N = 4**	**Lamine River RM 202 N = 13**	**Little Chariton River RM 227 N = 6**
Bighead Carp	–	–	–	–	2
Black Buffalo	–	–	–	–	–
Brook Silverside	–	–	6	–	–
Bluegill	–	–	1	–	–
Bigmouth Buffalo	–	–	1	2	4
Blue Sucker	–	–	–	–	–
Common Carp	–	2	–	1	–
Channel Catfish	–	–	2	–	1
Emerald Shiner	–	–	41	375	13
Flathead Catfish	–	–	–	–	–
Freshwater Drum	–	–	2	–	–
Goldeye	–	–	3	2	–
Grass Carp	–	–	–	–	1
Gizzard Shad	13	14	98	64	72
Longnose Gar	5	–	–	3	8
Mooneye	1	–	–	2	–
Western Mosquitofish	–	–	–	2	–
Orangespotted Sunfish	–	–	–	–	–
Paddlefish	–	–	–	–	–
Quillback	–	–	–	–	2
Red Shiner	–	–	3	6	–
River Carpsucker	–	–	2	–	–
Spotfin Shiner	–	–	–	–	–
Sauger	–	–	1	–	1
Skipjack Herring	–	–	–	1	–
Smallmouth Buffalo	–	–	3	–	9
Shortnose Gar	1	–	1	8	3
Shovelnose Sturgeon	–	–	–	–	–
Spottail Shiner	–	–	–	–	–
Silver Carp	–	–	30	127	14
Unidentified shiner	–	–	–	–	1
Walleye	–	–	–	–	–
White Bass	–	–	2	–	–
White Crappie	–	–	–	–	–
Total	**20**	**16**	**196**	**593**	**131**

Table A.1 continued

Fish Species	Grand River RM 250 N = 24	Crooked River RM 314 N = 6	Kansas River RM 367 N = 12	Platte River (MO) RM 391 N = 7	Nishnabotna River RM 542 N = 6
Bighead Carp	–	11	–	–	1
Black Buffalo	–	1	–	–	–
Brook Silverside	–	–	–	–	–
Bluegill	–	3	–	–	1
Bigmouth Buffalo	3	7	3	3	–
Blue Sucker	–	–	1	–	–
Common Carp	1	8	–	–	5
Channel Catfish	–	–	2	–	2
Emerald Shiner	102	87	336	134	182
Flathead Catfish	–	–	1	–	–
Freshwater Drum	–	2	9	–	4
Goldeye	3	1	2	1	3
Grass Carp	–	1	2	1	–
Gizzard Shad	18	98	62	25	199
Longnose Gar	4	2	4	1	2
Mooneye	–	–	–	10	–
Western Mosquitofish	–	–	1	–	–
Orangespotted Sunfish	–	1	–	–	–
Paddlefish	–	–	–	–	–
Quillback	–	–	–	–	–
Red Shiner	14	–	6	–	–
River Carpsucker	1	–	–	–	8
Spotfin Shiner	–	–	1	–	–
Sauger	–	–	–	–	2
Skipjack Herring	–	–	–	1	–
Smallmouth Buffalo	3	4	10	5	2
Shortnose Gar	4	5	3	1	11
Shovelnose Sturgeon	–	–	11	–	–
Spottail Shiner	5	–	–	–	–
Silver Carp	71	109	119	125	147
Unidentified shiner	–	–	–	–	–
Walleye	–	–	–	–	1
White Bass	–	3	–	–	–
White Crappie	–	1	–	–	–
Total	**229**	**344**	**573**	**307**	**570**

Table A.1 continued

Fish Species	Big Sioux River RM 734 N = 8	Vermillion River RM 772 N = 8	James River RM 800 N = 9	Total
Bighead Carp	2	1	–	**17**
Black Buffalo	–	–	–	**1**
Brook Silverside	–	–	–	**6**
Bluegill	–	–	–	**5**
Bigmouth Buffalo	5	16	1	**45**
Blue Sucker	–	–	–	**1**
Common Carp	–	4	–	**21**
Channel Catfish	–	–	–	**7**
Emerald Shiner	19	6	31	**1,326**
Flathead Catfish	–	–	–	**1**
Freshwater Drum	–	–	–	**17**
Goldeye	3	3	11	**32**
Grass Carp	–	–	–	**5**
Gizzard Shad	184	34	541	**1,422**
Longnose Gar	1	3	4	**37**
Mooneye	–	–	1	**14**
Western Mosquitofish	–	–	–	**3**
Orangespotted Sunfish	–	–	–	**1**
Paddlefish	–	–	6	**6**
Quillback	–	–	–	**2**
Red Shiner	–	–	9	**38**
River Carpsucker	8	11	1	**31**
Spotfin Shiner	–	–	–	**1**
Sauger	–	–	–	**4**
Skipjack Herring	–	–	–	**2**
Smallmouth Buffalo	2	3	1	**42**
Shortnose Gar	2	4	4	**47**
Shovelnose Sturgeon	–	–	–	**11**
Spottail Shiner	–	–	–	**5**
Silver Carp	274	342	103	**1,461**
Unidentified shiner	–	–	–	**1**
Walleye	–	–	–	**1**
White Bass	–	2	–	**7**
White Crappie	–	–	–	**1**
Total	**500**	**429**	**713**	**4,621**