

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:

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

Kansas: Kansas Department of Wildlife and Park (KDWP), Emporia State University

Missouri: Missouri Department of Conservation (MDC)

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

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), University of Idaho, Kennedy LIFE Lab

U.S. Fish and Wildlife Service (USFWS) - Columbia Fish and Wildlife Conservation Office, Bozeman Fish Health Lab, Missouri River Fish & Wildlife Conservation Office, and Great Plains Fish & Wildlife Conservation Office

Statement of Need:

The USFWS and the Aquatic Nuisance Species Task Force, in collaboration with multiple stakeholders, released a national invasive carp management and control plan (National Plan; Conover et al. 2007) to limit ecological and economic problems posed by these species. Despite tremendous progress towards achieving National Plan goals, there remains a great need to develop metrics to quantify the success of invasive carp management and inform control efforts, especially in the Missouri River Basin where funding for invasive carp research has been lacking. Defining the spatial distribution and demographics of invasive carp populations in the Missouri River Basin is fundamental to prescribing and assessing management actions as outlined in the National Plan Goals and Strategies related to prevention, containment and control, and extirpation. In addition, understanding the status and trends in abundance, size or age structure, maturity schedules, or fecundity of fish in a population is 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 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: KDWP, 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: KDWP, 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.
- Sub-Objective 4: Remove invasive carp below Bowersock Dam to provide a buffer against range expansion should dam be inundated during a high flow event.

Project Highlights:

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).

- **Nebraska Mainstem Sampling:**
 - Environmental DNA (eDNA) sampling indicated no new detections in the Missouri River outside the known distributions of the invasive carp species.
- **South Dakota Sampling:**
 - 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.
 - A total of 525 Silver Carp otoliths have been collected for otolith microchemistry analysis.
 - Issues with the mass spectrometry equipment have delayed results. A potential grant extension is being processed to allow for finalization of sample processing.

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).

- **Iowa Larvae Sampling:**
 - We collected nearly 800 ichthyoplankton samples across 13 site and two years.
 - We did not collect invasive carp in 98% of tows.
 - We collected nearly 6,000 larval fishes, including more than 4,500 carp larvae.
 - We captured most invasive carp larvae from the Big Sioux River on June 15, 2022 associated with a rising hydrograph and water temperatures.
- **Iowa Juvenile Sampling:**
 - We sampled fish communities at 81 sites in Missouri River tributaries and collected nearly 85,000 fishes of 57 species.
 - We did not collect juvenile invasive carp within any tributary.
 - We also sampled eight Missouri River off-channel habitats and collected juvenile invasive carp at three sites (Lower Hamburg Bend, Big Bear Park, Schilling Lake Wildlife Management Area).
- **Missouri Tributary and Mainstem Missouri River Sampling:**

- Silver Carp was the most abundant invasive carp with 761 sampled with a total weight of 1,429kg (3,150 lbs) of fish in 2023. Eleven Grass Carp and one Bighead Carp were also collected. No Black Carp were sampled.
 - Silver Carp catch rates were highest at Platte River Site 01 (171.6 fish/hour). Catch rates of Silver Carp at Platte River site 01 were significantly higher than Lamine River site 01 ($P < 0.05$). However, catch rates at the Missouri River Bend at the Platte River (14 fish/hour) was the lowest in 2023.
 - Size classes of Silver Carp were similar between all three tributaries with few fish below 500mm. However, individuals >600mm were more frequently sampled in the Platte than in the Lamine and Grand Rivers. Preferred size Silver Carp (560 - 739mm) comprised the highest proportion sampled from the Platte River in 2022 and 2023. The Platte River is also the only site to have memorable size Silver Carp (740 - 929mm) collected in all four sample years.
 - Silver Carp sex ratios of males to females tended to be close to a 1:1 ratio at most sites except for the Platte River and Missouri River Bends at the Lamine and Grand River, where a slightly higher ratio of males was sampled than females.
 - Size distributions and length at age data of Silver Carp in 2023 indicate limited reproduction and recruitment over the last three years in the three tributaries and associated Missouri River bends. This may be due to persistent low river levels in the lower Missouri River basin.
- Nebraska Mainstem Missouri River Sampling:
 - Invasive carp species collected below Gavins Point Dam include Silver Carp ($n = 184$), Bighead Carp ($n = 15$), and Grass Carp ($n = 48$).
 - No invasive carp were captured in the Ft. Randall tailwater as expected.
 - Otoliths for Invasive Carp were collected and indicated a relatively young age structure in the Gavins Point tailwater.
- Nebraska Platte River Sampling:
 - A total of 1,528 Silver Carp and 50 Bighead Carp have been sampled during FY 2022 and FY 2023. A total of 57 species have been encountered. Mean length of Silver Carp across all study sites was 642 mm TL (min = 461 mm TL, max = 966 mm TL). Mean length of Bighead Carp across all study sites was 707 mm TL (min = 521 mm TL, max = 955 mm TL). Ages ranged from 2 to 8. Catch per unit effort was highest in the Missouri River and in Salt Creek (a tributary of the Platte River). Invasive carp were captured at all locations sampled across the study reach indicating consistent occupancy across the lower Platte River basin including its tributaries.
 - Reproductively capable Silver Carp were sampled in all study reaches during the course of the study as evidenced by histological analysis and visual inspection of gonads. Reproductive individuals were present through each season indicating a potentially prolonged spawning period within the system. Visual identification based on morphometrics of eggs and larvae indicated no spawning in the Platte River basin but evidence of spawning within the Missouri River. Additional genetic validation of ichthyoplankton samples from the Platte River is ongoing.

- Nebraska Gear Assessment Project:
 - During FY 2023 extensive efforts were employed to develop and test herding as a potential mechanism to move Silver Carp and Bighead Carp downstream using established protocols developed in the literature (see Ridgeway et al. 2023). Experimental trials were conducted at the lower end of Salt Creek, NE (a tributary of the Platte River near Ashland, NE). Herding, using percussion sound emitted underwater, proved effective at moving carp downstream and results are being used to inform efforts in FY2024 where removal efforts will be implemented following herding of individuals into a capture area.
 - During FY 2023 a systematic review of the literature pertaining to sampling methods conducted across life-history stages for Silver Carp and Bigheaded Carp was initiated. Further, in addition to the assessment of herding techniques listed in Objective 1, we conducted larval and age-0 sampling efforts within the Platte River basin to locate potential areas of recruitment that would allow assessment of different gear types to sample those life stages. No larval or age-0 Silver Carp or Bigheaded Carp were detected at our sampling locations. As such, we intend to focus larval and age-0 sampling efforts to the confluence of the Platte River with the Missouri River in FY 2024 to optimize our chances of sampling those life stages given proximity to the Missouri River where we have documented young carps during previous projects.
- USFWS Sampling:
 - In 2023, the USFWS utilized the electrified dozer trawl to complete 214 transects across eight different tributary habitat confluences collecting 3,398 Silver Carp and 29 Bighead Carp.
 - Updated electrified dozer trawl sampling protocols to provide tributary invasive carp demographic information in potential areas of management throughout the basin.

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: KDWP, NGPC, MDC).

- Kansas sampling:
 - One water year of field sampling has been completed and project is on schedule to be completed as proposed.
 - Preliminarily, presence of carp is impacting native fish diets and measuring changes in stable isotopes may provide insight into the effects of continued invasive carp removal efforts.
- Missouri Fish Community Sampling:
 - Total of 4,833 fish collected from all sites in 2023.
 - 23 species in Lamine River, 26 spp. in Grand River and 29 spp. in Platte River (MO)
 - Missouri River at Lamine - 24 species, Missouri River at Grand – 25 spp., Missouri River at Platte – 19 spp.

- Nebraska Sampling: A total of 3,557 fish were caught below Fort Randall Dam, and a total of 6,290 were caught below Gavins Point Dam during the 2021-2022 field seasons.
- South Dakota Sampling:
 - No positive eDNA detections above the Sioux Falls on the Big Sioux River.
 - Positive detections above the East Vermillion Lake spillway are outside the known distribution of Silver or Bighead Carp and require additional sampling efforts.
 - The mean detection probability below known invasive carp barriers was 51% and 77% for the Big Sioux and Vermillion rivers.

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 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 (**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 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 to August 5th, 2022 and from April 27th to July 29th, 2023. Each sampling session lasted two consecutive days, where we sampled the Big Sioux and Floyd rivers one day and the Little Sioux and Boyer rivers the following day. We collected larval fishes with a conical ichthyoplankton net (0.5-m mouth diameter, 500- μ m 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 >80 meters to <7 meters). A General Oceanics flowmeter (model 2030R) 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. The nearest upstream USGS or NOAA stream gauge was used to obtain daily river discharge. We towed the net manually against the current when river discharge was too low to properly engage the flowmeter, once each at the FLD-UPR and LSR-UPR site in 2022 (August 5th and August 4th, respectively). In 2023, we conducted manual tows three times at the BYR-MID site (July 4th, July 18th, and July 28th), twice at the LSR-UPR site (July 18th and July 28th), and once each at the BSR-UPR and FLD-MID sites (both on July 29th). Furthermore, we did not sample the FLD-MID site on August 5th, 2022, the BSR-UPR site on July 19th, 2023, or the BYR-UPR site on July 18th and July 28th, 2023 due to low water levels, as the thalweg depth was less than half of the net's diameter. After each tow, we rinsed net contents into the cod end, filtered it through a 500- μ m sieve, and preserved the sample 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). The similarity between Bighead and Silver Carp larvae makes it difficult to distinguish between the two species; therefore, we identified bigheaded carps to genus.

Component 2: Assess detection probabilities and spatial distribution of juvenile invasive carp throughout Missouri River tributaries in Iowa.

We sampled fish assemblages at 81 stream sites across seventeen HUC8 watersheds in the Missouri drainage from May – October of 2022 and 2023 (**Figure 2**). We selected sites ranging from 2nd – 6th 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/chutes along the Missouri River proper during 2022 and four during 2023. 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 & Discussion

Component 1: Assess timing and magnitude of invasive carp reproduction in the Little Sioux River using larval densities

We conducted 787 larval tows across 13 sites on four rivers (Big Sioux, Little Sioux, Floyd, Boyer) during summer 2022 and 2023 to assess invasive carp reproduction throughout western Iowa (**Figure 1**). In total, we collected 5,878 larvae and over 500 eggs. We detected eggs and larvae at all sites, though not on all dates. Larval densities in individual tows ranged from 0 to 6,280 fish/100 m³. The majority of tows contained no invasive carp larvae (98.22% of tows) or larvae of any taxa (74.2% of tows). The highest density of age-0 fishes in a single tow (6,280 fish/100 m³) was recorded at the BSR-MID site on June 15th, 2022. This sample consisted almost entirely of invasive carp larvae, approximately 4,500 individuals. Invasive carp larvae were also collected at the LSR-UPR site on July 14th, 2022 (2 individuals) and the BYR-LWR site on July 14th, 2022 (1 individual), though they could not be identified to genus. In 2023, we collected 11 invasive carp at the BSR-MID and BSR-LWR sites on May 8th and at the LSR-UMD and LSR-LWR sites on June 16th and June 26th, respectively. Of the carp identified in both years, 50.5% were *Hypophthalmichthys* (2,324 individuals) and 20.7% were *Ctenopharyngodon* (953 individuals). An additional 1,321 carp larvae could not be identified to genus and were categorized broadly as “invasive carp”

All invasive carp larvae collected were yolk-sac and ranged in age from approximately less than 12 hours post-fertilization to 7 days (Chapman and George 2011). Larvae collected on June 15th, 2022 were likely spawned during a significant rise in flow from May 31st to June 1st (188 m³/sec) and from June 13th to June 16th (137 m³/sec). 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. All invasive carp from both years were collected during a peak in river discharge or a few days thereafter.

When captured, the highest density of native fish larvae was 102.2 fish/100 m³ in a single tow, while the average density was 14.07 ± 0.79 SE fish/100 m³ per tow. Native taxa consisted overwhelmingly of *Cyprinidae* (1,015) followed by *Catostomidae* (genera *Ictiobus* and *Carpionides*; 91), *Percidae* (68), *Centrarchidae* (genera *Pomoxis* and *Lepomis*; 12), *Sciaenidae* (3), and *Hiodontidae* (1). Native fish larval densities tended to peak in early to mid summer (**Figure 3a,b-4a,b**). Age-0 *Cyprinidae* reached the highest densities in late June (37.4 fish/100 m³ ± 13.8 SE), *Catostomidae* in early June (4.7 fish/100 m³ ±

2.5 SE), and *Percidae* in mid June ($4.9 \text{ fish}/100 \text{ m}^3 \pm 3.4 \text{ SE}$). Native larval and yolk-sac fishes were captured at water temperatures between 11.6 and 33.7°C and discharges between <1 and 150 m³/sec. Conductivity ranged from 448 to 1,149 µS and pH varied from 7.84 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 and years. However, preliminary visualization plots indicate potential differences in assemblages between river sites. *Cyprinidae*, *Percidae*, and *Catostomidae* were detected in all rivers; *Hiodontidae* was only captured in the Big Sioux River, *Sciaenidae* was only captured in the Little Sioux and Big Sioux rivers, and *Centrarchidae* was only captured in the Little Sioux and Floyd rivers. We detected three families in the Boyer River compared to four families in the Floyd, five in the Little Sioux, and six in the Big Sioux (**Figure 3a,b-41a,b**).

Late June, July, and August of both 2022 and 2023 were characterized by high water temperatures (recorded up to 33.9°C during sampling) and low water levels (less than 10 cm in depth at some sites). 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 both sampling years (**Figure 5**). It is therefore unsurprising we documented most invasive carp reproduction in the Big Sioux River, especially given the previous detection of young-of-the-year invasive carp in the lower Big Sioux (Hayer et al. 2014). However, the presence of invasive carp larvae in the upper sites of the Little Sioux River indicates spawning in river reaches where reproduction was not known to occur. Given the extremely low densities of carp larvae sampled, the high water temperatures, low water levels in those reaches, and lack of juveniles detected (see Component 2.2 below), it is unlikely that invasive carp spawning in 2022 or 2023 contributed significantly to population growth in the upper Little Sioux River.

Component 2: Assess detection probabilities and spatial distribution of juvenile invasive carp throughout Missouri River tributaries in Iowa

We sampled fish communities at 81 tributary sites throughout western Iowa during 2022 and 2023 to assess potential recruitment sources for invasive carp. We collected 84,892 individuals of 57 species during tributary sampling but did not detect juvenile invasive carp. We sampled 8 off-channel sites along the Missouri River proper during 2022 and four during 2023. 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 of 2022. Among the three sites, we only collected seven individuals, all were Silver Carp with a mean total length of $70 \pm 12 \text{ mm}$ and mean weight of $2 \pm 1 \text{ g}$. 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 composed of silty sand, depth was $\leq 0.5 \text{ m}$, 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 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 were lower than average during 2022 and 2023. We captured all juveniles late in the field season, suggesting sampling late-summer through early-autumn may increase captures; however, we also exclusively sampled off-channel habitats of the Missouri River late August – October. These results suggest juvenile invasive carp may preferentially use off-channel habitat opposed to tributaries in the Missouri River basin of Iowa. Continued research should be done to determine recruitment activity during years with normal and above average water levels.

In addition to juvenile invasive carp, we captured adult Silver Carp in the Little Rock River and Tyson Bend backwater (IA) during 2022 and the West Nishnabotna, Little Sioux, and Chariton Rivers during 2023. We observed but were unable to capture adult Silver Carp at one site in the Boyer River, one site in the Big Sioux River, the Decatur Bend backwater (IA), Louisville Bend backwater (IA), and Big Bear Park backwater (NE) during 2022. We also captured adult Grass Carp in three Iowa rivers: the Turkey Creek (Cass County), Wolf Creek Ditch (Woodbury County), and Floyd River (Sioux County) during 2022 and in Willow Creek (Harrison County), Little Sioux River (Clay County), and the Iowa Drainage Ditch (Fremont County) during 2023.



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.

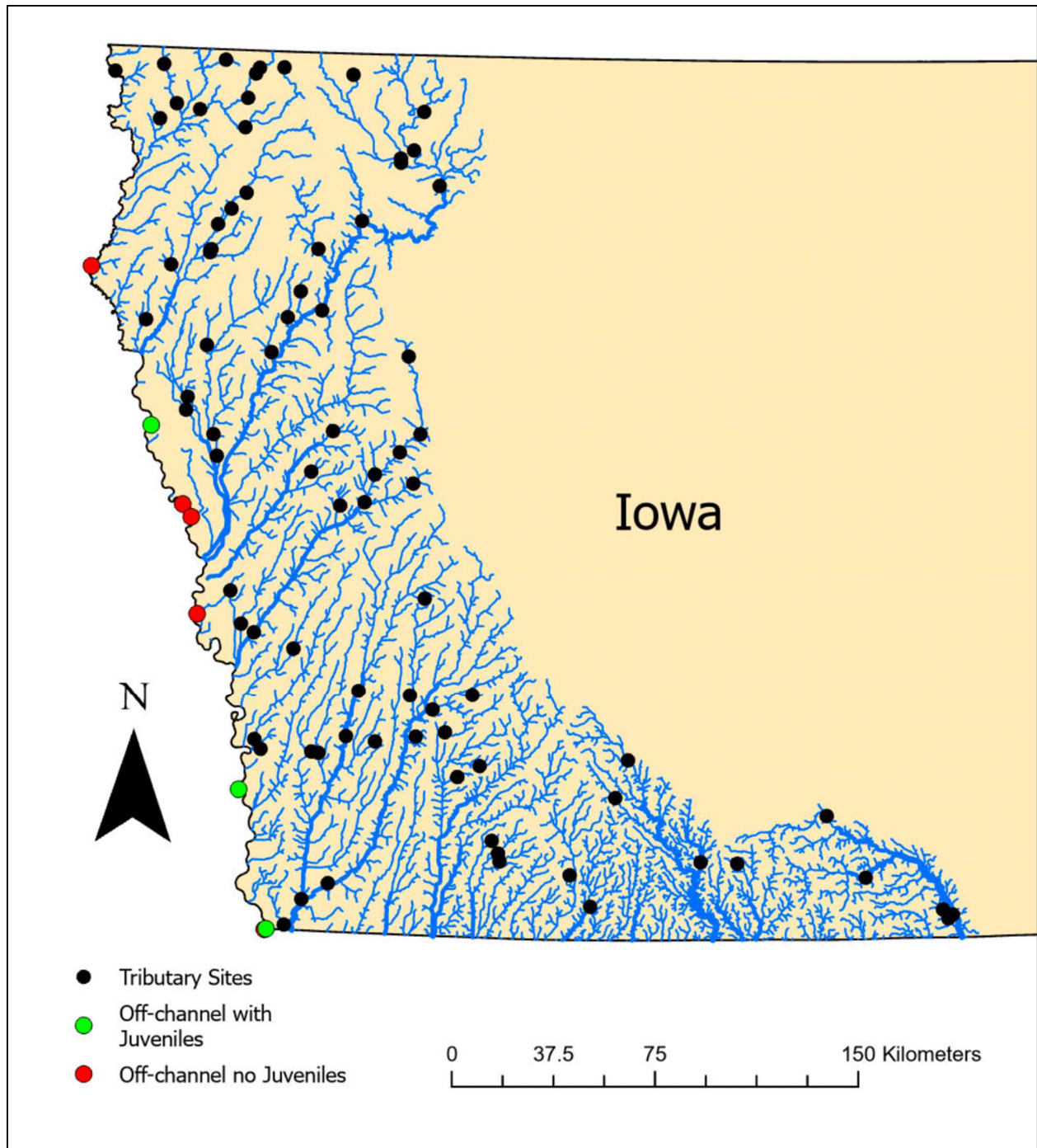


Figure 2. Locations of tributaries (n=81) and off-channel (n=8) sites in the Missouri River Basin where we sampled fish communities during 2022 – 2023. All juveniles were detected during 2022.

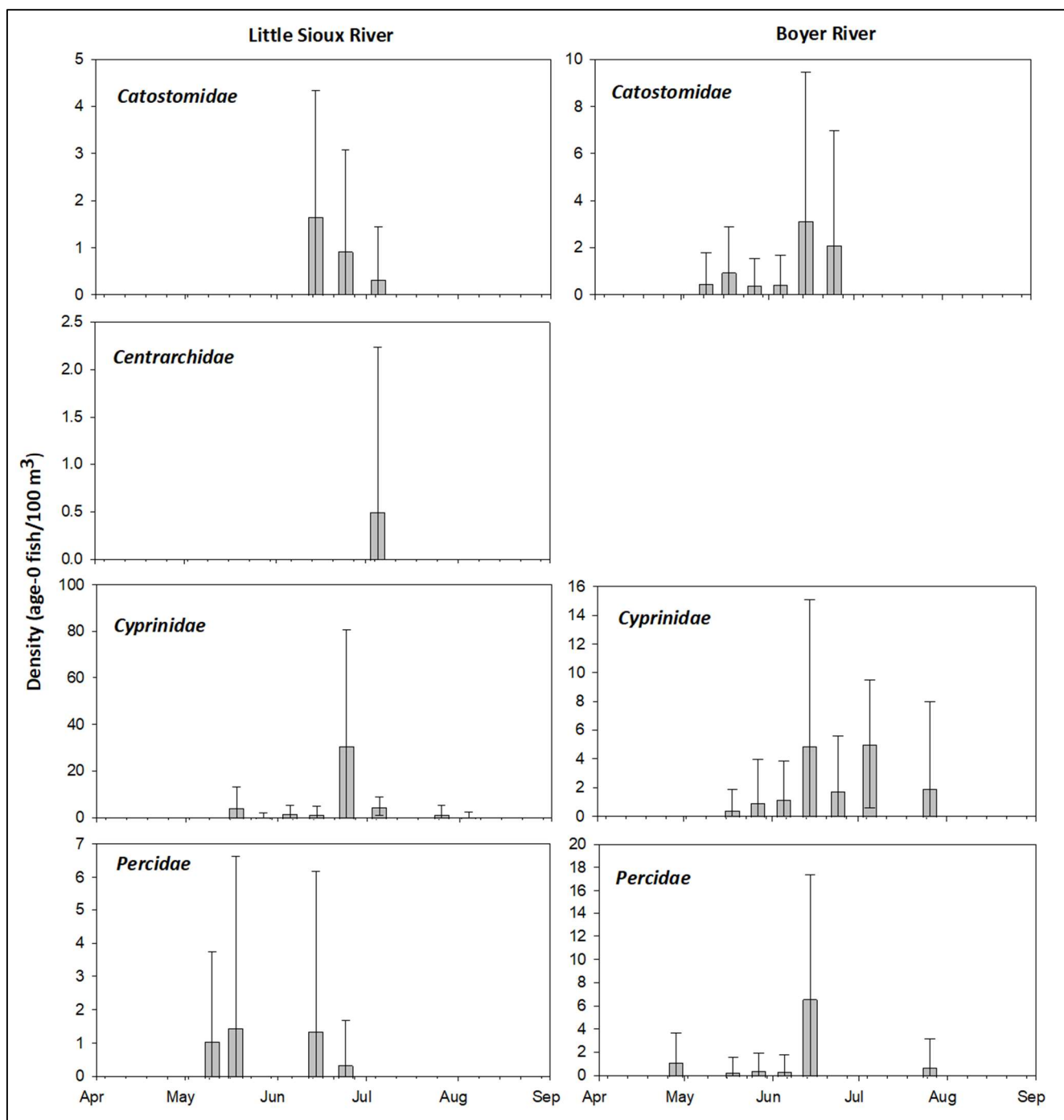


Figure 3a. Density (mean $\pm$ 1 SE) of age-0 fishes from four families collected on the Little Sioux River (left) and Boyer River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

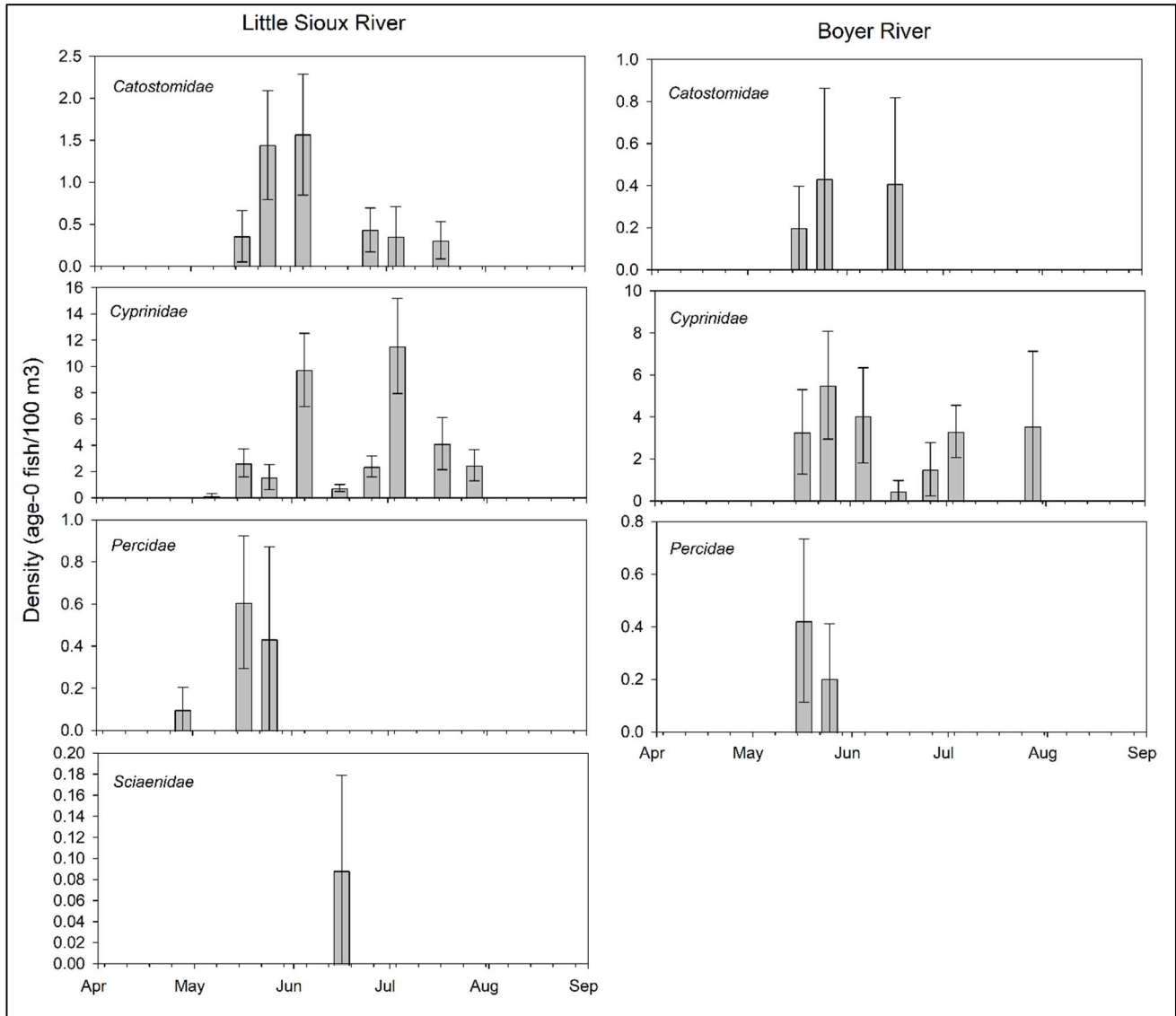


Figure 3b. Density (mean $\pm$ 1 SE) of age-0 fishes from four families collected on the Little Sioux River (left) and Boyer River (right) from April 27th to July 29th, 2023. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

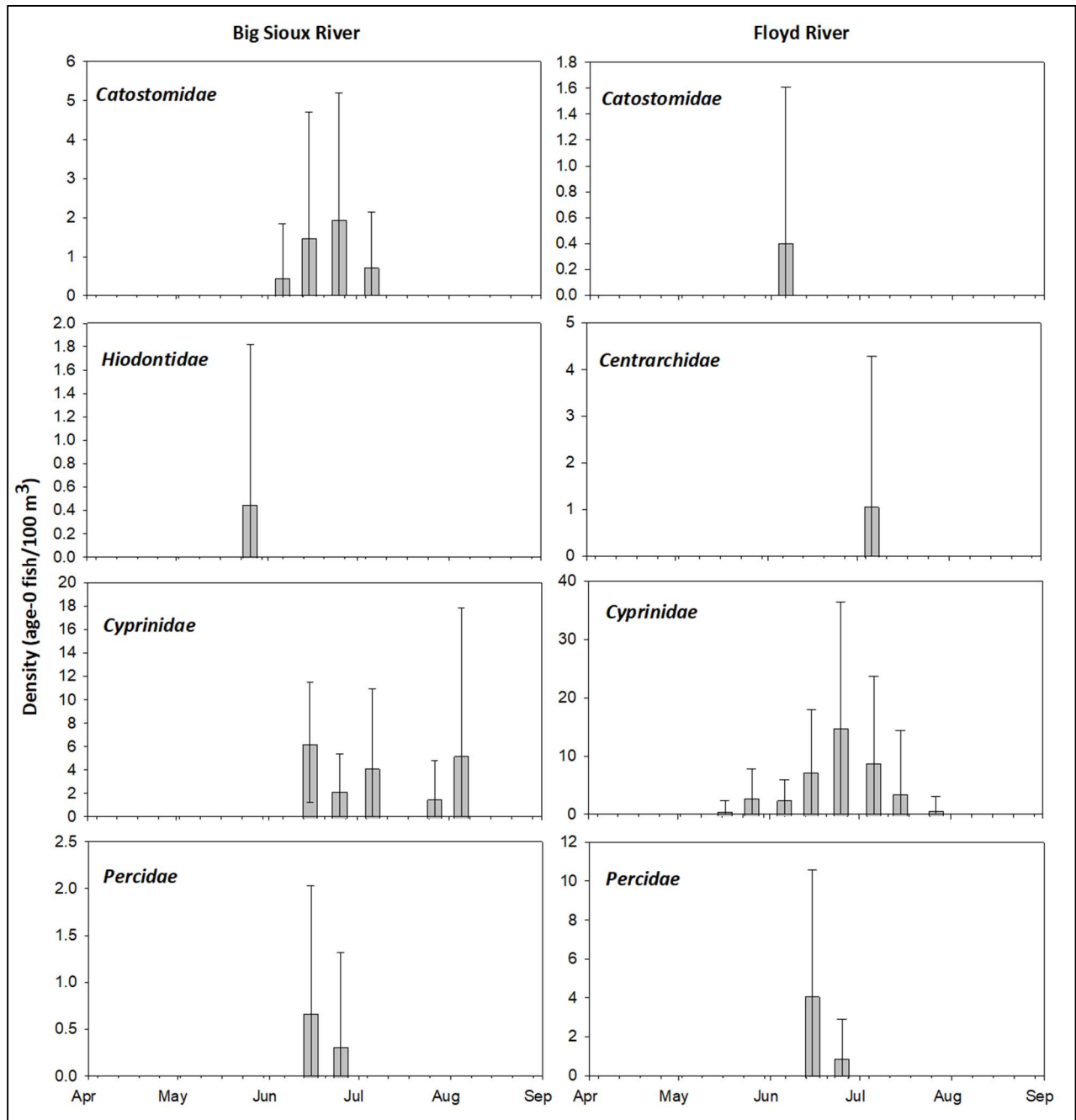


Figure 4a. Density (mean $\pm$ 1 SE) of age-0 fishes from five families collected on the Big Sioux River (left) and Floyd River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

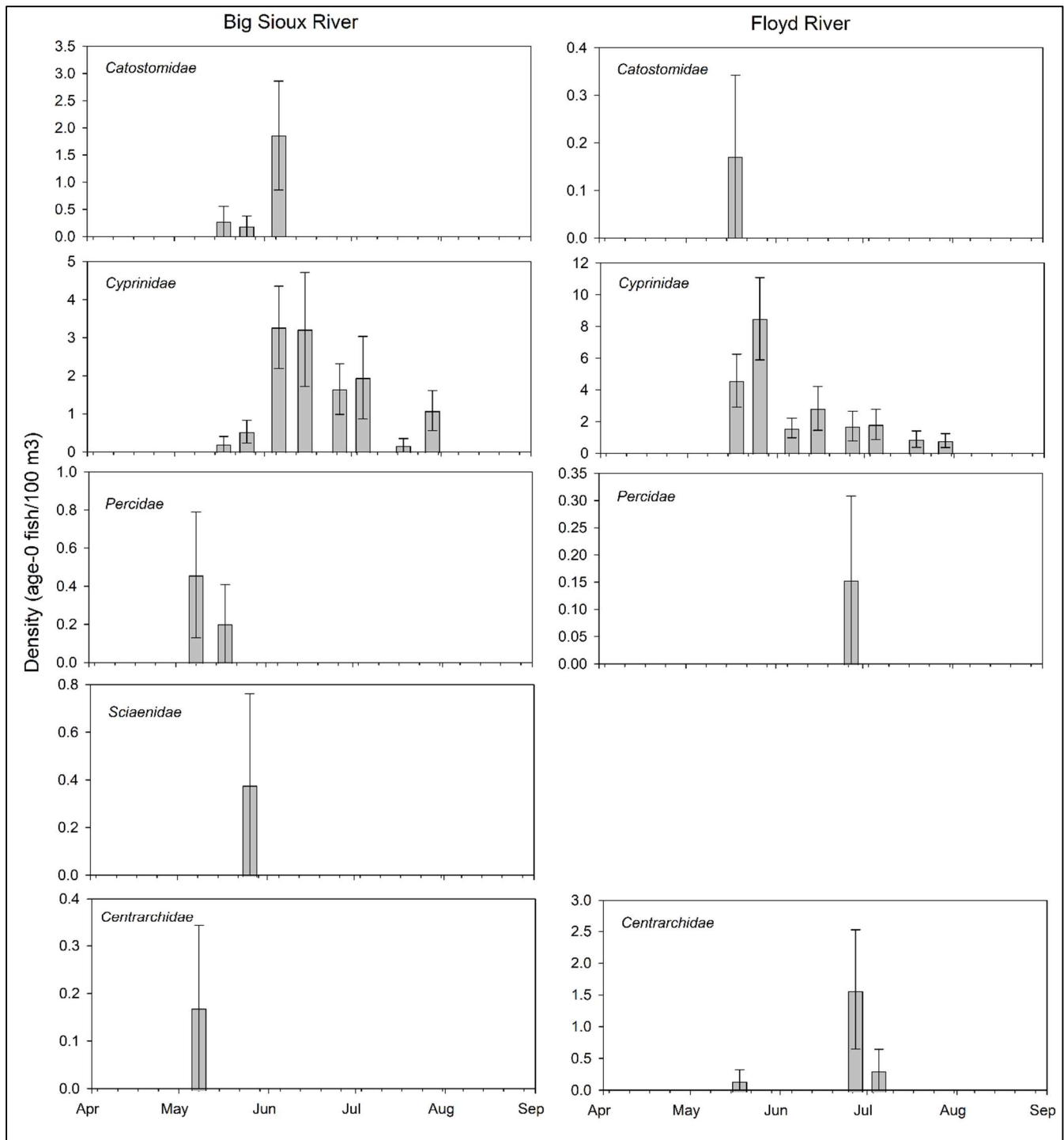


Figure 4b. Density (mean $\pm$ 1 SE) of age-0 fishes from five families collected on the Big Sioux River (left) and Floyd River (right) from April 27th to July 29th, 2023. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

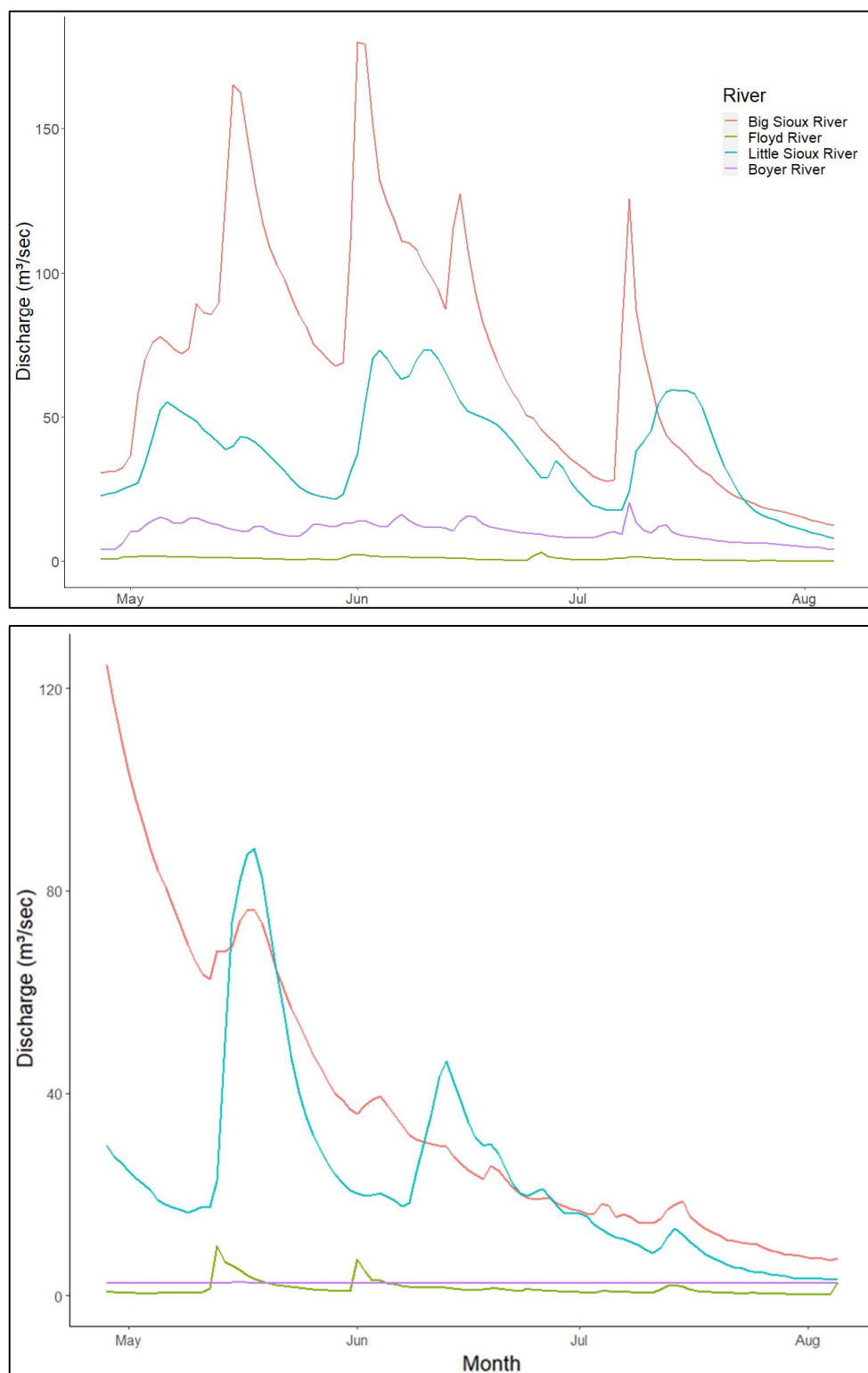


Figure 5. Discharge (m³/sec) of the Big Sioux, Floyd, Little Sioux, and Boyer River from April 28th to August 5th, 2022 (top panel) and 2023 (bottom panel).

Recommendation:

We collected invasive carp larvae in a number of small tributaries under drought conditions, but did not subsequently capture juveniles, suggesting these systems may support reproduction but not recruitment. However, because our two years of fieldwork occurred during drought conditions under low flow conditions, these systems may support higher reproduction and recruitment under more suitable conditions. Thus, additional work assessing the role these tributaries play in facilitating population growth under more favorable conditions for recruitment is warranted. If recruitment is low in tributaries, opportunities may exist to intensively manage tributaries to reduce or eliminate invasive carp from these systems.”

State Report: Kansas

Agency: Kansas Department of Wildlife and Parks

Project Title: Kansas River Invasive Carp Food Web Study

Methods:

Data was collected for one water year and will continue for one more consecutive year, in three seasons (spring [April], summer [July], fall [November]). Collection sites are above Bowersock dam (invasive carp absent), immediately below Bowersock dam, (moderate density invasive carp), and below the WaterOne dam (high density invasive carp). For each collection event, data was collected from up to 10 replicates of each of various native species and invasive carps, then paired down to representatives of three major feeding guilds (planktivores, detritivores, piscivores) using 10 native fishes, invasive carp, and invertebrates to establish baselines. Only species that could be found at all sites were used. Fish captured by electrofishing, gill netting, seining and angling. A tissue sample was collected for isotope analysis (muscle biopsy for large individuals [$>100\text{mm}$] and whole animals/muscle tissue for juveniles). Changes in consumers' isotopic niches will be determined using Layman's indices and Bayesian mixing models. Following collection and analyzation of isotope data, comparisons will be made to other river systems where similar data have been collected.

Results & Discussion

The planktonic baseline sampling in the first season was insufficient and without this baseline, mixing models are ineffective. Retroactive corrections will be attempted upon full dataset receipt. In the interim some of Layman's indices (Table 1), $\delta^{13}\text{C}$ range and $\delta^{15}\text{N}$ range, are used to describe niche breadth and distribution within and among species. Both metrics are the arithmetic range of the " δ " values. $\delta^{13}\text{C}$ range demonstrates niche diversification within a species and $\delta^{15}\text{N}$ range demonstrates the vertical food web structure of a species. Convex hull area (CL) is a representation of the full complexity of a dietary niche in isotope space. Data presented in this report is a reflection of the first season's sample (April), subsequent samples are pending analysis.

Planktivorous

Current and past studies often attempt to look at Bigmouth Buffalo (*Ictiobus cyprinellus*), Gizzard Shad (*Dorosoma cepedianum*), and American Paddlefish (*Polyodon spathula*) abundance and condition as metrics of impact from competition by filter-feeding invasive carp. In lieu of accessibility of paddlefish in our study, we assess juvenile sunfish who also rely on zooplankton. We hypothesized planktivores would be noticeably impacted between control (upper) and invaded (middle and lower) reaches due to intraspecific competition. All species within our sample demonstrated differences between sites with and without invasive carp, although these shifts were highly variable between species.

Bigmouth Buffalo presented a unique issue among the native planktivores in that our target of ten individuals per site was unattainable. Despite intensive, targeted efforts, only fourteen individuals were

captured; seven from the upper site, four from the middle site, and three from the lowest site. Given a small sample size that was differential between sites, it is inappropriate to compare the convex hull size. In lieu of baselines to ordinate the data from each site relative to one another, it is also impossible to conclusively determine niche movement, however; from upper site, to middle, to lower site it does *appear* that the $\delta^{15}\text{N}$ values are decreasing with means of 16.8, 16.4 and 15.0 respectively (Fig. 1). If this trend holds true in subsequent samples that are corrected for baselines, it will suggest that the trophic structure of plankton is being simplified in river reaches where the filter-feeding carp are present. Furthermore, the ability to capture a higher number of Bigmouth Buffalo at the upper site does lend credence to the suggestion that the species is being competitively excluded from the environment by direct competition.

American Gizzard Shad have a tight niche ($\delta^{13}\text{C}$ range = 4.63, $\delta^{15}\text{N}$ range = 3.09) in the control site within the iso-space expected of planktivores (Fig. 2). In the lower reach, this niche is expanded in all directions ($\delta^{13}\text{C}$ range = 9.80, $\delta^{15}\text{N}$ range = 6.85). This provides support for our hypothesis, as competitive exclusion from shad's preferred feed source (zooplankton) seems apparent.

Sunfish (*Lepomis sp.*) exemplified a generalist feeding strategy in the upper, carp-less reach with a broad isotopic niche ($\delta^{13}\text{C}$ range = 7.86) pointing to a variety of food sources. Conversely in the lower reach, their niche was constricted ($\delta^{13}\text{C}$ range = 2.57) and heavily overlapping the isotopic niche of filter-feeding carps (Fig. 3). The constricted niche shows the prey base available to *Lepomis sp.* is simplified. While the overlap in iso-space observed could be evidence of direct competition/shared diet in the lower reach, it may also be explained by *Lepomis sp.* being relegated to detrital food chains as detritivores (Fig. 5-9) occupy a similar iso-space.

Invasive Carp (*Hypophthalmichthys nobilis* and *H. molitrix*) are central to this study, however, are not present at the uppermost site. Like the Bigmouth Buffalo, Bighead Carp (*H. nobilis*) were not found in sufficient numbers ($n=2$) to make strong inferences and were only found at the lowest site. Silver carp were present at both the lower and middle site. They had similar niche breadth, barring a single outlier in the middle site, however the trophic diversity ($\delta^{15}\text{N}$ range) was greater in the middle site (Fig. 4). In lieu of sufficient baseline data, position in the biplot is not conclusive, however, carp in the middle site do have higher $\delta^{15}\text{N}$ values, so if this trend holds true in subsequent samples relative to respective baselines, it suggests that the highest density of carp has sufficiently impacted plankton communities to a level of simplifying trophic structure of plankton between the middle site (low-density of carp) and lowest site (high-density of carp).

Detritivores

We hypothesized detritivores may demonstrate a shift between sites if there were sufficient cascades to change the composition of the detritus in the river. Detritivores selected were Smallmouth Buffalo (*Ictiobus bubalus*), River Carpsucker (*Carpionodes carpio*), Bullhead Minnow (*Pimephales vigilax*), Red Shiner (*Cyprinella lutrensis*), and Sand Shiner (*Notropis stramineus*) (Fig 5-9 respectively), none of whose niche shifted drastically in isotopic space.

Smallmouth Buffalo and River Carpsucker (Fig. 5 and 6) isotopic niches were super-imposed over similar iso-space as the invasive carp, but their dietary preferences are dissimilar and there was no noteworthy effect on niche based on sample site. Competition is presumed unlikely, and we infer that the detrital food chains these fishes rely on derive from algal carbon sources.

Sand* and Red Shiners and demonstrated a weak trend downward in $\delta^{15}\text{N}$ with respect to site (Fig. 7 and 8), which is inconclusive in lieu of sufficient baseline data, however if subsequent samples concur relative to baselines it will provide further evidence for the simplification of the food web/trophic structure in the lower reach.

Bullhead Minnows* appeared to have a slight trend toward less negative $\delta^{13}\text{C}$ values for the invaded reaches of river (Fig. 9), which is inconclusive in lieu of sufficient baseline data. If subsequent samples concur relative to baselines it will provide evidence for a greater dependence on allochthonous inputs in the lower reach, which could be a response to the carp, or a reflection of greater availability of allochthonous nutrients in the lower reaches of river in habitats preferred by Bullhead Minnows.

*Sand Shiners and Bullhead Minnows were both plagued by apparent outliers who muddled the data and weakened inference. Trends referenced disregarded these values, but based on a single sample ($n=10$ per site) we do not have sufficient data to determine if these are true outliers that may have been compromised samples, or if they are an accurate reflection of the niche of these fishes, receipt of additional data should help clarify, re-submission of suspect samples is also possible as remaining tissue samples were retained.

Piscivores

We hypothesized piscivores should demonstrate any trophic cascades present as their prey sources can span the breadth of carbon sources in the river. Like the planktivores, shifts were evident, however they were species-dependant. Reliance on less negative $\delta^{13}\text{C}$ sources was observed in piscivores. Anecdotally, piscivores showed greater dominance in the lowest reach based on higher success in observation/capture. Further investigation into these phenomena is being discussed for the second year of sampling.

Channel Catfish displayed a similar trend to that of Gizzard Shad where they held a relatively tight niche in the upper reach ($\delta^{13}\text{C}$ range = 2.27) expanding to ($\delta^{13}\text{C}$ range = 5.34) in the lowest reach (Fig. 10). This reflects either shifts by the species on which they prey whose diets shifted relative to invasive carp e.g. Gizzard Shad, or more reliance on prey items that were not present in high concentrations in the upper reach e.g. Emerald Shiner.

Shortnose and Longnose Gar (*Lepisosteus platostomus* and *L. osseus*) presented a similar issue to Bigmouth Buffalo where collection in sufficient numbers was difficult and variable between sites. For this reason, niche size is being omitted from analysis. No gar were collected at the upper site, but the Gar at the middle and lower sites both demonstrated use of less-negative $\delta^{13}\text{C}$ sources (Fig. 11)

Flathead Catfish (*Pylodictus olivaris*) maintained similar carbon signatures but had reduced $\delta^{15}\text{N}$ values in the presence of carp (Fig. 12). This is inconclusive in lieu of sufficient baseline data. If subsequent samples concur relative to baselines, it will provide evidence that Flathead Catfish maintain a similar nutrient pathway, but the food web is subject to trophic simplification in the lowest reach where the invasive carp could be causing the shift.

Table 1 lists the Layman's indices calculated for selected species. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ range are the arithmetic ranges of " δ " values in a subset, MDC is the mean distance to centroid of a subset, MNND is the mean nearest-neighbor distance, SDNND is the standard deviation of nearest-neighbor distance, and CH is the

area of the minimum convex hull area that encapsulates all points in a subset. Data were collected from fishes of the Kansas River in April 2023.

Gizzard Shad	$\delta^{15}\text{N}$ range	$\delta^{13}\text{C}$ range	MDC	MNND	SDNND	CH
Lower	6.85	9.80	3.00	1.67	1.50	33.14
Upper	3.09	4.63	1.63	0.88	0.54	9.96
Sunfish						
Lower	3.12	2.57	1.12	0.61	0.39	4.3
Upper	8.73	7.86	3.36	1.64	0.68	33.07
Silver Carp						
Lower	0.7	1.55	0.44	0.21	0.15	0.56
Middle	2.74	2.58	0.81	0.56	0.49	2.1
Channel Catfish						
Lower	4.33	5.34	2.06	0.94	0.55	12.01

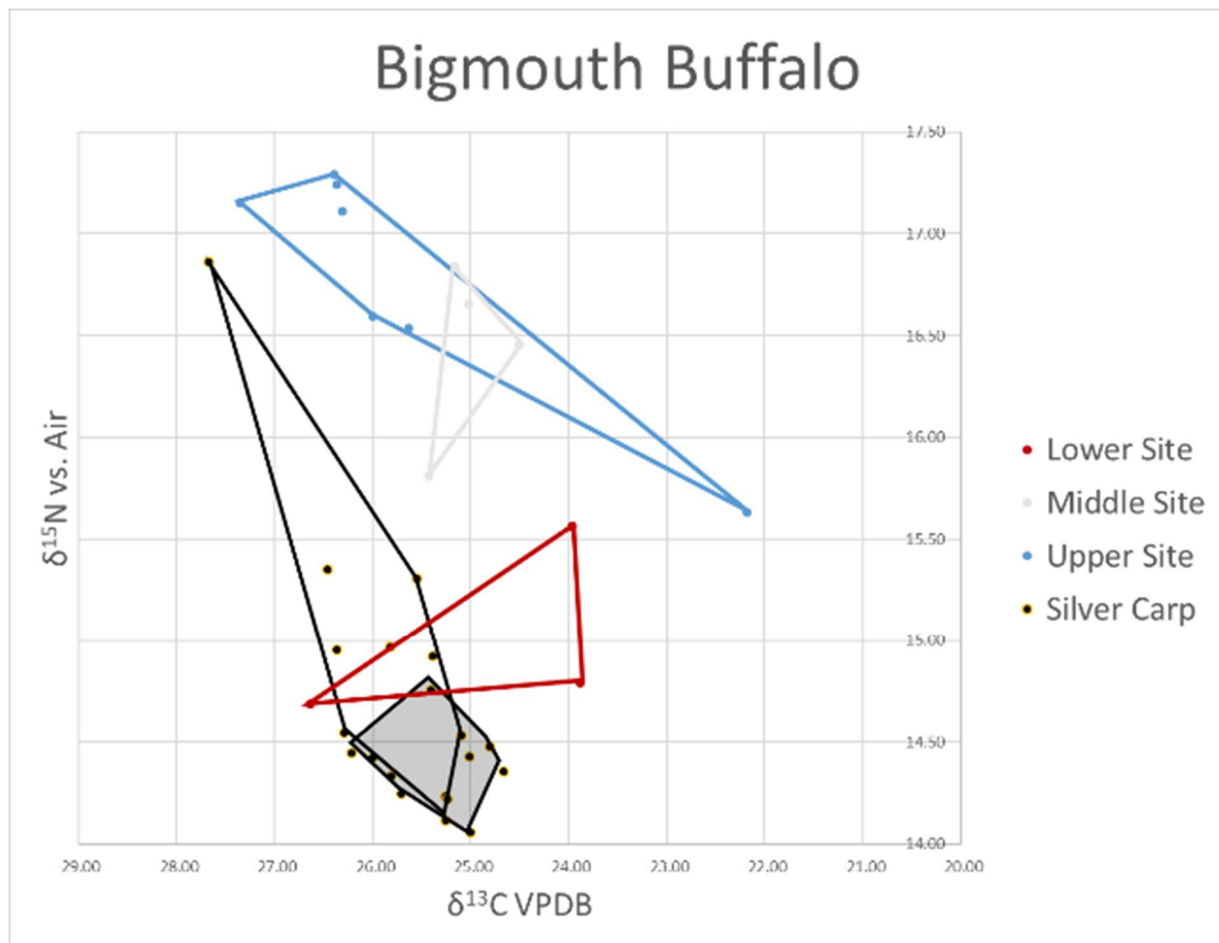


Figure 1 shows the isotopic niches of Bigmouth Buffalo based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

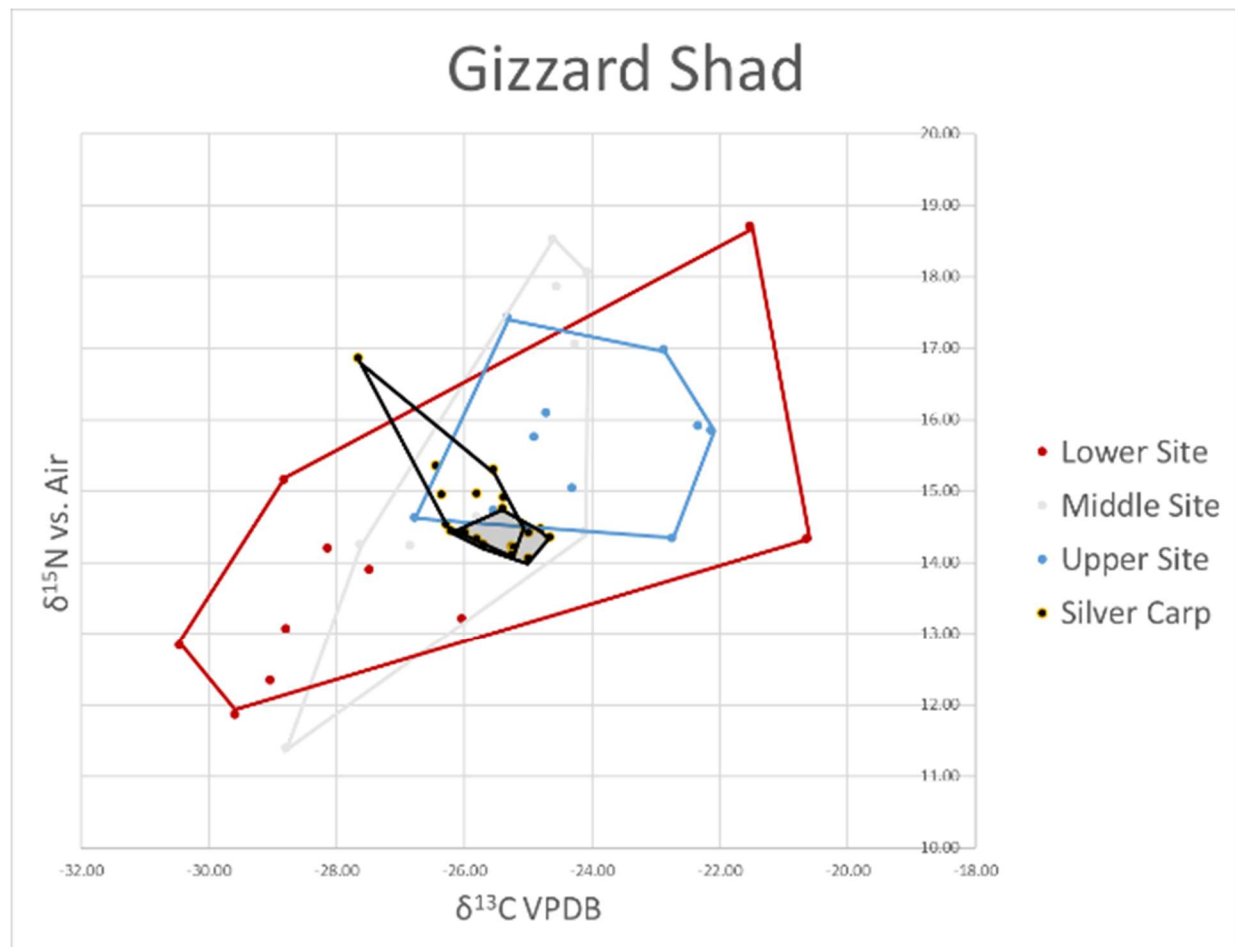


Figure 2 shows the isotopic niches of American Gizzard Shad based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. “Upper Site” refers to the reach of river directly upstream of the Bowersock Dam, “Middle Site” refers to the reach of river between the Bowersock and WaterOne dams, and “Lower Site” refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

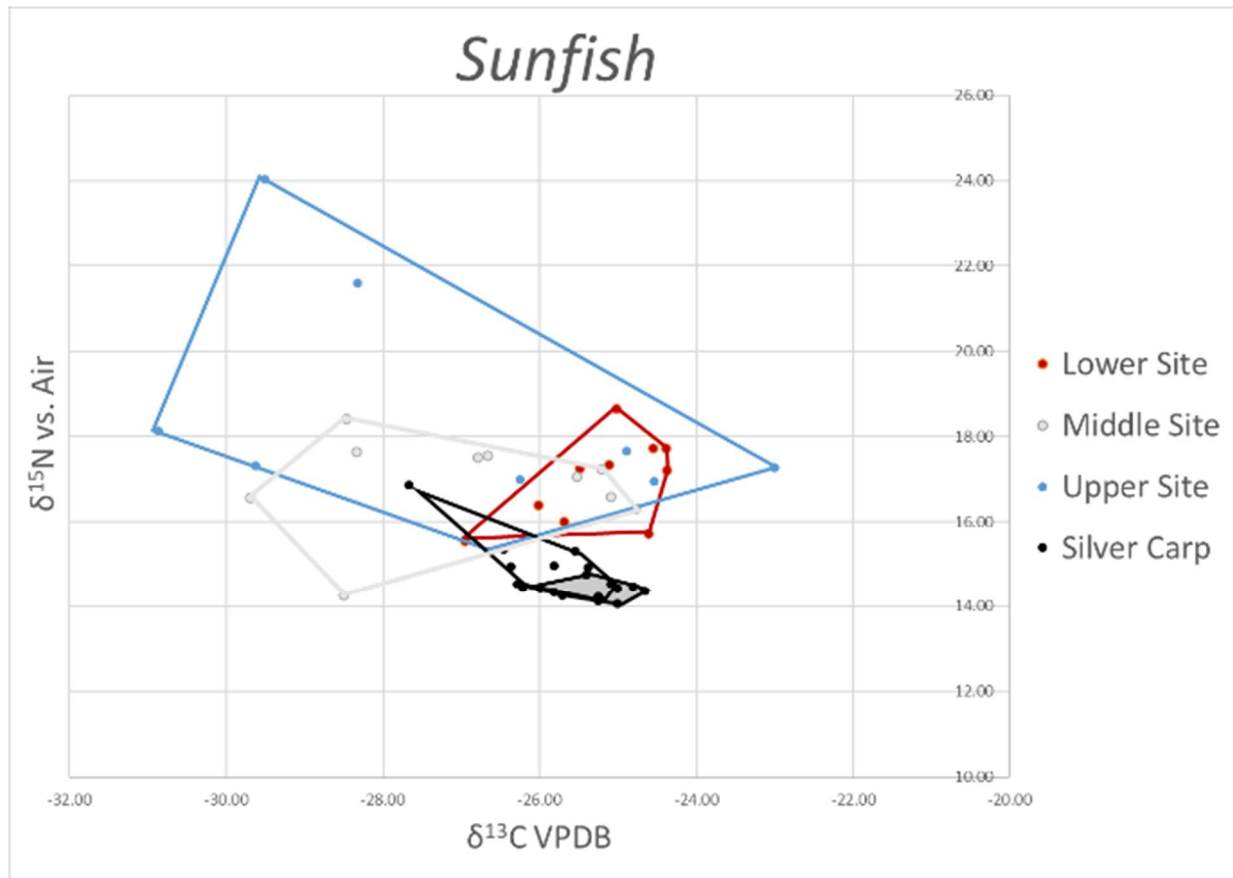


Figure 3 shows the isotopic niches of Juvenile Sunfish based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

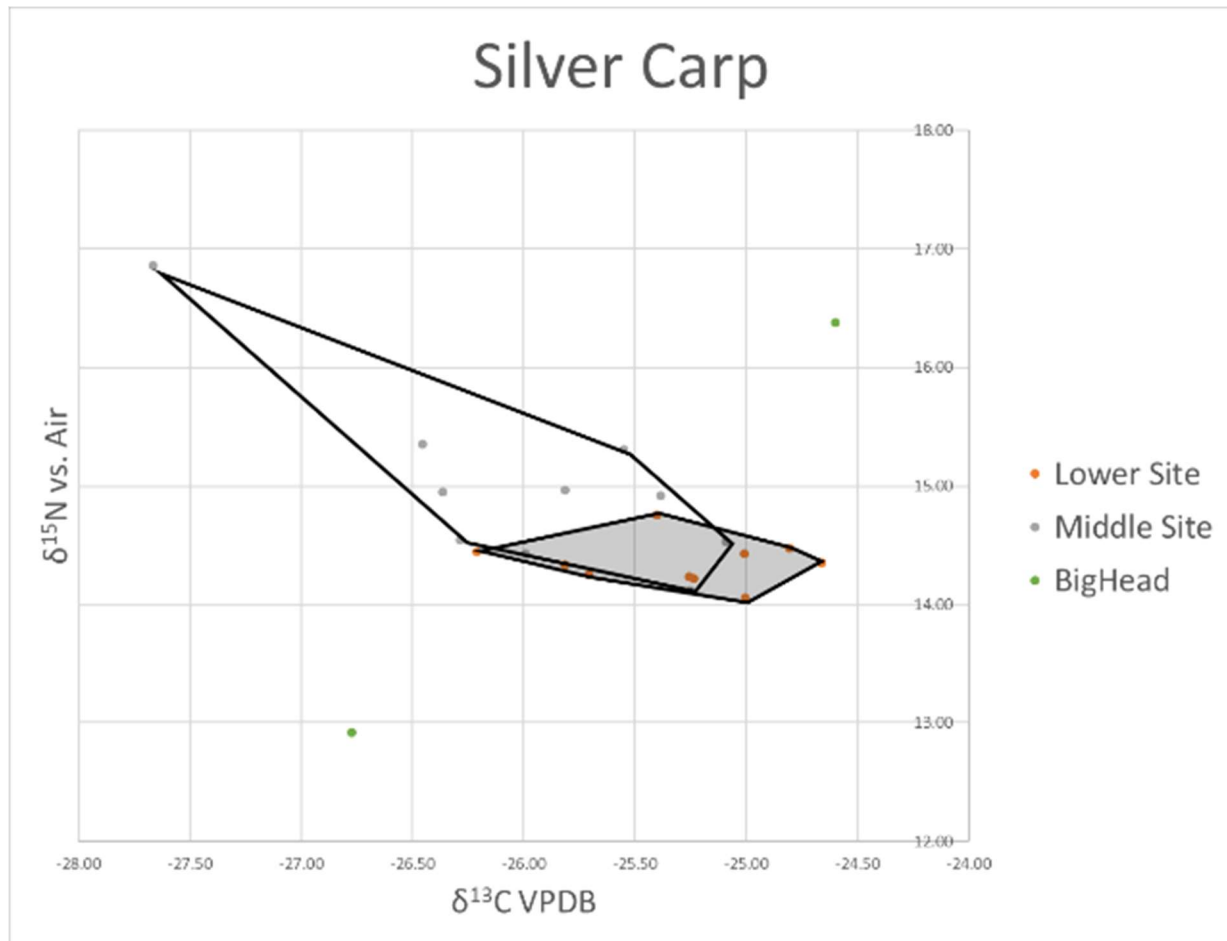


Figure 4 shows the isotopic niches of Silver Carp based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. “Middle Site” refers to the reach of river between the Bowersock and WaterOne dams, and “Lower Site” refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Bighead Carp from the “Lower Site” included for reference (n=2).

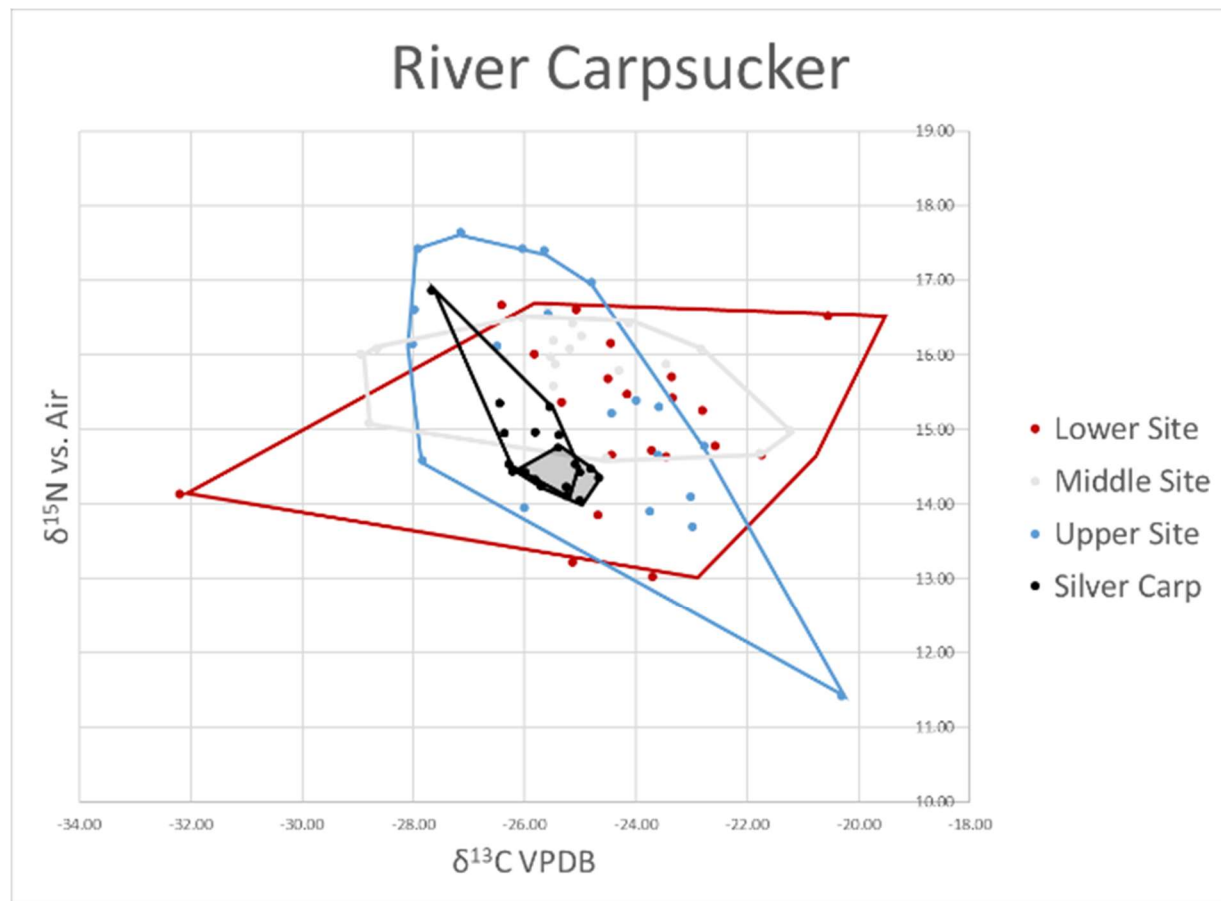


Figure 6 shows the isotopic niches of River Carpsucker based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

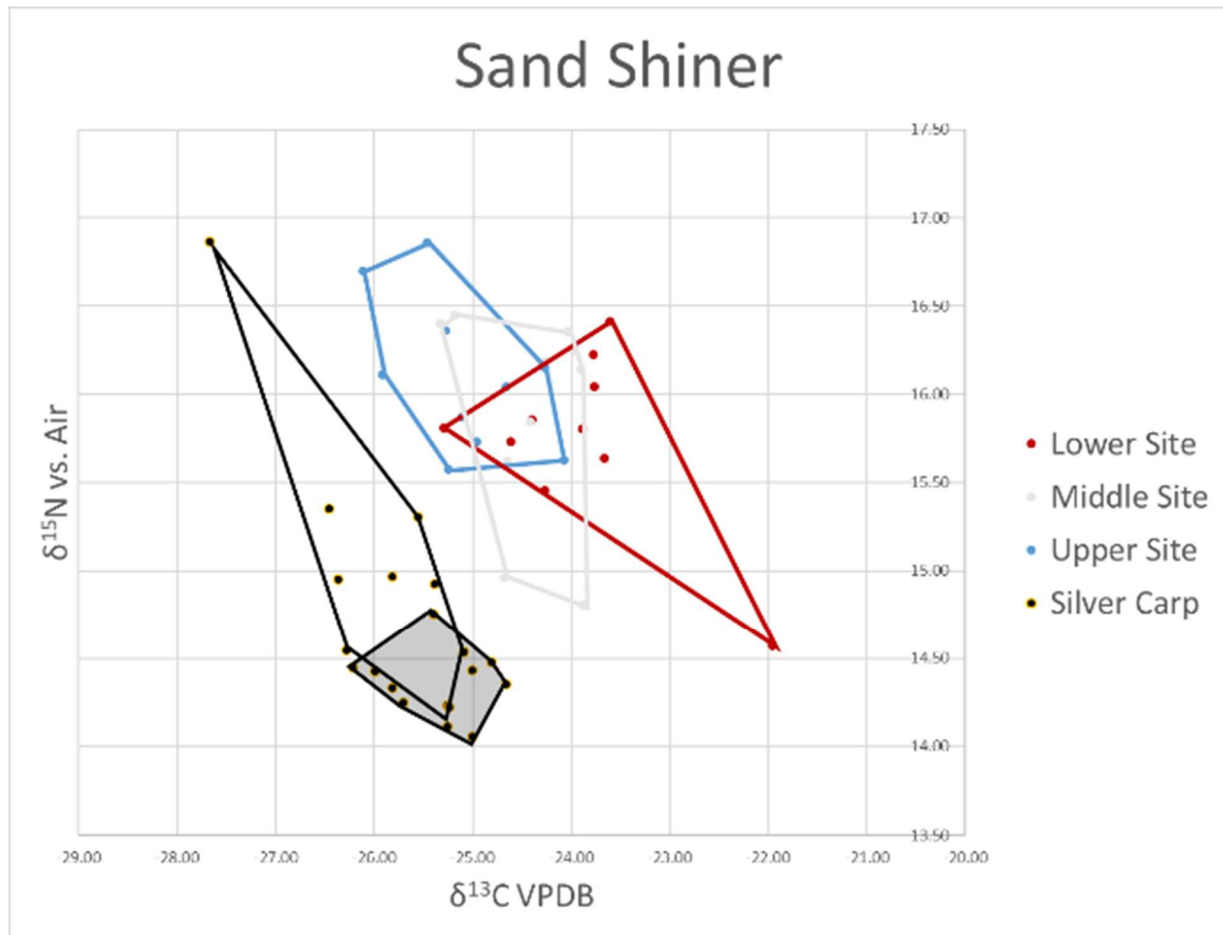


Figure 7 shows the isotopic niches of Sand Shiner based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

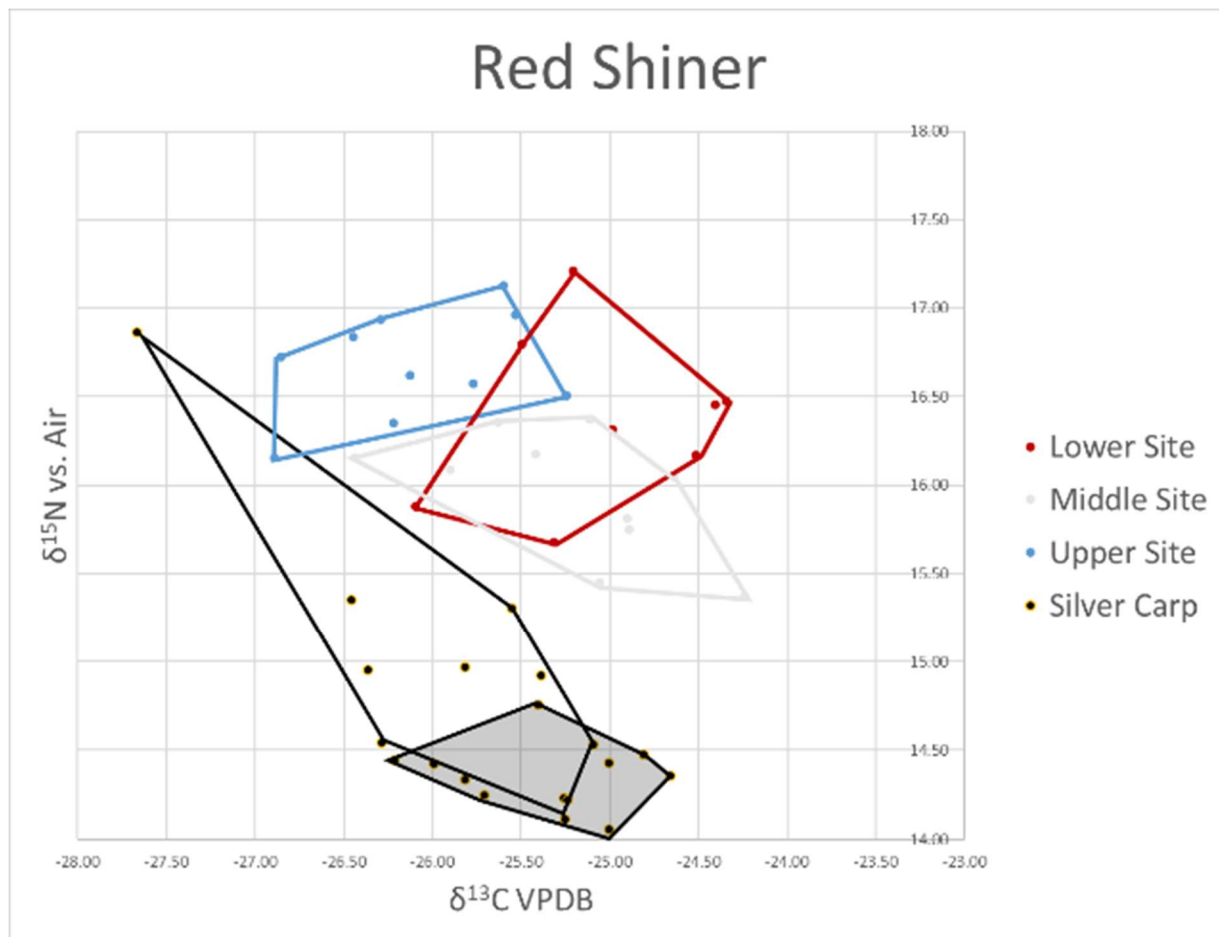


Figure 8 shows the isotopic niches of Red Shiner based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. “Upper Site” refers to the reach of river directly upstream of the Bowersock Dam, “Middle Site” refers to the reach of river between the Bowersock and WaterOne dams, and “Lower Site” refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

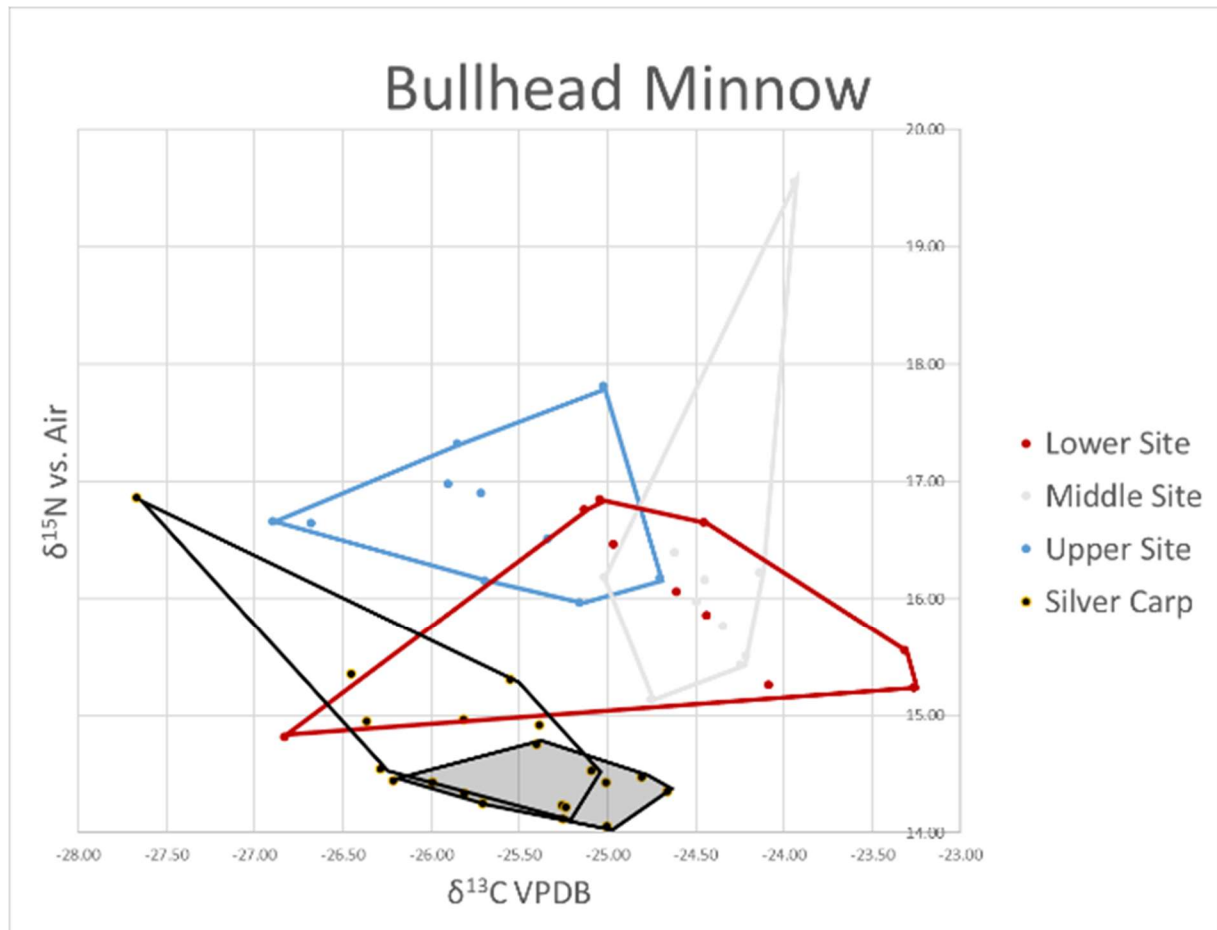


Figure 9 shows the isotopic niches of Bullhead Minnow based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

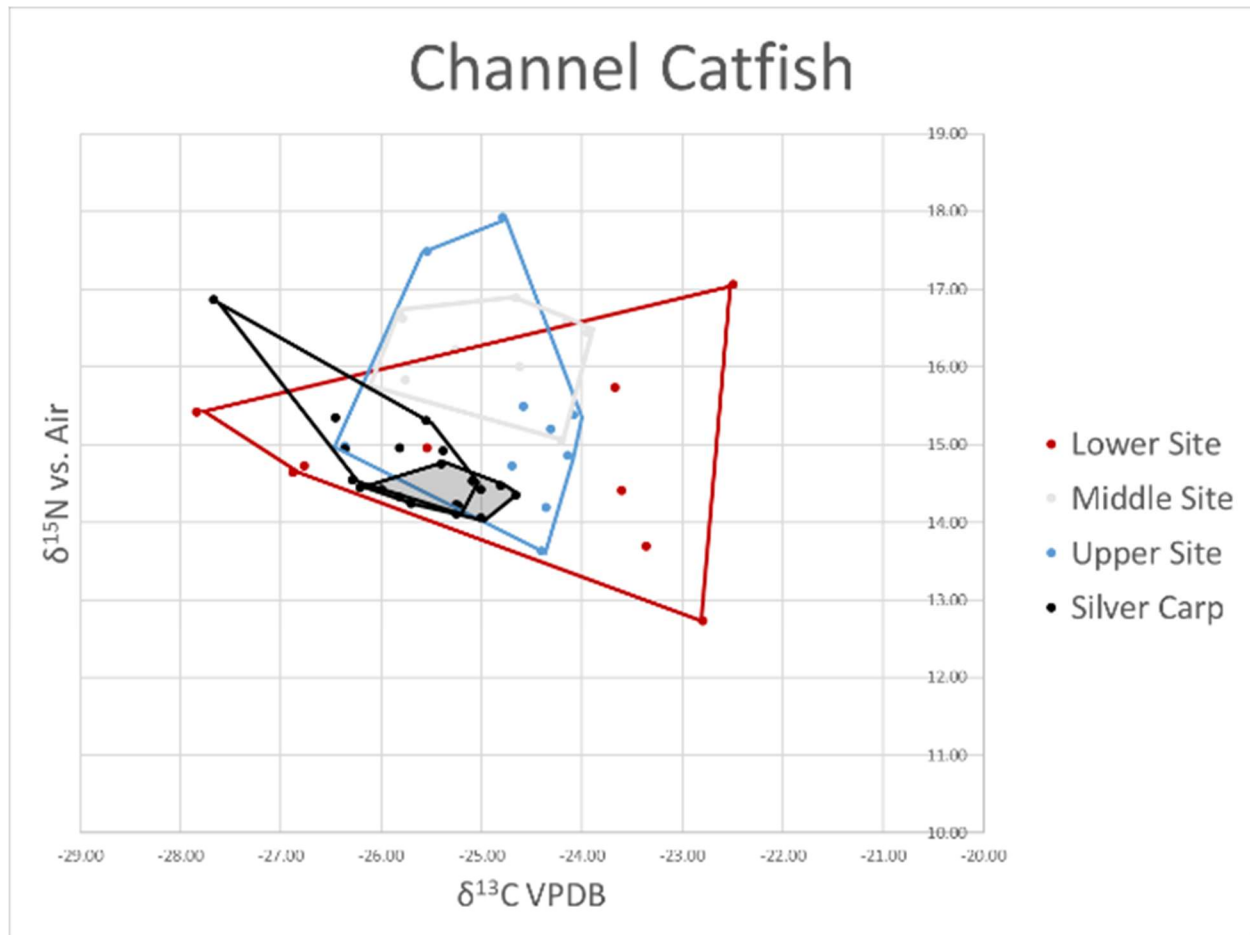


Figure 10 shows the isotopic niches of Channel Catfish based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. “Upper Site” refers to the reach of river directly upstream of the Bowersock Dam, “Middle Site” refers to the reach of river between the Bowersock and WaterOne dams, and “Lower Site” refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

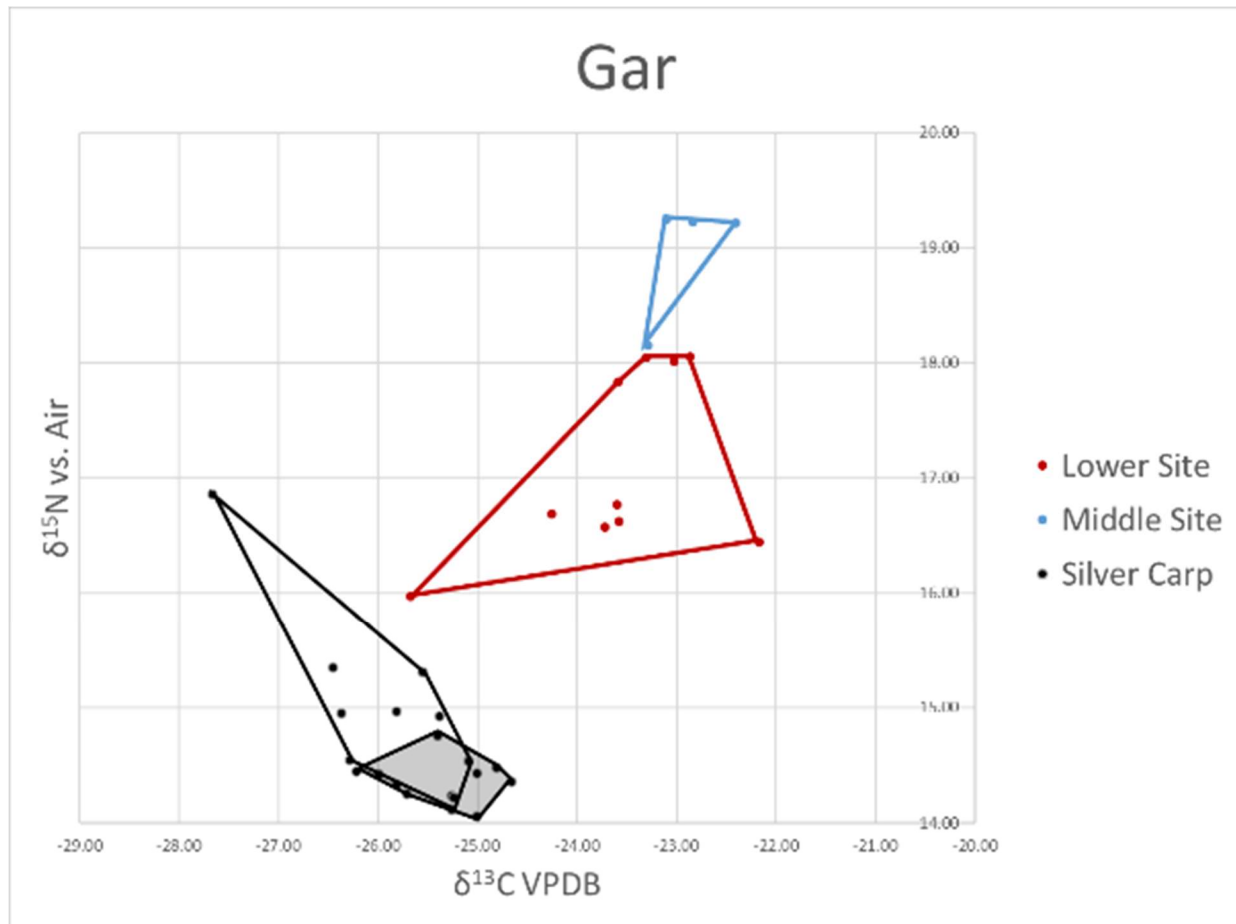


Figure 11 shows the isotopic niches of Long and Shortnose Gar based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. “Middle Site” refers to the reach of river between the Bowersock and WaterOne dams, and “Lower Site” refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

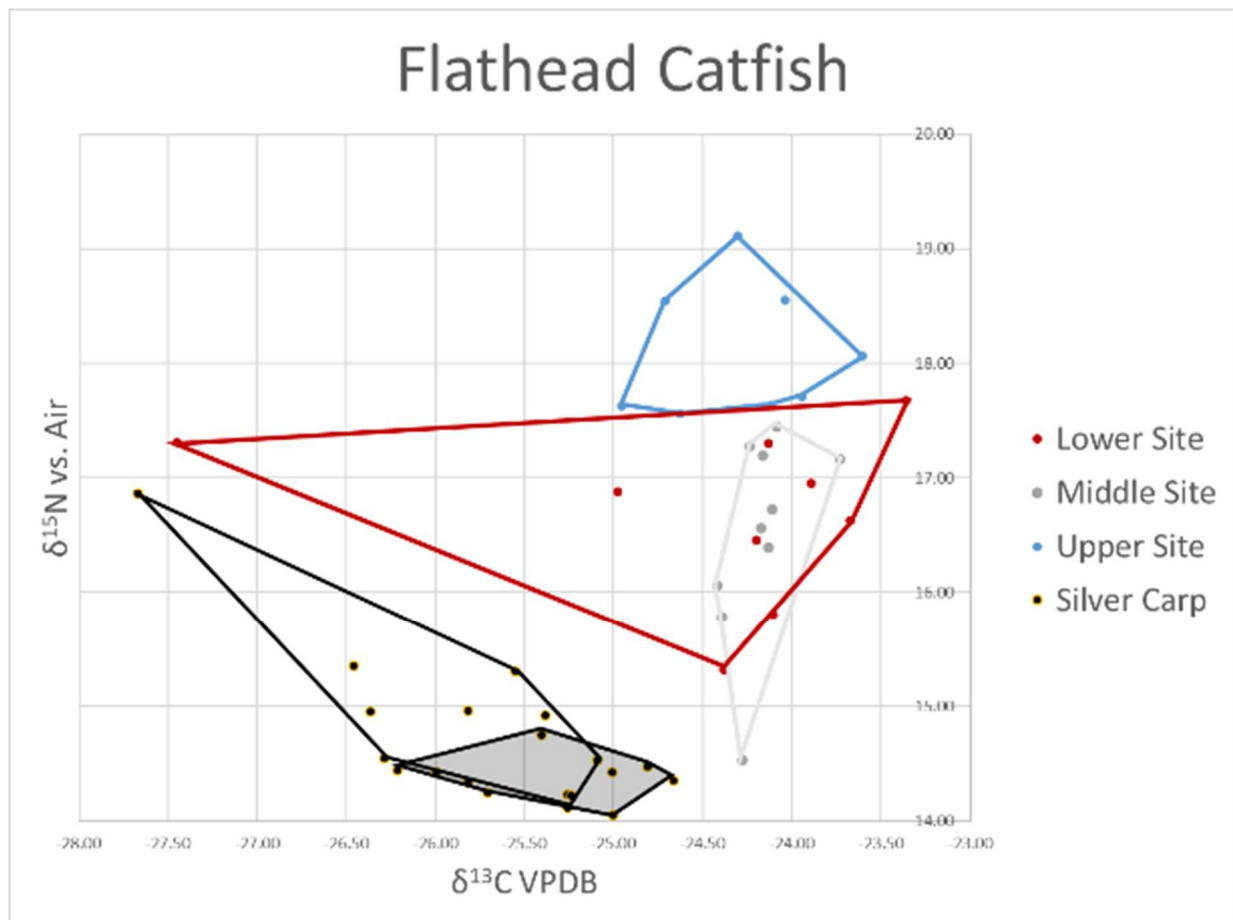


Figure 12 shows the isotopic niches of Flathead Catfish based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. Samples were collected from the Kansas River in April 2023. "Upper Site" refers to the reach of river directly upstream of the Bowersock Dam, "Middle Site" refers to the reach of river between the Bowersock and WaterOne dams, and "Lower Site" refers to the reach of river between the WaterOne Dam and the confluence with the Missouri River in Kansas City, KS. Silver Carp included for reference with the lower reach shaded.

Recommendation:

Continue project as scheduled to meet objectives and inform management and removal of invasive carp in the Kansas River.

State Report: Missouri

Agency: Missouri Department of Conservation

Project Title: Define the population demographics of invasive carp populations and the associated fish community in the Missouri River Basin - Year 3

Methods:

Three tributaries of the Missouri River with verified detections of Silver Carp were selected for sampling included: Lamine River (MO River kilometer 325), Grand River (MO River kilometer 338) and Platte River, MO (MO River kilometer 629). The three tributaries had varying drainage areas with the Grand River having the largest (20,429 km²). 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.

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

One electrofishing setting, 60Hz and 40% duty cycle with a goal of 20 amps was implemented for invasive carp electrofishing runs in sample year 2023. This setting was selected based on the three previous years' comparing different settings. A target of 10 runs per site was used for sampling. Each run was 5 minutes in length and involved a serpentine pattern going upstream in tributaries and downstream in the mainstem Missouri River. 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 cut close to the nucleus with an Isomet saw, sanded until the otolith nucleus was reached, and polished until the focus and all annuli were visible. Otoliths were read by three independent readers with aging experience and then ages compared, and 2/3 agreement reached. Population growth estimates and modeling was performed using FAMS version 1.64.4 (Slipke and Maceina 2014).

Fish community sampling was conducted from June through September. 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 mainstem 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: stock = 250 mm; quality = 450 mm; preferred = 560 mm; memorable = 740 mm; trophy = 930 mm as reported by Phelps and Willis (2013).

Results & Discussion

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 2023. A total of 30 electrofishing runs (10 - Missouri river bend, 10 - Lamine site 1, and 10 - Lamine site 2) and 2.82 gear hours of electrofishing resulted in 208 Silver Carp and 401 kg (884 lbs.) of fish. One Bighead Carp and three Grass Carp were also sampled in Lamine sites during standard sampling. One additional Grass Carp was collected in non-random sampling at the Missouri River Bend at the Lamine. Power output for electrofishing in the Lamine River samples ranged from 4250w-6750w. Power output for electrofishing in the Missouri River samples ranged from 5600w-6800w. Results from a Kruskal-Wallis one way analysis of variance on ranks indicated Silver Carp catch rates were not significantly different between sites ($P = 0.257$) in 2023 (Figure 1.) Silver Carp relative abundance is displaying a decreasing trend in Lamine River sites from 2021 to 2023 (Figure 2). However, Silver Carp catch rates were on an increasing trend from 2020 to 2022 at the Missouri River bend at the Lamine.

Silver Carp lengths ranged from 490mm – 764mm across all Lamine sites, and all three sites showed a bimodal size distribution. Lamine River sites were dominated by fish from 520mm to 560mm. Silver Carp lengths were not significantly different ($P = 0.203$) between Lamine River sites and Missouri River Bend at the Lamine River (Figure 3). Stock size Silver Carp (250-449mm) comprised the highest proportion sampled from 2020 to 2022 in the Lamine River. However, no stock size Silver Carp were collected in 2023 in the Lamine River (Figure 4). Quality size Silver Carp (450-559mm) comprised the highest

proportion in the Lamine River, while preferred size Silver Carp (560-739mm) encompassed the highest proportion in the Missouri River bend at the Lamine.

There were 110 otoliths were collected from Silver Carp sampled in Lamine River sites and 47 from the Missouri river bend at the mouth of the Lamine River. Additional otoliths were taken from one Bighead Carp and three Grass Carp across the Lamine sites. Otoliths are still being prepared for reading. Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 1.2: 1 while in the Lamine River sites it was 0.7: 1.

Lamine River – Fish Community

Forty electrofishing runs and 16 mini-fyke sets were conducted for all Lamine River sites. No sampling with otter trawls was conducted in 2023. Sampling resulted in a total of $n = 1,114$ fish (Missouri River = 821, Lamine site 1 = 134, Lamine site 2 = 159). Twenty-three species were sampled at the Lamine River sites (Lamine site 1 = 16, Lamine site 2 = 18) while 24 species were sampled at the associated Missouri river bend. Abundant species at Lamine site 1 included Orangespotted Sunfish (47.8%), Shortnose Gar (14.2%), Bluegill (8.9%), and Longnose Gar (6.7%). At site 2 dominant species included Silver Carp (29.5%), Gizzard Shad (11.9%), Orangespotted Sunfish (9.4%), River Carpsucker (8.8%), and Common Carp (6.9%). Sampling in the Missouri river at the mouth of the Lamine resulted in Red Shiner (45.8%) and Emerald Shiner (29.6%) comprising the highest proportion of the fish sampled and remaining species comprising less than 5% of the catch (Table 2).

Gizzard Shad mean relative weight was significantly higher at the Lamine Missouri River bend site (98.5) compared to the Grand River (78.2) and Missouri River bend at the Grand (85.1) ($P < 0.001$). Relative weights of River Carpsucker were not significantly different between the Lamine River and Missouri River bend at the Lamine ($P = 0.765$). However, River Carpsucker mean relative weight were significantly higher in the Lamine River (100.5) compared to the Grand River (88.6) ($P < 0.05$) (Figure 6).

Grand River – Invasive Carp Sampling

Invasive carp sampling for Grand River sites was conducted the last week of August in 2023. Thirty electrofishing runs (10 in the Missouri River bend, 10 Grand River site 1, 10 in Grand River site 2) and 3.41 electrofishing gear hours resulted in 246 Silver carp and 5 Grass carp and 456 kg (1,005 lbs) of fish. Power output for electrofishing runs in the Grand River ranged from 5700w-6820w, while power output for the associated Missouri River bend ranged from 6840w-7220w. Results from a Kruskal-Wallis one way analysis of variance on ranks indicated Silver Carp catch rates were not significantly different between Grand River sites in 2023 ($P=0.147$) (Figure 1).

Silver Carp lengths ranged from 382mm to 733mm for all sites combined. All sites displayed a bimodal length frequency distribution but were dominated with fish from 500mm to 550mm. Silver Carp lengths were not significantly different ($P=0.203$) between Grand River sites and Missouri River Bend at the Grand River (Figure 3). Stock size Silver Carp (250-449mm) comprised the highest proportion sampled from 2020 to 2022 in the Grand River. However, stock size Silver Carp accounted for 3% of the catch in 2023 (Figure 4). Quality size Silver Carp (450-559mm) comprised the highest proportion in the Grand River and Missouri River bend at the Grand.

Eighty-seven otoliths were collected from Silver Carp at the Missouri river bend at the mouth of the Grand River. One hundred twenty-two otoliths were collected from Silver Carp at the Grand River sites. No Age-0 to Age-2 Silver Carp were collected in 2023 and Silver Carp from Age-3 and 4 ranged from 384 to 561mm (Figure 5).

Grand River Silver Carp growth estimation parameters in 2023 from von Bertalanffy growth models using FAMS (2014) estimated an L_{inf} of 722 mm and growth coefficient (K) of 0.134. The catch curve regression estimated total annual mortality (AM) at 0.245, total annual survival (S) at 0.755 and a theoretical max age at 14.6. Silver Carp mortality estimates using Hoenig (1983) in FAMS (2014) estimated instantaneous natural mortality (M) at 0.291 and conditional natural mortality at 0.25 (Table 3).

Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 1.2:1 while in the Grand River sites it was 0.9:1.

Five Grass Carp were collected at Grand River sites in sample year 2023. Two Grass Carp measuring 560mm and 568mm were captured in the Missouri River bend at the Grand River. The remaining three Grass Carp were captured in the Grand River sites with sizes ranging from 459mm to 536mm with a mean length of 507mm.

Grand River – Fish Community

Forty electrofishing runs, 16 mini-fyke nets, and 12 otter trawl samples were collected in the Grand River sites including the associated Missouri River bend. This resulted in a total of 1,966 fish (Missouri River bend= 965, Grand River site 1 = 550, and Grand River site 2 = 451). Twenty-six species were sampled in the Grand River (Site 1 = 21 and Site 2 = 24). The Missouri River bend sampling resulted in 25 species. Missouri River samples were dominated by Emerald Shiner (46.8%) and Red Shiner (16.8%). Similarly, Grand River site 1 fish community was also dominated by Emerald Shiner (31.3%) and Red Shiner (20.4%). Grand River site 2 abundant species included Bullhead Minnow (37.3%), Red Shiner (18.4%), Silver Carp (9.5%), and Emerald Shiner (7.5%).

Bigmouth Buffalo mean relative weight from the Grand River was 83.6 and only one individual was collected in both the Lamine and Platte rivers. Gizzard Shad mean relative weight from the Grand River (78.2) were significantly lower than the Missouri River bend at the Grand (85.1) ($P < 0.05$) and the Missouri River bend at the Lamine ($P < 0.001$). River Carpsucker mean relative weight was not significantly different between the Grand River (88.6) and the Missouri River bend at the Grand (89.8) ($P = 0.956$). However, River Carpsucker mean relative weight at the Grand River was significantly lower than at the Lamine (100.5) ($P < 0.05$) (Figure 6).

Platte River – Invasive Carp Sampling

Invasive carp sampling for Platte River sites was conducted the fourth and fifth weeks of August in 2023. Thirty electrofishing runs (10 in the Missouri river bend, 10 at site 1, and 10 at site 2) and 2.18 electrofishing gear hours resulted in 183 Silver Carp, and 2 Grass Carp amounting to 467kg (1,030 lbs) of fish. No Bighead or Black Carp were sampled. Power for Platte River runs ranged 4383w – 6900w while power for the associate Missouri river bend was 3600w-4200w. Silver Carp mean catch per unit effort at the Platte River Site 01 (171.6 fish/hour) was significantly higher ($P < 0.001$) than the Missouri River bend site (14 fish/hour) and significantly higher ($P < 0.05$) than Platte River site 02 (39.6 fish/hour) (Figure 1).

Platte River site 01 Silver Carp catch rates in 2023 were significantly lower than 2022 ($P < 0.05$) regardless of being the highest catch rates between sites in 2023 (Figure 2).

Silver Carp lengths ranged from 479mm to 881mm for all sites combined (Figure 3). All sites displayed a bimodal length frequency distribution. Silver Carp lengths were not significantly different ($P=0.075$) between sites. Preferred size Silver Carp (560-739mm) comprised the highest proportion sampled from the Platte River and Missouri River bend at the Platte in 2022 and 2023. The Platte River is also the only site to have memorable size Silver Carp (740-929mm) collected in all four sample years. However, stock size Silver Carp accounted for the highest proportion of the catch in 2020 and 2021 (Figure 4).

One hundred thirty-eight otoliths were collected from Silver Carp in the Platte River sites. Thirty-eight otoliths were collected from Silver Carp in the Missouri River bend at the mouth of the Platte River. No Age-0 to Age-2 Silver Carp were collected in 2023 (Figure 5).

Platte River Silver Carp growth estimation parameters in 2023 from von Bertalanffy growth models using FAMS (2014) estimated an L_{inf} of 624 mm and growth coefficient (K) of 0.817. The catch curve regression estimated total annual mortality (AM) at 0.111, total annual survival (S) at 0.889 and a theoretical max age at 29.5. Silver Carp mortality estimates using Hoenig (1983) in FAMS (2014) estimated instantaneous natural mortality (M) at 0.141 and conditional natural mortality at 0.13 (Table 3).

Sex ratios (Male: Female) in the Missouri river site was 0.9:1 compared to 1.3:1 in the Platte River sites combined.

Platte River – Fish Community

Forty electrofishing runs, 14 mini-fyke nets, and 12 otter trawl samples were completed in the Platte River sites including the associated Missouri river bend. This resulted in 1,753 fish sampled (Missouri river = 1,113, Platte River site 1 = 215, and Platte River site 2 = 425). Nineteen species were sampled in the Missouri river at the mouth of the Platte River. Twenty-nine species were sampled at the Platte River sites (Site 1 = 23, Site 2 = 23). Missouri River samples were dominated by Emerald Shiner (84.9%). Platte River site 1 fish community included Emerald Shiner (36.3%), Shortnose Gar (20.9%), and Freshwater Drum (13.9%). The Platte River site 2 samples were dominated by Emerald Shiner (46.6%) with remaining species comprising less than 5% of the catch (Table 2).

River Carpsucker collected in the Missouri River bend at the Platte had the highest mean relative weight (100.8) in 2023, although not significantly different from other sites (Figure 6).

Discussion

Silver Carp was the overwhelmingly dominant invasive carp species in all tributaries and their associated Missouri River bends. Silver Carp relative abundance was variable between sites and abundance increases moving upstream in the larger tributaries such as the Lamine and Grand. The smaller Platte tributary did not follow this trend in 2023, possibly due to low water in the upper Platte site 02. The lower Platte site 1 still maintains the highest Silver Carp abundance annually from 2020 to 2023 possibly due to the lower wetted width compared to the Grand and Lamine and the abundance of snags and woody debris for Silver Carp to congregate.

Size distributions and length at age data of Silver Carp in 2023 provide evidence to limited reproduction and recruitment over the last three years in the three tributaries and associated Missouri River bends. This is probably due to low river levels in the lower Missouri River basin witnessed these past three years.

Silver Carp growth, mortality and survival estimates were interesting between the Grand and Platte (MO) rivers in 2023. The Grand River had an invasive carp removal in 2022, while the Platte has had little exploitation other than invasive carp euthanized during population demographic sampling. Mortality estimates were higher in the Grand than the Platte, however the growth coefficient was lower in the Grand than the Platte.

Eleven Grass Carp and one Bighead Carp were also collected in sample year 2023. 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 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. The addition of experimental gill nets in late fall may provide better estimates for Bighead and Grass Carp. Gill nets may also provide more consistent captures annually of native species such as Bigmouth Buffalo, Gizzard Shad, Paddlefish and River Carpsucker if population indices for those species such as abundance and relative weight metrics were analyzed to evaluate invasive carp management actions.

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 Grand river had two short term rapid invasive carp removals conducted in 2022 and 2023 so demographic data is important to understanding impacts from the removals. The Lamine river is scheduled to have a removal in Fall of 2024 so demographic data will also be beneficial preceding and following the removal.

Also, consideration for future sampling may include one or more random or fixed mainstem Missouri River bend(s) in proximity to each tributary but geographically distanced to gather abundance data outside the potential influence of a tributary.

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

Site ¹	# of Samples	60Hz 40% Mean CPUE (± S.E.) ²	Total # Silver Carp	Total Weight kilograms(lbs)	Male : Female
MOLR	10	87.1 (78.0)	208	401 (884)	1.2 : 1
LR1	10	56.7 (22.2)			0.7 : 1
LR2	10	95.6 (41.2)			
MOGR	10	71.9 (22.9)	246	456 (1,005)	1.2 : 1
GR1	10	55.9 (17.2)			0.9 : 1
GR2	10	86.1 (29.8)			
MOPR	10	14.0 (27.0)	183	458 (1,010)	0.9 : 1
PR1	10	171.6 (59.6)			1.3 : 1
PR2	10	39.6 (40.5)			

¹MOLR = 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,

²Catch 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	8	10	--	821	24
LR	8	30	-	293	23
MOGR	8	10	--	965	25
GR	8	30	12	1,001	26
MOPR	8	10	--	1,113	19
PR	6	30	12	640	28

¹MOLR = 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.

Table 3. Silver Carp growth estimation parameters (L_{inf} and K) from von Bertalanffy growth models, total annual mortality (AM), total annual survival (S), theoretical max age from catch curve regression, instantaneous natural mortality (M) and conditional natural mortality (cm) estimates using Hoenig (1983) in FAMS (Fishery Analysis and Modeling Simulator 1.64) for Grand and Platte rivers in 2023.

Site	L_{inf}	K	AM	S	Max Age	M	cm
Grand River	722	0.134	0.245	0.755	14.6	0.291	0.25
Platte River	624	0.817	0.111	0.889	29.5	0.141	0.13

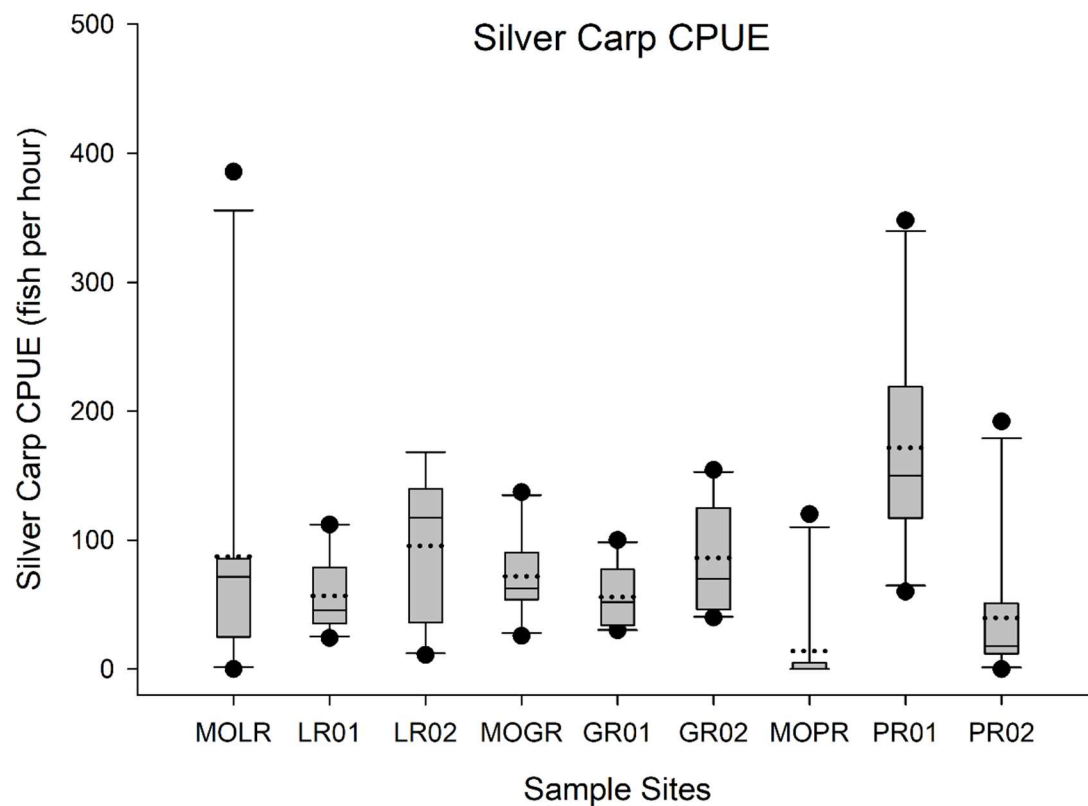


Figure 1. Box plots of Silver Carp catch per unit effort at each site in Lamine (LR), Grand (GR), Platte (PR), and associated Missouri River bends (MO) at each tributary in sample year 2023. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

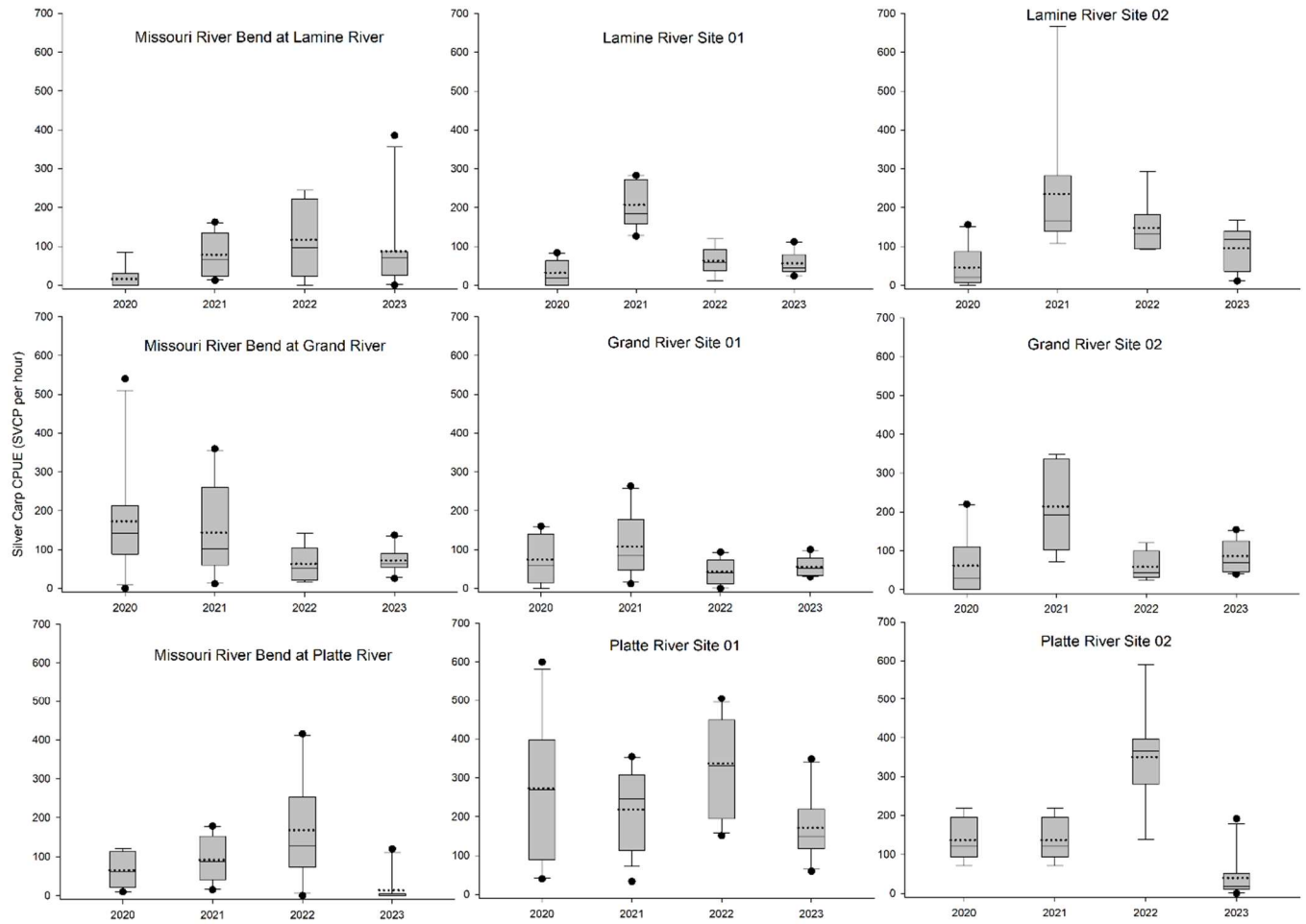


Figure 2. Box plots of Silver Carp catch per unit effort at each site in Lamine, Grand, Platte, and associated Missouri River bends (MO) at each tributary from 2020 to 2023 using 60Hz and 40% Duty Cycle. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

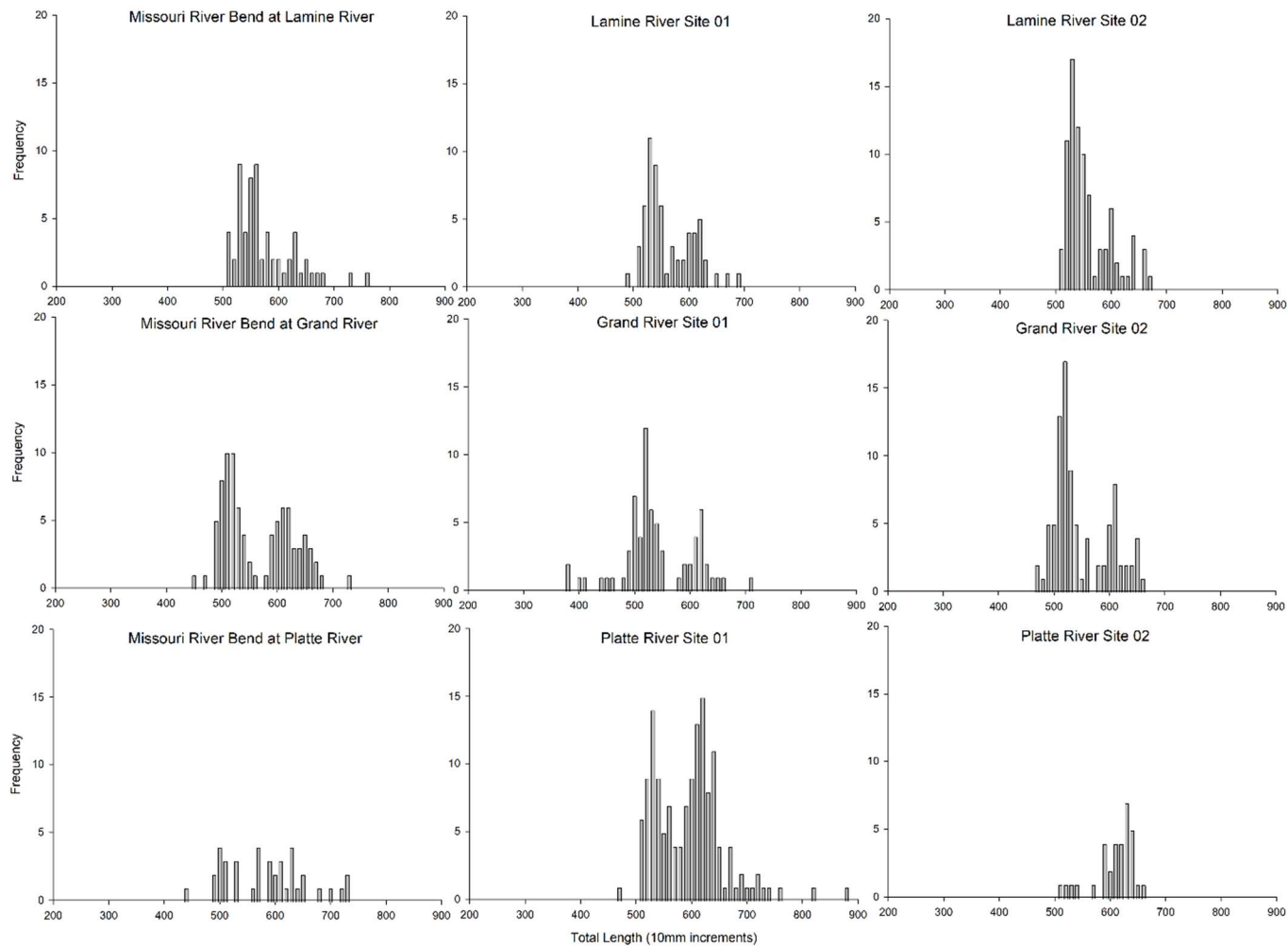


Figure 3. Length frequencies of Silver Carp in 10mm increments for all sites in 2023.

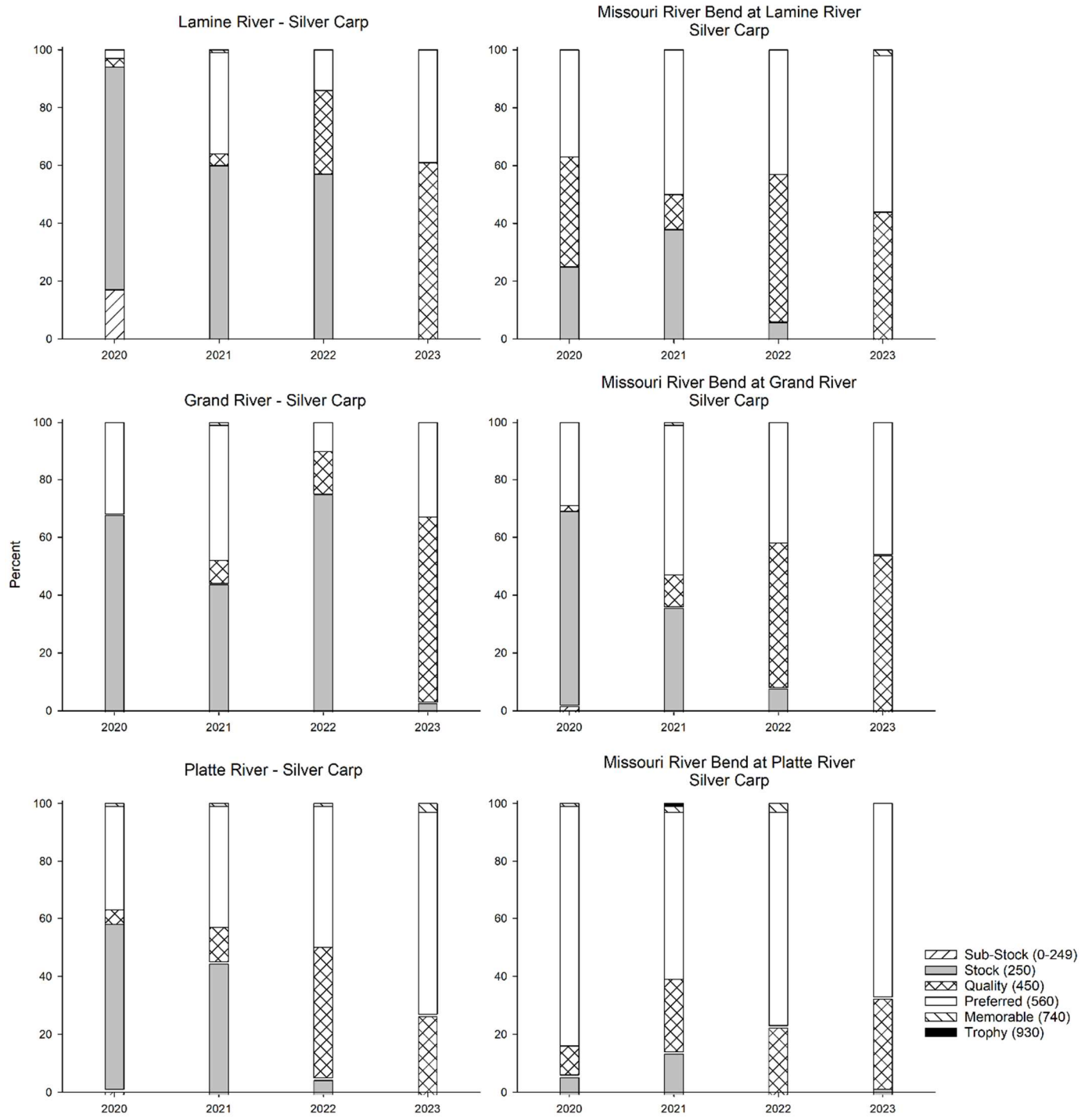


Figure 4. Incremental relative stock densities of Silver Carp based on Phelps and Willis (2013) from each tributary and associated Missouri River bend.

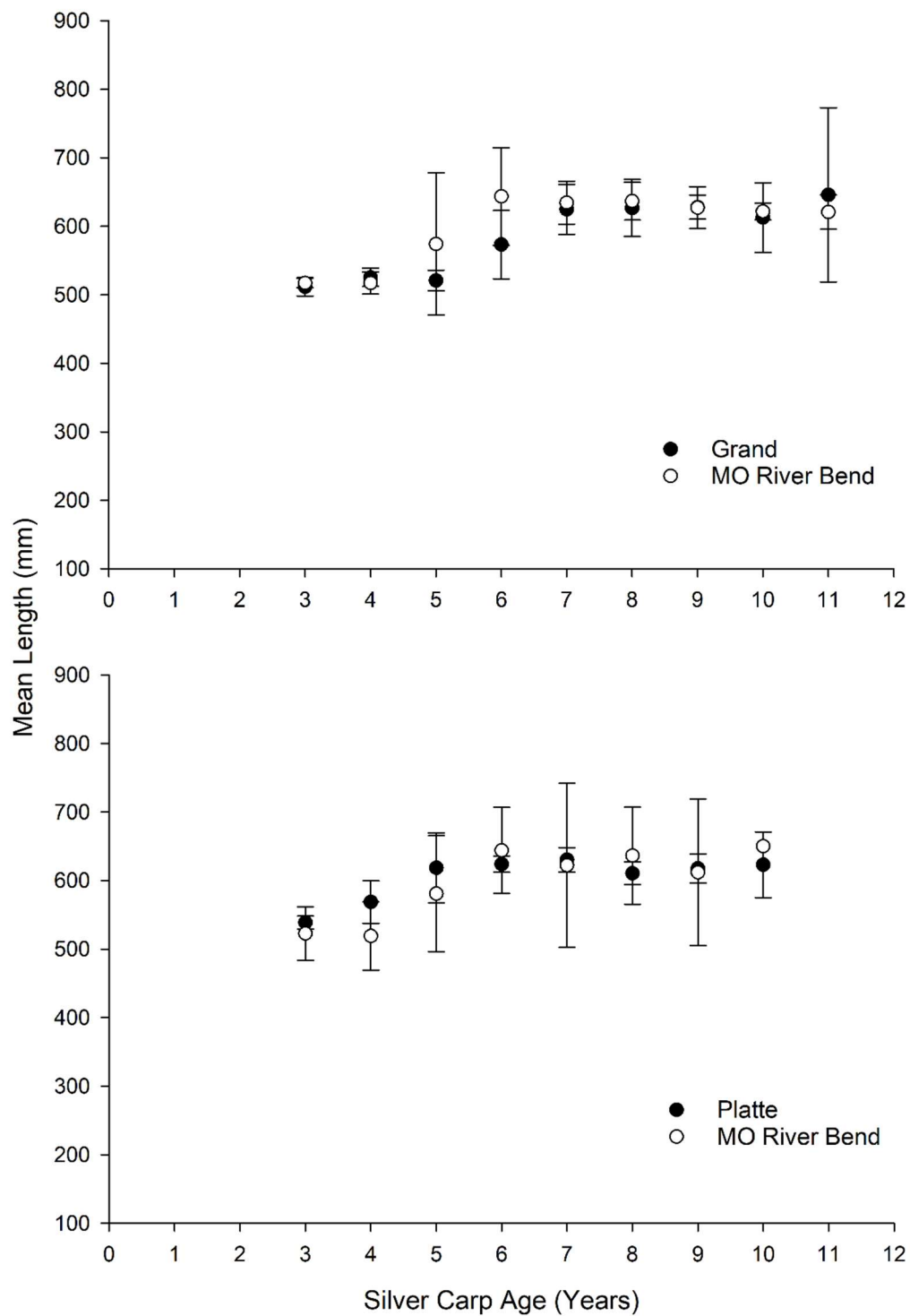


Figure 5. Silver Carp mean length at age ± 2 standard error for Grand and Platte River and associated Missouri River bend in 2023.

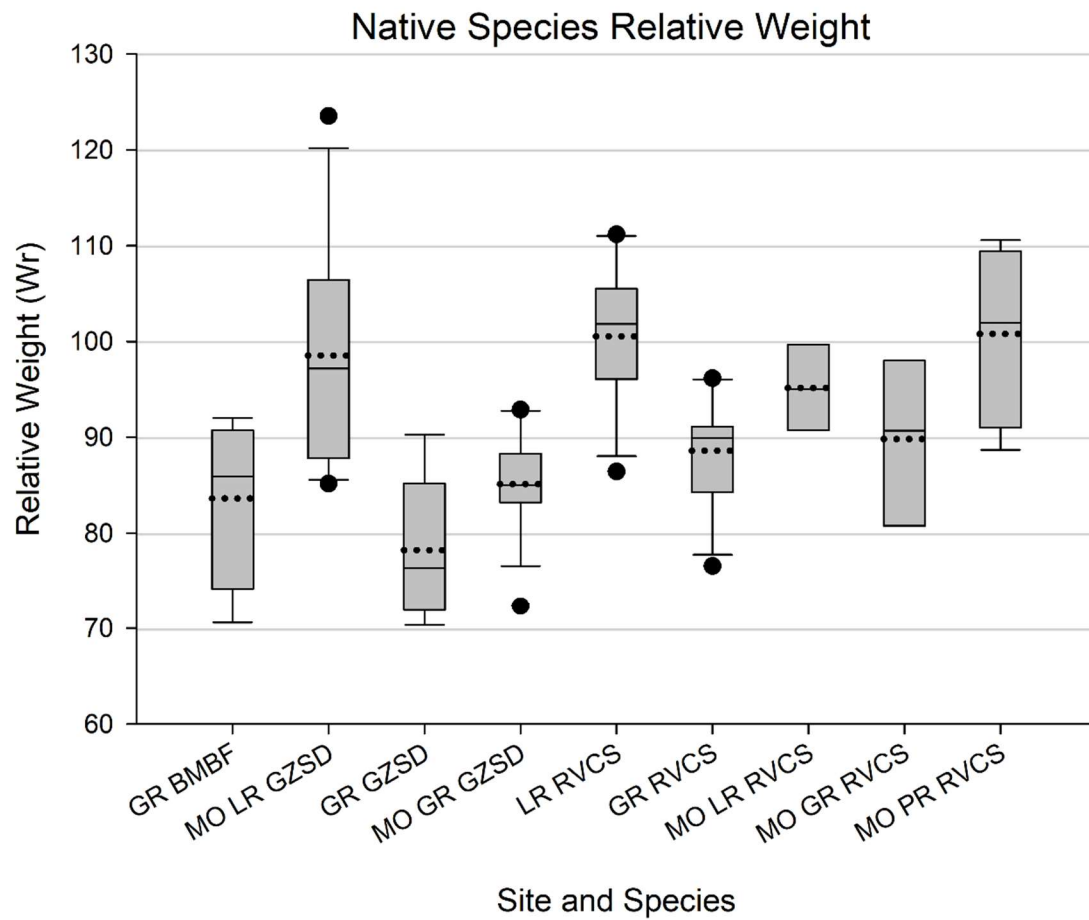


Figure 6. Box plots of relative weights for Bigmouth Buffalo (BMBF), Gizzard Shad (GZSD) and River Carpsucker (RVCS) at associated sites in Lamine (LR), Grand (GR), Platte (PR) and associated Missouri River bends (MO) at each tributary. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

State Report: Nebraska

Agency: Nebraska Game and Parks Commission (NGPC) and University of Nebraska – Lincoln, School of Natural Resources (UNL)

Project Title: Spatial distribution and population demographics of Invasive Carp in the Missouri River Basin, Nebraska

Methods:

Invasive Carp (Bighead, Silver, and Grass Carp) have been detected in the Missouri River as far upstream as Gavins Point Dam. Black Carp have also been detected in the lower Missouri River but have not been detected as far upstream as Gavins 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.

Sampling was completed in early fall 2022 below Gavins 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 each dam were sampled. Concurrently, 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. Finally, we received additional samples from anglers in the area that captured an Invasive Carp species during the annual Paddlefish snagging season (1-31 October). Captured fish were measured for total length (mm) and mass (g). Sturgeon species were measured to fork length and Paddlefish were measured from eye to fork. Additionally, sagittal otoliths were removed from Invasive Carp for 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 through 2022. 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 were collected across the state by partners involved in concurrent research that are providing an additional layer of potential Invasive Carp detection across the interior Nebraska rivers and streams.

Results & Discussion

Sampling in 2021-2022 captured a total of 9,847 fish across both tailwater reaches. Of these, 3,557 were captured below Fort Randall Dam and 6,290 were captured below Gavin's Point Dam. The total number of Carp caught during the 2021-2022 field seasons was 15 Bighead Carp, 184 Silver Carp, and 48 Grass Carp (Table 1). No Black Carp were sampled below either dam, and no Invasive Carp were sampled within the reach below Fort Randall Dam.

Catches in both tailwaters were dominated by native Cyprinid and Catastomid species – namely Emerald Shiner, Shorthead Redhorse, and River Carpsucker. However, there were differences between the two communities (ANOSIM: Global $R = 0.66$; $P < 0.01$; Figure 2). Further analyses to evaluate potential influence of biomass of each species are ongoing and will be reported in the final report.

Age-frequency of Silver Carp collected in the Gavins Point tailwater suggest a relatively young age structure (Figure 3). Most fish captured by all three methods were typically age-4 or younger. Similar results were observed for Bighead Carp (Figure 4) and also for Grass Carp (Figure 5). Sufficient data to calculate Von Bertalanffy growth curves were lacking for Bighead Carp but were calculated for Silver Carp (Figure 6) and Grass Carp (Figure 7). These data are being compared to population dynamics parameters from elsewhere in the current distribution of each species.

Water samples collected for eDNA analyses found no additional range expansion upstream in the Missouri River during 2021-2022. Specifically, no positive detections for Black Carp in Nebraska's waters have occurred. Similarly, Bighead Carp and Silver Carp have not been detected above Gavin's Point Dam using eDNA techniques.

Table 1. Species counts of fish sampled using boat electrofishing, mini-fyke nets, and hoop nets below each dam during 2021 and 2022.

Species	2021		2022	
	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
Total	2289	3820	1268	2470



Figure 1. Sample reaches for each tailwater area (Ft. Randall tailwater – top; Gavins Point tailwater – bottom).

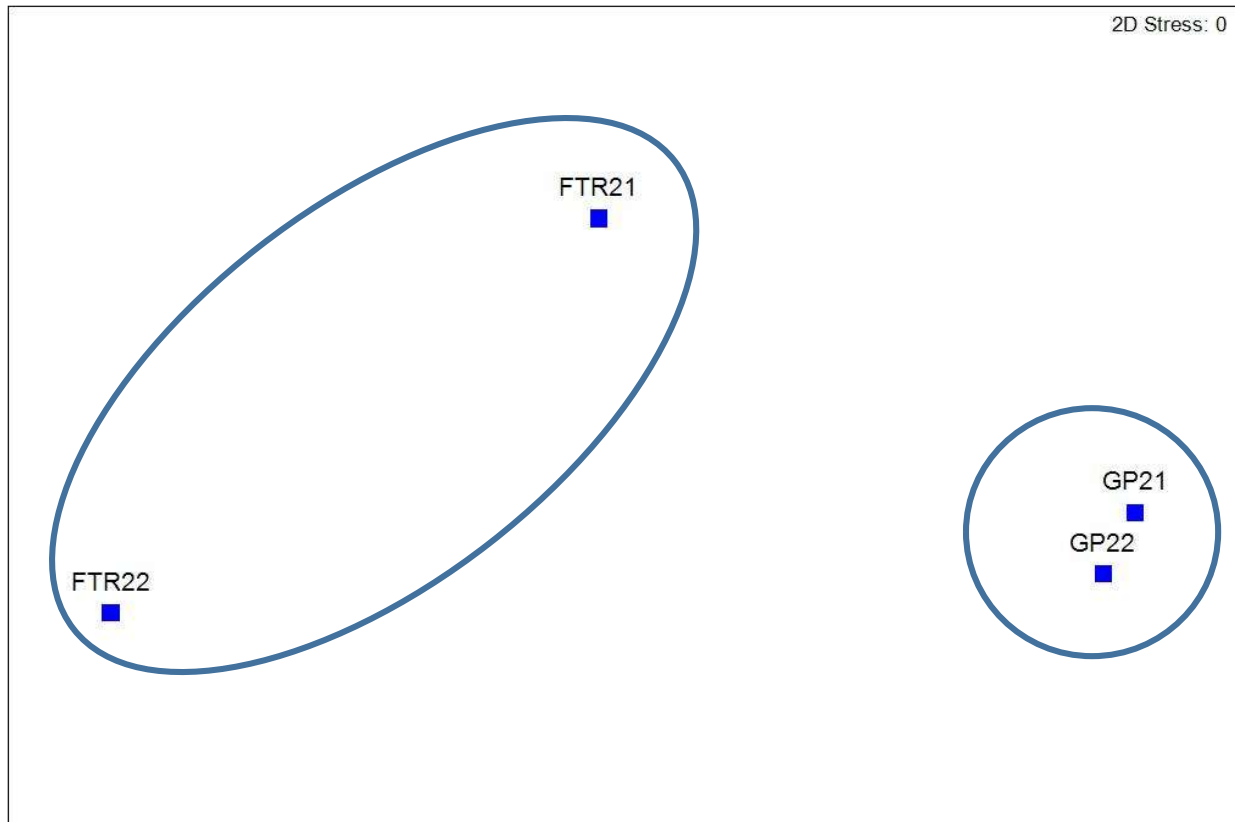


Figure 2. Multidimensional Scaling plot representing fish communities from the Fort Randall (FTR**) and Gavins Point (GP**) tailwaters during 2021 -2022 sampling. Fish communities were different between tailwaters (ANOSIM: Global R = 0.66; $P < 0.01$).

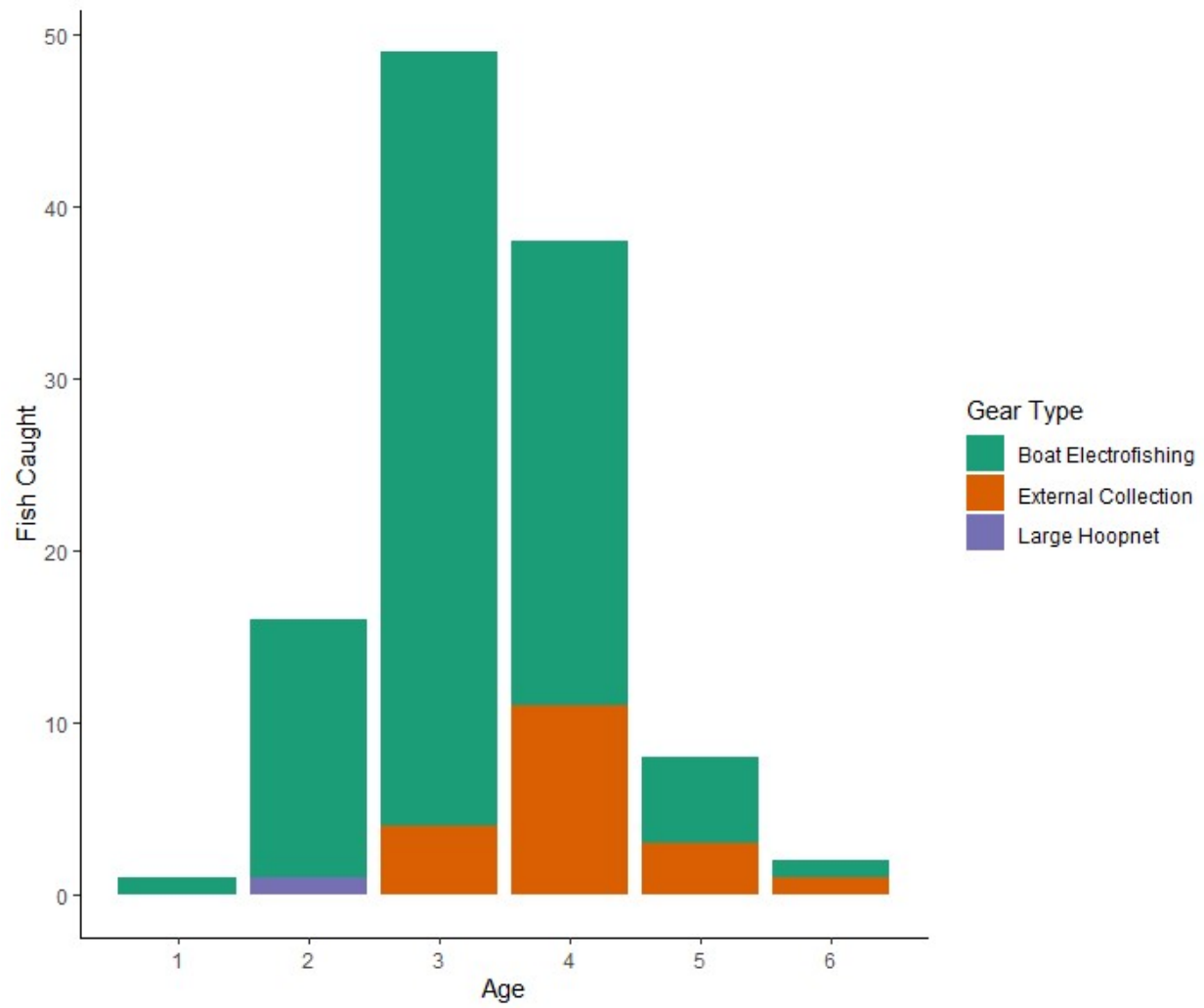


Figure 3. Age-frequency of Silver Carp captured in the Gavins Point tailwater 2021-222.

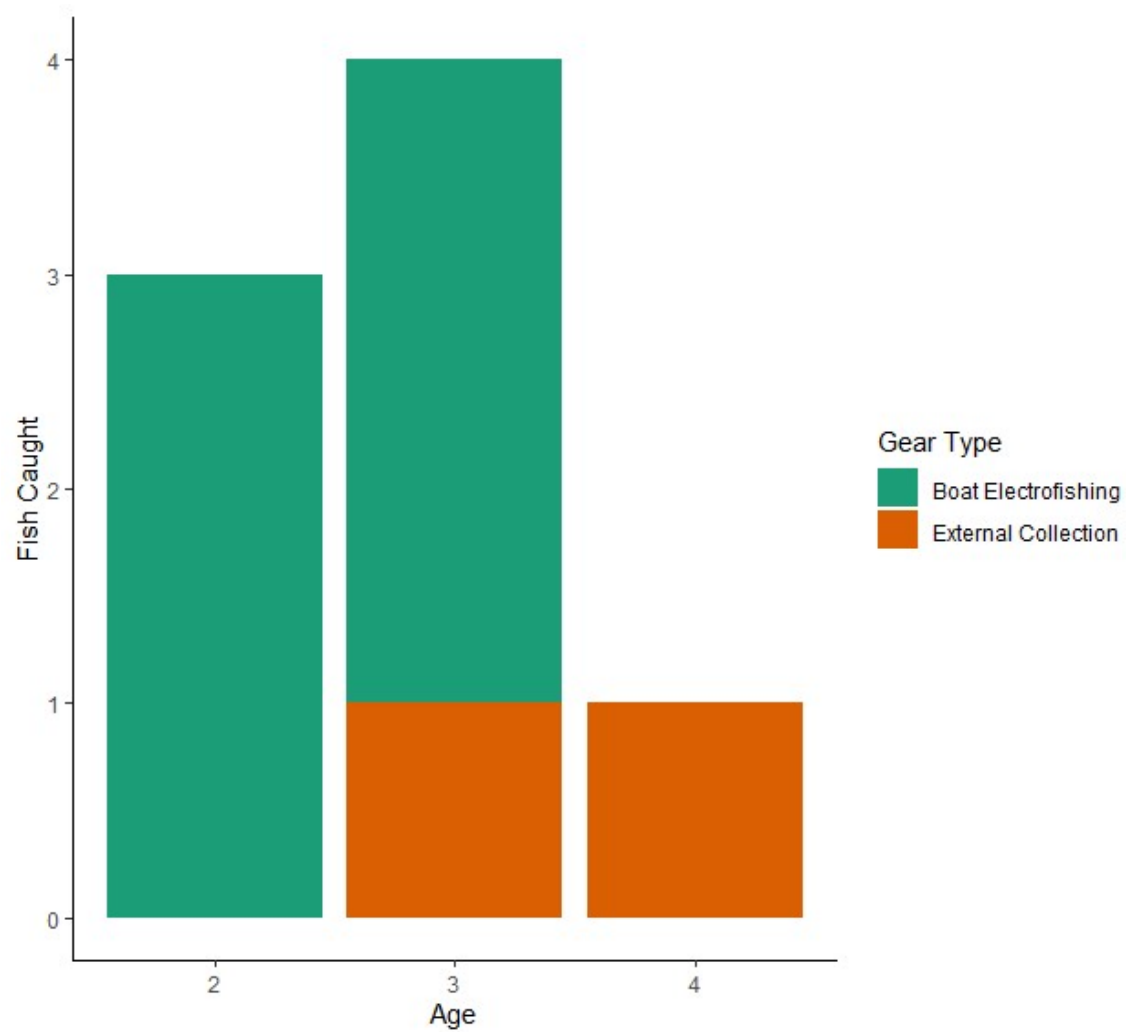


Figure 4. Age-frequency of Bighead Carp captured in the Gavins Point tailwater 2021-222.

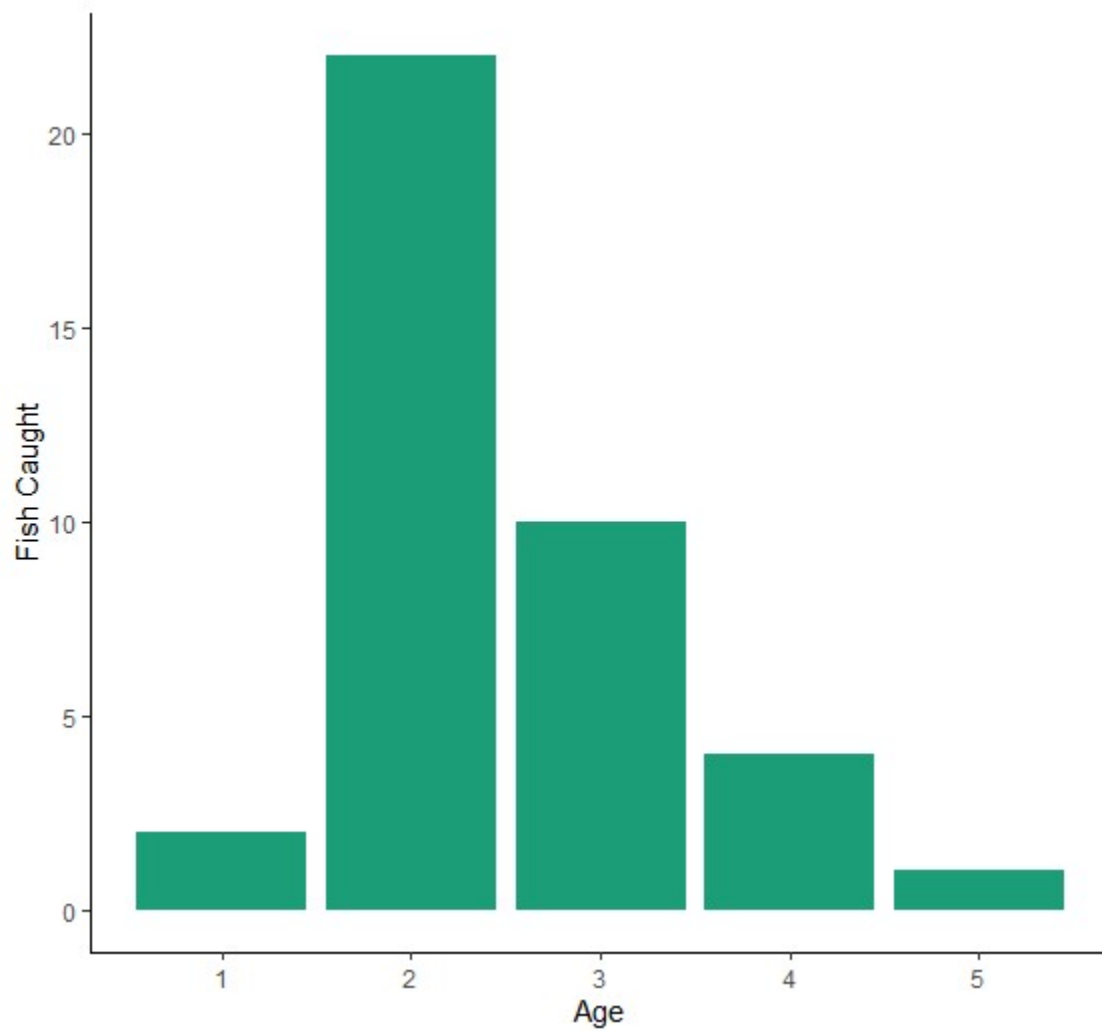


Figure 5. Age-frequency of Grass Carp captured by boat electrofishing in the Gavins Point tailwater 2021-222.

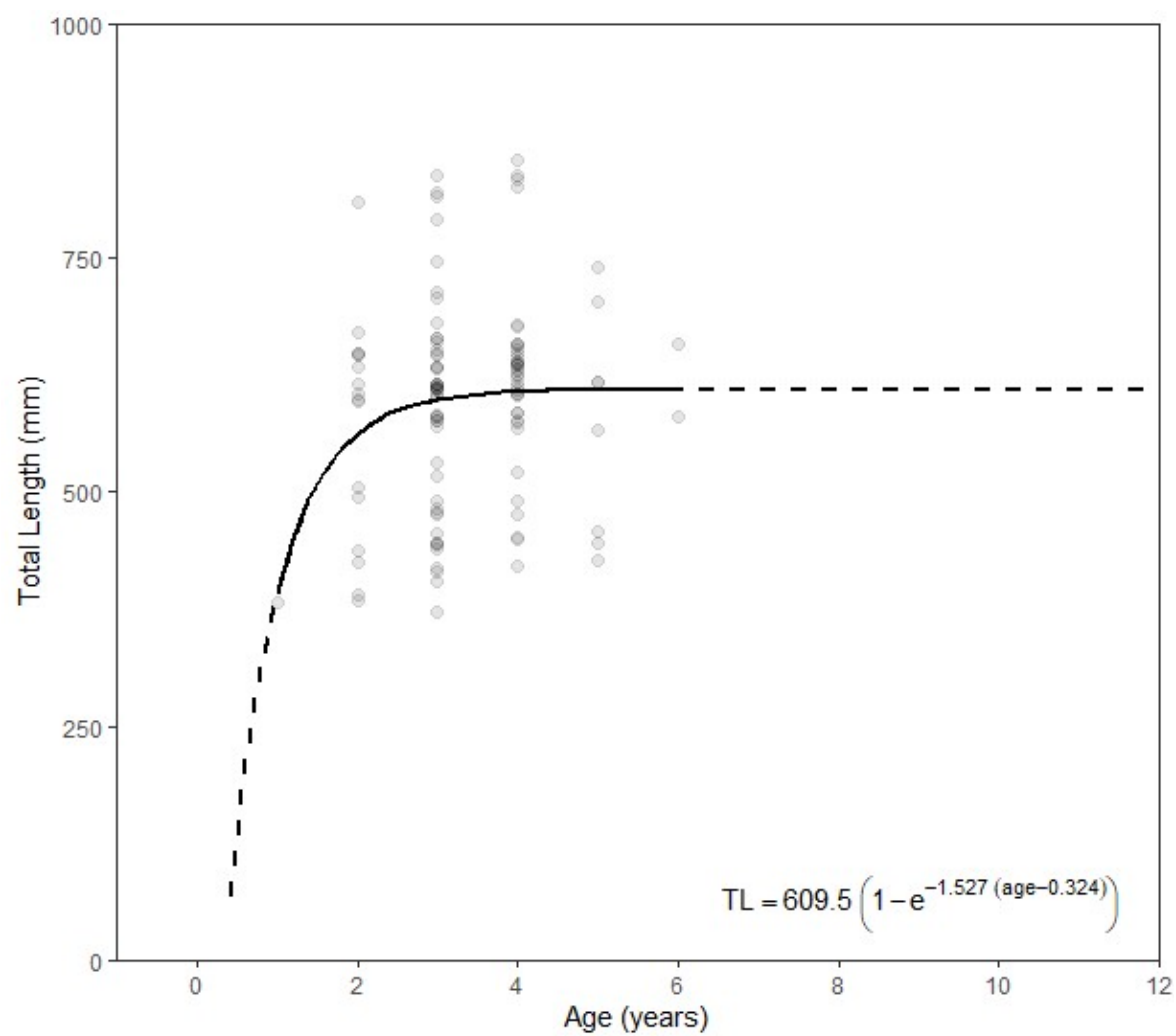


Figure 6. Von Bertalanffy growth curve fitted to Silver Carp captured below Gavins Point Dam, Yankton, SD in 2021 and 2022.

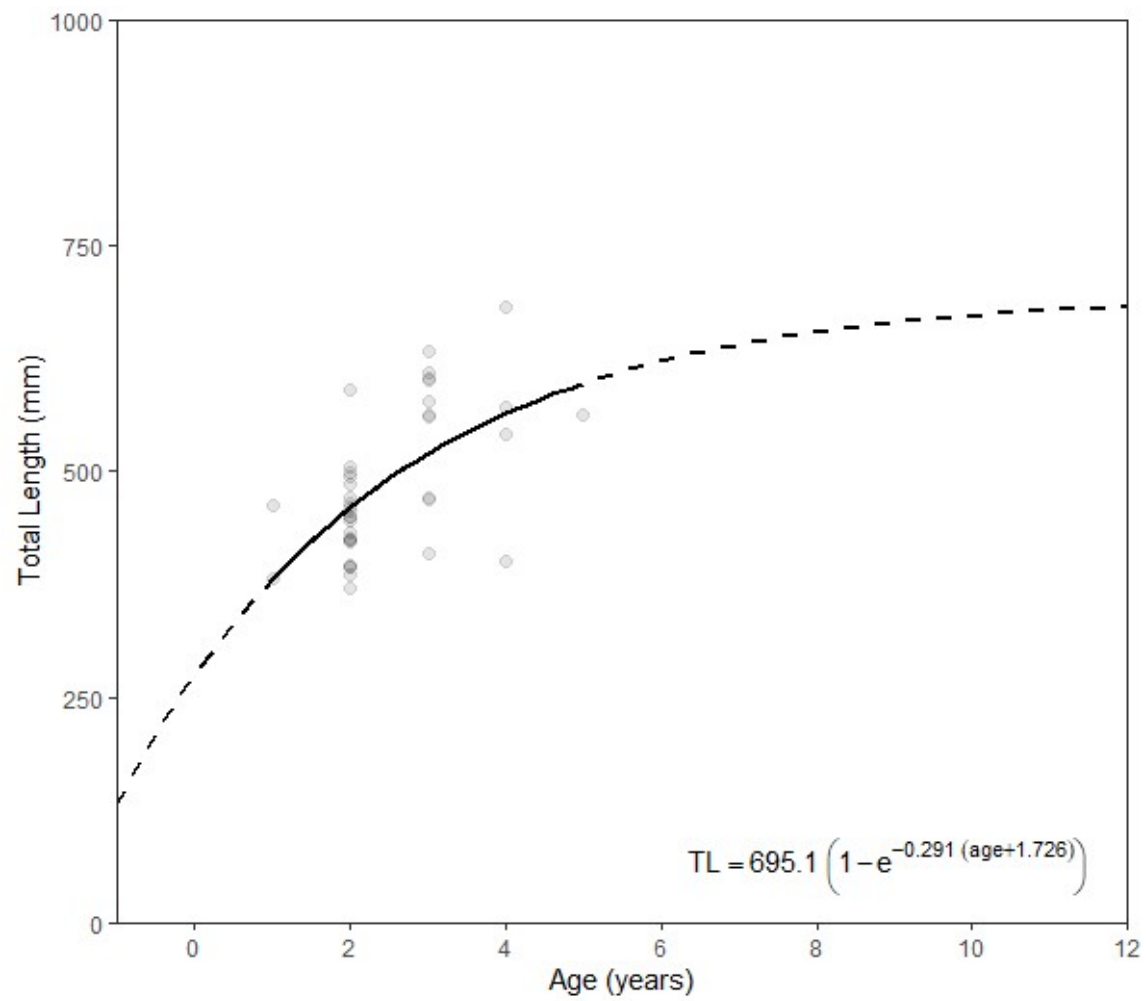


Figure 7. Von Bertalanffy growth curve fitted to Grass Carp captured below Gavins Point Dam, Yankton, SD in 2021 and 2022.

Recommendation: None

State Report: Nebraska

Agency: Nebraska Game and Parks Commission, University of Nebraska-Lincoln, School of Natural Resources and USGS Nebraska Cooperative Fish and Wildlife Research Unit

Project Title: Population distribution, population dynamics, reproduction and recruitment of Silver Carp and Bighead Carp in the Platte River, Nebraska

Methods: The study area included 16 sites throughout the Platte River basin from Elm Creek, Nebraska to the confluence of the Platte River and the Missouri River (365 river kilometers (rkm)). Sampling was conducted during the spring, summer, and fall of 2022 and 2023. Each site was divided into 4 independent, secondary sites that were visited once per season. This sampling framework allowed generation of encounter histories and subsequent occupancy model development. A 15-minute electrofishing run was conducted at each secondary site. Fish were collected using boat electrofishing or tote barge electrofishing, which was dependent on river discharge. Boat electrofishing was conducted using an ETS Electrofishing Systems (Madison, Wisconsin) MBS-2D control box mounted on a 4.9 m Aluminum flat-bottom boat. The control box was paired with a 3500-watt Champion Power Equipment (Milwaukee, Wisconsin) generator. The tote barge was fabricated by Midwest Lake Electrofishing Systems (Polo, Missouri) and featured an Infinity HC-80 control box paired with a similar 3500-watt generator. We used high frequency, pulsed direct current (60 hz, 25% duty cycle) and adjusted voltage to elicit a satisfactory fish reaction based on water conductivity.

Total length (mm) and body weight (g) were measured for each Silver Carp and Bighead Carp captured. An Analysis of Covariance was used to test for differences in Silver Carp $\log_{10}$ length and $\log_{10}$ weight between river and sex. Sex of Silver Carp and Bighead Carp was determined by feeling the texture (rough/smooth) of the posterior surface of the pelvic fin and was then verified by dissection and observation of gonads. Otoliths were collected from each Silver Carp and Bighead Carp for age and growth analysis. A Kruskal-Wallis test was used to assess differences in mean length at age between river reaches and sexes.

For the purposes of some statistical analyses, sites were consolidated into seven hydrologically unique groups or river reaches. These groups were defined as: Missouri (approx. MO River kilometer 630), Lower Platte (confluence of Platte/Missouri to the confluence of Platte/Elkhorn), Middle Platte (confluence of Platte/Elkhorn to the confluence of Platte/Loup), Central Platte (confluence of Platte/Loup and upstream on the Platte River) Elkhorn (confluence of Platte/Elkhorn and upstream on the Elkhorn River), Loup (confluence of Platte/Loup and upstream on the Loup River), and Salt Creek (confluence of Platte/Salt Creek and upstream on Salt Creek).

Catch per unit effort (CPUE) was calculated as the number of Silver Carp caught per sampling effort (i.e., each 15-minute electrofishing run). The CPUE data were tested for normality using a Shapiro-Wilks test. After determining data were nonnormal, differences in CPUE between river reaches and season were assessed using a nonparametric Kruskal-Wallis test. This test was followed by a Pairwise Wilcoxon Rank Sum Test with a Bonferroni p -value correction. Differences in length frequency distributions and age distributions of Silver Carp between season and river reach were assessed using Kolmogorov-Smirnov tests with Bonferroni corrections. Silver Carp were grouped into one of two river groups: Platte River Basin, or Missouri River. Sex proportions (m:f) of Silver Carp were tested against a null distribution (1:1)

using a test of equal or given proportions. A Pearson's Chi-squared test and subsequent post hoc analysis was used to assess differences in sex proportions between river reach and season. Differences in length frequency distributions between males and females were assessed using a Kolmogorov-Smirnov Test. A one-tailed Mann-Whitney U Test was used to determine the relationship between sex and total length. All analyses were performed using Program R.

Female gonads were removed, weighed in grams, then preserved in 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 the largest clutch of eggs from 0.50g sampled from the anterior, middle, and posterior of one ovary. Batch fecundity was analyzed for only spawning capable females. Egg diameter was measured for the first 80 eggs in each ovarian subsample to the nearest 0.1 mm to determine the mean egg size in each batch count. Histological analyses were conducted at the Nebraska Veterinary Diagnostic Center at UNL to categorize reproductive phases for spawning phenology assessments.

The associations between both batch fecundity and GSI with total lengths and weights of female Bighead Carp and Silver Carp were assessed using generalized linear regression with identity link. Seasonal differences in GSI and in batch fecundity for female Bighead Carp and Silver Carp as well as spatial trends between river reaches were assessed using two-way ANOVAs with Tukey's post-hoc test.

Larval sampling was conducted once a week per site from mid-May to mid-August of 2022 and 2023. Ten-minute stationary ichthyoplankton nets were set at eight randomly selected locations at each site, where each site is a 5km river reach on the Elkhorn River at Waterloo, NE, the Missouri River at Omaha, NE, and the Platte River upstream its confluence with the Missouri River. Eggs and larvae were sorted from debris, then preserved in ethanol for identification. Water quality (i.e., water temperature, dissolved oxygen, pH) and habitat data (i.e., water depth, macrohabitat, mesohabitat) were collected for each subsample.

Results & Discussion

A total of 1,528 Silver Carp and 50 Bighead Carp were sampled during FY 2022 and FY 2023. A total of 57 species were encountered. Mean length of Silver Carp across all study sites was 642 mm TL (min = 461 mm TL, max = 966 mm TL). Mean length of Bighead Carp across all study sites was 707 mm TL (min = 521 mm TL, max = 955 mm TL). The length distribution of carp was shifted between the Missouri River and Platte River basin where a larger proportion of smaller fish were found in the Missouri River (Figure 1).

Length-Weight Regressions

All river reaches in the Platte River basin were consolidated. Intercepts were significantly different between river, where Silver Carp in the Platte River were heavier at a given length than Silver Carp in the Missouri River ($F_1 = 57.07$, $P < 0.001$). Further, the slope of the length-weight regression of Silver Carp in the Platte River was significantly higher than that of the Missouri ($F_2 = 9.73$, $P = 0.002$). Slopes of length-weight regressions were similar between sexes ($F_2 = 0.26$, $P = 0.61$). However, intercepts were

significantly different, where female Silver Carp were heavier at a given length than male Silver Carp ($F_1 = 77.59, P < 0.001$).

Age Structure

Ages of Silver Carp varied from age-2 to age-8 with a mean age of 4 across the population. Preliminary age data of Silver Carp was tested for normality using a Shapiro-Wilks test. After determining that the data was nonnormal, a Kruskal-Wallis test was used to assess differences in age structure between river reach and sex. Age structure varied significantly between river reach ($P < 0.001$) but did not vary significantly between sexes ($P = 0.07$). A Dunn's test for pairwise comparisons indicated that ages of Silver Carp in the Elkhorn River were greater than ages of Silver Carp in the Platte River ($P = 0.01$) and Missouri River ($P = 0.001$). Ages of Silver Carp in Salt Creek were greater than ages of Silver Carp in the Platte River ($P = 0.02$) and Missouri River ($P = 0.004$) as well.

Growth

Mean length at age was similar between river reaches ($P = 0.68$) and between sexes ($P = 0.14$).

Reproduction and Recruitment

Overall mean GSI for female Silver Carp was 9.76 ± 6.18 ($n=336$). Mean GSI was highest in the spring (12.9 ± 6.09 ; $n=79$) followed by summer (11.0 ± 6.45 ; $n=148$) and fall (5.86 ± 3.25 ; $n=109$). Spring and summer mean GSI were significantly greater than mean GSI in the fall ($p < 0.05$), but spring and summer lacked significant difference ($p=0.167$). Mean GSI was highest in the Lower Platte (16.0 ± 4.16 ; $n=5$), followed by Elkhorn (13.6 ± 6.40 ; $n=47$), Salt Creek (12.0 ± 5.83 ; $n=75$), Loup (9.08 ± 5.99 ; $n=9$), Platte Confluence (8.09 ± 5.28 ; $n=170$), and Middle Platte (6.76 ± 6.76 ; $n=30$).

The main effects of season and of HUs within each season, with the covariate body weight, showed significant differences in GSI. In the spring, estimates of the marginal means of Silver Carp GSI values in the Platte Confluence were significantly lower than those in Salt Creek, Elkhorn, Loup and the Middle Platte ($p < 0.05$), but other pairwise comparisons lacked significant difference. In the summer, estimated marginal means of GSI values in the Platte Confluence were significantly lower than those in Lower Platte, Salt Creek, and the Elkhorn ($p < 0.05$), but other pairwise comparisons lacked significant difference. In the fall, GSI values in the Platte Confluence were significantly lower than those in Salt Creek, Elkhorn, and significantly greater than that in the Loup ($p < 0.05$). Estimated marginal means of GSI values in Salt Creek in the fall were significantly greater than those in the Platte Confluence, Middle Platte, and Loup ($p < 0.05$), but not the Elkhorn. Other pairwise comparisons in the fall lacked significant difference. GSI values were consistently lower in the Platte Confluence.

Batch Fecundity

Overall mean and standard deviation of batch fecundity was 203338.6 ± 124069.7 ($n=208$). Longer and heavier females had higher batch fecundity estimates ($p < 0.05$). Mean batch fecundity was highest in the spring (mean $\pm$ standard deviation: 246638 ± 130528 ; $n=61$), followed by the summer (202697 ± 122315 ; $n=111$) and the fall (131947 ± 79381 ; $n=36$). Fall batch fecundity was significantly lower compared to the spring and summer ($p < 0.05$), but spring and summer batch fecundity did not significantly differ ($p=0.06$; Table 5). Regardless of season, the Elkhorn had the highest batch fecundity (274526 ± 136452 ; $n=27$), followed by the Middle Platte (257851 ± 137773 ; $n=6$), Salt Creek (230371 ± 120355 ; $n=40$), the Lower

Platte (201257 ± 103652 ; $n=5$), the Platte Confluence (178302 ± 115069 ; $n=123$), and the Loup (168969 ± 138342 ; $n=7$).

The main effects of season and of HUs (Platte Confluence and Salt Creek) within each season with the covariate total length showed significant differences in batch fecundity estimates. In the spring, estimated marginal means of Silver Carp batch fecundity collected in the Platte Confluence were significantly lower than that of Salt Creek, Elkhorn, and Middle Platte ($p < 0.05$), but other pairwise comparisons lacked significance. In the summer, estimated marginal means batch fecundity was significantly lower in the Platte Confluence than in the Lower Platte, Salt Creek, and Elkhorn ($p < 0.05$), but other pairwise comparisons lacked significance. In the fall, estimated marginal means of batch fecundity was significantly lower in the Platte Confluence compared to Salt Creek, but not significantly different from the Elkhorn, Middle Platte, and Loup. However, in the fall, Salt Creek was significantly greater compared to the Middle Platte and the Loup ($p < 0.05$), but not significantly different from the Elkhorn ($p = 0.072$). Batch fecundity remained lower in the Platte Confluence across all seasons compared to HUs in the Platte River Basin.

Developing female Silver Carp were only captured at the Platte Confluence ($n=7$) and Salt Creek ($n=2$; Table 1). One hermaphrodite was collected at the Platte Confluence during the spring. Spawning capable females were collected at every HU in each season, except at the Elkhorn and Lower Platte in the fall. The majority females in the spring (85%) and summer (75%) were in the spawning capable phase at every HU. Regressing and regenerating fish were only captured in both the spring ($n=7$, $n=2$) and summer ($n=10$, $n=8$) at the Platte Confluence. In the spring, one regenerating fish was caught in the Elkhorn. In the summer, regressing fish were collected at Salt Creek ($n=12$) while regenerating fish were collected at the Elkhorn ($n=1$).

In the fall, spawning capable phase comprised about 35% of phases in the Platte Confluence, Salt Creek and Loup. The majority (65.5%) of fish in the fall were in the regressing phase among all HUs, except the Loup. Regenerating females were collected in the fall at Middle Platte and among all seasons in the Platte Confluence and Elkhorn.

The proportion of reproductive phases varied significantly across seasons within each HU (the Platte Confluence, Salt Creek, and Elkhorn ($p < 0.05$) except at Middle Platte ($p > 0.05$). The proportion of individuals in the prior spawn subphase did not differ among seasons (X-squared = 1.5471, $df = 2$, p -value = 0.4614). Evidence of prior spawning was present among all seasons and sites. The Elkhorn had significantly greater proportions of individuals with evidence of prior spawning compared to the Platte Confluence and Salt Creek. The proportions of female Silver Carp in the reabsorbed subphase (e.g., mass atresia present) significantly varied from the fall compared to the spring and summer (X-squared = 23.72, $df = 2$, $p < 0.001$) (Table 11). After adjusting with the Bonferroni method, the proportion of fish in the reabsorbing subphase varied significantly between Salt Creek and Middle Platte in the fall (Fisher's exact test: $p = 0.007$). Other pairwise comparisons were not statistically significant after adjusting. The presence of mass atresia is higher in the fall than spring. Compared to the Platte Confluence, mass atresia appears greater in the Elkhorn River and the Middle Platte.

Table 1. Counts of female Silver Carp among HUs and seasons in each reproductive phase (1=developing; 2=spawning capable; 3=regressing; 4=regenerating).

Hydrologic Unit	Spring				Summer				Fall			
	1	2	3	4	1	2	3	4	1	2	3	4
Platte Confluence	1	33	7	2	6	63	10	8	0	12	20	1
Lower Platte	0	1	0	0	0	4	0	0	0	0	0	0
Salt Creek	0	12	0	0	1	15	12	0	1	13	20	0
Elkhorn	0	11	0	1	0	19	0	1	0	0	12	1
Middle Platte	0	5	0	0	0	1	0	0	0	1	17	6
Loup	0	1	0	0	0	2	0	0	0	3	3	0

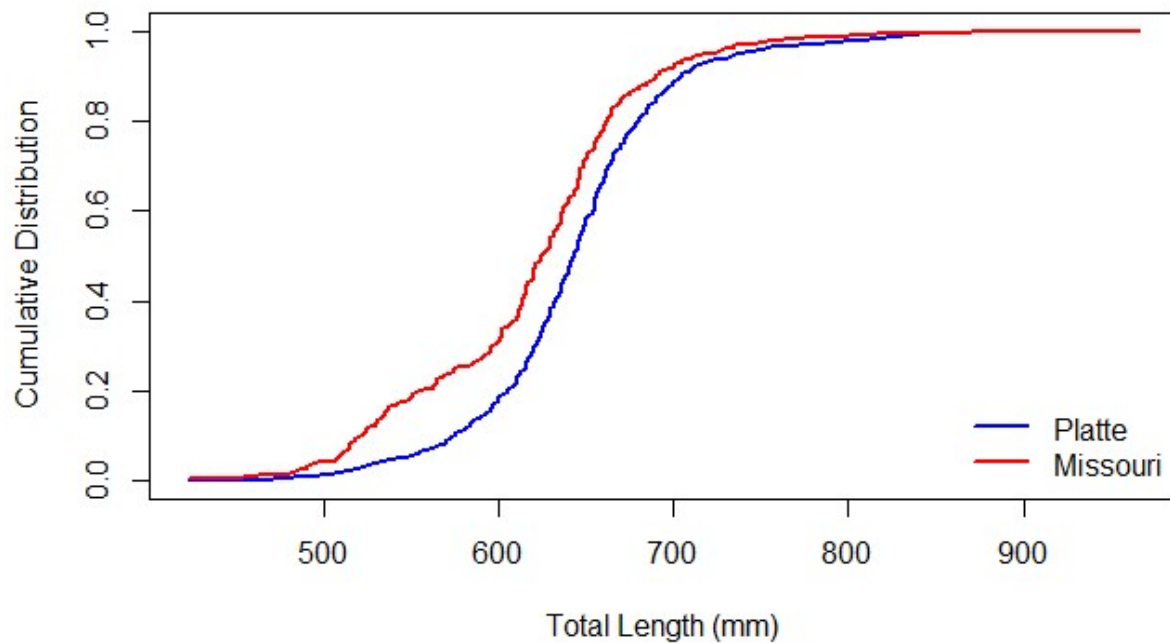


Figure 1. Cumulative distribution plot comparing Silver Carp total length between the Platte River (including tributaries) and the Missouri River near the Platte River confluence.

Recommendation:

Telemetry efforts will enable a better understanding of patterns of movement and direction of movement. Continued efforts to document and understand the spatial and temporal dynamics of reproduction in the system will be critical to inform any future population modelling efforts for the Platte River basin.

State Report: Nebraska

Agency: Nebraska Game and Parks Commission, University of Nebraska-Lincoln, School of Natural Resources and USGS Nebraska Cooperative Fish and Wildlife Research Unit

Project Title: Sampling Efficiency Assessment for Silver Carp and Bighead Carp in Nebraska Mid-Order Streams and Rivers

Methods:

The effectiveness of four herding techniques, including sound administered through underwater speakers, electric stimulus, a combination of sound and electric stimulus, and boat and motor only was assessed based on the net directional movement of bigheaded carp (i.e., Silver Carp and Bighead Carp combined). Directional movement with no added stimulus as a control was also assessed to compare with the four herding techniques. Each herding technique was applied using a 4.9-m (16ft) aluminum flat-bottomed boat equipped with a 25-hp outboard motor. The electric stimulus included pulsed-DC electrofishing administered using an MBS-2D control box (ETS Electrofishing, Madison, Wisconsin) with 60 Hz, and 25% duty cycle (adjusting settings to keep fish mobile) and powered by a 3,500-W generator (Champion, Santa Fe Springs, California). An underwater speaker (TOA CA160R; Lubell Labs, Whitehall, Ohio) was used to emit 160 dB of loud percussive sound (Ridgway et al. 2023). The combination technique included the use both sound and electrofishing using the settings above. The study area included 6 sites (150 m in length) located along the lower 5.3 km of Salt Creek (Figure 1). The first site was about 3.7 km upstream of the Platte River confluence. The study area spanned 1.6 km upstream of the first site. The study area was open between trials, enabling fish movement among sites. Sites were sampled using a systematic random method in which a herding technique was randomly selected each day to sample 2 sites. Sites were sampled working from downstream to upstream and skipping neighboring sites to reduce interference between herding treatments. A minimum buffer of 900 m was placed between sites sampled on the same day to help ensure independence. Sampling was omitted from the entire study area for at least three days out of the week and sites were given at least three days to recover before being sampled again.

Sampling at a site consisted of three 5-min runs with block nets deployed across the upper and lower end of the site (Figure 2). The lower block net was set at a 20° angle extending downstream towards the opposite shoreline leaving an approximately 6-m gap (Figure 2). This gap was left open during all 3 herding runs to allow fish immigration and emigration from the site. A dual-frequency identification sonar (DIDSON; Sound Metrics, Bellevue, Washington) was positioned near the open end of the downstream block net to record fish movement. The start and end times were recorded for each run. While herding, the boat moved downstream in a slow (3 km/h) zig-zag pattern to enable fish movement downstream and ensure site coverage (Figure 2). The boat continued to maneuver downstream until it reached the lower block net opening. Sampling continued throughout June and July of 2023 until each herding technique and the control had 8 replications. Physical characteristics and water quality measurements will be recorded for each treatment. Dissolved oxygen, conductivity, water temperature, and turbidity data will be gathered from the United States Geological Survey (USGS) gaging station near Ashland, Nebraska (gage 06805000), approximately 223 m downstream of the study area within Salt Creek. Discharge data will be collected from the USGS gauge near Greenwood, Nebraska (gauge 06803555), approximately 14.6 km upstream of the study area within Salt Creek.

Community composition sampling was conducted after the completion of all herding replications and occurred over a 600 m reach in Salt Creek that spanned previously sampled sites. Block nets were set in a similar fashion as during herding replications and 3 150-m trammel nets were set below the downstream block net in a zig-zag pattern with bank-to-bank coverage. Once the boat had passed through the downstream block net opening, it was closed off to prevent escapement. The trammel nets were retrieved after 30 minutes. All fish were identified to species, measured in total length (mm), and enumerated.

To document presence of and assess potential areas of recruitment of Silver Carp and Bighead Carp within the Platte River basin we conducted 80 seine hauls at 16 sites in October 2023. Occurrence of larval or age-0 carp was intended to inform future placement of gears to sample and compare those gear types.

Analyses

Sonar recordings were analyzed using Mark Fish tool provided with DIDSON software (V5.27.54). Bigheaded carp were enumerated for each herding run where a bigheaded carp was identified based on size (>400 mm TL) and body morphology (e.g., fusiform, large head, narrow caudal peduncle). Adult bigheaded carp are predicted to mature sexually at age 2, translating to approximately 400 mm TL. The reliability of DIDSON software in measuring fish length was assessed by Hightower et al. (2013). Community sampling and prior data collected from electrofishing on Salt Creek will be used to form length frequency histograms for comparison with perceived Silver Carp lengths. Generalized linear models were used to assess net fish count data for each herding technique.

Results & Discussion

A total of 40 herding treatments (120 runs) were completed during the summer of 2023. A total of 1,441 fish (>400 mm TL) were counted for all herding techniques and runs. 77.00% of all fish observed on the DIDSON recordings were moving downstream. Community composition sampling concluded that Silver Carp made up 64% of the catch during a herding run. Sound recordings are being used to analyze sound pressure levels for each technique. All treatments showed significant increases in net downstream movement compared to control (no stimulus). The combination of sound and electrofishing and sound alone each appeared to move more fish downstream compared to boat motor alone ().

A total of 32 species and 16,421 fish were collected between September 5, 2023 and October 3, 2023. No age-0 bigheaded carp were sampled or observed during the fall of 2023, however, 3 adult Silver Carp were captured. Red Shiners (8,044), Sand Shiners (4,135), and River Shiners (2,034) were the most abundant species sampled. A few species of note include 1 Plains Topminnow, 36 Plains Minnows, 2 Western Silvery Minnows, 1 Iowa Darter, 2 Johnny Darters, 7 Silver Chub, and 4 Shoal Chub.



Figure 1. Map of sites located on Salt Creek near Ashland, NE where 5 herding techniques were implemented during the summer of 2023.

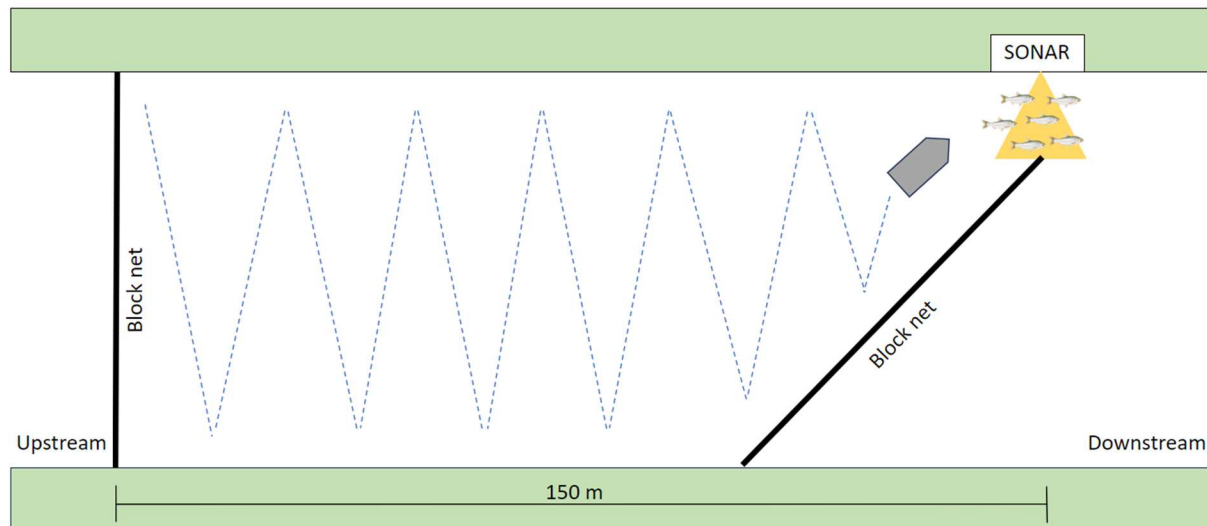


Figure 2. Spatial representation of herding methods employed on Salt Creek during the summer of 2023.

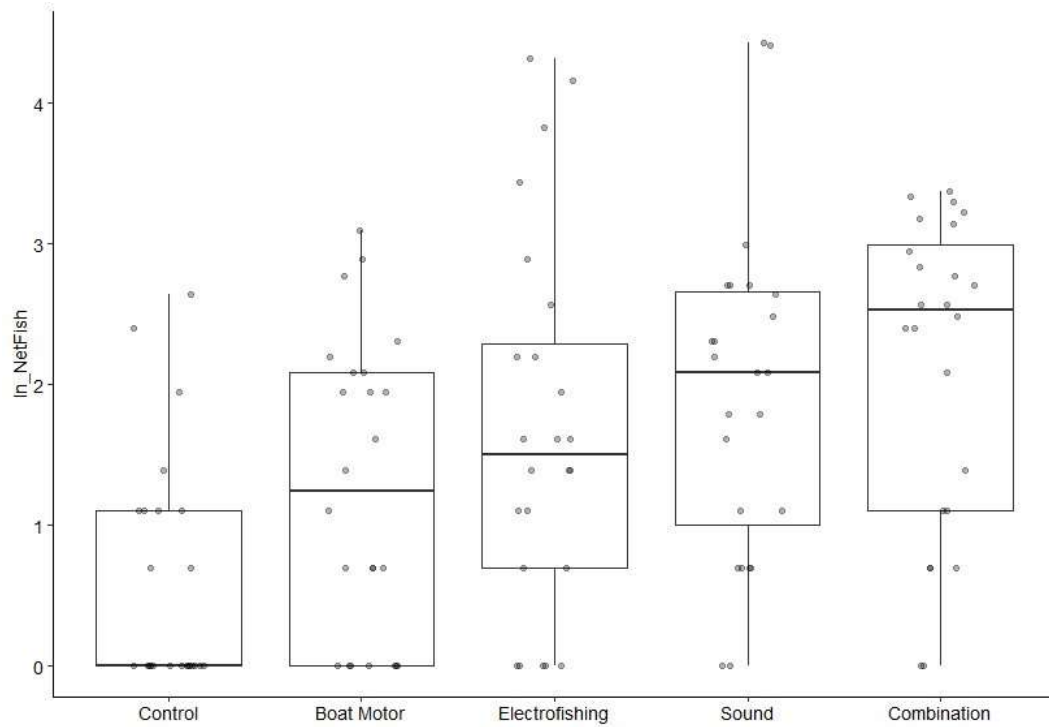


Figure 3. Box plot depicting the log transformed net number of fish (fish moving upstream subtracted from fish moving downstream) per run observed on the DIDSON recordings for each herding technique during the summer of 2023. The horizontal solid line represents the median, upper and lower limits of the box are the quartile ranges, and the whiskers are 95% confidence intervals.

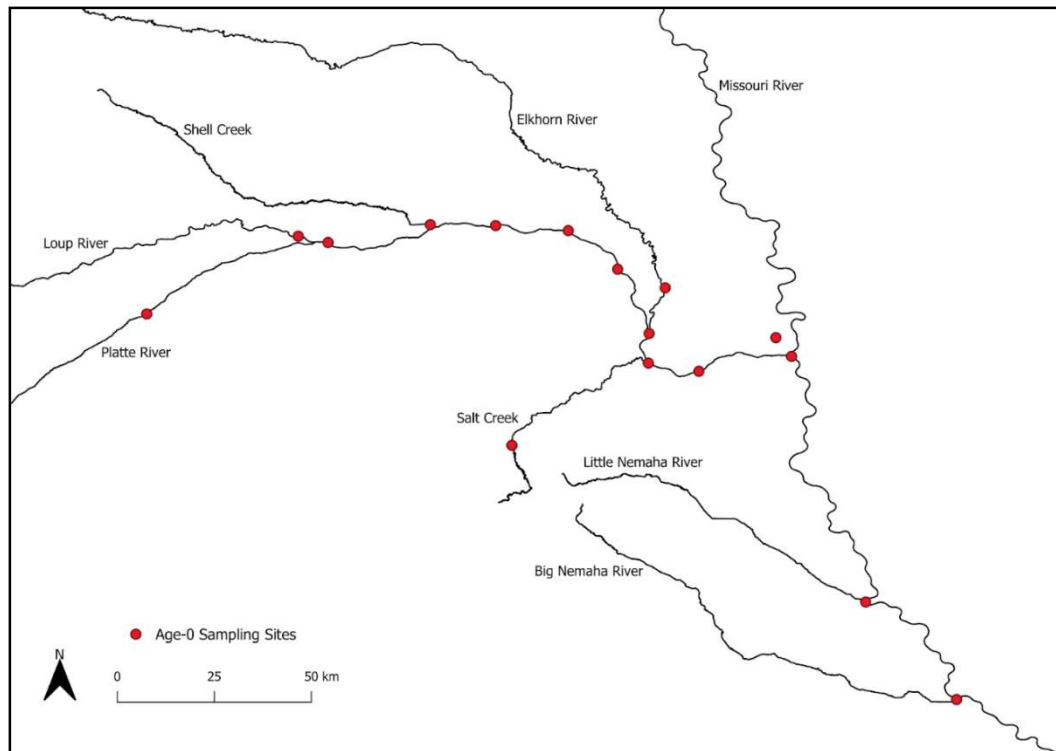


Figure 4. Map of sites located across the Lower Platte River and Big Nemaha River basins where seining for age-0 bigheaded carp occurred during the fall of 2023.

Recommendations:

Sampling in 2024 will include efforts to effectively remove carp following herding (using most effective herding technique). Initially, a combination of seine hauls and electrofishing will be used where conditions permit. Other gear types including trap nets, gill nets, or trammel nets may be used.

Development of appropriate age-0 sampling protocols will be dependent on locating age-0 individuals within the Platte River basin. To date, we have not detected age-0 bigheaded carp during a drought period within the basin. Efforts to identify areas where age-0 carp are present using active gears including seine hauls will continue into 2024.

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:

Study sites

We tested for the presence of environmental DNA (eDNA) of Bighead and Silver Carp in two tributaries to the Missouri River in eastern South Dakota: the Big Sioux River and the Vermillion River. Both rivers experience intermittent patterns of flooding and drought, and flows are highly dependent on the amount of precipitation, including snowmelt, and agricultural runoff (Underhill, 1959).

Sampling methods

Water sampling took place over two field sampling seasons. In the first field season, at each sample location, two water samples were collected using two, 2-Liter high-density polyethylene (HDPE) bottles. In the second field season at each sample location, one, 2L sample was taken. Samples were collected according to procedures described in Coulter et al., 2019. The most downstream location in each river was sampled first, and each successive sample was collected upstream of the previous sample to avoid eDNA cross-contamination. All samples were collected while standing on the shoreline to reduce the risk of boots or waders contaminating the water with invasive carp eDNA. One negative control sample (one, 2-L bottle of distilled water) was “collected” above and below each barrier. After sampling, all bottles were placed in coolers on ice until filtration was carried out no more than 8 hours after sample collection.

Water samples (2 L) were filtered through 1.5- μ m glass microfiber filters (Cytiva Whatman Binder-Free Glass Microfiber Filters; grade 934-AH circles; diameter outside: 47mm; borosilicate glass; grade 934-AH filter; 47 mm circle) (Eichmiller et al., 2014) using a magnetic polyphenylsulfone filter funnel (Eichmiller et al., 2014; Nukazawa et al., 2018; Coulter et al., 2019). In 2021, one-half of the water samples were filtered in the field using a 7.2-L, manual pump MityVac MIT7400 fluid evacuator. The other half of the samples were transported back to the lab and filtered using a 1000-mL Büchner flask. In 2022, samples were exclusively filtered using the field filtering method. For both filtering techniques, water samples were drawn through the filter until the pores were clogged and water could no longer pass through the filter. The volume of water that passed through was recorded for each filter. Each filter was stored in 95% ethanol in a 15-mL polypropylene Falcon tube in a lab freezer at -20°C until eDNA extraction could occur. All supplies (filter funnels, forceps, coolers, etc.) were sterilized with 20% bleach for ≥ 10 seconds, rinsed with distilled water, and dried with a new paper towel before and after each use and between samples.

eDNA extraction

Prior to extraction, filters were cut in half, with one half returned to the 15-mL Falcon tube in the freezer and the other half was cut into three smaller pieces. The three pieces were placed in 1.5-mL microcentrifuge tubes and allowed to air dry in a fume hood over a period of ≥ 4 hours until all ethanol

had evaporated. Tools were sterilized with 20% bleach for ≥ 10 seconds, rinsed with distilled water, and then rinsed with nanopure water to ensure no contamination between filters. Then, eDNA extraction was carried out in a designated sequencing room at the University of South Dakota and according to the Qiagen DNeasy Blood and Tissue Extraction protocol (Qiagen, 2023) with one modification at the end step to centrifuge 100- μ L of elution buffer through the spin columns twice instead of 200- μ L once (i.e., “kristie” protocol (Schmidt et al., 2021)). Extracted samples were stored in 1.5-mL or 2-mL microcentrifuge tubes at -20 °C.

Quantitative Polymerase Chain Reaction (qPCR) analysis

The real-time quantitative polymerase chain reaction (qPCR) analysis was conducted using an Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System thermocycler (Thermo Fisher). The forward primer, reverse primer, and gBlock used in this study were developed by Erickson et al. (2017) and targeted the mitochondrial gene *nd5*, where the primer sequence binds to an area of the mitochondrial genome that is conserved between both Bighead and Silver Carp, and the probe sequence binds to an area of the mitochondrial genome that is specific to either species (Erickson et al., 2017). Each well had a volume of 20 μ L, containing 7- μ L molecular grade water, 10- μ L TaqMan™ Fast Advanced Master Mix (Thermo Fisher), 1 μ L of 10 nanomole forward and reverse primer mix, 1 μ L of 5 nanomole probe mix, and 1 μ L of extracted eDNA sample. A 313-base pair Megamer ssGene Fragment, or gBlock, containing DNA sequences conserved between both Bighead and Silver Carp was used to create a standard dilution series which served as the eDNA calibration curve for each qPCR run (Erickson et al., 2017). The 96-well plates contained four replicates of each of the following: eight field samples, one field blank, one extraction blank, and one positive control. For positive qPCR controls, we collected tissue from silver carp in the James River and extracted DNA using the DNeasy Qiagen Blood and Tissue Kit. Each plate also contained three spiked replicates of each sample (extracted sample plus 0.1 μ L of the 10^3 dilution per well). Spiked samples were added to indicate the presence of qPCR inhibition in cases where invasive carp eDNA was not detected in the regular field samples. Ten, no-template control wells (1- μ L nanopure water) and two replicates of a 10^6 standard dilution series derived from the gBlock megamer were included on each plate (Table 1). The qPCR cycling conditions followed the protocol in Erickson et al., (2017). We considered each sample to have been “positive” if at least one sample replicate amplified during qPCR, and “negative” if none of the replicates amplified.

Statistical analysis

We evaluated the probability of a positive detection for each species, river, and location in respect to the barriers in each river. We used Program R (version 4.1.1; R Core Team 2022) with the *brms* package (Bürkner, 2017) to fit a Bayesian generalized linear mixed model (Song et al., 2017; Song et al., 2020) to estimate the probability of a positive detection using a Bernoulli likelihood with a logit link using the following equation:

$$\begin{aligned} E &\sim \text{Bernoulli}(p), \\ \text{logit}(p) &= \alpha + \beta X + \gamma_{\text{sample_name}} + \gamma_{\text{species}} \\ \alpha &\sim \text{Normal}(-10, 1) \\ \beta X &\sim \text{Normal}(0, 1) \end{aligned}$$

$$\gamma_{\text{sample_name}} \sim \text{Normal}(0, \sigma_{\text{sample_name}})$$

$$\gamma_{\text{species}} \sim \text{Normal}(0, \sigma_{\text{species}})$$

$$\sigma_{\text{sample_name}} \sim \text{Half-Student-t}(3, 0, 2.5)$$

$$\sigma_{\text{species}} \sim \text{Half-Student-t}(3, 0, 2.5)$$

Where E is the presence or absence of eDNA, p is the proportion of positive detections, α is the intercept, $\beta\mathbf{X}$ is a vector of 7 parameters representing three predictors (quantity, location_{below}, river_{vermillion}) and their interactions, γ is the random intercept effect for sample name and species, and σ is the hyperprior for the random effects.

The model was run using a Hamiltonian Monte Carlo No-U-Turn sampler with 4 Markov chains, 2,000 iterations per chain with a warm-up of 1,000 iterations. We evaluated the fit of the model by running posterior probability checks and determined it properly converged if Gelman-Rubin statistics were less than 1.1 (Brooks & Gelman, 1998). We sampled from the posterior and estimated the relationships for the model and predicted the mean with 95% credible intervals.

We ran a sensitivity analysis to assess the impact of the priors on our presence/absence model by doubling the standard deviation values to Normal (-10, 2) for the intercept, Student's t (3, 0, 5) for the hyperpriors, and Normal (0, 2) for the beta values.

Results & Discussion

Water sampling

A total of 213 water samples and 38 field blanks were collected after two years of field sampling (98 water samples and 20 field blanks in 2021, 115 water samples and 18 field blanks in 2022). The average filtered volume was 382 mL (range 100 to 1650 mL). In 2021, Big Sioux River samples were taken on August 17 and September 24 at three below-barrier sites and six above-barrier sites. Due to low water levels downstream of the Falls, the third round of water samples were collected only at sites upstream of the Falls on October 29, 2021. In 2022, Big Sioux River samples were taken on May 26, July 11, and August 17, at five below-barrier sites and eleven above-barrier sites (Figure 1). In 2021, Vermillion River samples were taken on August 5 and September 22, at two below-barrier sites and seven above-barrier sites. Due to low water levels below the spillway, the third round of water samples were collected only at sites upstream of the spillway on October 28, 2021. In 2022, 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 2).

Invasive Carp were observed swimming below the barriers at almost all sampling sessions, except for the below-barrier Vermillion River samples in October and September 2021, where only fish carcasses of various species, including Bighead and Silver carp, could be observed floating in the water below the spillway following a large fish kill event. A rain event washed most of the carcasses downstream prior to

the second sampling event, but some bones and other carcass remnants were still present in and around the water at the time of sampling. In 2022, we visually observed carp of undetermined species at all water sampling sessions below both barriers.

We also collected six water samples (five samples and one field blank) on June 3, 2022, 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 Bighead and/or Silver Carp scattered along the shoreline of Silver Lake and live Common Carp swimming in the lake, but we observed no live Bighead or Silver Carp in the water during sampling.

qPCR results

Bighead and Silver Carp eDNA was detected below both barriers in 2021 and 2022 in varying quantities (Table 2). Bighead Carp and Silver Carp eDNA was detected in two and three samples respectively above the Lake Vermillion Spillway (Figure 3) in samples taken in July 2022 but not in previous or successive samples. The samples collected at Silver Lake near Freeman, SD, in response to a fish kill were all negative for eDNA detection.

The probability of detecting eDNA below the barrier in the Big Sioux River was 51% (95% Credible Interval (CrI): 2 to 97%) and in the Vermillion River was 77% (95% CrI: 8 to 99%). The probability of detecting eDNA above the barriers was significantly smaller: 1% (95% CrI: 0.02 to 22%) in the Big Sioux River and 1% (95% CrI: 0.02-24%) in the Vermillion River. The probability that detection was higher below the barriers than above the barriers was greater than 99% (Figure 4).

Future directions

We recommend future, expanded eDNA water sampling above the barriers in the Big Sioux and Vermillion Rivers to further elucidate the status of these species above the barriers, coupled with physical sampling efforts to gauge invasion presence and risk into the future. Future studies could even be broadened to other small tributaries (i.e., tributaries of the James, Big Sioux, and Vermillion Rivers, or other Missouri River tributaries) to assess the presence of these fish in finer detail throughout eastern South Dakota. It may also be necessary to eventually reconsider the effectiveness of these barriers, especially during flooding events due to the capability of these fish to surpass the barriers in times of flooding.

Table 1. A general 96-well plate set up for detecting Bighead and Silver Carp eDNA. “NTC” stands for “no template control.”

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10 ⁶	10 ⁶
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10 ⁵	10 ⁵
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10 ⁴	10 ⁴
D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10 ³	10 ³
E	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	10 ²	10 ²
F	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	10 ¹	10 ¹
G	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	Positive control	Positive control
H	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	Positive control	Positive control

Table 2. Average eDNA quantification (copies/ μ L) for each river, above and below barriers, for each species in both years.

2021			
		Bighead Carp	Silver Carp
Vermillion River	Above	0	0
	Below	555	0.009
Big Sioux River	Above	0	0
	Below	18.4	1.54

2022			
		Bighead Carp	Silver Carp
Vermillion River	Above	0.302	0.00960
	Below	24.1	1.53
Big Sioux River	Above	0	0
	Below	2.74	0.391

Figures

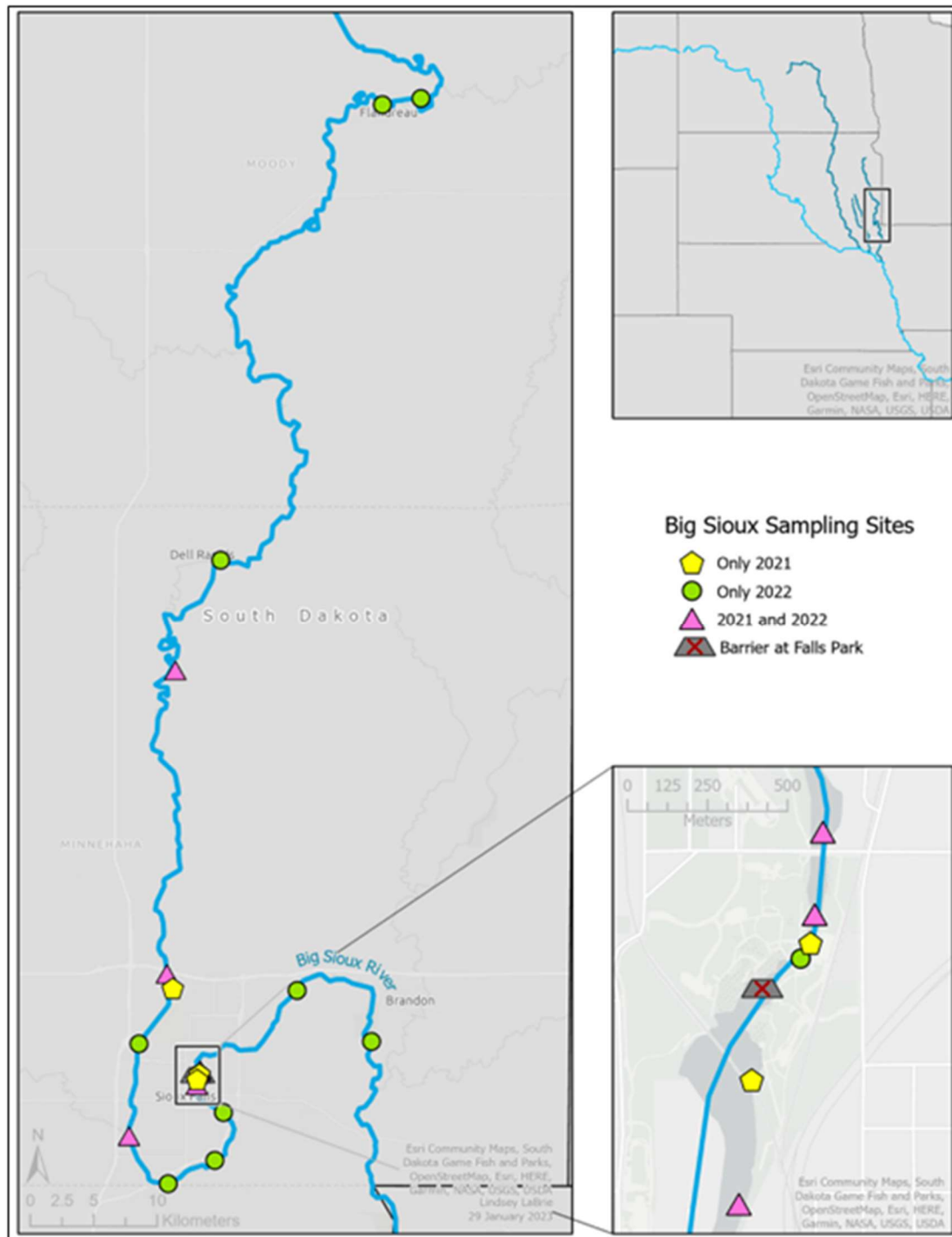


Figure 1. eDNA water sampling locations in the Big Sioux River. The inset map depicts a zoomed in portion of the river below and above Falls Park in Sioux Falls, SD. Yellow pentagons represent areas sampled only in 2021, pink triangles represent areas sampled in both years, green circles represent sampling locations that were added in 2022.

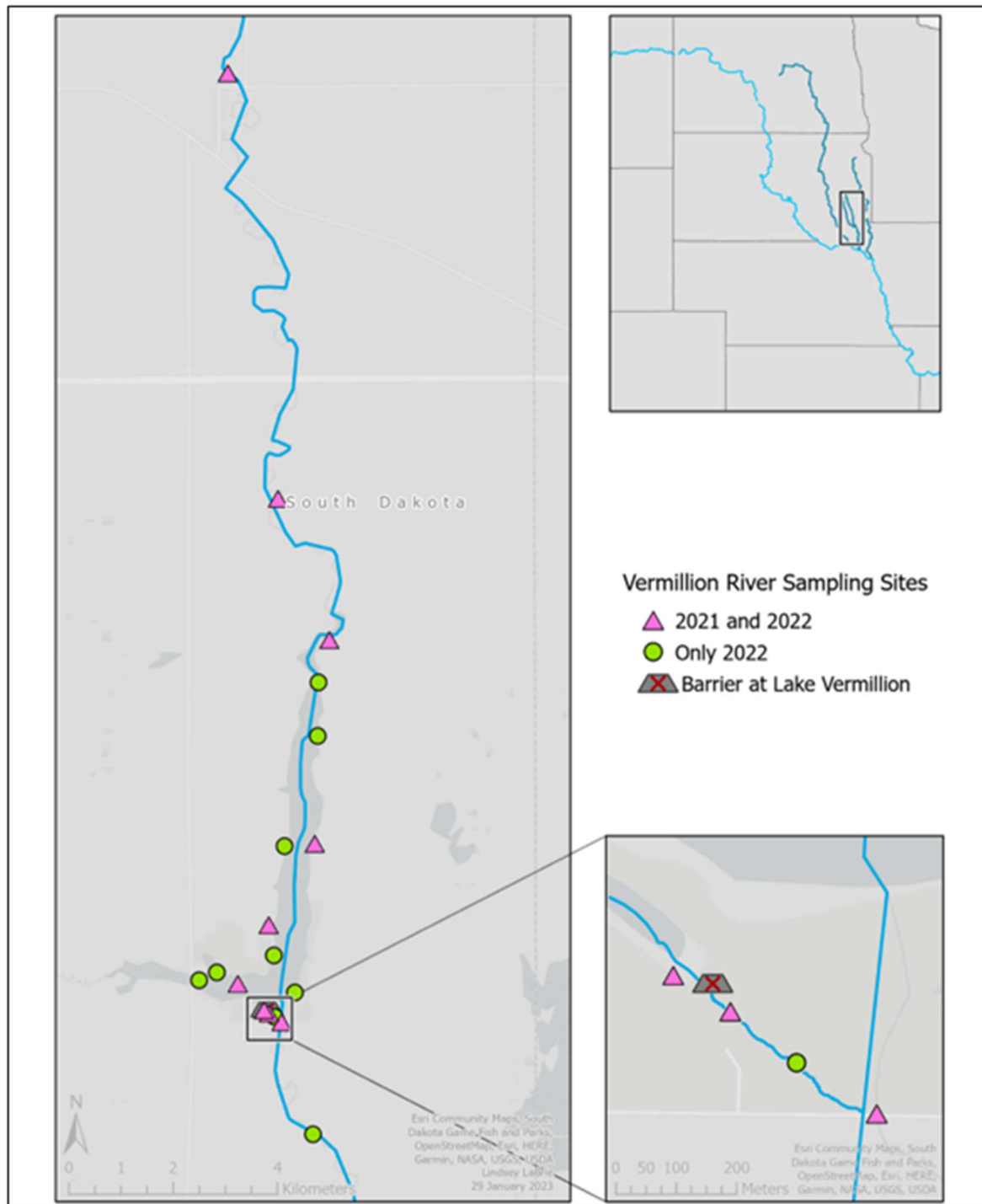


Figure 2. eDNA water sampling locations in the Vermillion River. The inset map on the bottom right depicts a zoomed-in portion of the river below the spillway at Lake Vermillion. Pink triangles represent areas sampled in both years, and green circles represent areas that were only sampled in 2022 (in addition to the locations denoted by pink triangles).

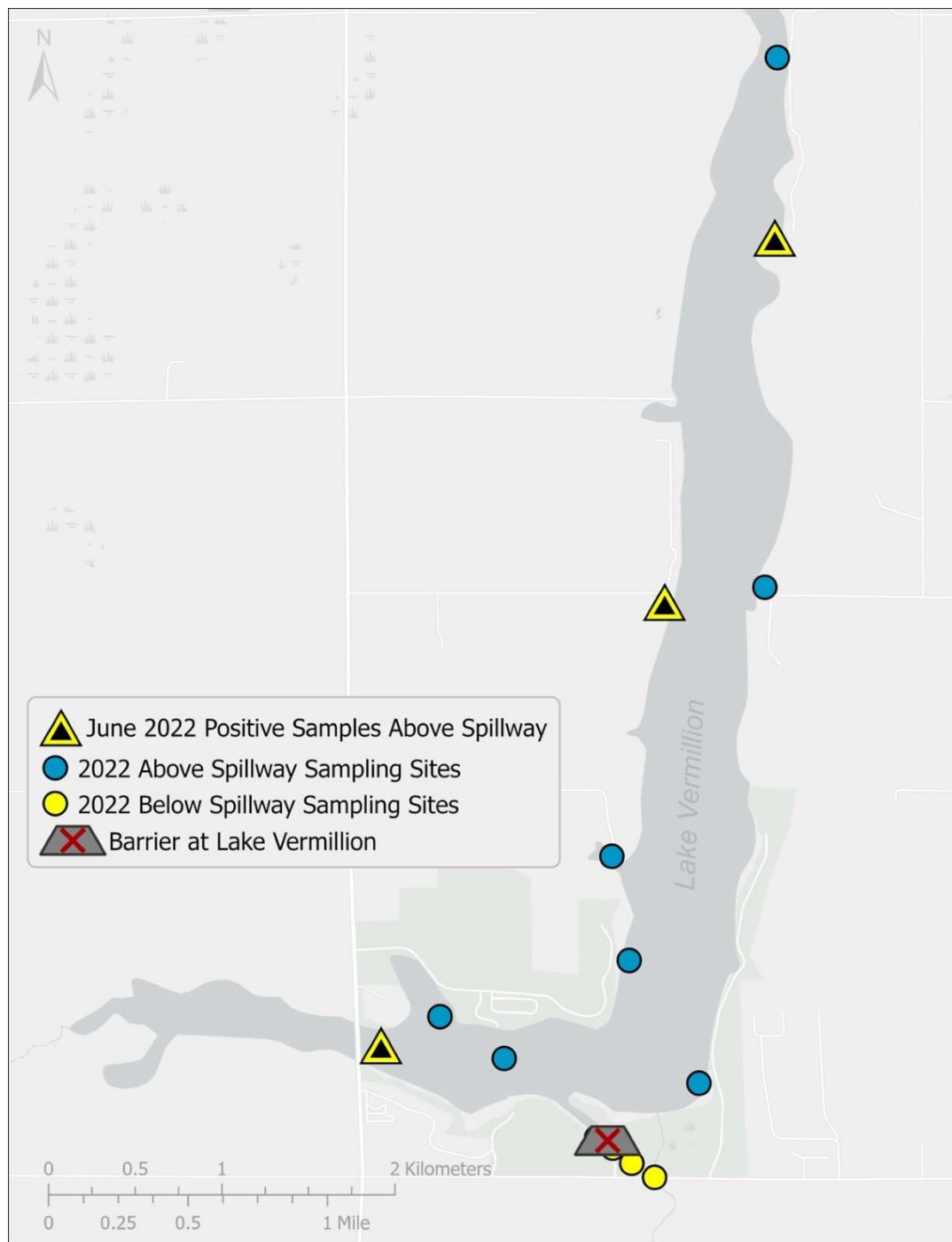


Figure 3. Locations of positive eDNA detections (represented by yellow triangles) in Lake Vermillion. Samples were collected in June 2022.

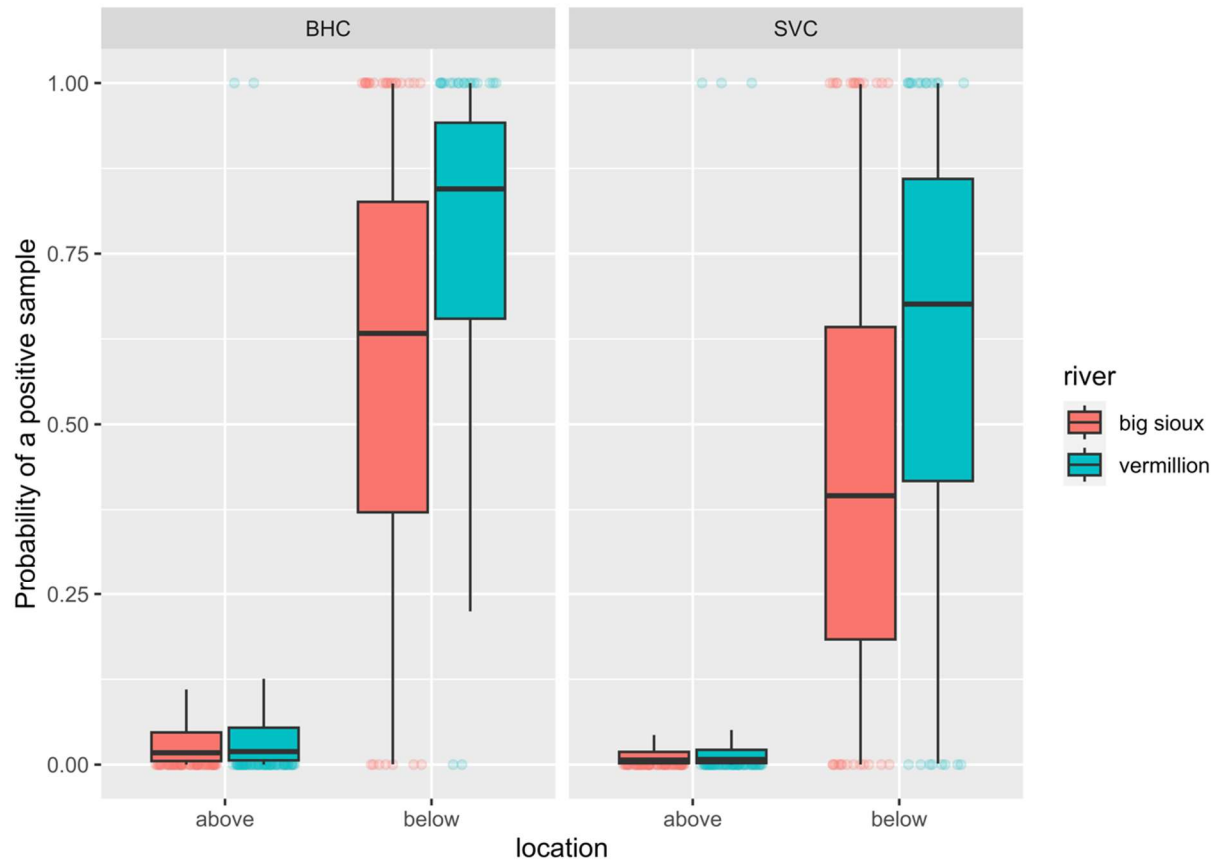


Figure 4. Posterior predictions for the probability of a positive sample for Bighead Carp (BHC) and Silver Carp (SVC) in the Big Sioux and Vermillion Rivers above and below the major barriers to upstream fish movement. The hollow, colored circles represent actual data points, with zero (0) on the Y-axis indicating no eDNA detection and one (1) on the Y-axis indicating a positive eDNA detection. The probability of a difference between above and below barrier samples was greater than 99%.

Recommendation:

Work with collaborators to identify strategies for follow-up evaluations on positive eDNA detections in areas outside the known range of invasive carp.

State Report: South Dakota

Agency: South Dakota Game, Fish and Parks, South Dakota State University; U.S. Geological Survey Cooperative Fish and Wildlife Unit; Kennedy LIFE Lab

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

Methods:

Water chemistry

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. In 2023, sampling was conducted in late May-early June and late July-early August at the same locations as in 2022, but oxygen isotopes were only processed in the latter sample.

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 $\delta^{18}\text{O}$.

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 *Hypophthalmichthys molitrix* were collected from 2020-2023 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 all 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. A total of 47, 37, and 38 Silver Carp otoliths were collected from the Big Sioux, James, and Vermillion rivers in 2020-2021. 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. In 2023, Silver Carp were sampled from the Big Sioux, James, and Missouri rivers to bolster sample sizes. We collected a total of 53, 17, and 43 fish from the Big Sioux, James, and Missouri rivers, respectively. Total lengths of fish collected in 2023 ranged from 429-743 mm. Additional juvenile

(presumed age-0) Silver Carp were collected in the upper James River in fall 2021 ($n = 2$) and lower James River in fall 2022 ($n = 18$), and several juvenile Silver Carp were collected by USFWS staff in 2020 in the mainstem Missouri River. All collected Silver Carp otoliths will be sent for processing to Southern Illinois University for LA-ICPMS.

Results & Discussion:

Trace element and $\delta^{18}\text{O}$ 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 5). 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 $\delta^{18}\text{O}$ ratios had substantial overlap among the three eastern SD tributaries, but the Missouri River signatures were substantially lower (Figure 6).

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 3). 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.

Issues with equipment have prevented LA-ICPMS processing, so results are pending. A small sample ($n=18$) of otoliths have been ablated, and results demonstrate that otolith chemistry differs enough to allow for differentiation between tributary (James, Vermillion, and Big Sioux rivers) and mainstem Missouri River Sr:Ca molar ratio signatures (Figure 7). Additionally, the limited analysis of Ba:Ca molar ratios appears to demonstrate that differentiation of the Big Sioux and James rivers will be possible.

Table 3. Strontium isotope water chemistry results for each site on the three eastern SD tributaries and the Missouri River sampled in June 2021.

River	Site	<i>⁸⁷Sr/⁸⁶Sr</i>	<i>±2SE (ratios included)</i>
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

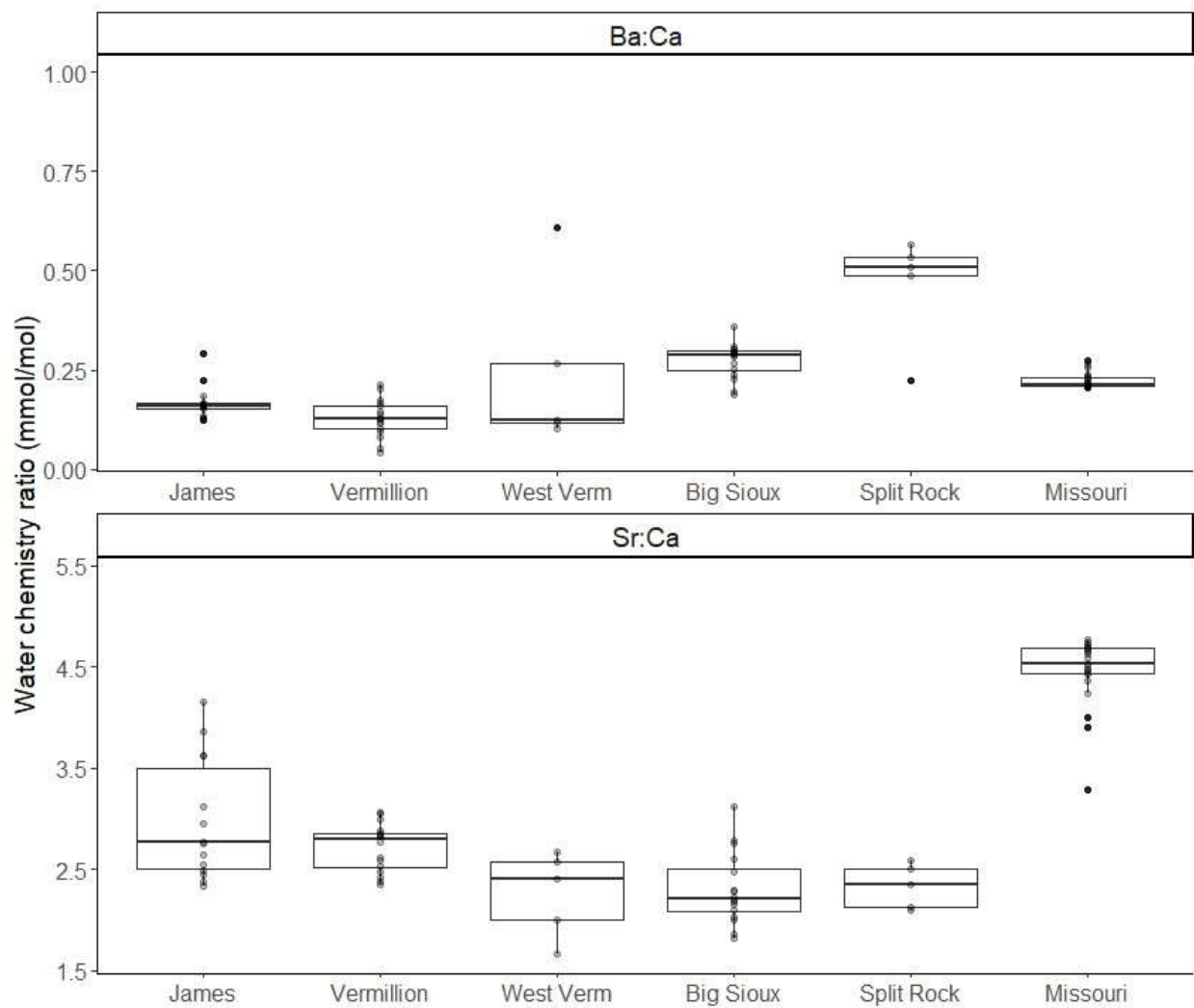


Figure 5. Boxplots of water Sr:Ca and Ba:Ca ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021-2023.

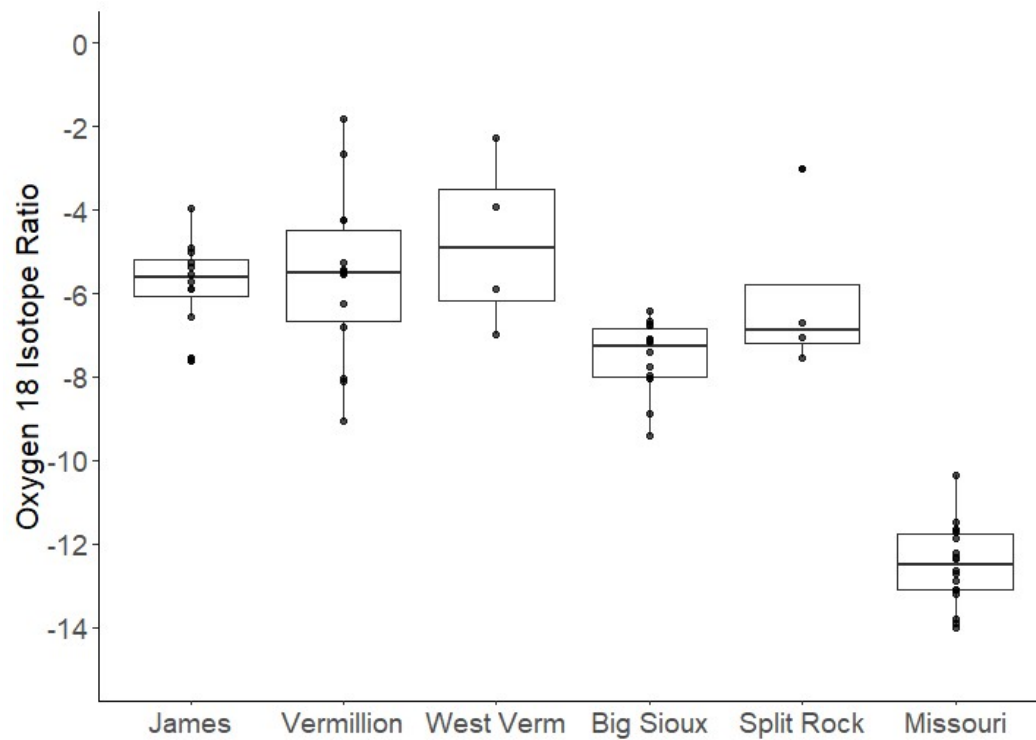


Figure 6. Boxplot of water oxygen-18 ($\delta^{18}\text{O}$) isotope ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021-2023.

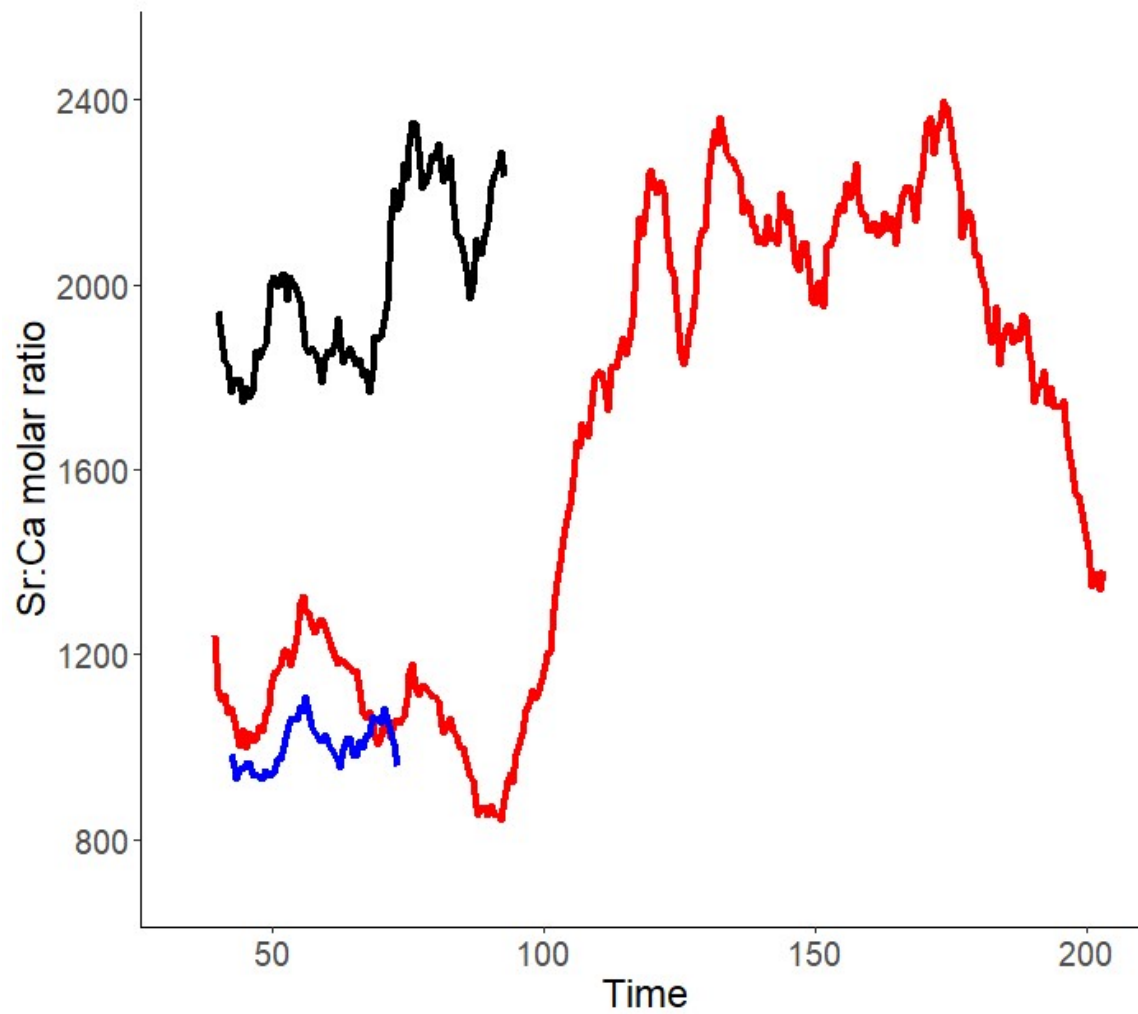


Figure 7. Strontium-to-calcium (Sr:Ca) molar ratio (mmol/mol) for Silver Carp lapillus otoliths collected from a juvenile collected in the Missouri River in July 2020 (black), an age-0 from the James River in October 2022 (blue), and an adult collected in the James River in May 2022 (red).

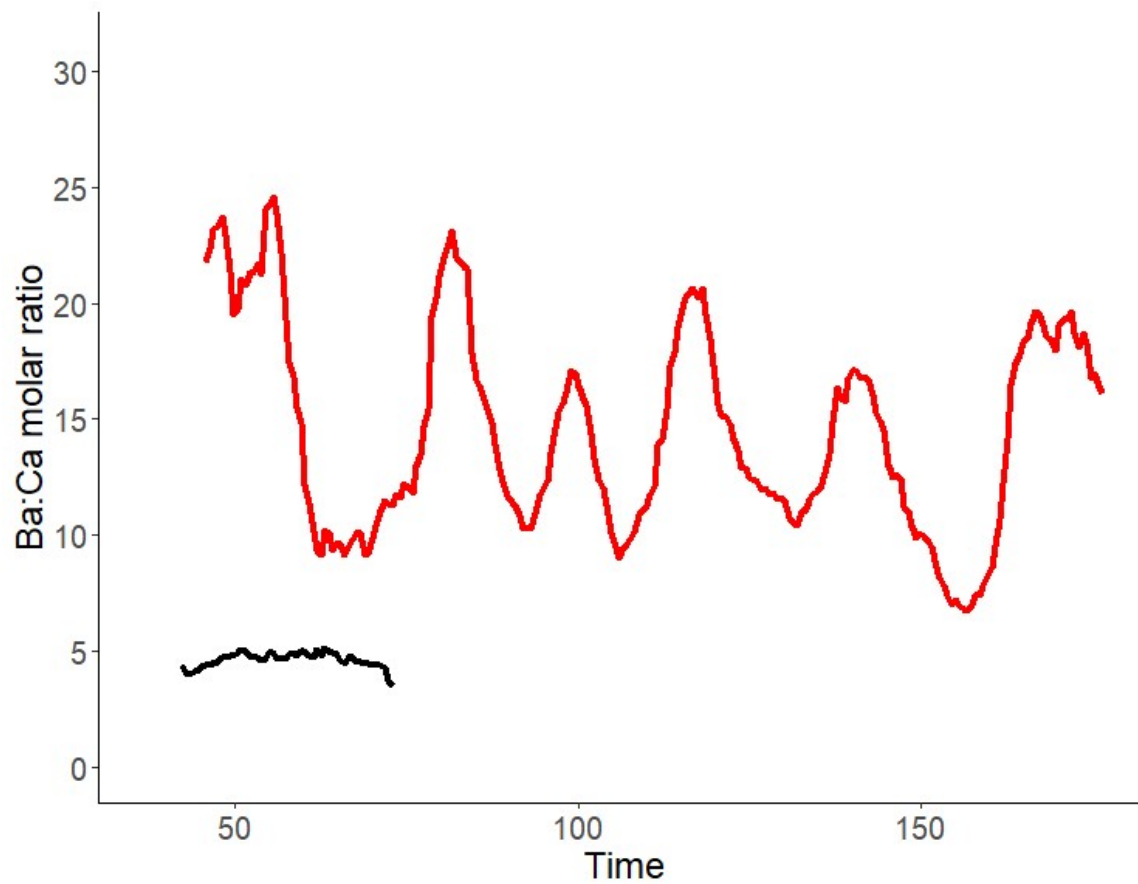


Figure 7. Barium-to-calcium (Ba:Ca) molar ratio (mmol/mol) for Silver Carp lapillus otoliths collected from a juvenile collected in the Big Sioux River in June 2020 (Red) and an age-0 from the James River in October 2022 (black).

Recommendation: None

State Report: US Fish and Wildlife Service

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

Project Title: Missouri River Tributary Invasive Carp Population Assessment

Methods:

Data Collection

The Columbia and Great Plains FWCO implemented an updated sampling protocol in 2023 to collect invasive carp demographics in tributaries throughout the sub-basin. Data collected from 2020-2023 was analyzed for spatial and temporal patterns to inform protocol updates (Appendix A). In 2023, Columbia FWCO sampled eight tributaries along the lower Missouri River with an electrified dozer trawl (Hammen et al. 2019; Drews 2020). The tributaries sampled were the James River, Vermillion River, Big Sioux River, Nishnabotna River, Kansas River, Grand River, Lamine River, and a small tributary category (Perche Creek and Crooked River). The small tributary category combines two randomly selected tributaries with limited sampleable habitat as one tributary, selected from Perche Creek and the Platte, Crooked, and Little Chariton rivers in Missouri.

Sampling occurred in late August through October 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). Crews used an electrified dozer trawl to sample the lower 10 river kilometers (5 river kilometers for each small tributary) of each tributary confluence. Transects were completed along the contour of the shoreline of either the right or left descending bank (randomly assigned as depth allowed) and starting from the lower most kilometer working upstream with one transect per river kilometer. Target effort was 10 five-minute electrified dozer trawl transects at 4.8 kph (3 mph) to be repeated across three sample events throughout the fall. Each sampling event was a minimum of two weeks apart.

All fish sampled were identified to species, enumerated, and then measured for total length (TL; mm) and weight (g). All individuals less than 200 mm TL were not weighed due to scale sensitivity. Sex of Silver Carp was determined by roughness of pectoral fin rays (Wolf et al. 2018) and verified by visual observation of gonads. Lapilli otoliths were extracted from the first 100 Bighead and Silver Carp sampled per tributary. For the small tributary category, the lapilli otoliths were extracted from the first 50 otoliths from each individual tributary to reach a total of 100. After the first 100 otoliths, additional otoliths were collected to fill length bin requirements (20 Bighead or Silver Carp per 50 mm length bin) for each tributary. Silver Carp that jumped into the boat during standard sampling (herein referred to as volunteers) were not used in calculating relative abundances but were used in length-frequencies and growth models.

Otoliths were stored in coin envelopes during collection then transferred to the Columbia Fish and Wildlife Conservation Office laboratory. Otoliths dried for at least two weeks 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.

Data Analysis

Catch per unit effort (CPUE; fish per hour) was used to measure relative abundances of stock size (250mm; Phelps and Willis 2013) and larger Silver Carp for each tributary. Relative standard error (RSE) of CPUE was calculated to estimate precision of catches, with a target RSE of ≤ 25 (Dumont and Schlechte 2004). Because of multiple sampling events, the average CPUE was calculated for each river kilometer which was then used to calculate overall relative abundance and RSE in each tributary. Body condition and proportion of sex were calculated for all stock-sized Silver Carp. Sex ratio was calculated for each transect with a minimum of three Silver Carp and then transects were averaged according to tributary. Relative weight was calculated using the observed weight and the standard weight equation specific to Silver Carp (Lamer 2015). The relative weight equation was developed using a 50th percentile approach (Wege and Anderson 1978; Lamer 2015), which defines a Silver Carp in average condition as having a relative weight of 100.

Growth parameters were estimated per tributary using a von Bertalanffy growth model (von Bertalanffy 1983). The growth models provide: L_{inf} – the theoretical average maximum total length, K – Brody's growth coefficient, and t_0 – the theoretical time at which a fish's length is zero. Due to missing lower year classes, t_0 was set to zero to produce parameters that are within reason based off other growth models in different basins (MICRA 2022), acknowledging this can affect L_{inf} and K . Instantaneous mortality (M_{est}) was calculated using the updated Pauly (1980) empirical mortality estimator described in Then et al. (2015) as:

$$M_{est} = 4.1189K^{0.73}L_{inf}^{-0.333}$$

The von Bertalanffy parameters, L_{inf} (cm) and K , are used to calculate mortality. To have comparable information, only tributaries outlined in the updated protocols were used for demographic comparisons across years. All analyses were done in R Studio (R Core Team 2022).

Results & Discussion

In 2023, 214 electrified dozer trawl transects were conducted across 8 different waterbodies, collecting a total of 8,267 fish (Table 1). Efforts collected 34 unique species with the most prevalent being Silver Carp, Gizzard Shad, and Emerald Shiner (Appendix B). A total of 3,398 Silver Carp (41% of total catch) were sampled across all tributaries, with an additional 47 Silver Carp being collected in nonstandard sampling to increase aging structure numbers. All tributaries met the target of sampling 10 river kilometers per sampling event except for the Nishnabotna (8 transects), Big Sioux (7-9 transects), Vermillion (5-9 transects), and James (7-9 transects) rivers due to water levels or barriers (Table 1). A total of 29 Bighead Carp were collected across three waterbodies: small tributary category ($N = 25$), Grand River ($N = 2$), and Nishnabotna River ($N = 2$; Table 2). Bighead Carp total lengths ranged from 360 to 765mm from ages 4 to 11 (Table 2). Given the paucity of Bighead Carp captures, no further analyses were conducted for this species.

Silver Carp CPUE was variable across tributaries with the highest average CPUE in the Vermillion River and the lowest average CPUE in the Grand River (Table 1; Figure 1). Observationally, the Big Sioux, Vermillion, and James rivers had high relative abundances compared to other tributaries (Figure 1). In 2023, the average RSE values was below 25 for all tributaries except the James River (Table 1). Annual mean CPUE varied across years for all tributaries (Figure 2).

Total length of Silver Carp ranged from 353 to 859mm with an average length of 586 ± 1 mm (Table 1). No sub-stock sized (<250 mm TL) Silver Carp were collected in any of the tributaries. Size structure varied across tributaries, but more than half of all Silver Carp sampled (57%) were between 550 mm and 659 mm in total length (Figure 3). The small tributary category had the highest proportion (51%) of smaller sized Silver Carp, 450-499 mm, comparatively across tributaries (Figure 3).

Condition of Silver Carp was near or slightly below average for all tributaries in 2023 (Figure 4). In previous years, average relative weight has ranged from 90 to 110 with the current sample year values falling within in this range. The average proportion of male Silver Carp varied, 41-73%, among tributaries in 2023 (Figure 4). All tributaries were within 5% of expected 50% male female ration except for the Big Sioux, Kansas, and Lamine rivers. The Big Sioux and Kansas rivers exceeded this ratio by greater than 10% higher with values of 74% and 63%, respectively. The Lamine River was 9% lower than the expected 50% male average. In prior years, average proportion male ranged from 32 to 69%.

The 2023 age data showed that Silver Carp age ranged from 3 to 12 in the first 100 Silver Carp sampled with weak and strong cohorts present in all tributaries. There was variability in the age proportions across tributaries but the 2019-year class, age 4, are the most prevalent throughout the system (Figure 5). Additionally, an age 7-8 (2015-2016) year class is prevalent in tributaries higher in gradient on the Missouri River (Figure 5). No recruitment has been observed in any of the tributaries since the 2019-year class. von Bertalanffy growth models were calculated for all tributaries in 2023 and the average theoretical maximum length was similar across all tributaries ranging from 615 to 686 mm (Table 3; Figure 6). The Brody growth coefficient, K, ranged from 0.38 to 0.58 across tributaries (Table 3). The mortality estimates of tributaries in 2023 ranged from 0.52 to 0.69 whereas in 2022, tributary mortality estimates ranged from 0.10 to 0.31. Growth parameters were estimated using all years of age data, instead of just the collection year, which along with different empirical estimators could explain the changes in mortality values.

To further inform and expand invasive carp management in the Missouri River, quality data is required so stakeholders can make informed decisions and quantify management effects over time. Through a data informed process, the updated sampling protocol in 2023 provided higher resolution on invasive carp populations in the tributaries than previous years. Multiple sampling events in tributaries doubled the number of Silver Carp collected when compared to the previous year in similar hydrological conditions. Even though the target number of transects was not met in every waterbody due to water depth or barriers, increased efforts produced an increase in quality demographic data that is useful for monitoring relative abundance, growth, and mortality in all tributaries.

The overall relative abundance as well as the variability in catch-per-unit-effort across transects was highest in the Big Sioux, Vermillion, and the James rivers. Due to the behavioral responses of Silver Carp,

high variability in catch is expected which is why the electrified dozer trawl transect time is standardized to a consecutive five minutes to mitigate an over or under inflation of relative abundance. This sample year provided a total of 214 transects across all tributaries, 34 of which had reduced effort times due to barriers, gear failure, or other factors. The Big Sioux, Vermillion, and the James rivers accounted for 28 of the 34 transects that did not meet the target five minutes which meant 35%, 49%, and 38% of the total transects in each tributary did not meet the standard, respectively. The invasive carp density in these tributaries is undoubtedly high, but the relative abundance values could be inflated.

Furthermore, throughout the basin no sub-stock sized Silver Carp were collected in 2023, indicating no detectable recruitment has occurred from the prior sampling efforts. In the fall of 2022, juvenile Silver Carp were captured in four tributaries (Crooked, Big Sioux, Vermillion, and the James rivers) but failed to be collected in 2023 sampling indicating they did not recruit to the population. Tributaries in the Missouri River basin have a variety of age classes, but the 2019 cohort is annually the strongest year class present.

Sampling efforts have provided a thorough understanding of Silver Carp demographics and relative abundance in tributaries throughout the basin but the importance of Silver Carp residing in the mainstem are not fully understood. Commercial harvest programs are being initiated in the mainstem river, but the knowledge of the invasive carp population is limited. In previous years, limited mainstem sampling was piloted with an electrified dozer trawl in select areas with variable results. Further efforts and methods of sampling are crucial to fully understand the overall invasive carp population that resides in the mainstem river and their connection to these tributary habitats.

Table 1. Summary of 2023 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 ‘Small Tributary’ waterbody is a combination of Perche Creek (RM 170) and Crooked River (RM 314). The total number of all fish and Silver Carp sampled are listed. Also listed are the maximum number of transects and total amount of trawls completed in each tributary over three sampling events. An asterisk indicates that the maximum transect was not met in all sampling events. Average catch per unit effort (CPUE) of stock sized SVCP is reported with standard error in parentheses, as is average relative standard error (RSE) of SVCP CPUE for all tributaries. The average, minimum and maximum total length (TL) 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)	Total Trawls (N)	All Fish (N)	SVCP (N)	CPUE (SVCP/hr)	CPUE RSE	Mean TL (mm)	Min -Max TL (mm)
Small Tributary	170 & 314	-	10	30	1,662	396	160 (26)	16	483	390 - 843
Lamine River	202	6,880	10	30	431	266	106 (13)	13	562	484 - 683
Grand River	250	20,429	10	30	307	151	64 (8)	13	562	430 - 837
Kansas River	367	288,343	10	30	835	373	153 (21)	14	628	496 - 775
Nishnabotna River	542	7,730	8	24	946	462	244 (58)	24	597	435 - 859
Big Sioux River	734	19,257	9*	23	862	620	502(117)	23	627	353 - 760
Vermillion River	772	6,784	9*	23	1,193	626	624 (151)	24	583	440 - 781
James River	800	48,092	9*	24	2,031	504	561 (234)	42	599	425 - 817
Summary				214	8,267	3,398	269(36)	13	586 (1)	353 - 859

Table 2. Bighead Carp collections for 2023 electrified dozer trawl sampling. Each row represents a single fish.

Date	Waterbody	Species	Length (mm)	Weight (g)	Sex	Age
8/31/2023	Crooked River	Bighead Carp	725	3,490	Female	8
8/31/2023	Crooked River	Bighead Carp	520	1,520	Female	5
8/31/2023	Crooked River	Bighead Carp	550	1,830	Female	5
8/31/2023	Crooked River	Bighead Carp	414	730	Female	5
8/31/2023	Crooked River	Bighead Carp	397	580	Female	5
8/31/2023	Crooked River	Bighead Carp	390	520	Female	5
8/31/2023	Crooked River	Bighead Carp	545	1,680	Female	5
8/31/2023	Crooked River	Bighead Carp	415	730	Female	4
8/31/2023	Crooked River	Bighead Carp	385	550	Female	4
8/31/2023	Crooked River	Bighead Carp	415	690	Female	4
8/31/2023	Crooked River	Bighead Carp	404	670	Female	4
8/31/2023	Crooked River	Bighead Carp	497	1,180	Female	4
8/31/2023	Crooked River	Bighead Carp	495	1,270	Female	4
9/25/2023	Crooked River	Bighead Carp	434	790	Male	5
9/25/2023	Crooked River	Bighead Carp	500	1,300	Male	4
9/25/2023	Crooked River	Bighead Carp	413	680	Male	4
9/25/2023	Crooked River	Bighead Carp	391	610	Male	4
9/25/2023	Crooked River	Bighead Carp	360	480	Male	4
10/10/2023	Crooked River	Bighead Carp	472	1,007	Female	4
10/3/2023	Grand River	Bighead Carp	460	1,050	Female	4
10/3/2023	Grand River	Bighead Carp	450	990	Female	4
9/6/2023	Nishnabotna River	Bighead Carp	765	3,980	Male	11
9/26/2023	Nishnabotna River	Bighead Carp	463	1,120	Male	7
8/30/2023	Perche Creek	Bighead Carp	485	1,190	Female	6
8/30/2023	Perche Creek	Bighead Carp	460	1,040	Female	5
8/30/2023	Perche Creek	Bighead Carp	507	1,390	Female	4
8/30/2023	Perche Creek	Bighead Carp	478	1,200	Female	4
8/30/2023	Perche Creek	Bighead Carp	495	1,250	Female	4
9/21/2023	Perche Creek	Bighead Carp	560	1,990	Female	5

Table 3. Growth estimation parameters (L_{inf} and K) from von Bertalanffy growth models and instantaneous annual mortality (M_{est}) estimates for each waterbody. All Silver Carp were collected 2020-2023 and the total number of individuals aged per tributary to estimate growth and mortality are listed under SVCP (N). The Small Tributary includes all four tributaries: Perche Creek, Crooked River, Little Chariton River, and the Platte River (MO).

Waterbody	SVCP (N)	L_{inf}	K	M_{est}
Small Tributary	640	627	0.38	0.52
Lamine Rver	321	615	0.50	0.63
Grand River	258	622	0.49	0.61
Kansas River	331	642	0.58	0.69
Nishnabotna River	313	686	0.41	0.52
Big Sioux River	330	661	0.43	0.55
Vermillion River	347	616	0.48	0.61
James River	309	640	0.48	0.60

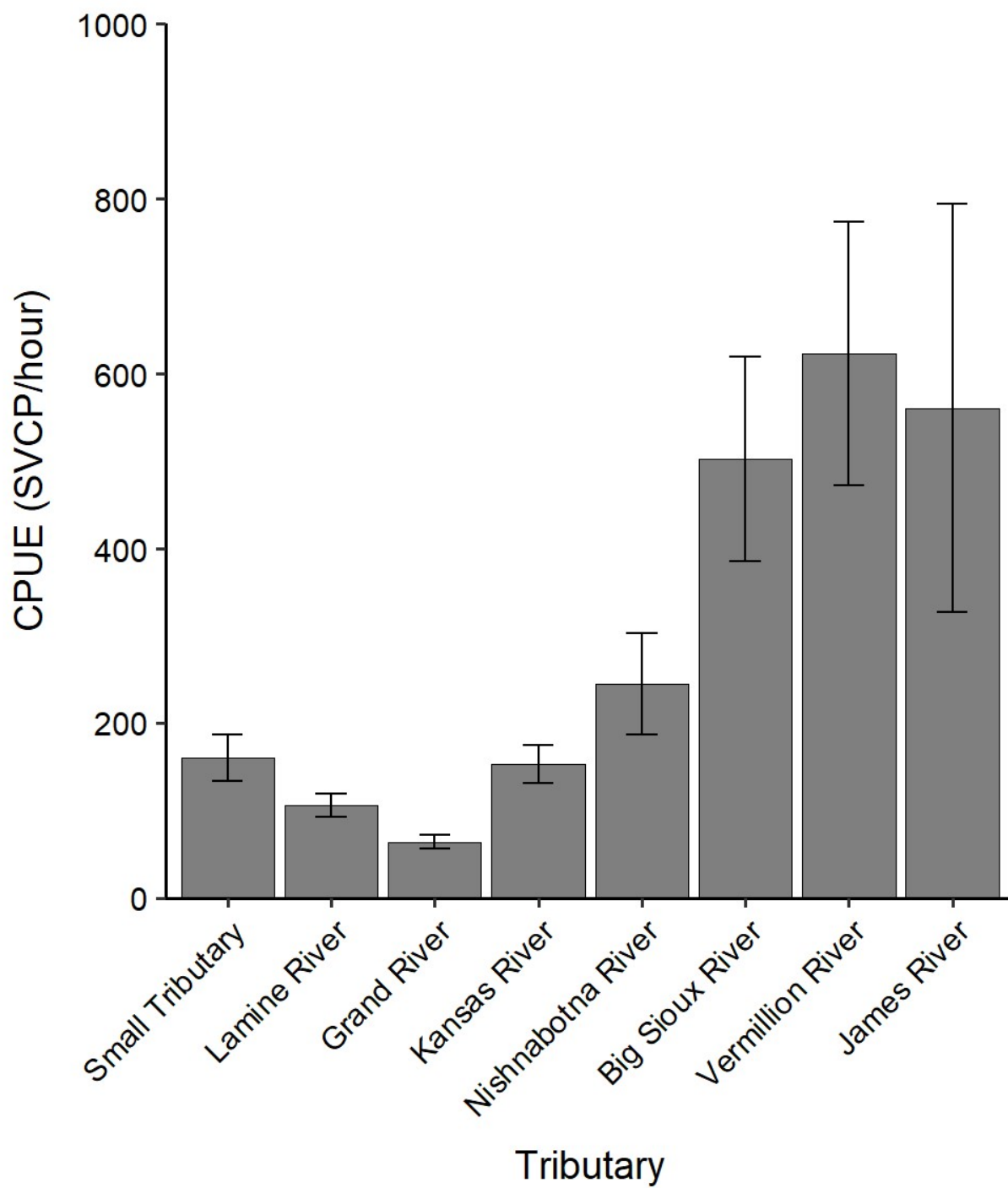


Figure 1. Mean catch per unit effort (CPUE; SVC/hour) with standard errors of Silver Carp sampled in each tributary with an electrified dozer trawl in 2023.

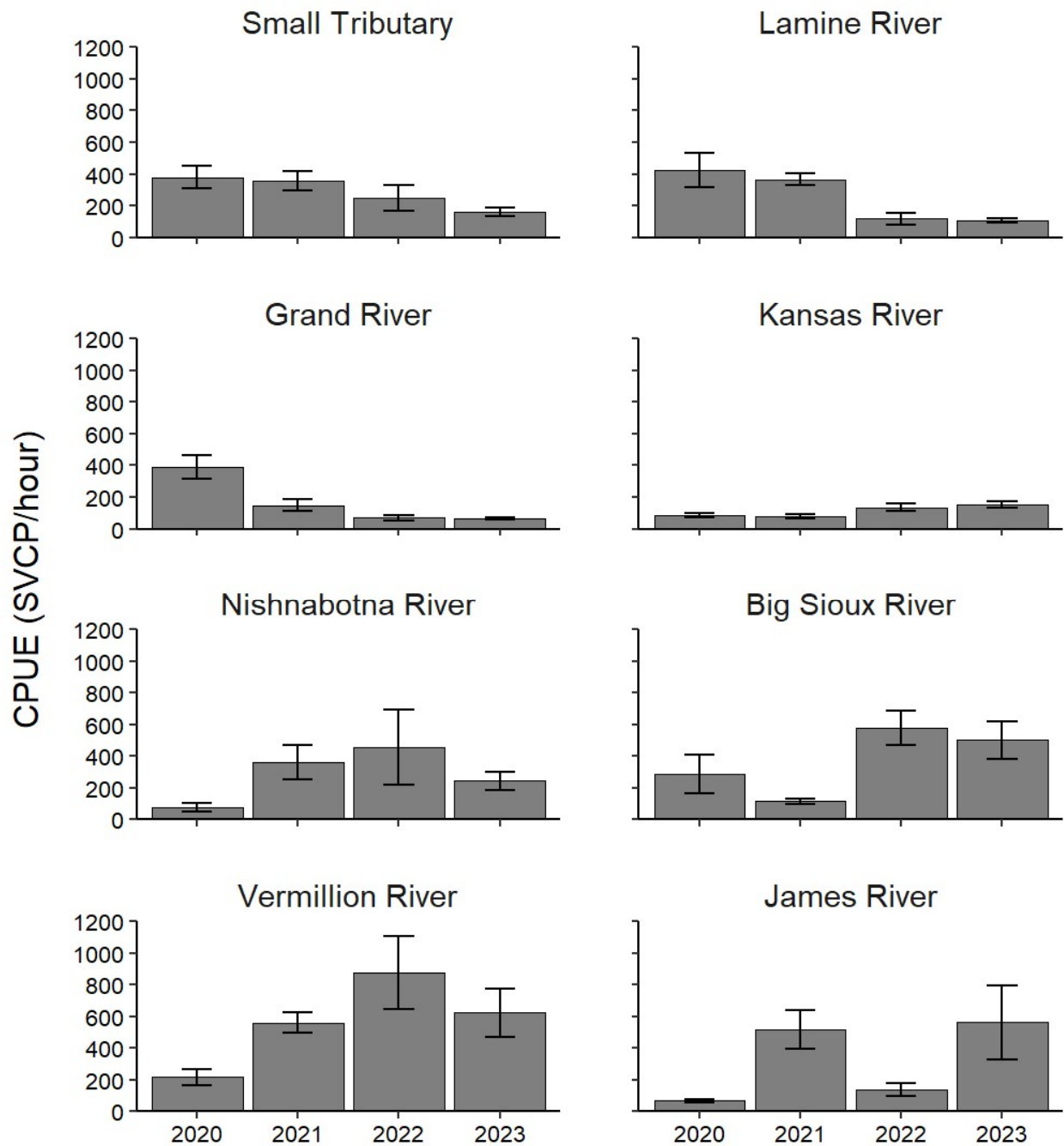


Figure 2. Mean catch per unit effort (CPUE; SVCP/hour) with standard errors of Silver Carp sampled with an electrified dozer trawl in each tributary by year, 2020 - 2023.

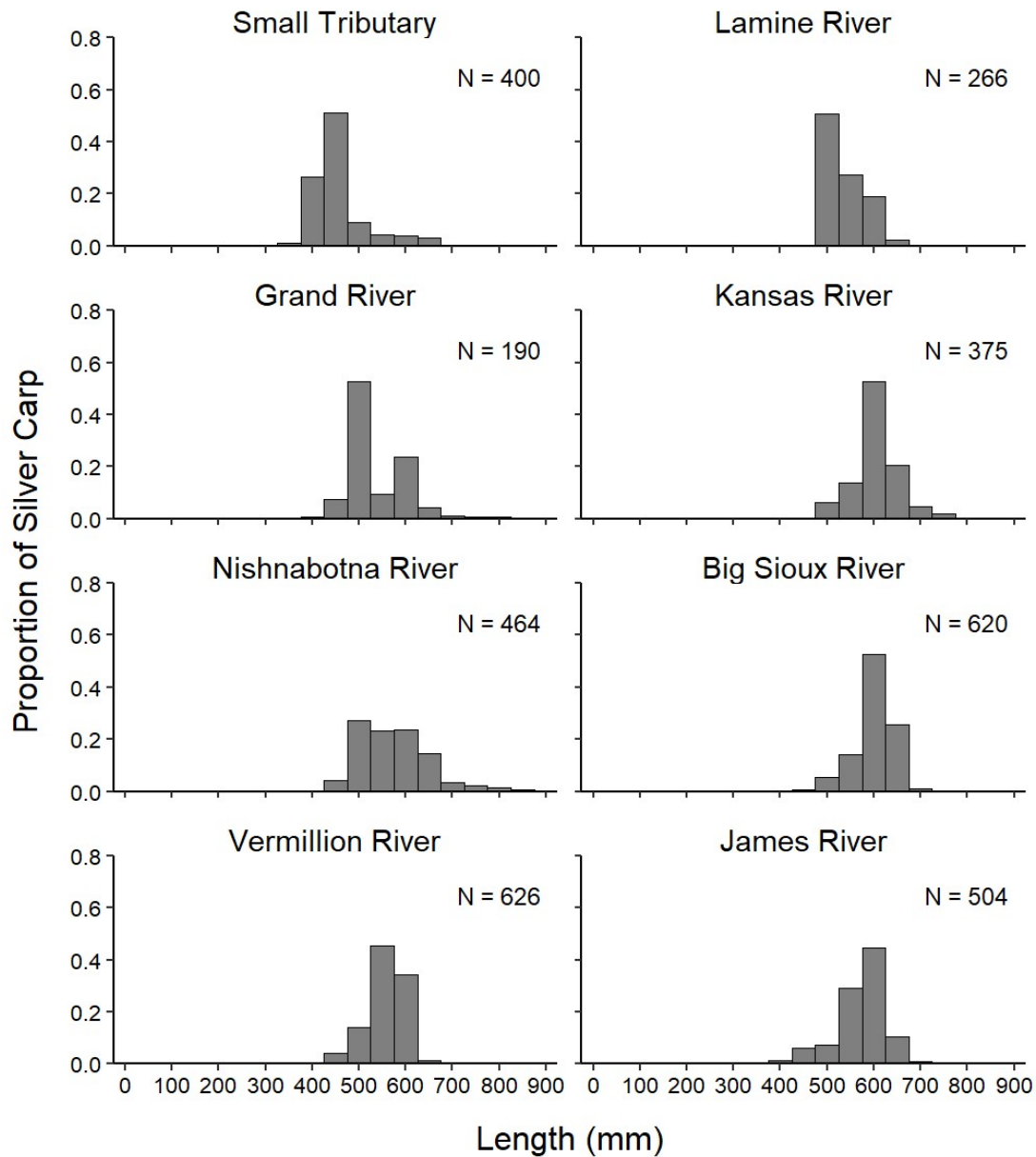


Figure 3. Relative length frequency distribution by 50 mm length bin of Silver Carp captured with an electrified dozer trawl in 2023 in each Missouri River tributary. The number (N) of Silver Carp includes all lengths collected during standard protocols, SVCP volunteers, and extra samples to collect age data so the totals may not match the totals listed in Table 1 that were used to calculate CPUE.

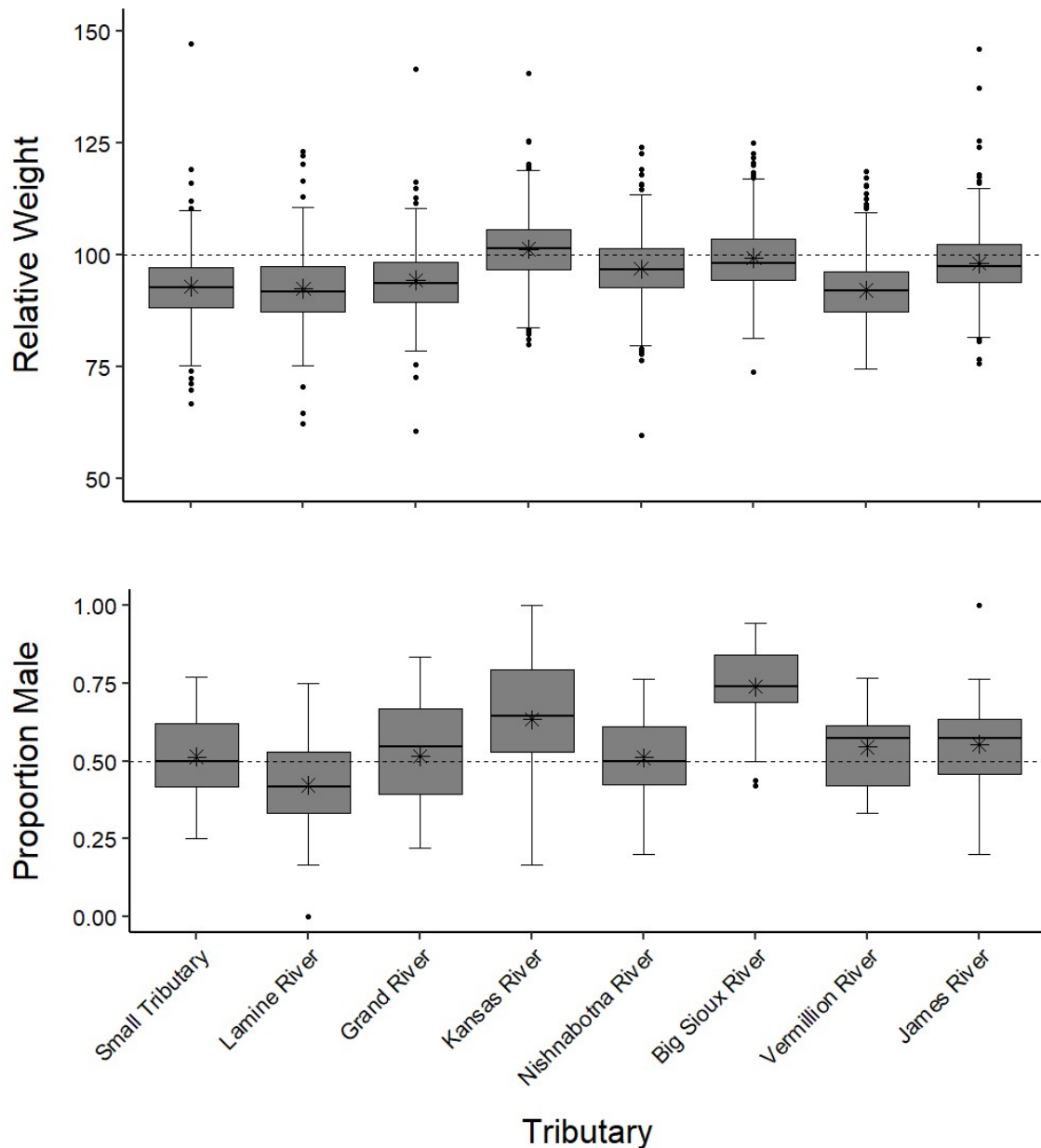


Figure 4. Mean relative weight (top graph) and proportion male (bottom graph) of Silver Carp sampled in 2023 by tributary. A value of 100 is the average body condition of Silver Carp and represented by a dashed line (top graph). Any samples that had less than four Silver Carp were excluded from the data when calculating proportion male. Boxplot interpretation: asterisk represents the mean, the solid black line represents the median, the upper and lower limits of the box represent the first and third quartile, the upper and lower whiskers represent the largest and smallest value within range (1.5x) of the quartiles, and the black dots represent outside values.

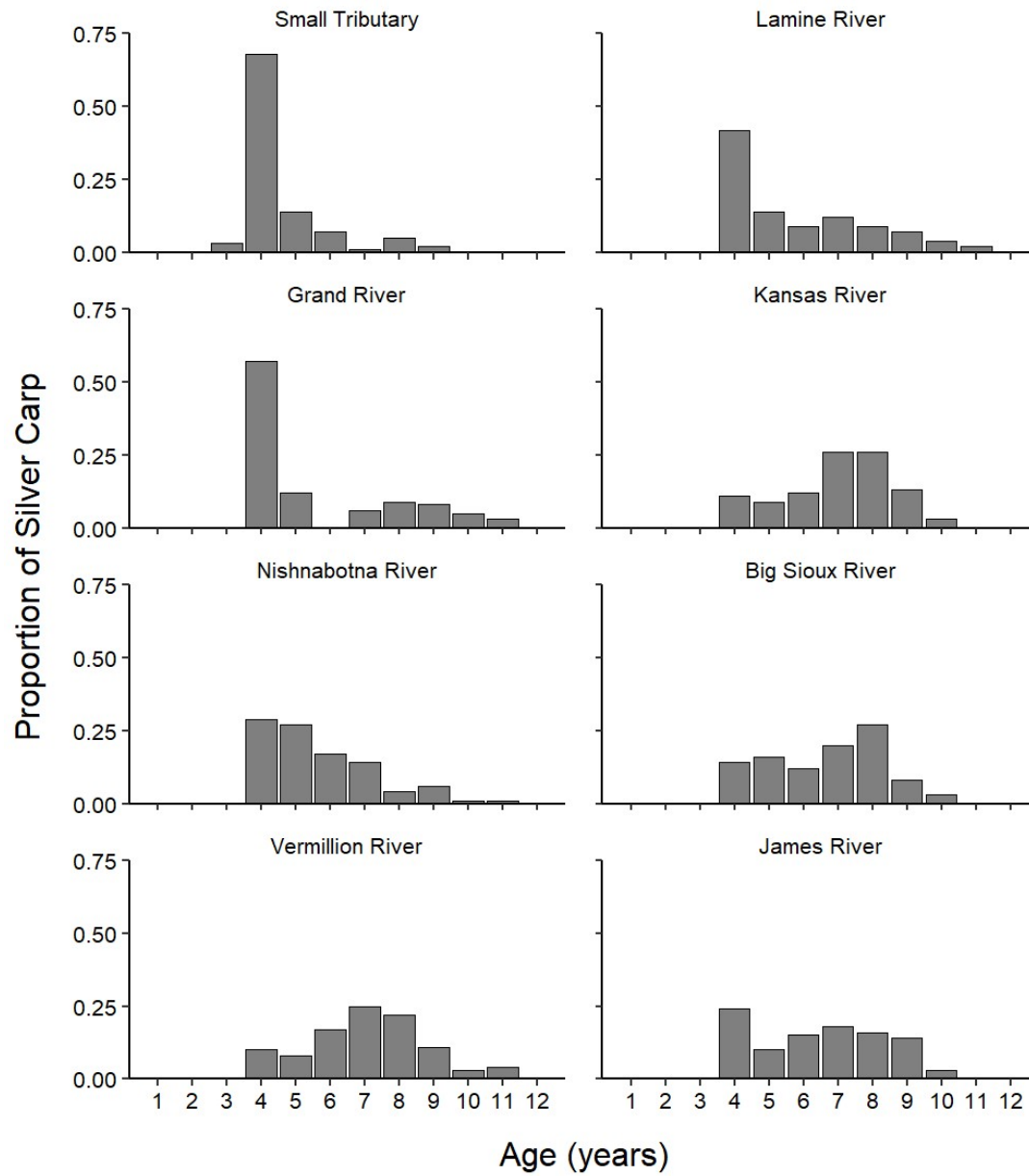


Figure 5. Relative age frequency of Silver Carp captured with an electrified dozer trawl in 2023 in each tributary. Each tributary had the first 100 Silver Carp otoliths extracted and aged. Otoliths were extracted from any length bin not filled by the first 100 (20 Silver Carp per 50 mm increments) and used for von Bertalanffy growth estimates (Figure 6).

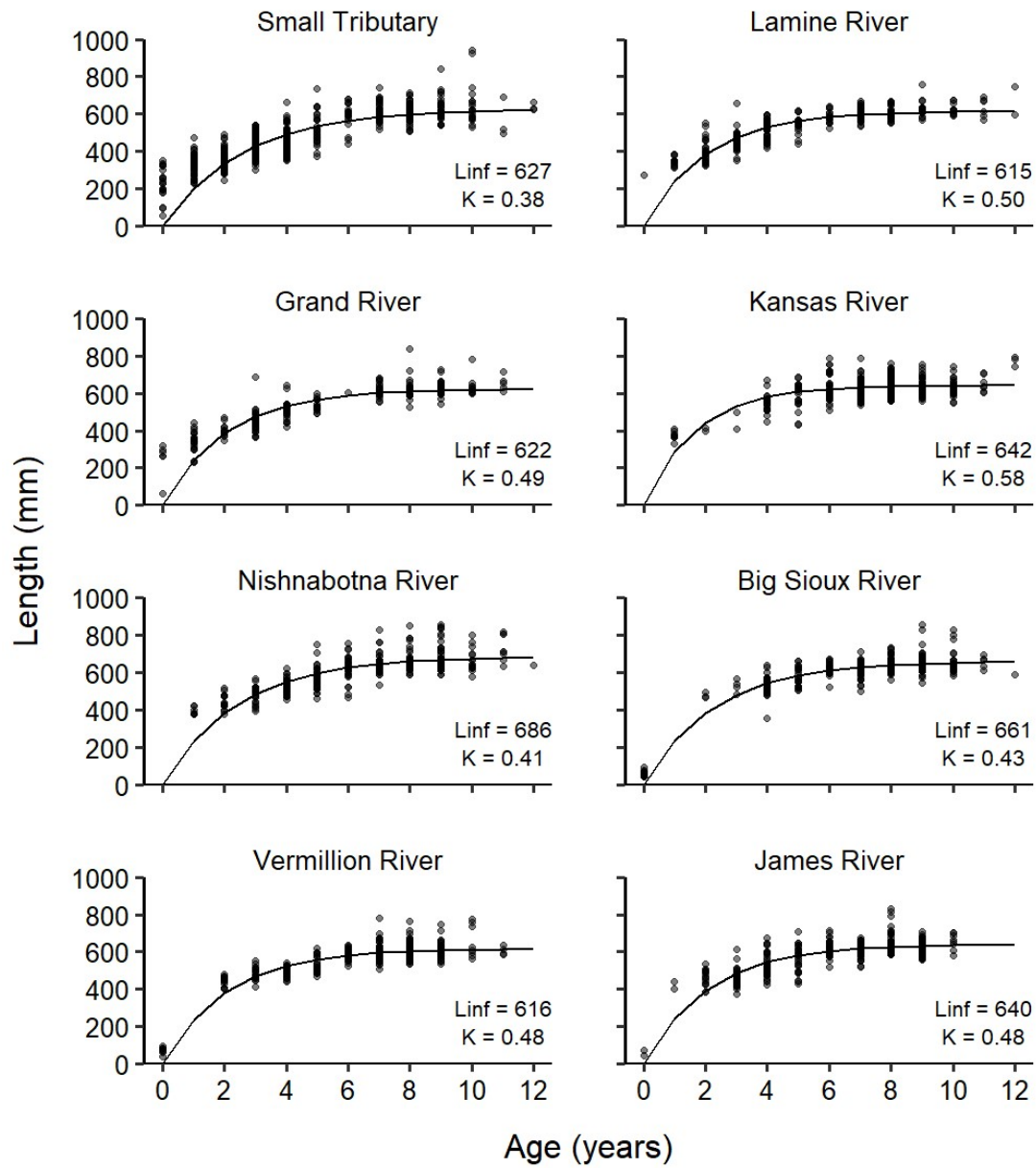


Figure 6. Length at age with for all Silver Carp collected by electrified dozer trawl from 2020-2023, as represented by dots. For all von Bertalanffy growth curves, t_0 was set to zero. L_{inf} is the theoretical average maximum length and K is Brody's growth coefficient.

Recommendation:

- 1.** Continue to collect invasive carp data and evaluate protocols in tributaries to further inform demographic rates to help evaluate management efforts.
- 2.** Explore the feasibility of sampling the mainstem river to supplement invasive carp demographic information and identify high abundance areas.
- 3.** Continue to monitor relative abundances of invasive carp pre and post removals as management actions continue to be implemented.

Appendix A.

Tributary Sampling Protocol Updates

Introduction

In 2020, the Columbia Fish and Wildlife Conservation Office (FWCO) designed and implemented a multi-year assessment to quantify relative abundance, recruitment, growth, and mortality of Bighead and Silver carp in tributary confluences throughout the lower portion of the Missouri River. To develop initial population assessment protocols, Columbia FWCO staff incorporated existing knowledge from other Mississippi River sub-basins as well as regional, state, and local knowledge of the Missouri River Basin.

Thirty Missouri River tributary confluences (defined as the lower 20 river km; Thornbrugh and Gido 2010) were identified as sampleable with an electrified dozer trawl based off watershed size and navigability (>1,000 km²; Flotemersch et al. 2006). Target effort was 20 five-minute electrified dozer trawl transects (one dozer trawl transect per one kilometer segment to allow a buffer between samples) conducted at 4.8 kph (3.0 mph) along the contour of the shoreline from the downstream-most site in an upstream fashion per tributary. Fall (September – October) sampling was selected to meet recommended water temperatures for consistent catch of Silver Carp and to allow annual annuli formation on otoliths (Sullivan et al. 2017; Thompson and Beckman 1995). The target metrics for each tributary confluence were to collect 125 stock size or larger invasive carp, 100 otoliths, and to have a catch per unit effort (CPUE) relative standard error (RSE) of 25 or less (Phelps and Willis 2013; Dumont and Schlechte 2004).

After three years (2020 – 2022) of data collection in low water conditions, many of the sampling metrics were unable to be met for individual tributaries. To adapt protocols to better fit management needs and provide reliable trend data across a wide range of hydrological conditions, an evaluation of data collections was undertaken. Data was analyzed for: (1) spatial trends within and across tributaries; (2) temporal trends within tributaries and across years; (3) trends between mainstem and tributary confluences; (4) how often target metrics were met for each tributary. The overall objective of this effort was to refine standard sampling protocols based on prior year information to allow for consistent data collection across the Missouri River Basin across a wide range of hydrological conditions and ensure maximum value to partner management efforts.

Methods

To understand spatial trends within a tributary for each year, patterns were explored in relative abundance, relative weight, and sex ratios along the 20 km gradient. Linear regressions were used to test the relationship of the variable of interest plotted against river kilometer, and the associated coefficient of determination (R^2) was used to describe variability explained by that relationship. Similarly, linear regressions were also used to observe longitudinal trends in relative abundance, relative weight, and sex ratio for the tributaries across the basin using Missouri River river mile as well as drainage size. Length-frequency histograms were used to visually compare size structures across tributaries within each year. A clustered Kolmogorov-Smirnov tests (KS test) with an alpha value set at

0.05 for significance was utilized to statistically compare size structures between tributaries. All analyses were conducted using R Studio (R Core Team 2022).

Patterns in relative abundance (CPUE), relative weight, and size structure were compared across years within each tributary and across tributaries to look for relationships using both linear regressions and length-frequency histograms across time. In 2020, there was data collected twice in the fall (once in September and once in October) for two tributaries using standardized protocols, which allowed for limited intra-seasonal variability in CPUE and size structure to be explored using linear regressions and a clustered KS test, respectively.

To compare mainstem Missouri River fish collections to paired tributary confluences, three data sources were used. Limited mainstem (within 5 km of tributary mouth) and paired tributary confluence data were collected in pilot studies by Columbia FWCO in 2020 (two sites) and 2022 (four sites) using the electrified dozer trawl. In addition, Missouri Department of Conservation (MDC) annually (2020 – 2022) sampled select tributaries and the associated mainstem river bend using standard electrofishing and gill nets. MDC found no difference in CPUE of Silver Carp based on habitat type (mainstem vs. tributary) or electrofishing settings, with one exception (MICRA 2021). Based on those findings, data were grouped from different electrofishing settings to explore differences in CPUE and size structure between habitat types. To compare CPUE of Silver Carp from the mainstem and associated tributary, linear regressions and box plots were used. All available data were used to compare the size structures of Silver Carp sampled between mainstem and the tributary across years using length-frequency histograms.

Data collection metrics were evaluated to assess whether the metrics were still valid for this population assessment and how many samples would be needed to meet standard metrics. Through literature review and past data collection throughout the basins, our goal was to collect 125 stock and larger sized invasive carp, 100 random otoliths to estimate growth and mortality, and achieve an average CPUE RSE of 25 or less in 80% in samples for each tributary.

Results

Spatially there was no patterns when assessing CPUE, relative weight or sex ratio within in each tributary. Variability of CPUE was present in every tributary within the sampleable habitat. Longitudinally across the basin, there was no consistent trends among the tributaries in relation to CPUE or sex ratio. The linear regressions did indicate that northern latitude tributaries (Nishnabotna, James, Vermillion, and Big Sioux rivers) tended to a higher relative weight and larger sized individuals, which is consistent when comparing length-frequencies. Size structure was significantly different when comparing between tributaries depending on the tributary being compared. Significance of size structure comparisons were variable but overall results indicate larger and smaller sized drainage areas could be driving the significant comparisons. Linear regressions and length-frequencies also indicate that tributaries with smaller or larger drainage areas when in comparison typically have an increased number of smaller and larger Silver Carp, respectively.

When comparing across the three years there were no consistent trends in CPUE within each tributary. Relative weight can be variable among individuals, but the range of values remained constant throughout time. No strong relationships were found across the tributaries when comparing average CPUE within the basin for each year. Average length tended to increase through time due to an aging cohort. When comparing size structures of the two tributaries sampled twice in one year, there was a

significant difference in only one of the tributaries. Relative abundance was variable depending on transect but the overall relationship was similar between the tributaries for the two sample events. Additionally, Silver Carp CPUE in tributaries tended to be higher when compared to the associated mainstem for electrofishing but can be variable depending on location and year. Similarly, large Silver Carp (>600 mm) are captured in higher frequencies in the mainstem river, but the overall size structure of the population does not differ between the mainstem and tributary for both electrofishing and dozer trawling.

From 2020 – 2022, 43-53% of the 30 selected tributaries were successfully sampled in any given year with the electrified dozer trawl. Tributaries which could be sampled with the electrified dozer trawl often did not have the required depth to reach a target of 20 transects. The Kansas River was the only tributary in which 20 transects could be completed annually except for 2022 when only 12 km could be sampled. Other target metrics (125 stock or larger invasive carp, 100 otoliths, $RSE \leq 25$) were met 31-57% of the time depending on sampling year. In 2020 and 2021, the goal of collecting 100 otoliths was not met in any tributary because otoliths were targeted per length bins whereas in 2022, the first 100 individuals had otoliths extracted. Based on prior years data, the number of transects required to meet a $RSE \leq 25$ in 80% of sample years ranged from 7 to 33, with over half requiring above 15 samples. The number of samples needed to reach a target of 125 stock or larger individuals needs also ranged from 4 to 17 samples.

Discussion

Historically low discharges impeded the ability to consistently sample all targeted Missouri River tributaries in 2020 – 2022, leading to a low success rate of meeting CPUE RSE, length, and age structure target goals for each tributary sampled. Climate change models predict an increased frequency of 100-year droughts in the Midwest (Zhao et al. 2020) and an increase in major flood frequencies in temperate areas (Slater et al. 2021); therefore, standardized sampling approaches should accommodate a wide range of discharges and water levels. By using three years of low water conditions to refine protocols, the new proposed locations and methods should be able to accommodate higher water discharges except in extreme flooding where conditions are hazardous for navigation and would prevent any sampling.

Spatially, there were no trends in CPUE or size structure within a tributary, indicating that the lower 10 km of a tributary will produce similar data as the lower 20 km. Targeting the lower 10 km allows sampling to occur in depths more suitable to the dozer trawl in low water conditions and allows crew to be selective in where to safely sample during high water events. Similarly, there were no patterns in CPUE or size structure within a tributary across years, though there was high variability. Silver Carp catch rates are expected to be highly variable due to schooling and swimming behaviors that contribute to an ability to evade gear capture (Rakowitz et al. 2012). Because of high variability in CPUE, multiple lines of evidence, such as length and relative weight, should be used to support interpretations of data.

Tributaries farther upstream in the Missouri River Basin, typically above Kansas River (RM 367), tended to have higher frequencies of larger (> 600 mm) Silver Carp with larger average relative weights. Similarly, a limited number of smaller tributaries tended to hold higher numbers of small (< 450 mm) Silver Carp. These small tributaries were characterized by drainage size as well as shallow depths or narrow wetted widths that limited dozer trawl effectiveness to fewer than 10 river km of sampleable habitat. This demonstrated the need to ensure geographical coverage throughout the basin. Limited

data showed that repeat sample events within a season could produce statistically different catch rate or size structure estimates. Also, data showed there could be higher frequencies of larger fish collected in mainstem Missouri River habitats compared to tributary confluences. These results were considered carefully along with target metrics to refine sampling protocols.

Based on results, it was clear that several factors were important to sampling design, including location selection, timing of sampling, and ensuring there is enough data to accurately represent Silver Carp populations. Tributaries where 10 km was possible to sample with a dozer trawl were selected to ensure consistent effort, but a couple exceptions (such as the James River, where only 9 km are sampleable downstream of a passage barrier) were included to ensure geographic representation across the basin. For the small tributary habitat, two small tributaries will be randomly selected each year from a list of four where at least 5 river km were available to be sampled, and data will be combined for reporting purposes. The list of small tributaries was selected based off a combination of sampleability in low water conditions, wetted width, and drainage size. Repeat sampling in the fall will allow length frequency and age structure collection targets to be met based off sample size estimators as well as incorporate intra-seasonal variation in the data. The number of transects needed to meet the target RSE was often higher than the available sampleable habitat in most tributaries.

Furthermore, previous years of sampling provided a comprehensive baseline of achievable outcomes when sampling tributaries of the Missouri River. Using that data, the information provided was analyzed and used to determine how the data could be used to benefit the partners in the basin. Through this process it was found that focusing the number of tributaries sampled with increased sampling events will allow the sub-basin partnership to have a better understanding of the invasive carp population in the tributaries throughout the basin and help inform potential control areas. Based on this work, additional efforts should be focused on understanding how varying hydrological conditions affect catchability and data collections, whether incorporating mainstem Missouri River habitats is essential to understanding the basin population, and how these data could be combined with other data sources such as telemetry or genetics to best inform management of Silver Carp in the Missouri River Basin.

Revised Protocols

1. Seven tributaries and one small tributary category were selected to allow for consistent sampling across hydrological conditions, provide geographical coverage of the Missouri River Basin, and tend to be areas where management is likely to occur:
 1. James River
 2. Vermillion River
 3. Big Sioux River
 4. Nishnabotna River
 5. Kansas River
 6. Grand River
 7. Lamine River
 8. Small tributary category (two small tributaries randomly selected from Crooked River, Little Chariton River, Perche Creek and Platte River in Missouri)
2. Target of two or three sampling events in each tributary to increase sample size and incorporate intra-seasonal variability within the fall.

1. Target of 10 dozer trawl transects per each sampling event (5 dozer trawl transects for each small tributary to be combined to 10 total).
2. Sample events should have at least one week difference.
3. Target metrics were further defined to ensure confidence in the ability to provide a representation of the population of interest, as well as to detect trends through time.
 1. Target RSE of CPUE is 25 or less, though results with higher RSE will still be reported with any potential explanatory variable that may have influenced variability in catch rates.
 1. For each tributary, 125 stock and larger invasive carp are targeted to accurately represent the size structure of the population.
 2. A random collection of 100 otoliths allows for exploration of age frequency in the population of interest while filling additional 50 mm length bins up to 20 individuals per bin, allowing for more accurate von Bertalanffy growth coefficient estimates that are used for growth and mortality estimates.

Appendix B.

Table B.1. All fish collected by electrified dozer trawl in fall 2023 by species and tributary. River mile (RM) is listed under each tributary. Totals included all sampled fish during standard sampling as well as volunteers and extra runs completed to collect aging structures.

Species	Small Tributary RM 170 & 314	Lamine River RM 202	Grand River RM 250	Kansas River RM 367	Nishnabotna River RM 542	Big Sioux River RM 734	Vermillion River RM 772	James River RM 800	Total
Bighead Carp	25	-	2	-	2	-	-	-	29
Brook Silverside	2	-	-	-	-	-	-	-	2
Blue Catfish	-	-	-	1	-	-	-	-	1
Bluegill	3	-	-	-	-	-	-	-	3
Bigmouth Buffalo	17	4	4	1	3	2	6	1	38
Blue Sucker	-	-	-	10	-	7	-	2	19
Common Carp	49	1	4	2	71	1	2	1	131
Channel Catfish	5	-	-	-	-	-	-	-	5
Emerald Shiner	314	50	36	313	68	113	402	438	1,734
Flathead Catfish	-	-	-	2	2	-	-	-	4
Freshwater Drum	8	3	2	6	34	-	-	4	57
Goldeye	21	9	4	5	4	16	16	32	107
Grass Carp	1	-	1	-	22	1	-	-	25

Gizzard Shad	666	64	81	59	80	53	89	827	1,919
Largemouth Bass	-	-	-	-	-	-	1	-	1
Longnose Gar	19	8	4	10	29	4	7	51	132
Mooneye	3	1	4	9	5	-	-	-	22
Orangespotted Sunfish	1	-	-	-	-	-	-	-	1
Paddlefish	3	1	1	-	-	-	-	15	20
Quillback	1	-	-	-	-	-	-	-	1
Red Shiner	1	-	-	1	2	17	5	4	30
River Carpsucker	25	2	1	8	61	-	12	2	111
Spotfin Shiner	-	-	-	-	-	1	-	1	2
Sauger	3	-	-	-	1	-	-	-	4
Skipjack Herring	7	5	6	-	-	-	-	-	18
Smallmouth Buffalo	53	12	2	23	27	14	12	6	149
Shortnose Gar	32	5	4	6	72	9	11	140	279
Shovelnose Sturgeon	-	-	-	1	-	-	-	-	1
Sand Shiner	-	-	-	1	-	-	-	2	3
Spottail Shiner	-	-	-	-	-	1	-	-	1
Silver Carp	400	266	190	375	464	620	626	504	3,445
Unidentified Cyprinidae	-	-	-	1	-	-	-	-	1

Unidentified	1	-	-	-	-	-	-	-	1
Unidentified <i>Notropis</i> sp.	-	-	-	-	-	2	-	-	2
Walleye	-	-	-	-	-	-	1	1	2
White Bass	6	-	-	3	1	1	2	-	13
White Crappie	-	-	-	-	-	-	1	-	1
Total	1,666	431	346	837	948	862	1,193	2,031	8,314

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