

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); South Dakota State University (SDSU)

U.S. Fish and Wildlife Service (USFWS) - Columbia Fish and Wildlife Conservation Office, Great Plains Fish & Wildlife Conservation Office, and Gavins Point National Fish Hatchery

Statement of Need:

In collaboration with multiple stakeholders, the USFWS and the Aquatic Nuisance Species Task Force released a national invasive carp management and control plan (National Plan; Conover et al. 2007) to limit ecological and economic problems posed by these species. Despite tremendous progress towards achieving National Plan goals, there remains a great need to develop metrics to quantify the success of invasive carp management and inform control efforts. 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 for prevention, containment and control, and extirpation. In addition, understanding the status and trends in abundance, size or age structure, maturity schedules, or fecundity of fish in a population are central to informed decision-making.

Currently, more information on the abundance and distribution of Silver *Hypophthalmichthys molitrix*, Bighead *Hypophthalmichthys nobilis*, and Black Carp *Mylopharyngodon piceus* is needed to inform the

strategic placement, development, and assessment of management actions across the Missouri River Basin as population assessments provide baseline population data to inform management decisions. Early detection sampling is used to detect new introductions and the spread of existing populations and can provide managers with critical information about the speed and mechanisms of spread. By detecting new populations early, actions can more effectively be implemented to control the population.

Missouri River Basin partners identified tributaries of the Missouri River as high-priority areas as they provide access to state inland waters of high recreational, economic, and ecological value. The Platte River is a major tributary to the Missouri River and Nebraska's largest river system. The Platte River contains multiple large tributaries and a network of diversion canals for an array of water usage. Furthermore, the Platte River does not contain large main-stem impoundments to block fish movement and may act as a source for both native and non-native fishes to the Missouri River. As such, understanding the population characteristics and recruitment of Silver Carp and Bighead Carp within the Platte River system is imperative to prevent further expansion and mitigate the risks to human interests and the native fish communities.

Larval invasive carp have been documented and collected at several locations throughout their invaded range. However, juvenile invasive carp are rarely captured, and little is known about nursery habitats that juveniles occupy and how they support recruitment to the adult stage. Utilizing tools to identify natal origins can help identify spawning and recruitment sources and management actions targeting this behavior and life stage. 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.

Managers must understand the factors influencing population dynamics to effectively guide efforts to manage and control invasive carp in the Missouri River Basin. 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), otolith microchemistry, and hydroacoustics are examples that are 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 effective protocols using these tools to answer high-priority questions.

Otolith microchemistry is an important tool for determining the natal origin of fishes from environments with distinct water chemistries (Campana et al 2000; Gibson-Reinemer et al. 2009; Zitek et al. 2010). An important first step is determining the variation in water chemistry among water bodies of interest. Specifically, the trace elements of barium (Ba), strontium (Sr), and calcium (Ca) along with oxygen (^{16}O ,

¹⁸O) isotopes are commonly used to differentiate between waterbodies. Once the unique water chemistries are determined, signatures within fish tissues such as otoliths can be assessed for the same trace elements and isotopes to inform where a fish spent portions of their life history, including natal origins. This technique may be best applied over a large spatial scale where differences in water chemistry are evident and can retroactively determine where fish from multiple-year classes captured in a single sampling season spent time, informing habitat use, movement, and, subsequently, future management actions.

The tasks outlined in this document represent the 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: USFWS).

- Sub-Objective 1: Participate in the US Fish and Wildlife Service Invasive Carp eDNA Community of Practice to increase understanding of environmental DNA (eDNA) as a tool for the detection and measurement of invasive carp populations. This workgroup hosts informational webinars/workshops to provide education and learning opportunities.
- Sub-Objective 2: Implement a strategy for information sharing on the methods needed to successfully analyze eDNA samples for invasive carp primers, and coordinate efforts with Genetic Labs to integrate methods with partners already using eDNA for the detection of invasive carp.
- Sub-Objective 3: Continue to assess 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: Continue to assess the presence/absence of Bighead and Silver 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, relative abundance, recruitment) while developing standard operating procedures that are specific for the lower Missouri River Basin to prescribe and assess population control measures (Agencies involved: ISU, MDC, NGPC, SDGFP, SDSU, 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 eDNA sampling sites to validate eDNA results.
- Sub-Objective 4: Evaluate the natal origin and movement of Silver Carp collected in the Missouri River and its tributaries using microchemistry analysis.

Objective 3: Characterize the historic and current fish community in 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, MDC).

- Sub-Objective 1: Deploy fish community assessment gears 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.
- Sub-Objective 3: Empirically examine direct and indirect effects of invasive carps on native species and food webs through the use of stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) to examine seasonal food webs of high, low, and zero invasive carp density sections of the Kansas River.
- Sub-Objective 4: Compare Kansas River isotope results with similar studies in other river systems (Wabash and Illinois Rivers; Meta-analysis).

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: USFWS).

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, relative abundance, recruitment) while developing standard operating procedures that are specific for the lower Missouri River Basin to prescribe and assess population control measures (Agencies involved: IA DNR/ISU, MDC, NGPC/UNL, SDGFP/SDSU, USFWS).

- Iowa Sampling:
 - We collected invasive carp larvae in the Big Sioux, Little Sioux, and Boyer rivers.
 - Invasive carp larvae were rarely detected, but densities were very high in the Big Sioux River (up to 6,280 fish/m³) whereas only a few individuals were collected in the Little Sioux and Boyer rivers.
 - Successful invasive carp reproduction occurring in these small tributaries during drought conditions is concerning for population expansion.
 - We did not detect juvenile invasive carp at 81 sites in tributaries but did collect juveniles in 3 of 8 backwaters of the Missouri River, indicating recruitment is occurring in this reach of the Missouri River.
 - Mean length of the seven juvenile carp was 70 mm.
- Missouri Sampling:
 - Silver Carp was the most abundant invasive carp with 1,072 sampled. Five Grass Carp and one Bighead Carp were also collected for a total weight of 2,280 kg (5,027 lbs.) of fish removed. No Black Carp were sampled.
 - Silver Carp catch rates were highest at Platte River (MO) Site 01 (158.8 fish/hour). However, catch rates at the Missouri River Bend at the Platte River (31.5 fish/hour) was the lowest in 2024.
 - Size classes of Silver Carp were similar between all three tributaries with few fish below 500mm. However, Silver Carp median length at Grand River Site 03 (543mm) were significantly different ($P < 0.05$) than multiple sites including Lamine Site 02 (598mm), Grand Site 02 (599mm), Platte Site 01 (598mm) and Missouri River bend sites at the Grand (581mm) and Platte River (611mm).
 - Silver Carp sex ratios of males to females tended to be close to a 1:1 ratio at most sites except for the Missouri River Bend at the Lamine, where a higher ratio of males was sampled than females (1.7: 1).
 - Silver Carp young of year were collected with mini-fykes in all three tributaries and all Missouri River bend sites in 2024. Bighead Carp young of year were collected at all three Grand River sites and one individual was captured at Platte River Site 02.

- Size distributions and length at age data of Silver Carp in 2024 indicate limited recruitment over the last four years in the three tributaries and associated Missouri River bends
- Nebraska Sampling:
 - The herding technique (a combination of sound and electrofishing) was determined to be the most effective method to move bigheaded carp in a downstream direction toward capture nets.
 - A total of 6,943 fish were collected using this method, consisting of 6,205 bigheaded carp (14,079 kg or 31,038 lbs.), which were removed from the system.
 - Assessment between the heading technique and standard electrofishing showed that electrofishing was more cost-effective and removed more fish per personnel hour.
 - Age-0 fish were opportunistically collected throughout the summer while sampling adult fish. The catchability of age-0 fish declined throughout the summer, with a notable decrease in abundance during late August.
- 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 delayed results. Final sample results were received in late 2024, and data processing will occur in early 2025.
 - Partners across the Missouri River Basin contributed 996 otoliths from 13 different locations to help assess natal origins of Invasive Carp across the basin.
- USFWS Sampling:
 - In 2024, the USFWS completed 147 electrified dozer trawl transects across nine tributary confluences, where 2,975 Silver Carp were collected.
 - A large spawning event occurred across the basin as age-0 Silver Carp were collected in seven out of nine tributaries sampled (N = 920).

Objective 3: Characterize the historic and current fish community in 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, MDC).

- Kansas Sampling:
 - All 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.

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:

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 (*H. 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.

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 and Discussion:

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 (98.2% 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 31 to June 1 (188 m³/sec) and from June 13 to June 16 (137 m³/sec; Figure 3). Water temperatures between May 31 and June 15 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 4-7). 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 fish/100 m³ ± 3.4 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 4-7).

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 8). It is therefore unsurprising that 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 the 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.

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 ± 12 mm and mean weight of 2 ± 1 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 ≤ 0.5 m, there was little to no flow, little macrophytes and woody debris, and no canopy cover. We collected all juvenile Silver Carp within samples that had moderate to high abundances of Gizzard Shad (*Dorosoma cepedianum*). Our captures of juvenile Silver Carp only in backwaters and side channel habitats of the Missouri River and not in 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.

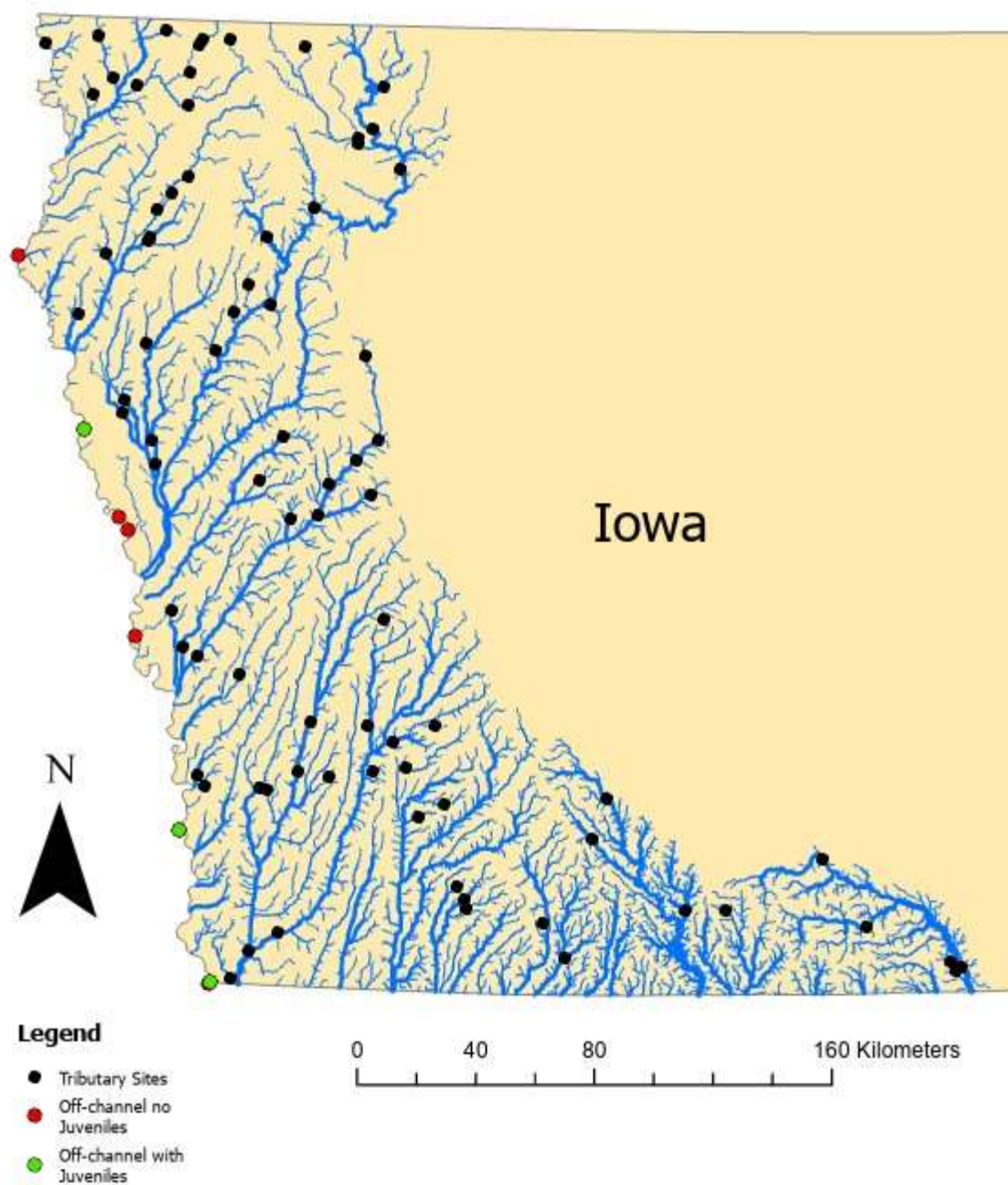


Figure 2. Locations of tributaries ($n = 81$) and off-channel ($n=8$) sites in the Missouri River Basin where we sampled fish communities during 2022 – 2023. All juveniles were detected during 2022.

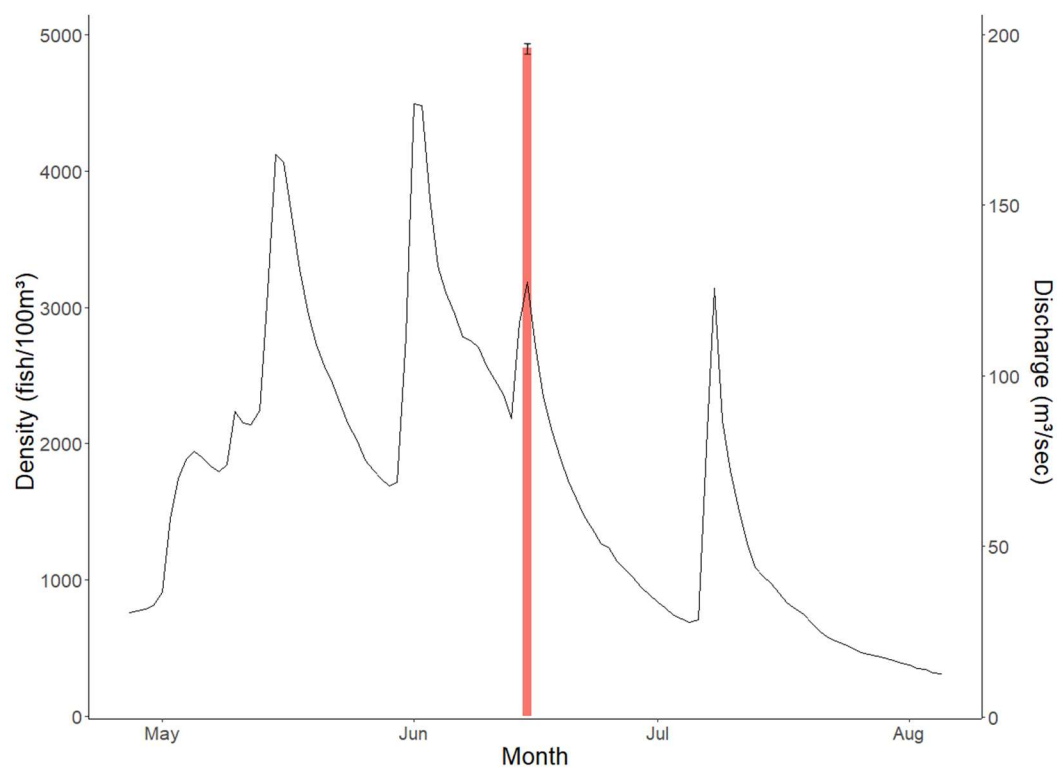


Figure 3. Larval and yolk-sac invasive carp densities (red bar; mean $\pm$ standard error) at the Big Sioux River middle site (BSR-MID) on June 15th, 2022, in relation to river discharge (m³/sec).

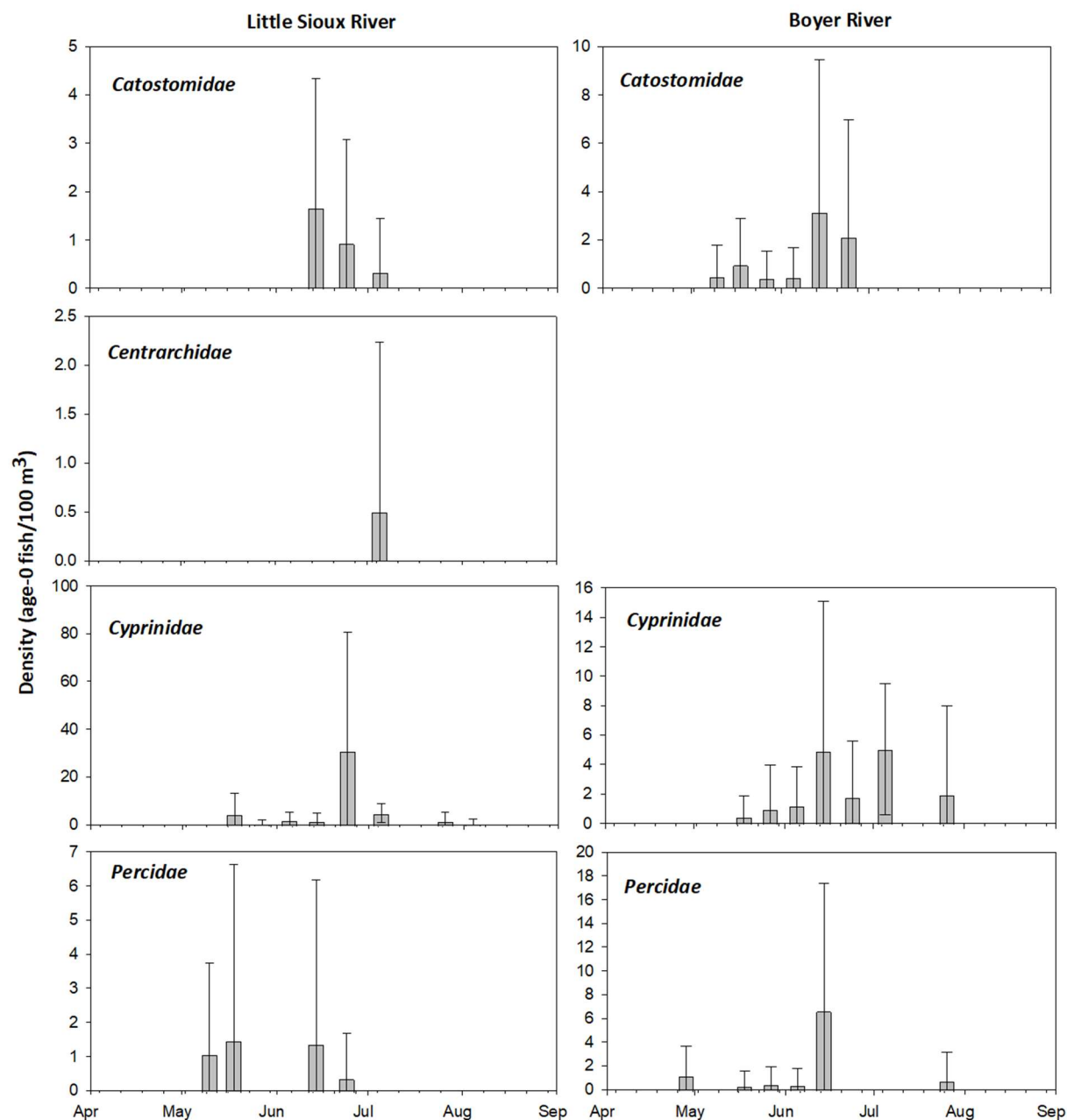


Figure 4. Density (mean $\pm$ 1 SE) of age-0 fishes from four families collected on the Little Sioux River (left) and Boyer River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

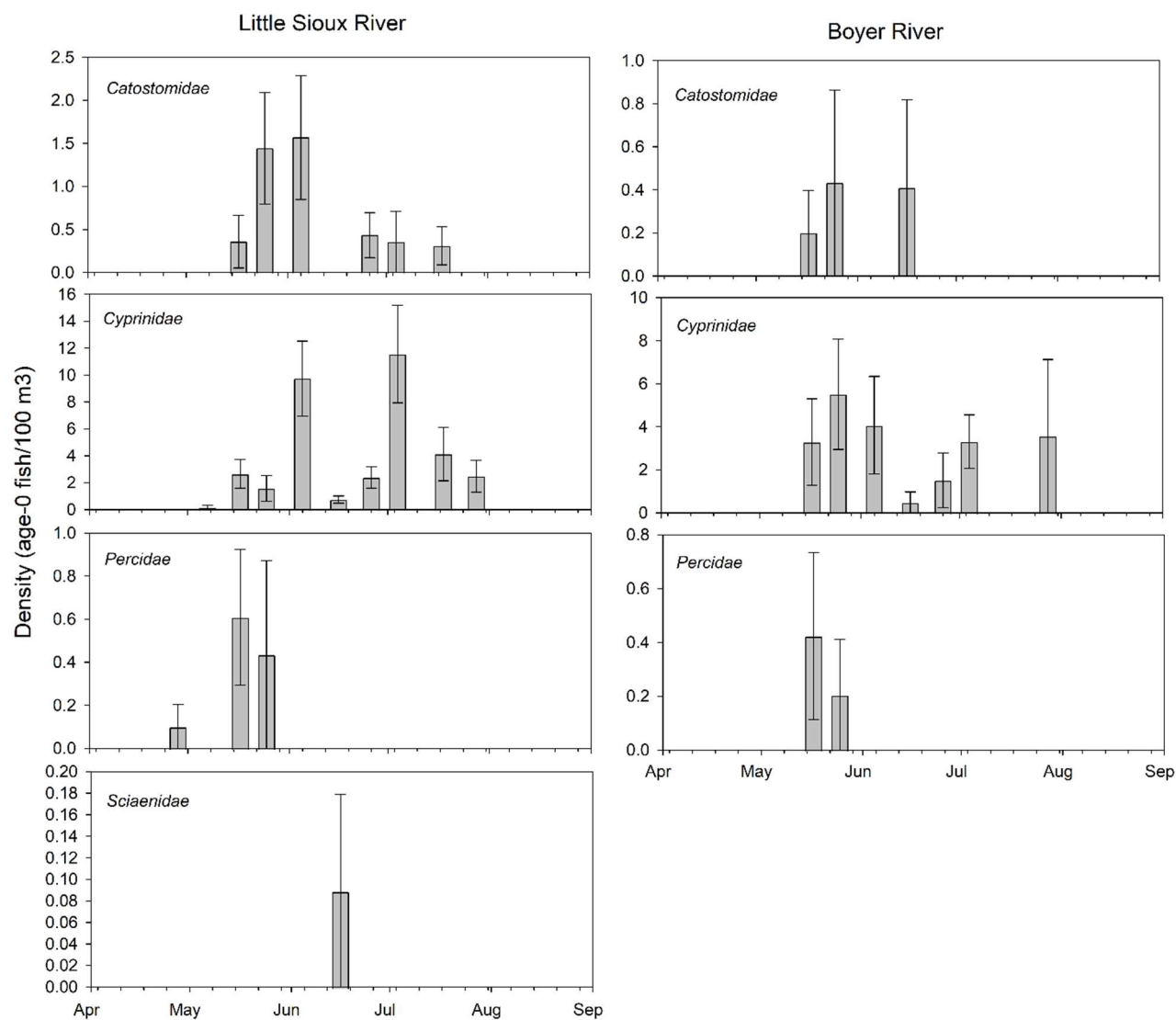


Figure 5. Density (mean $\pm$ 1 SE) of age-0 fishes from four families collected on the Little Sioux River (left) and Boyer River (right) from April 27th to July 29th, 2023. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

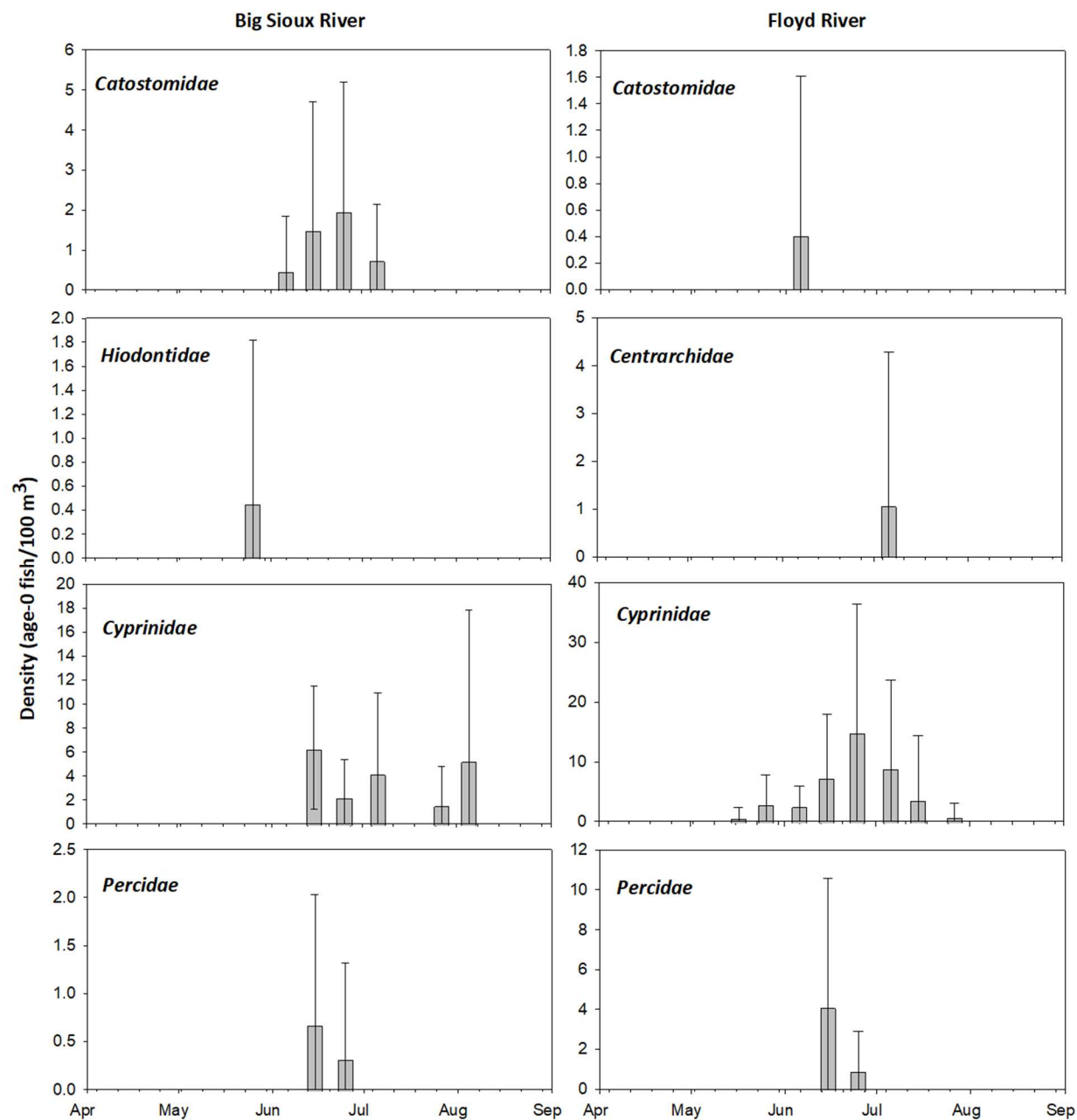


Figure 6. Density (mean $\pm$ 1 SE) of age-0 fishes from five families collected on the Big Sioux River (left) and Floyd River (right) from April 28th to August 5th, 2022. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

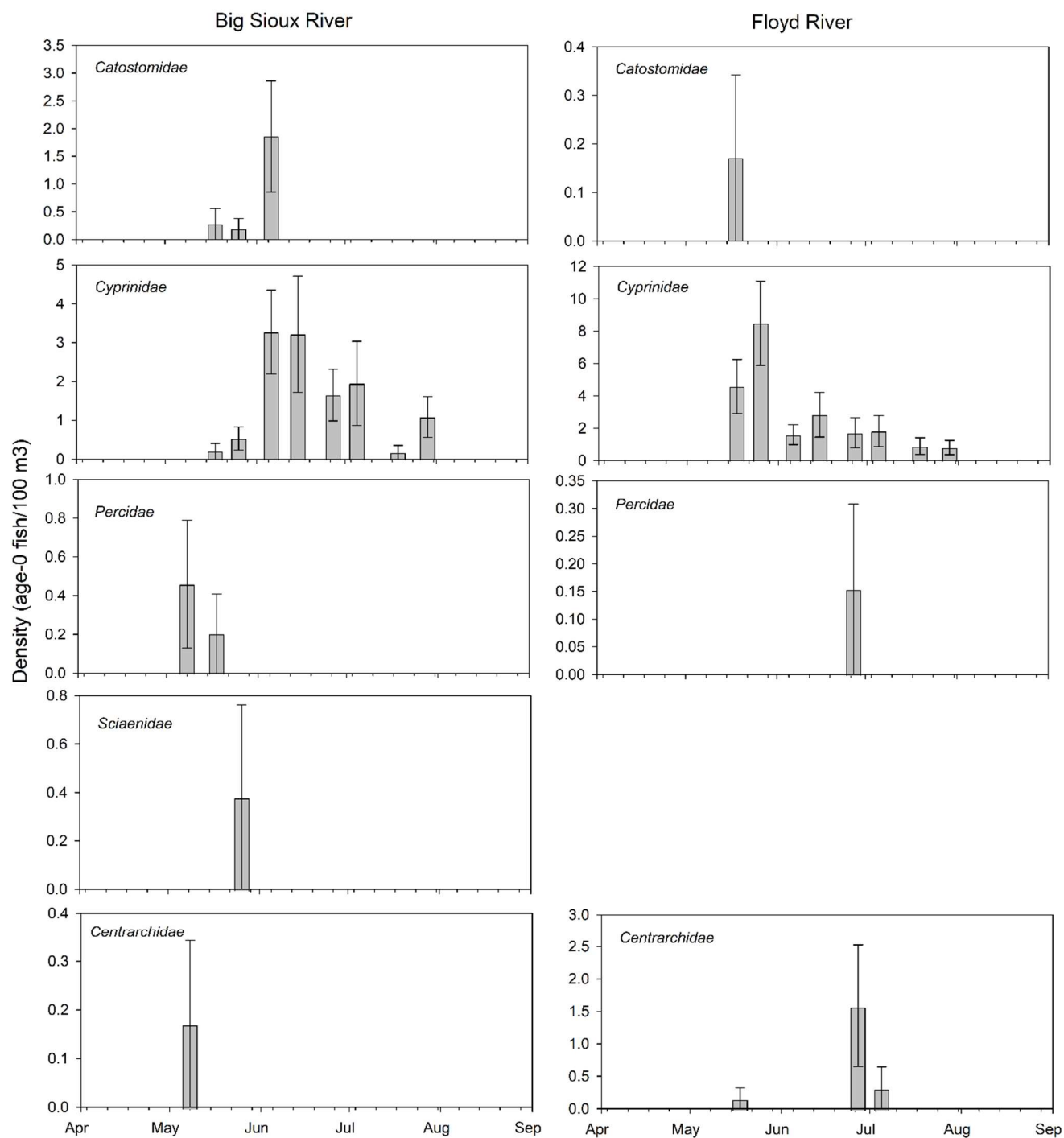


Figure 7. Density (mean $\pm$ 1 SE) of age-0 fishes from five families collected on the Big Sioux River (left) and Floyd River (right) from April 27th to July 29th, 2023. Note differences in y-axis scale among figure panels. No other taxa were detected on these rivers.

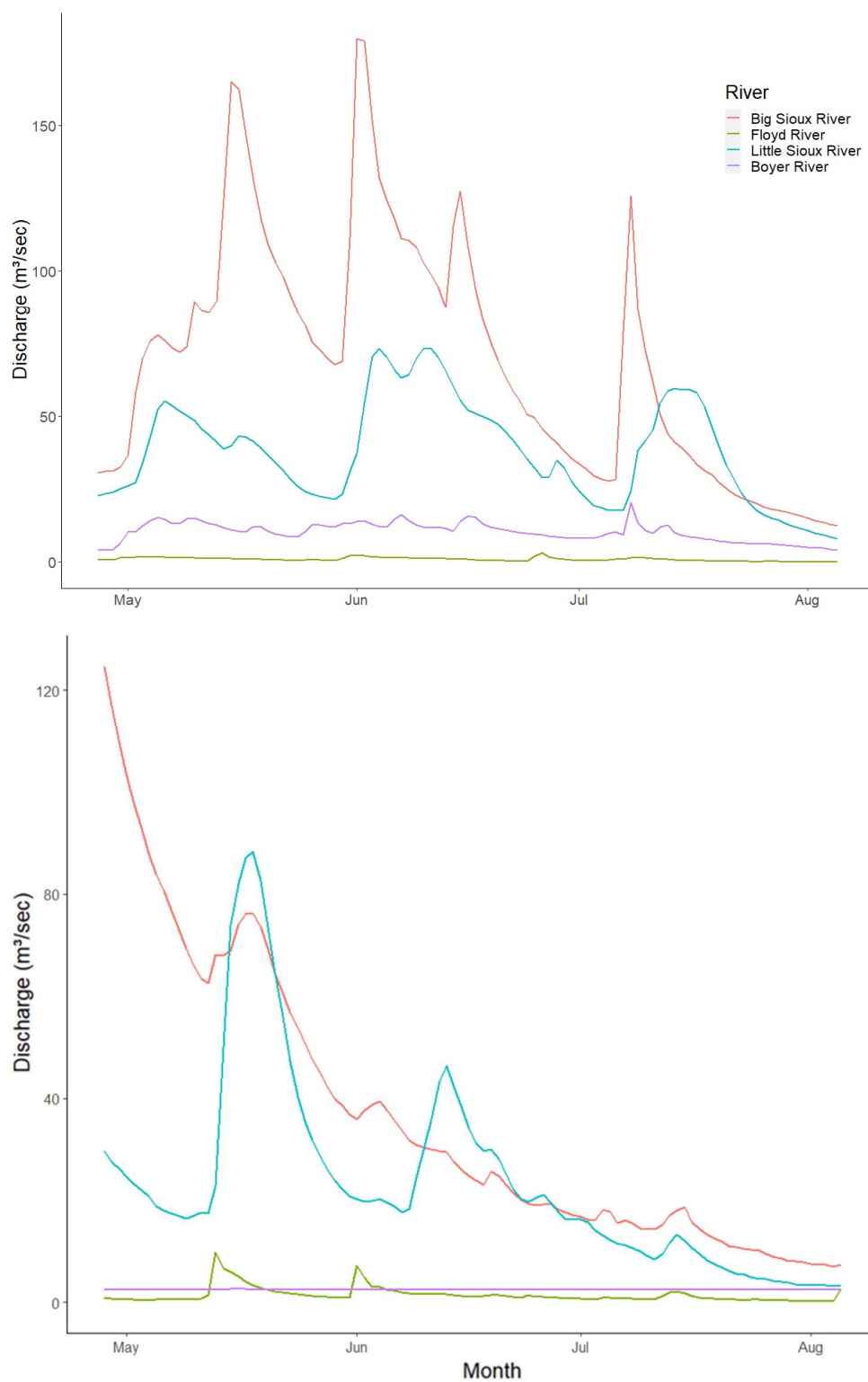


Figure 8. Discharge (m^3/sec) of the Big Sioux, Floyd, Little Sioux, and Boyer River from April 28th to August 5th, 2022 (top panel) and 2023 (bottom panel).

State Report: Kansas**Agency:** Kansas Department of Wildlife and Parks**Project Title:** Kansas River Invasive Carp Food Web Study**Methods:**

Data collection is complete and was collected for two full water years in three seasons (spring [April], summer [July], fall [November]) of 2023 and 2024. 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 (with the exception of bighead carp and silver carp which are absent upstream of Bowersock Dam) were used. Fish were 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 are being determined using Layman's indices and Bayesian mixing models. Data analysis is ongoing with a final report fully describing methods, results, and conclusions due in 2025. Following completion of analyzation of isotope data, comparisons will be made to other river systems where similar data have been collected.

Results & Discussion:

Among the planktivores: Bigmouth Buffalo isotopic signatures are depleted in $\delta^{15}\text{N}$ in the presence of invasive carp, (Fig. 1), suggesting their preferred food of large predatory zooplankton has been depleted, relegating them to a lower, less-preferred trophic level and food sources, likely benthos. Similarly juvenile sunfish (Fig. 2) have shifted to a heavily constricted niche in the presence of the invasive carp in terms of $\delta^{13}\text{C}$ and slight reduction in $\delta^{15}\text{N}$. This indicates a simplified trophic structure of food sources available, indicative of zooplankton suppression by invasive carp. Conversely, Gizzard Shad (Fig. 1) had a broadly expanded $\delta^{13}\text{C}$ niche, suggesting they are being competitively excluded from their preferred niche of phytoplankton, and instead are relying on a less-specific range of planktonic food sources. Other studies have suggested that detritivores are not as negatively impacted by carp, instead the shunting of nutrients by carp to the benthos may increase their success. In terms of isotopic niche, our study demonstrates no change for these fishes (Fig. 3). Piscivores, especially catfish, have expanded their isotopic niche in $\delta^{13}\text{C}$ signatures with slight reduction in $\delta^{15}\text{N}$, following the trend of Gizzard Shad. This would suggest either a continued reliance on the impacted Gizzard Shad and/or a heavier reliance on much broader prey sources e.g. benthivores.

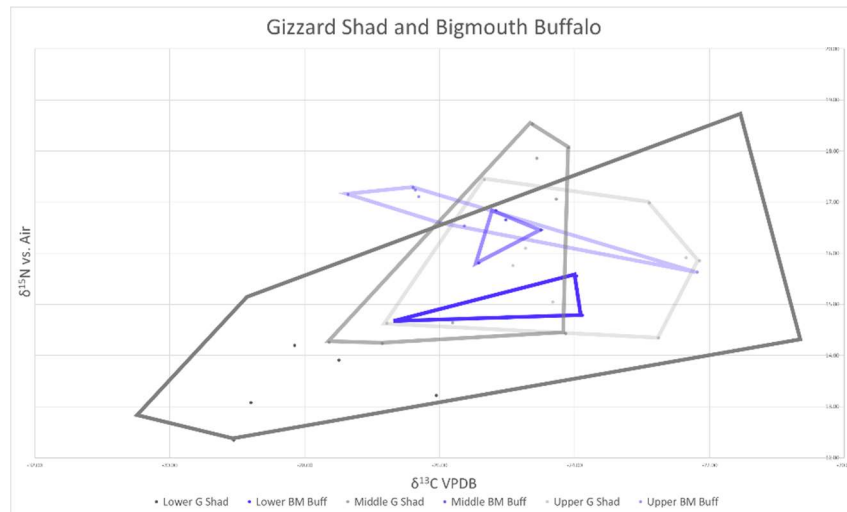


Figure 1: Carbon and Nitrogen Isotope signatures of primary planktivores American Gizzard Shad (*Dorosoma cepedianum*) and Bigmouth Buffalo (*Ictiobus cyprinellus*) taken from the Kansas River showing trophic reduction of Bigmouth Buffalo, and niche expansion of Gizzard Shad relative to the *Hypophthalmichthys* carp invasion gradient. “Lower” denotes fish taken from the river reach downstream of WaterOne Dam in Edwardsville, KS to the confluence with the Missouri River in Kansas City, KS; “Middle” denotes fish taken from the river reach downstream of the Bowersock Dam in Lawrence, KS to the WaterOne Dam; “Upper” denotes fish taken from the river reach extending upstream of the Bowersock Dam to Lecompton, KS.

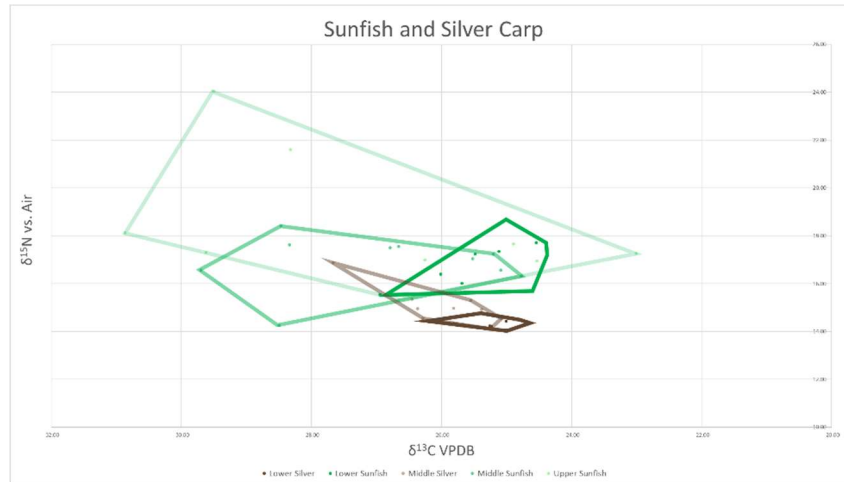


Figure 2: Carbon and Nitrogen Isotope signatures of primary planktivores juvenile sunfish (*Lepomis sp.*) and Silver Carp (*Hypophthalmichthys molitrix*) taken from the Kansas River showing niche constriction of sunfish, and trophic reduction of Silver Carp relative to the *Hypophthalmichthys* carp invasion gradient. “Lower” denotes fish taken from the river reach downstream of WaterOne Dam in Edwardsville, KS to the confluence with the Missouri River in Kansas City, KS; “Middle” denotes fish taken from the river reach downstream of the Bowersock Dam in Lawrence, KS to the WaterOne Dam; “Upper” denotes fish taken from the river reach extending upstream of the Bowersock Dam to Lecompton, KS. Silver Carp are not present in the “upper” reach.

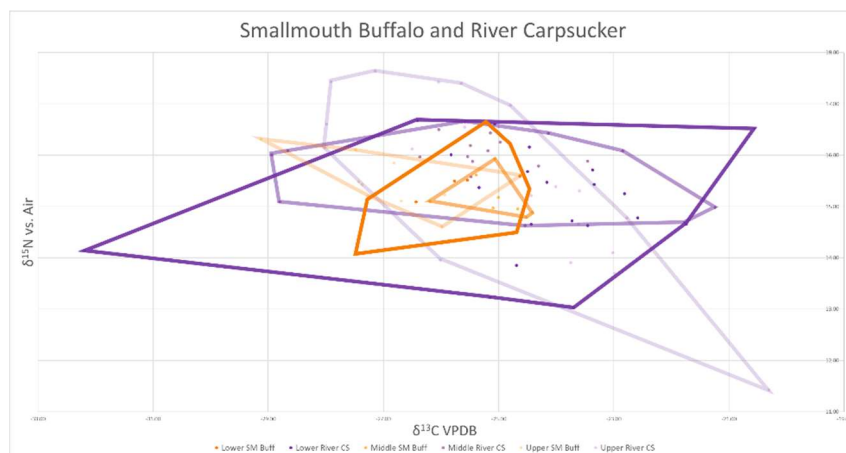


Figure 3: Carbon and Nitrogen Isotope signatures of detritivore/benthivores Smallmouth Buffalo (*Ictiobus bubalus*) and River Carpsucker (*Carpionodes carpio*) taken from the Kansas River showing similar niche size and region in isotope space across the *Hypophthalmichthys* carp invasion gradient. “Lower” denotes fish taken from the river reach downstream of WaterOne Dam in Edwardsville, KS to the confluence with the Missouri River in Kansas City, KS; “Middle” denotes fish taken from the river reach downstream of the Bowersock Dam in Lawrence, KS to the WaterOne Dam; “Upper” denotes fish taken from the river reach extending upstream of the Bowersock Dam to Lecompton, KS.

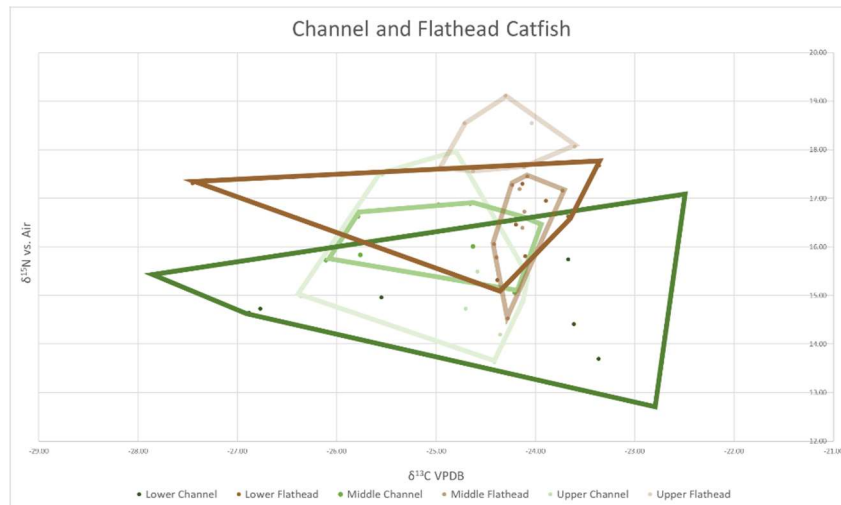


Figure 4: Carbon and Nitrogen Isotope signatures of primary piscivores Channel Catfish (*Ictalurus punctatus*) and Flathead Catfish (*Pylodictis olivaris*) taken from the Kansas River showing niche expansion with respect to the *Hypophthalmichthys* carp invasion gradient. “Lower” denotes fish taken from the river reach downstream of WaterOne Dam in Edwardsville, KS to the confluence with the Missouri River in Kansas City, KS; “Middle” denotes fish taken from the river reach downstream of the Bowersock Dam in Lawrence, KS to the WaterOne Dam; “Upper” denotes fish taken from the river reach extending upstream of the Bowersock Dam to Lecompton, KS.

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 4

Sites

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.

Methods:

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. This setting was selected based on 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.

Mini-fyke nets were set in tributary and Missouri River bend sites to collect invasive carp young of year to estimate the amount of spawning or reproduction on an annual basis. This was conducted from July through September with number of nets per site based on tributary wetted width and watershed drainage similar to Dunn and Paukert 2020. Three to four 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. Eight mini-fyke nets were set in each Missouri River bend.

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

Lamine River – Invasive Carp Sampling

Invasive carp sampling for Lamine River sites was conducted the third week of August and the Missouri River bend at the Lamine was sampled the first week of September in 2024. A total of 30 electrofishing runs (10 - Missouri river bend, 10 - Lamine site 1, and 10 - Lamine site 2) and 2.76 gear hours of electrofishing resulted in 299 Silver Carp collected. All electrofishing runs including non-random runs resulted in 408 Silver Carp and 886 kg (1,953 lbs) of fish removed from the Lamine River. One Grass Carp was also sampled in Lamine sites during standard sampling. Results from a one-way analysis of variance indicated Silver Carp catch rates were not significantly different between sites ($P = 0.409$) (Figure 1.) Silver Carp relative abundance in Lamine River sites was similar over the last three years and below the highest catch rates from 2021 (Figure 2). Silver Carp catch rates were also similar the last two years at the Missouri River bend at the Lamine.

Silver Carp lengths ranged from 426mm – 709mm across all Lamine sites with fish from 560mm to 620mm dominating the catch (Figure 3). Silver Carp median length at Lamine Site 02 (598mm) were significantly different ($P < 0.05$) between Lamine River Site 01 (581mm) and Missouri River Bend at the Lamine River (577mm) with larger individuals at Lamine River Site 02. 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 or 2024 in the Lamine River (Figure 4). Preferred size Silver Carp (560-739mm) encompassed the highest proportion in Lamine River sites and the Missouri River bend at the Lamine.

Otoliths were collected from 98 Silver Carp sampled in Lamine River sites and 65 from the Missouri river bend at the mouth of the Lamine River. Additional otoliths were taken from one Bighead Carp in the Lamine River and one Grass Carp in the Missouri River bend. No age-1 to age-3 Silver Carp were collected in 2024 and Silver Carp from age-4 ranged from 519 to 632mm (Figure 5). Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 1.7: 1 while in the Lamine River sites it was 0.9: 1.

Lamine River – Mini-fyke Nets

Sixteen mini-fyke sets were conducted for all Lamine River sites in 2024. Sampling resulted in a total of 390 fish (Missouri River = 340, Lamine site 1 = 11, Lamine site 2 = 39). Twelve species were sampled at

the Lamine River sites (Lamine site 1 = 4, Lamine site 2 = 11) while 19 species were sampled at the associated Missouri river bend. Silver Carp young of year (N = 3) were collected in Lamine Site 2 and the Missouri River bend at the Lamine. No Bighead Carp young of year were collected in Lamine River sites. (Table 2).

Grand River – Invasive Carp Sampling

Invasive carp sampling for Grand River sites was conducted the last two weeks of August and the first week of September in 2024. Forty electrofishing runs (10 in the Missouri River bend, 10 Grand River site 1, 10 in Grand River site 2 and 10 in Grand River site 3) and 3.59 electrofishing gear hours resulted in 434 Silver carp and 899 kg (1,982 lbs) of fish removed from the Grand River. Results from a Kruskal-Wallis one way analysis of variance on ranks indicated Silver Carp catch rates were not significantly different between sites ($P=0.304$) (Figure 1). The overall Silver Carp relative abundance in 2024 for Grand River sites increased from 2023 and 2022 (Figure 2). However, Silver Carp catch rates at the Missouri River Bend site remained stable the last three years.

Silver Carp lengths ranged from 428mm to 791mm for all sites combined with fish from 530mm to 630mm accounting for most of the catch (Figure 3). Silver Carp median lengths were significantly different ($P < 0.05$) between Grand River Site 03 (543mm) and Grand River Site 02 (599mm) in addition to the Missouri River Bend at the Grand River (581mm). 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 1% of the catch in 2024 (Figure 4). Preferred size Silver Carp (560-739mm) comprised the highest proportion in the Grand River and Missouri River bend at the Grand. Memorable size Silver Carp (740-929mm) were also collected in the Lamine River sites and Missouri River Bend site for the first time since 2021.

Otoliths were collected from eighty-one Silver Carp at the Missouri river bend at the mouth of the Grand River. Sampling at the Grand River sites collected otoliths from one hundred seventeen Silver Carp. Otoliths were also collected from one Bighead Carp on the Grand River. No age-1 to age-2 and only 1 age-3 Silver Carp were collected in 2024 and Silver Carp from age- 4 ranged from 438 to 735mm (Figure 5). Sex ratios (Male: Female) for Silver Carp sampled in the Missouri river bend was 0.8: 1 while in the Grand River sites it was 1: 1.

Grand River – Mini-fyke Nets

Sixteen mini-fyke nets were completed in the Grand River sites including the associated Missouri River bend. This resulted in a total of 2,057 fish (Missouri River bend= 1,082, Grand River site 01 = 248, and Grand River site 02 = 727). Twenty species were sampled in the Grand River (Site 01 = 14 and Site 02 = 15). The Missouri River bend sampling resulted in 22 species. Silver Carp young of year (N = 400) were collected in all three Grand River Sites. Mean lengths of young of year Silver Carp ranged from 32 mm at Grand Site 01 to 40 mm at Site 02 and 33 mm at the Missouri River bend at the Grand River. Bighead Carp young of year (N = 15) were also collected in all three Grand River sites. Mean lengths of Bighead Carp ranged from 33 mm at the Missouri River bend of the Grand River to 46 mm at Site 2. Site 1 only collected one individual measuring 58 mm.

Platte River – Invasive Carp Sampling

Invasive carp sampling for Platte River sites was conducted the last week of August in 2024. Thirty electrofishing runs (10 in the Missouri river bend, 10 at site 01, and 10 at site 02) and 2.13 electrofishing gear hours resulted in 230 Silver Carp, 4 Grass Carp and 1 Bighead Carp amounting to 495kg (1,091 lbs) of fish removed from the Platte River (MO). Silver Carp mean catch per unit effort at the Platte River Site 01 (158.8 fish/hour) and Platte River Site 02 (114.6 fish/hour) was significantly higher ($P < 0.05$) than the Missouri River bend site (31.5 fish/hour) (Figure 1). Silver Carp relative abundance in 2024 at Platte River sites was similar to 2023 and below catch rates from 2020 to 2022 (Figure 2). Silver Carp catch rates at the Missouri River bend site at the Platte River (MO) were low in 2024 and 2023.

Silver Carp lengths ranged from 470mm to 750mm for all sites combined with fish from 550mm to 630mm dominating the catch (Figure 3). Silver Carp lengths were not significantly different ($P = 0.158$) between sites. Preferred size Silver Carp (560-739mm) comprised the highest proportion sampled from the Platte River and Missouri River bend at the Platte for the last three years (Figure 4). Sample year 2024 is the first year memorable size Silver Carp (740-929mm) were not collected in Platte River. However, memorable size Silver Carp were collected at the Missouri River bend site at the Platte.

Otoliths were collected from one hundred eighty Silver Carp in the Platte River sites. Fifty-three otoliths were collected from Silver Carp in the Missouri River bend at the mouth of the Platte River. No age-1 to age-3 Silver Carp were collected in 2024 and age-4 fish ranged from 494 to 674mm (Figure 5).

Sex ratios (Male: Female) in the Missouri river site was 1.1 : 1 and 1.1 : 1 in the Platte River sites combined.

Platte River – Mini-fyke Nets

Fourteen mini-fyke nets were completed in the Platte River sites including the associated Missouri river bend. This resulted in 1,644 fish sampled (Missouri river = 1,442, Platte River site 01 = 67, and Platte River site 02 = 135). Twenty-seven species were sampled in the Missouri river at the mouth of the Platte River. Nineteen species were sampled at the Platte River sites (Site 01 = 12, Site 02 = 16). Silver Carp young of year ($N = 142$) were collected in all three Platte River Sites. Mean lengths of young year Silver Carp ranged from 38 mm at Platte Site 1 to 48 mm at Site 02 and 55 mm at the Missouri River bend at the Platte River. One young of year Bighead Carp was captured in Platte River Site 02 and measured 41 mm.

Discussion

Silver Carp was the overwhelmingly dominant invasive carp species in all tributaries and their associated Missouri River bends. Catches of Silver Carp were variable between sites, however tributary sites had higher catch rates than Missouri River bend sites. The lower Platte River (MO) site 01 still maintains the highest Silver Carp abundance annually from 2020 to 2024 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 mean length at age data of Silver Carp in 2024 provide evidence to limited recruitment over the last four years in the three tributaries and associated Missouri River bends. However, there were a substantial number of young of year Silver Carp collected in mini-fyke nets from

multiple sites in 2024 indicating a significant level of reproduction in the lower Missouri River basin. The question will be how many fish from this year class recruits to the population next year. Bighead Carp young of year were also detected in 2024, however, in much fewer numbers than Silver Carp.

Five Grass Carp and one Bighead Carp were also collected in sample year 2024. 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 are paramount to plan for control, 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. Another invasive carp removal was conducted on the Lamine River in September of 2024 so demographic data is important to understanding potential impacts from removals. The Platte River (MO) is scheduled to have a removal in Fall of 2025 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 upstream or downstream 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 ($\pm$ 2S.E.)	Total # Silver Carp	Total Weight kilograms(lbs)	Male : Female
MOLR	10	91.1 (42.2)	408	886 (1,953)	1.7 : 1
LR1	10	130.8 (46.7)			0.9 : 1
LR2	10	105.6 (52.3)			
MOGR	10	81.9 (49.3)	434	899 (1,982)	0.8 : 1
GR1	10	129.5 (52.1)			
GR2	10	99.9 (45.9)			1 : 1
GR3	10	101.2 (51.4)			
MOPR	10	31.5 (29.9)	230	495 (1,091)	1.1 : 1
PR1	10	158.8 (57.6)			1.1 : 1
PR2	10	114.6 (66.3)			

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. Mini-fyke sampling effort and number of species and fish at each tributary and associated Missouri River bend.

Site ¹	Mini Fyke #nets	Number of Fish	Number of Species	# YOY Invasive Carp Silver/Bighead
MOLR	8	340	18	2
LR	8	50	12	1
MOGR	8	1,082	22	187 / 2
GR	8	975	20	213 / 13
MOPR	8	1,442	27	122
PR	6	202	19	20 / 1

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.

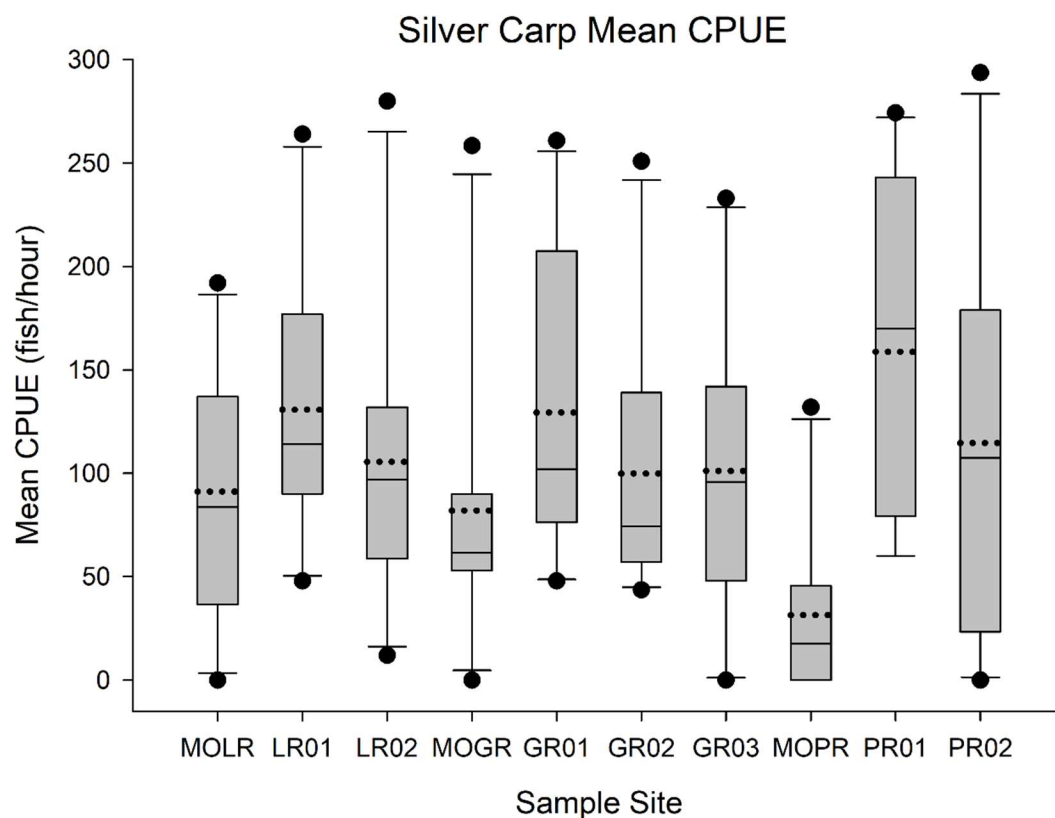


Figure 1. Box plots of Silver Carp catch per unit effort at each site in Lamine (LR), Grand (GR), Platte (PR), and associated Missouri River bends (MO) at each tributary in sample year 2024. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

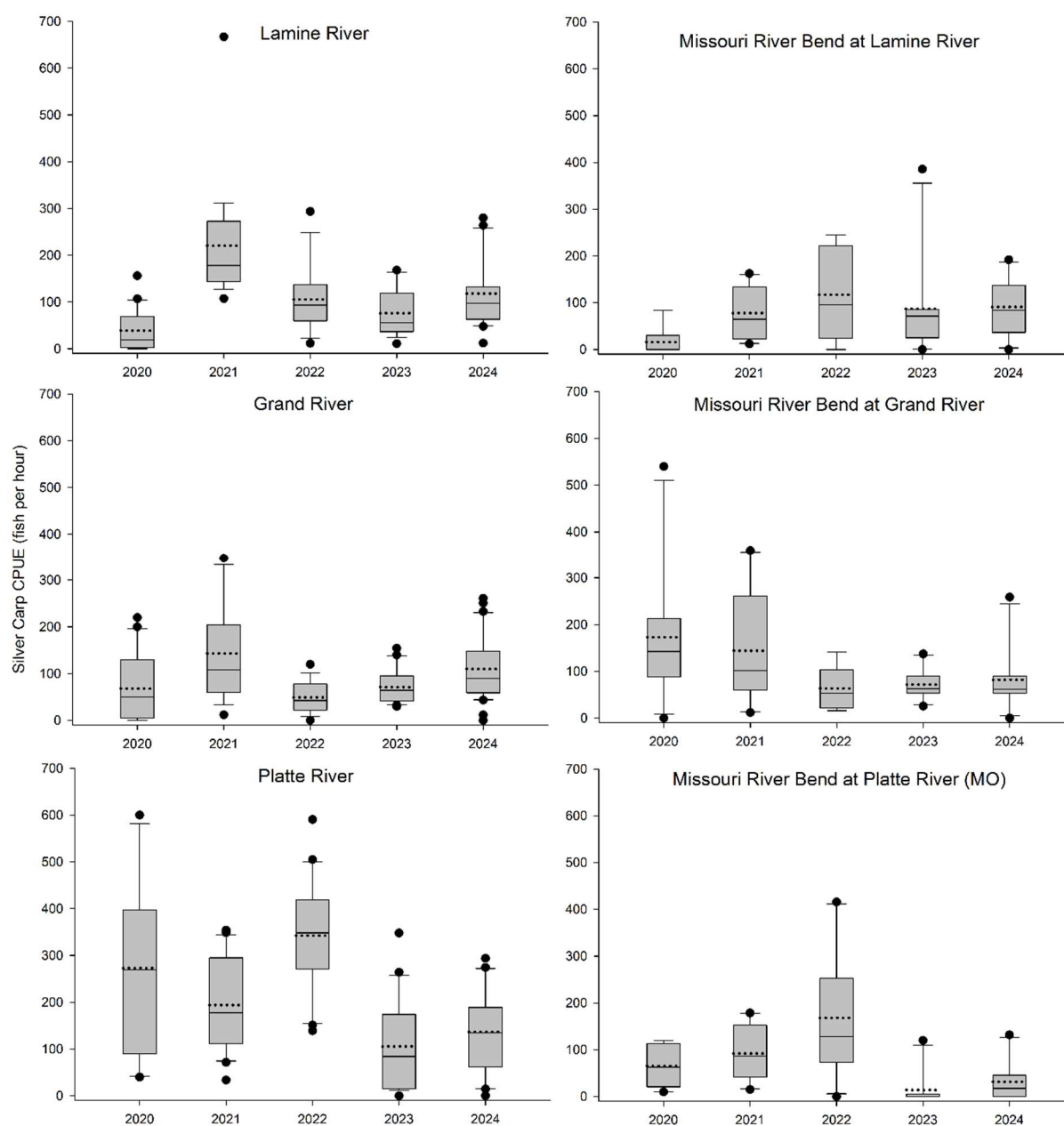


Figure 2. Box plots of Silver Carp catch per unit effort combined annually for Lamine, Grand, Platte, and associated Missouri River bends (MO) at each tributary from 2020 to 2024 using 60Hz and 40% Duty Cycle. Boxes represent the upper and lower quartiles, solid line represents the median, dotted line represents the mean, whiskers represent the 10th and 90th percentiles and black circles indicate outliers.

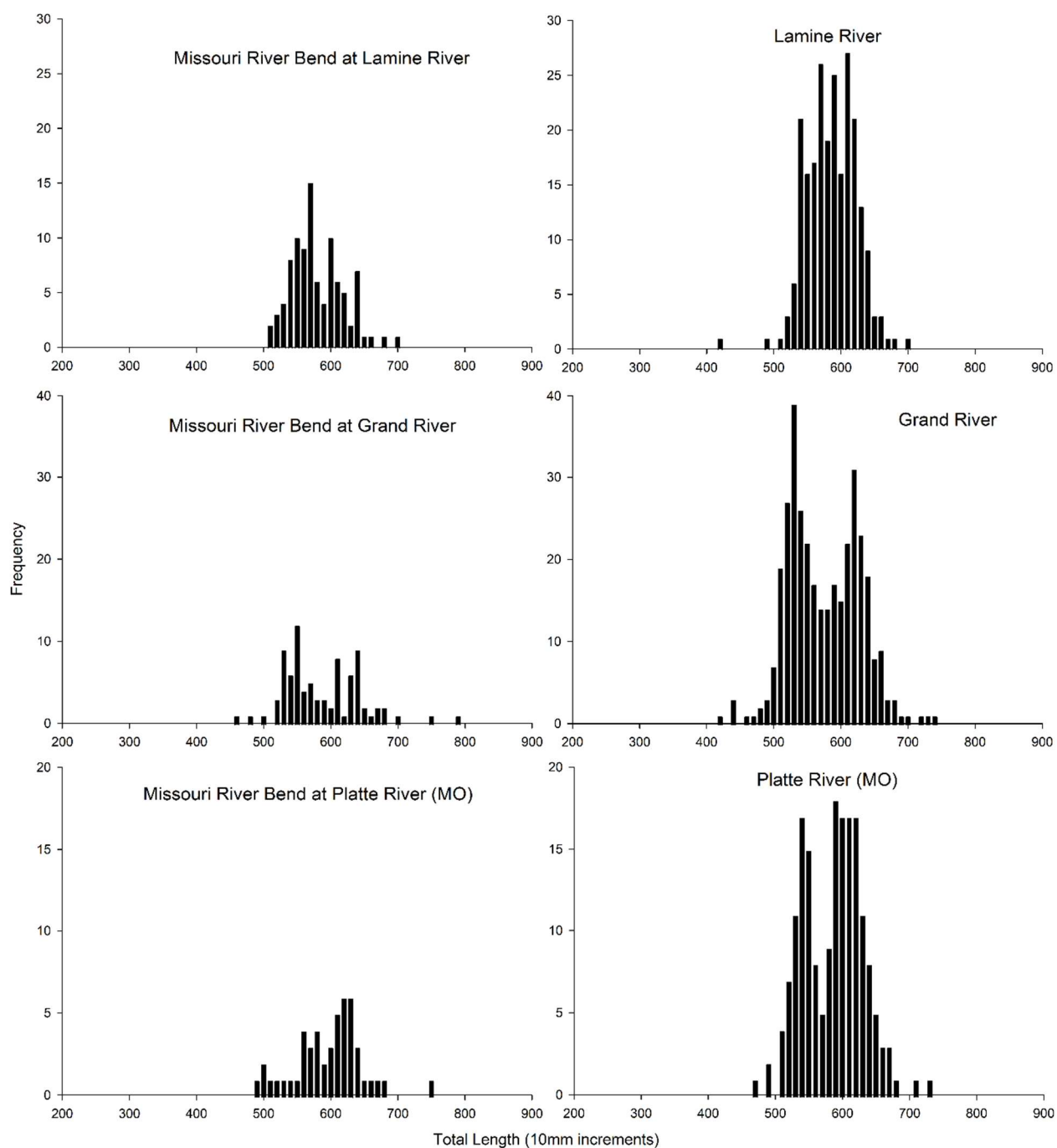


Figure 3. Length frequencies of Silver Carp from electrofishing in 10mm increments for Missouri River bend sites at each tributary (left panel) and tributary sites combined (right panel) in 2024.

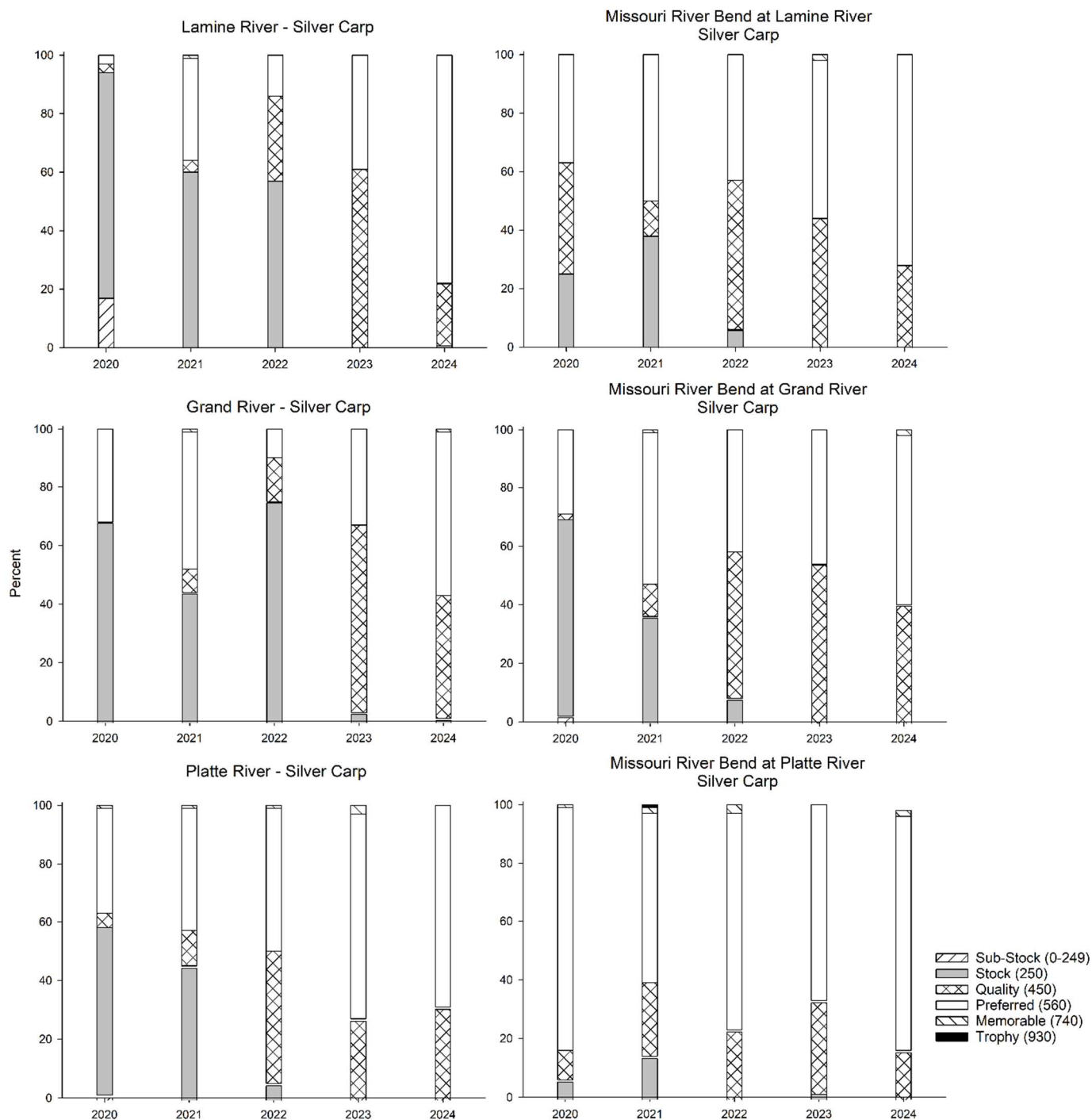


Figure 4. Incremental relative stock densities of Silver Carp based on Phelps and Willis (2013) from electrofishing in each tributary and associated Missouri River bend.

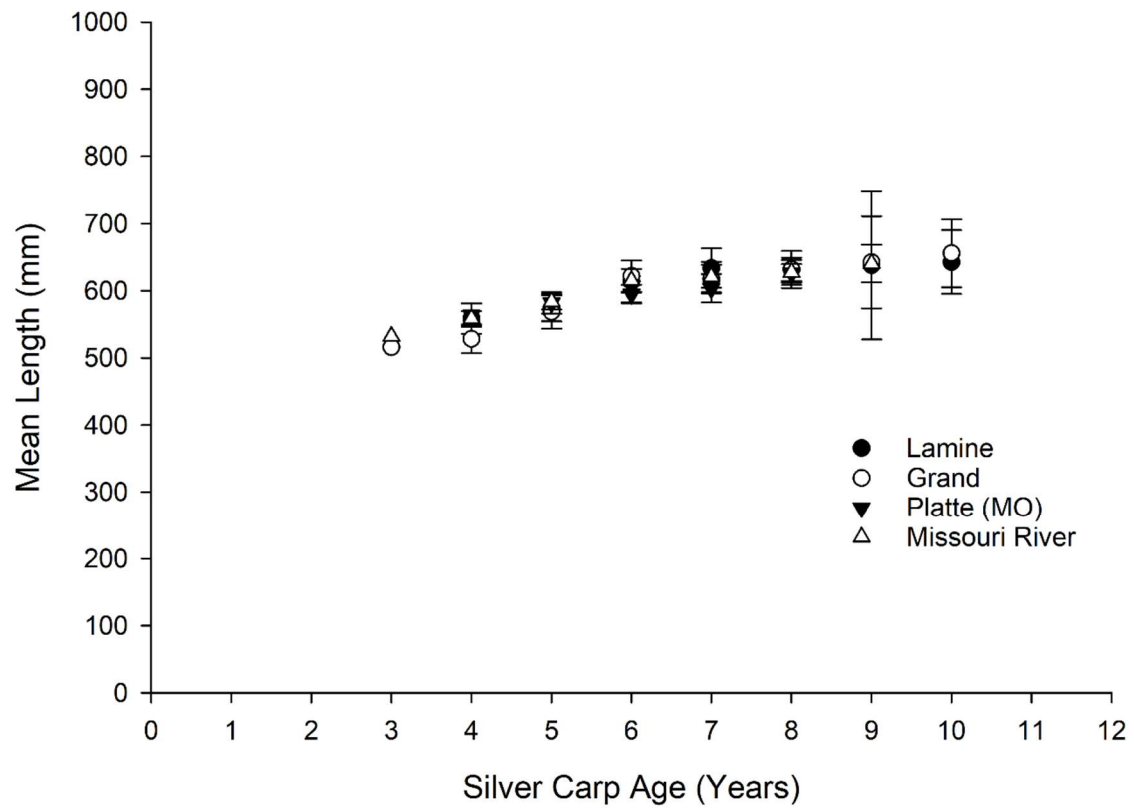


Figure 5. Silver Carp mean length at age $\pm$ 2 standard error for tributaries and Missouri River sites from electrofishing in 2024.

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:

2024 Adult Sampling:

Bigheaded carp were collected from Salt Creek, Papillion Creek, Little Nemaha River, and Big Nemaha River during May to August of 2024 (Figure 1). Sampling gears consisted of herding and boat electrofishing. Herding included the combination (sound and electrofishing) herding technique from 2023 that was determined to most effectively move bigheaded carp in a downstream direction. In addition, attempts to capture fish while herding was employed in 2024 and included the use of passive gill net sets in a 75m capture area. Block nets were used to enclose the upstream end of the site and create a downstream capture area. The downstream block net was set at a 30° angle leaving a gap (~5 m) at the opposite bank. Passive gill netting included surface-to-bottom gill nets (91-m x 12-ft hobbled to 10-ft, 3-in mesh) set in a zig-zag pattern throughout the capture area with one gill net (40- to 60-m x 12-ft hobbled to 10-ft, 3-in mesh) spanning the entire downstream end (Figure 2). Sampling continued until 5 replicates of each gear were completed.

A total of 6,943 fish were caught during May-August of 2024. 6,205 bigheaded carp were captured which equated to approximately 14,079 kg (31,038 lbs). Due to adjustments in sampling methodology, gear comparisons were limited to 5,926 bigheaded carp (5,884 Silver Carp and 42 Bighead Carp) that were captured from June-August. There were 4,374 bigheaded carp (4,342 Silver Carp and 31 Bighead Carp; Table 1) collected with herding and 1,553 bigheaded carp (1,542 Silver Carp and 11 Bighead Carp) captured while electrofishing from May to August 2024 (Table 2; Figure 3). Herding resulted in a relatively low amount of bycatch (8%; Table 1). Herding (mean fish/hr = 437.3, 95% CI = 346.4-528.2) was more efficient than electrofishing (mean fish/hr = 155.0, 95% CI = 115.0-195.0) based on gear effort (Figure 4). However, electrofishing was more efficient (mean fish/person-hr = 31.4, 95% CI = 21.2-42.5) than herding (mean fish/person-hr = 15.5, 95% CI = 10.9-20.1) (Figure 5). While herding, the bank-to-bank set gill net at the bottom of the site caught approximately 2x more bigheaded carp (Figure 6). Electrofishing required 1.89-2.02 person hours while herding required 7.08-32.58 person hours each day (Table 3). Therefore, electrofishing was considered to be more cost effective than herding based on weekly cost (Table 4). Electrofishing was shown to encompass a broad size range of Silver Carp while herding tended to capture relatively large fish (Table 5; Figure 7).

Table 1. Count and percentage of species caught while herding during June-August 2024.

Species	Count	Percentage
Silver Carp	4342	90.95
Smallmouth Buffalo	115	2.41
River Carpsucker	87	1.82
Bigmouth Buffalo	58	1.21
Common Carp	55	1.15
Grass Carp	37	0.78
Bighead Carp	31	0.65
Paddlefish	13	0.27
Channel Catfish	6	0.13
Gizzard Shad	6	0.13
Black Buffalo	5	0.10
Longnose Gar	5	0.10
Blue Catfish	4	0.08
Blue Sucker	2	0.04
Freshwater Drum	2	0.04
Flathead Catfish	2	0.04
Quillback	2	0.04
Shortnose Gar	2	0.04

Table 2. Count and percentage of species caught while electrofishing during June-August 2024.

Species	Count	Percentage
Silver Carp	1542	87.81
River Carpsucker	49	2.79
Common Carp	31	1.77
Shortnose Gar	24	1.37
Smallmouth Buffalo	23	1.31
Longnose Gar	20	1.14
Bigmouth Buffalo	17	0.97
Gizzard Shad	15	0.85
Bighead Carp	11	0.63
Freshwater Drum	5	0.28
Channel Catfish	4	0.23
Goldeye	3	0.17
Largemouth Bass	3	0.17
Flathead Catfish	2	0.11
Grass Carp	2	0.11
White Crappie	2	0.11
Black Crappie	1	0.06
Shorthead Redhorse	1	0.06
Walleye	1	0.06

Table 3. Crew size, effort (i.e., run time), assembly time (i.e., deployment and retrieval of gear), and person hours [crew size × (effort + assembly)] required for electrofishing and herding during June-August 2024.

Gear	Crew Size	Effort (min)	Assembly (min)	Person Hours
Electrofishing	3	30	8–10	1.89–2.02
Herding	4–6	30	55–361	7.08–32.58

Table 4. Estimated person hours and costs required to achieve equivalent weekly (Monday–Friday) bigheaded carp catches in mid-order tributaries of the Platte and Missouri Rivers. Herding had a mean daily catch of 219 (weekly = 1,095), while electrofishing had a mean daily catch of 78 (weekly = 390). Electrofishing person hours were adjusted to achieve equivalent catches. Cost per fish was calculated based on cost per week divided by weekly catch (n = 1,095).

Gear	Crew	Person-hr/day	Person-hr/week	Cost/week	Cost/fish
Electrofishing	3	6	30	600	0.55
Herding	4-6	7-33	35-163	735-3300	0.67-3.01

Table 5. Length categories of Bighead Carp and Silver Carp captured while electrofishing and herding during June-August 2024.

Gear	Length Category	Species	Min	Mean (SE)	Max
Electrofishing	> 400 mm	BHC	620	726 (19.21)	825
		SIC	402	587 (1.94)	892
	≤ 400 mm	BHC	-	-	-
		SIC	321	371 (5.07)	400
Herding	> 400 mm	BHC	504	694 (16.70)	940
		SIC	413	623 (1.08)	822
	≤ 400 mm	BHC	-	-	-
		SIC	350	379 (7.74)	393

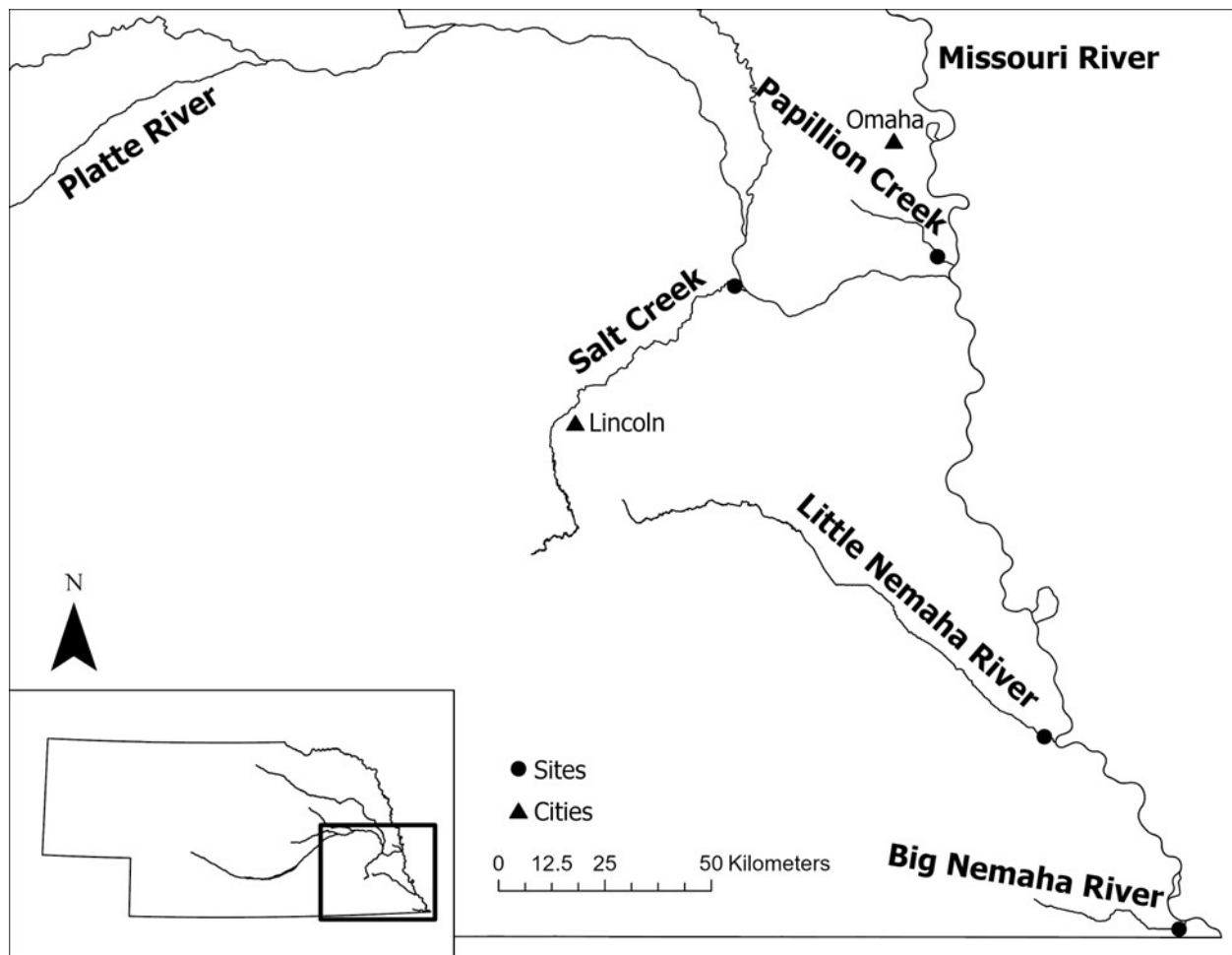


Figure 1. Sites for electrofishing and herding on Salt Creek, Papillion Creek, Little Nemaha River, and Big Nemaha River during 2024.

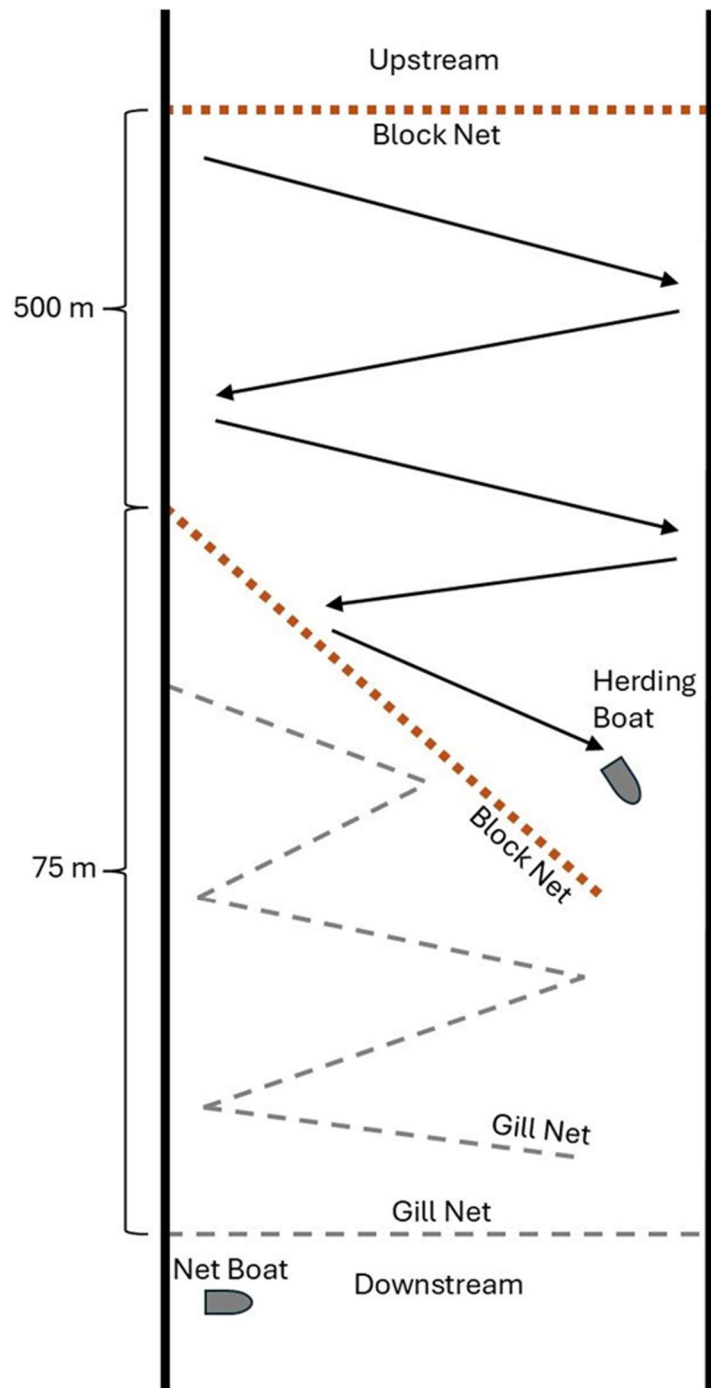


Figure 2. Diagram of herding methods implemented during June-August 2024.

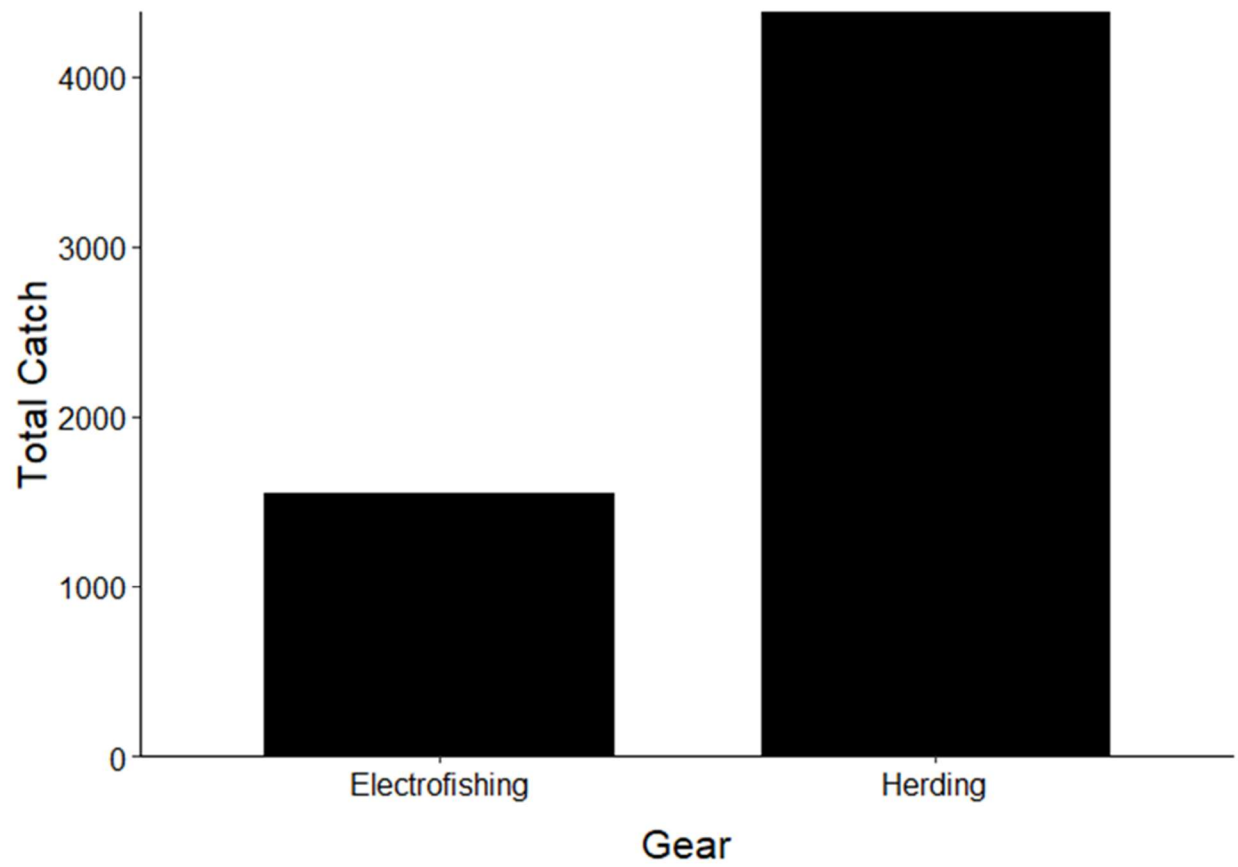


Figure 3. Total amount of bigheaded carp captured by electrofishing and herding during June-August 2024.

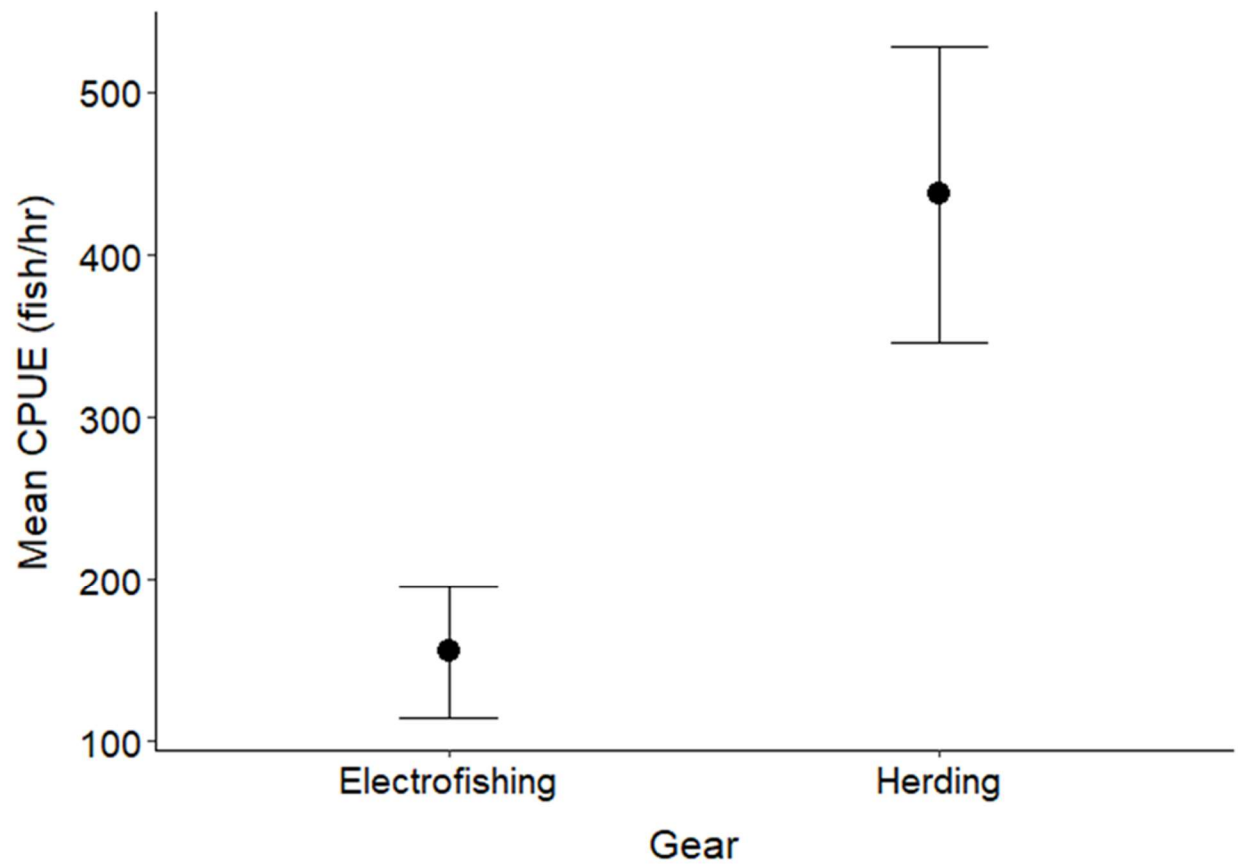


Figure 4. Mean CPUE (fish/hr) and 95% confidence intervals based on gear effort (i.e., run time) for electrofishing and herding during June-August 2024.

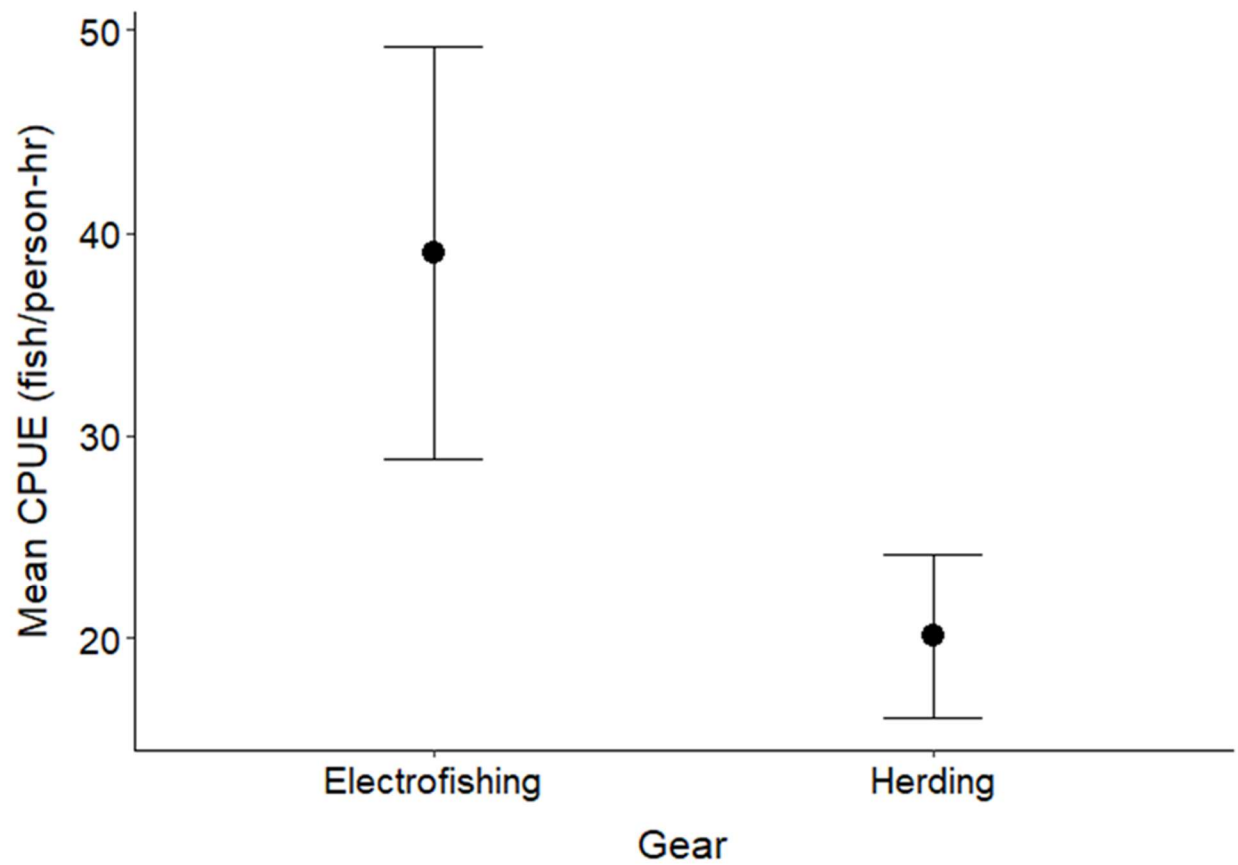


Figure 5. Mean CPUE (fish/person-hr) and 95% confidence intervals based on total effort (person-hr = crew size $\times$ (effort + assembly)) for electrofishing and herding during June-August 2024.

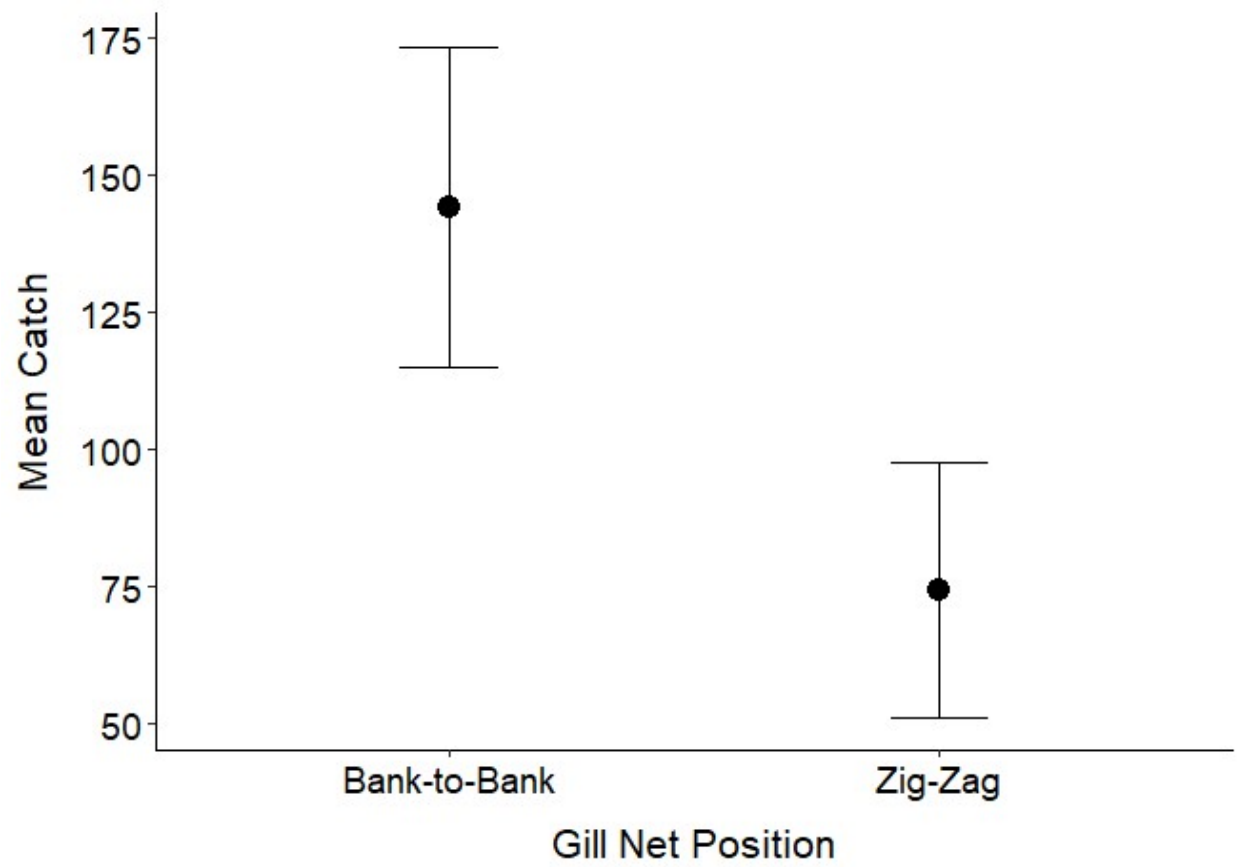


Figure 6. Mean catch and 95% CI for gill net positions while herding during June-August 2024.

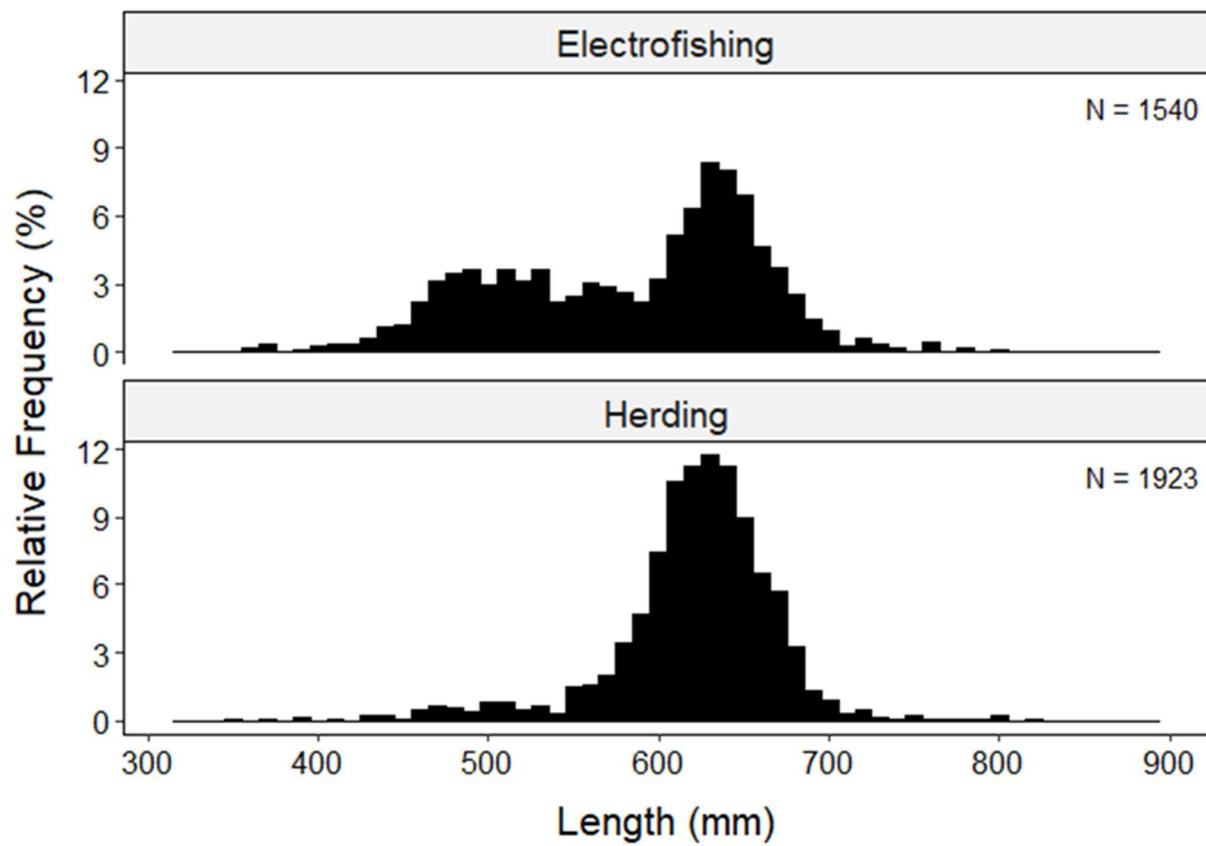


Figure 7. Relative length frequency (%) of Bighead Carp caught while electrofishing and herding during June-August 2024.

2024 AGE-0 SAMPLING:

Electrofishing captured all life-stages (YOY, Juvenile/Age-1, and Adult) of bigheaded carp. Age-0 bigheaded carp were immobilized at times but were primarily captured by dip netting areas where they had been observed near the surface or jumping. Age-0 Silver Carp were first collected on July 8, 2024, on Papillion Creek approximately 3 miles upstream of the confluence with the Missouri River. Schools of age-0 Silver Carp were observed near the surface and congregated near obstructions, such as the boat hull (particularly when anchored to shore). Age-0 Silver Carp were subsequently collected at the Little Nemaha River and Big Nemaha River during mid-July. Similarly, they were observed in numerous schools and quite often jumping in response to disturbances (e.g., adult bigheaded carp movement, predation, boat activity). Age-0 fish were opportunistically collected throughout the summer while conducting sampling for adult fish. This primarily involved using electrofishing dip nets with sufficiently small mesh diameter. The catchability of age-0 fish declined throughout the summer with a notable decrease in abundance during late-August. All age-0 bigheaded carp collected during adult sampling were preserved.

Seining was conducted during the summer and fall to locate areas of recruitment for bigheaded carp. 188 seine hauls were completed across 20 sites during 2023-2024 (Figure 8). A total of 23,272 fish and 38 species were captured (Table 6). No age-0 bigheaded carp were collected in 2023. There were 146 age-0 Silver Carp collected from the mouth of the Elkhorn River, the Platte River at Louisville and Plattsmouth, Papillion Creek near Fort Crook Rd, Big Nemaha River, and Little Nemaha River during 2024. Age-0 Silver Carp ranged from 29-81 mm in length.

Table 6. Frequency of species collected while seining for age-0 bigheaded carp during September-October of 2023 and July-September 2024.

Common Name	Scientific Name	Frequency
Bigmouth Shiner	<i>Notropis dorsalis</i>	40
Black Crappie	<i>Pomoxis nigromaculatus</i>	7
Bluegill	<i>Lepomis macrochirus</i>	153
Brassy Minnow	<i>Hybognathus hankinsoni</i>	33
Brook Silverside	<i>Labidesthes sicculus</i>	199
Channel Catfish	<i>Ictalurus punctatus</i>	195
Common Carp	<i>Cyprinus carpio</i>	5
Creek Chub	<i>Semotilus atromaculatus</i>	1
Emerald Shiner	<i>Notropis atherinoides</i>	1183
Fathead Minnow	<i>Pimephales promelas</i>	1144
Freshwater Drum	<i>Aplodinotus grunniens</i>	60
Gizzard Shad	<i>Dorosoma cepedianum</i>	460
Green Sunfish	<i>Lepomis cyanellus</i>	21
Iowa Darter	<i>Etheostoma exile</i>	1
Johnny Darter	<i>Etheostoma nigrum</i>	14
Largemouth Bass	<i>Micropterus salmoides</i>	20
Longnose Gar	<i>Lepisosteus osseus</i>	8
Orangespotted Sunfish	<i>Lepomis humilus</i>	45
Northern Plains Killifish	<i>Fundulus kansae</i>	42
Plains Minnow	<i>Hybognathus placitus</i>	162
Plains Topminnow	<i>Fundulus sciadicus</i>	1
Quillback	<i>Carpodes cyprinus</i>	20
Red Shiner	<i>Cyprinella lutrensis</i>	9883
River Carpsucker	<i>Carpodes carpio</i>	700
River Shiner	<i>Notropis blennioides</i>	2951
Sand Shiner	<i>Notropis stramineus</i>	5045
Shoal Chub	<i>Macrhybopsis hyostoma</i>	9
Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>	1
Shortnose Gar	<i>Lepisosteus platostomus</i>	11
Silver Carp	<i>Hypophthalmichthys molitrix</i>	149
Silver Chub	<i>Macrhybopsis storeriana</i>	12
Suckermouth Minnow	<i>Phenacobius mirabilis</i>	12
Western Mosquitofish	<i>Gambusia affinis</i>	582
Western Silvery Minnow	<i>Hybognathus argyritus</i>	3
White Bass	<i>Morone chrysops</i>	1
White Crappie	<i>Pomoxis annularis</i>	10
White Perch	<i>Morone americana</i>	10
	<i>Hybognathus</i> spp.	73
	<i>Notropis</i> spp.	5

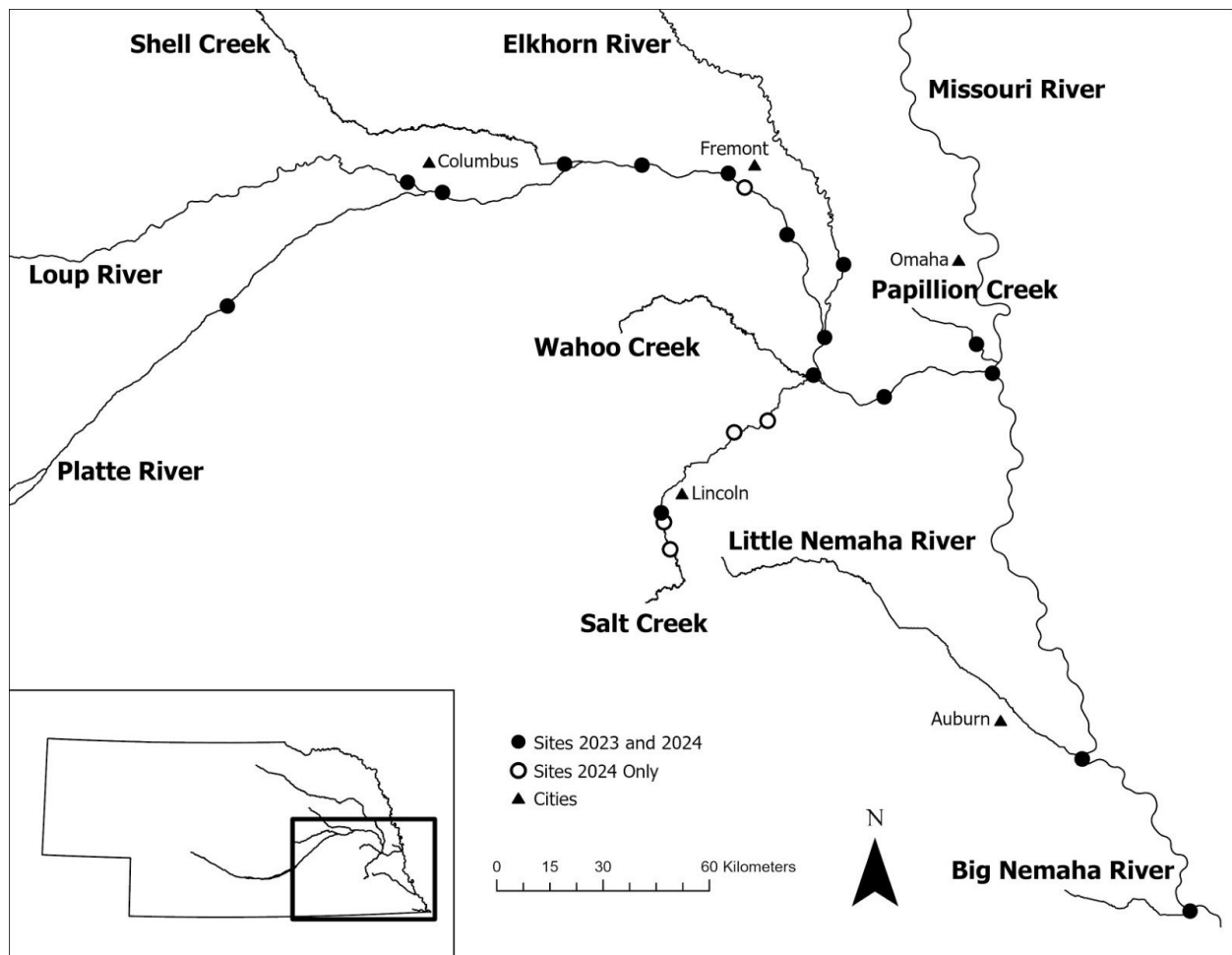


Figure 8. Sites located across the Lower Platte River and Big Nemaha River basins in eastern Nebraska where seining for age-0 bigheaded carp occurred during September-October of 2023 and July-September 2024.

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 and 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 1). Further, there appears to be a substantial difference in mean Ba:Ca ratios between the Big Sioux River and the James and Vermillion rivers (Figure 1). Mean $\delta^{18}\text{O}$ ratios had substantial overlap among the three eastern SD tributaries, but the Missouri River signatures were substantially lower (Figure 2).

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

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 3). Additionally, the limited analysis of Ba:Ca molar ratios appears to demonstrate that differentiation of the Big Sioux and James rivers could be possible (Figure 4). Results from the laboratory for the remaining samples were received in late 2024, so data analysis will be completed in 2025. Finalized data analysis will be presented in a future Missouri River basin invasive carp annual technical report.

Tables and Figures:

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

River	Site	<i>$^{87}\text{Sr}/^{86}\text{Sr}$</i>	<i>$\pm 2\text{SE}$ (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

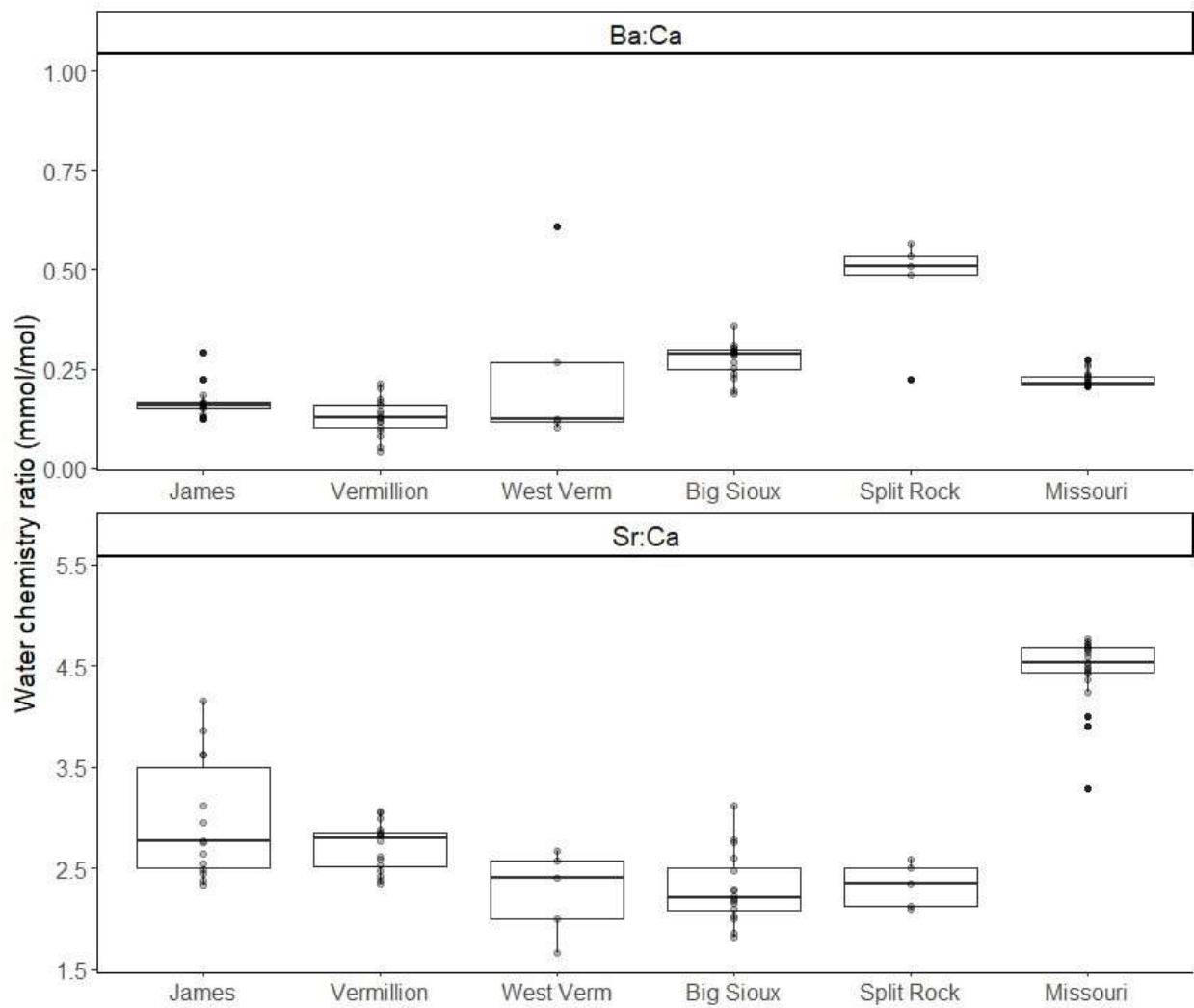


Figure 1. Boxplots of water Sr:Ca and Ba:Ca ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021-2023.

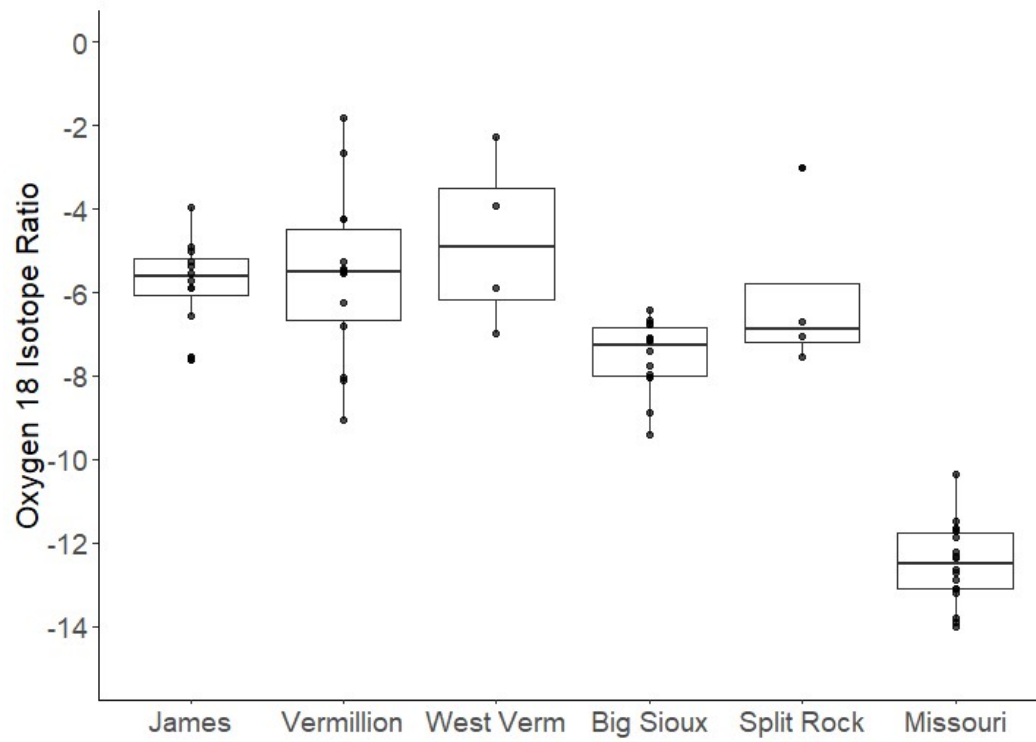


Figure 2. Boxplot of water oxygen-18 ($\delta^{18}\text{O}$) isotope ratios for three eastern SD tributaries and the Missouri River from samples collected in 2021-2023.

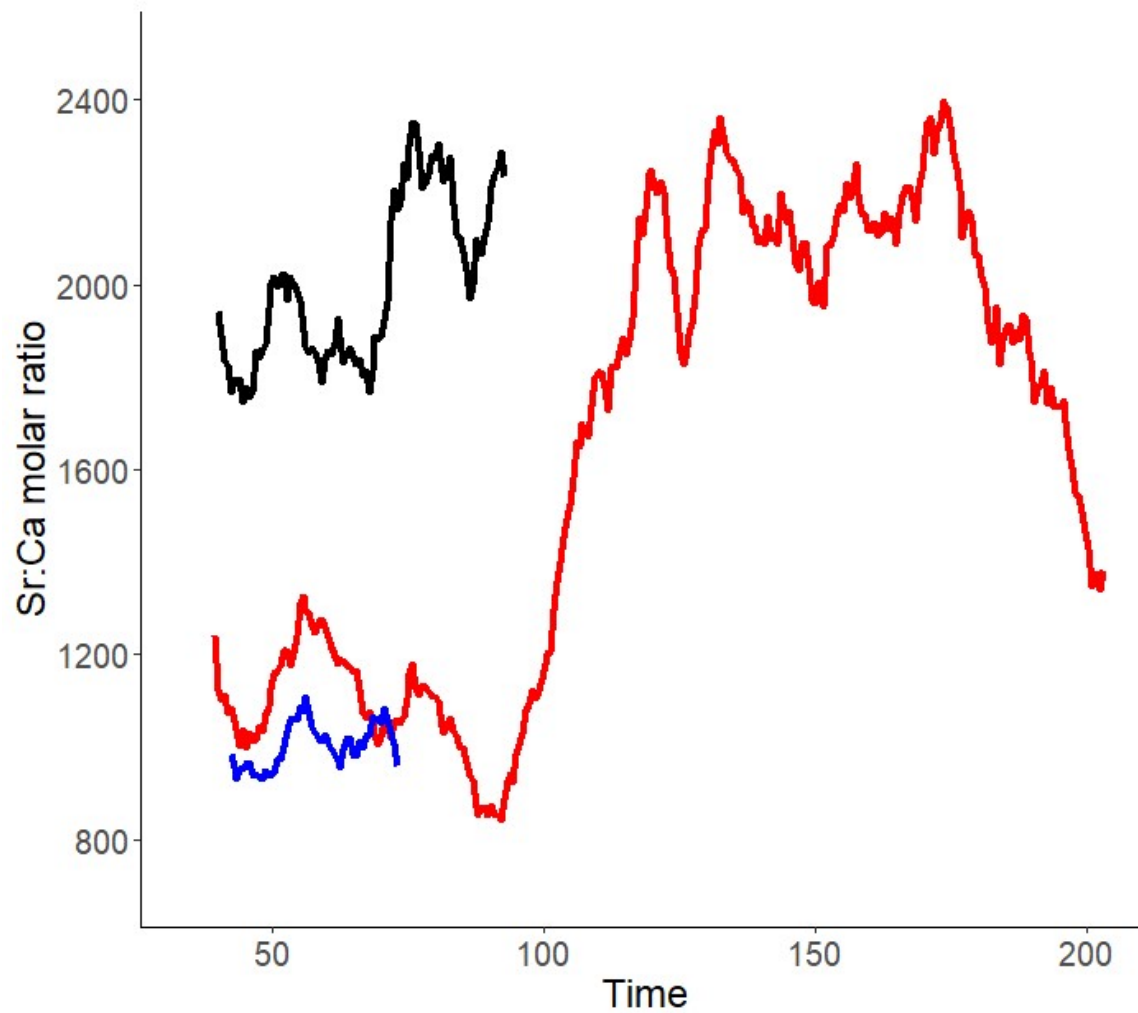


Figure 3. Strontium-to-calcium (Sr:Ca) molar ratio (mmol/mol) for Silver Carp lapillus otoliths collected from a juvenile collected in the Missouri River in July 2020 (black), an age-0 from the James River in October 2022 (blue), and an adult collected in the James River in May 2022 (red).

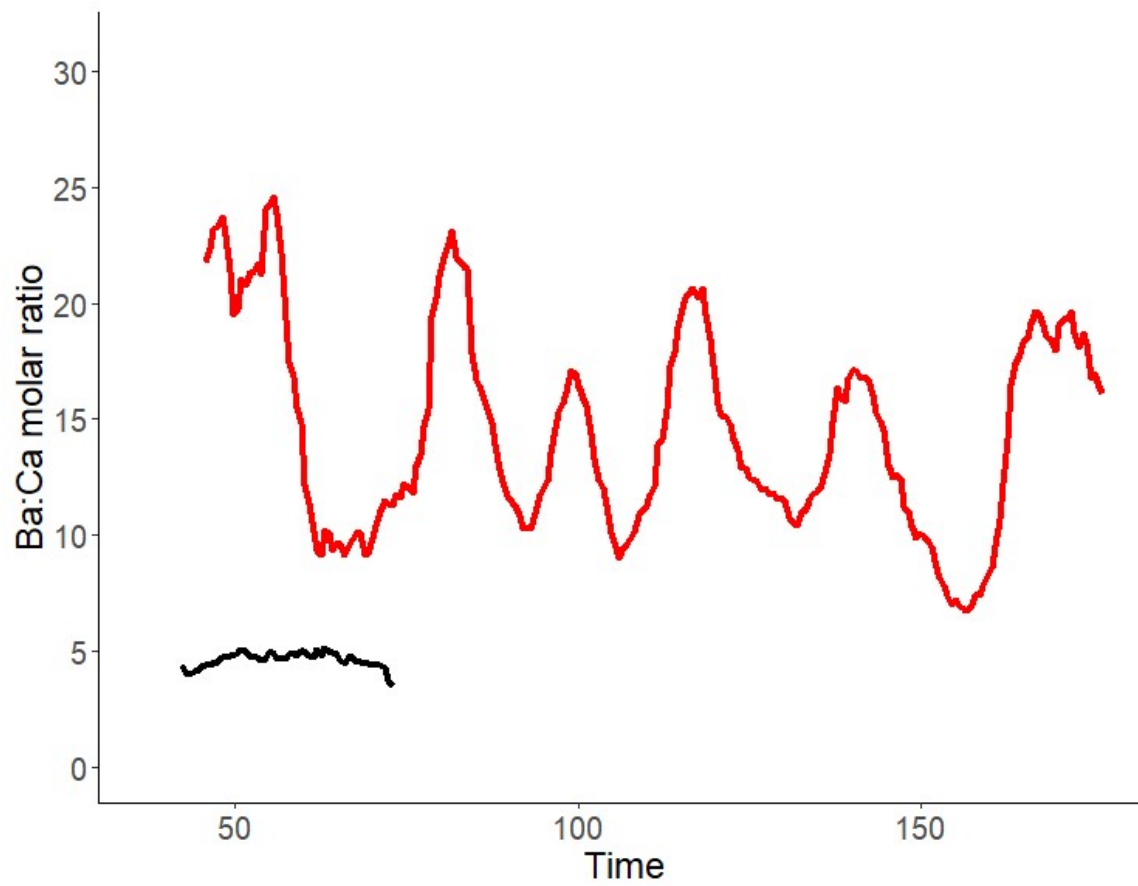


Figure 4. Barium-to-calcium (Ba:Ca) molar ratio (mmol/mol) for Silver Carp lapillus otoliths collected from a juvenile collected in the Big Sioux River in June 2020 (Red) and an age-0 from the James River in October 2022 (black).

State Report: South Dakota

Agency: South Dakota Game, Fish and Parks, South Dakota State University

Project Title: Using otolith microchemistry to identify the natal origin and movement of invasive carp in the Missouri River Basin

Methods:

Objective 2 (Sub-objective 4): SDGFP in conjunction with SDSU will work to determine invasive carp recruitment sources throughout the Missouri River Basin. This work will build upon a prior study by Southern Illinois University that identified differences in water chemistry throughout the Missouri River Basin (stable oxygen isotope ratio and strontium, barium, and calcium concentrations). Since otoliths are chemically inert, the chemical composition of the environment is collected in otoliths as fish grow and move among locations with different water chemistry. We can then analyze the chemical composition from the core of an adult's otolith to identify the location where it was spawned. This allows us to determine whether adults collected from a location were produced in that area or if they were spawned in another location and subsequently moved later in life. This knowledge can identify potential locations from which adult Missouri River Basin invasive carp are recruiting while informing source-sink dynamics and decisions regarding the location of management actions (e.g., placement of movement deterrents or removal effort).

Otolith microchemistry can provide an important tool for determining the natal origin of freshwater fishes from environments with distinct water chemistries (Campana et al 2000; Gibson-Reinemer et al. 2009; Zitek et al. 2010). Water samples were previously collected and analyzed from the Missouri River main channel and tributaries (Listed in Table 1 of Whitledge 2022). This prior study indicated that many tributaries and main channel reaches have distinct chemical signatures that can be used to identify the natal origins of invasive carp (Whitledge 2022). To account for possible changes in water chemical signatures between this study and prior data reported by Whitledge (2022), 46 additional water samples will be collected and analyzed for trace elements (barium, Ba; strontium, Sr; and calcium, Ca) and oxygen isotope ratios ($\delta^{18}\text{O}$). These water samples will be collected using 250-mL, acid-washed, polyethylene bottles that are pre-rinsed with river water, filtered through a Whatman Puradisc PP filter (0.45 μm), and stored in sealed, acid-washed polyethylene bottles. Trace elements of Ba, Sr, and Ca will be analyzed using inductively-coupled mass spectrometry (ICP-MS) at Southern Illinois University. Water samples will be sent to the University of Arizona for stable oxygen isotope ratio ($\delta^{18}\text{O}$) analysis.

In 2024, a maximum of 75 adult Silver Carp and Bighead Carp were collected from up to 23 locations (tributaries and main channel reaches) throughout the Missouri River Basin in collaboration with state partners (maximum 1,725 otoliths). Lapilli otoliths will be removed from these individuals and processed for trace element analysis. Otoliths will be mounted in epoxy, and transversely sectioned to create a thin section exposing the nucleus, and thin sections will be mounted to a glass slide. With one otolith, laser-

ablation ICP-MS will be used along a transect of the otolith beginning at the nucleus and ending at the margin to measure chemical signatures throughout the lifespan of the fish. Microchemistry will be analyzed for strontium, barium, and calcium concentrations. If needed to differentiate the origin, a core sample will be extracted from the second otolith using micro-milling and sent to the University of Arizona for $\delta^{18}\text{O}$ analysis. Chemical composition data will ultimately determine the proportion of adults present at a location that was spawned in that location versus the proportion of adults spawned in other specific tributaries or main channel reaches. These results will also allow us to quantify the amount of movement occurring among tributaries and main channel reaches a temporal scale that complements ongoing telemetry work and spawning assessments in the basin.

The natal origin of Silver Carp will be evaluated by comparing trace-elemental markers and their ratios (Sr:Ca, Ba:Ca) measured from otoliths to chemical signatures from water samples. To screen for potential markers allowing the separation of chemical signatures from different rivers, we will use correlation analysis to evaluate relationships between element concentrations in water samples and otoliths. Trace elements and $\delta^{18}\text{O}$ in lapilli otoliths that are correlated to water concentrations will be included in a model-based discriminant function or cluster analysis, such as *k*-nearest neighbor (KNN; Rosing et al. 1998). To evaluate classification error for assigning natal origin to carp, we will use a cross-validation approach. Age-0 Silver Carp may be targeted from tributaries across the basin to validate results from otolith microchemistry. Microchemical signatures from the otoliths of these age-0 individuals from known locations may be used as a training dataset for discriminant function analysis alongside the available water data (Hegg and Kennedy 2021).

Results and Discussion:

Water samples were collected from 21 locations to add to previously collected data (Whitledge 2022). Water samples were sent to the University of Southern Mississippi for processing of Ba, Sr, Ca, and $\delta^{18}\text{O}$ and results are expected in early 2025. Additional water samples will be collected in 2025 to account for the site-level variation in these metrics. Otoliths were collected from multiple partner agencies (i.e., the U.S. Fish & Wildlife Service - Columbia Fish and Wildlife Conservation Office, Iowa Department of Natural Resources, Kansas Department of Wildlife and Parks, and Missouri Department of Conservation) averaging 5 – 92 otoliths from 13 out of the 23 target locations. Otoliths were subsetting to represent individuals across different lengths available at each site. We also ensured we had multiple years represented and equal representation of males and females where feasible. Otoliths could not be obtained from 6 locations (Big Nemaha, Gasconade, Hinkson, Loutre, Missouri River – Nebraska, and Platte – Nebraska) due to sampling limitations (e.g., low water levels). We excluded the Niobrara River as it does not contain Silver Carp or Bighead Carp. We will also incorporate previously collected data from the James River, Vermillion River, and several Nebraska Rivers into future analyses. For microchemistry analysis validation, Age-0 Silver Carp were obtained from the following locations: Vermillion River, James River, Kansas River, Nishnabotna River, Lamine River, Grand River, and the Platte River. Otoliths have been mounted in epoxy and are currently being sectioned. Otolith microchemistry will be processed summer 2025.

Recommendation:

Continue to collect new otoliths through time and curate existing collections so that changes in reproductive contributions among locations can be tracked through time.

Tables and Figures:

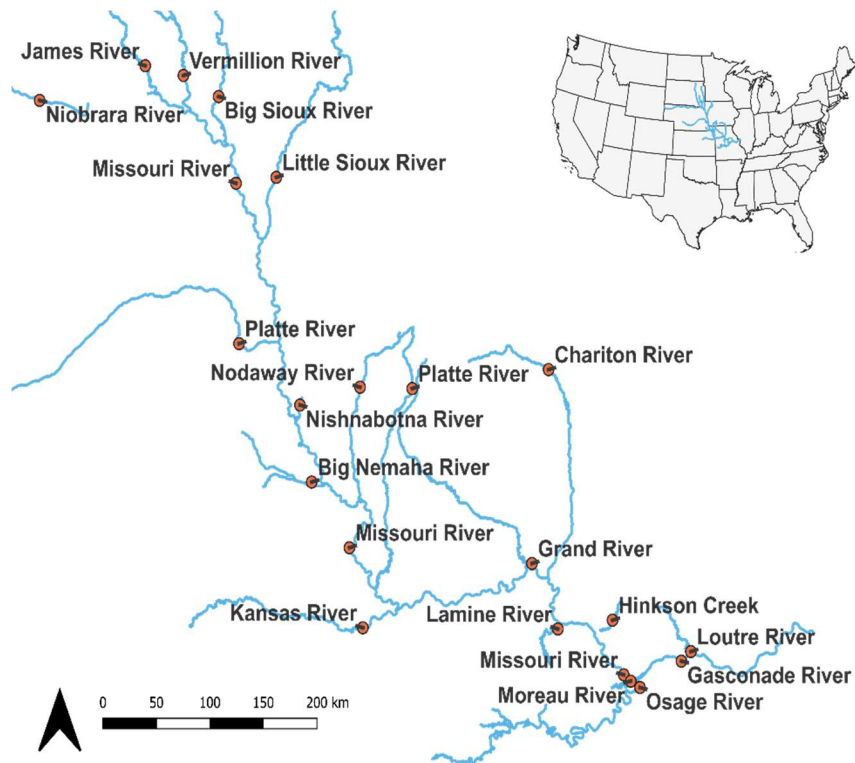


Figure 1. Map of study area, showing the locations of otoliths and water sample locations in the main reaches and tributaries of the Missouri River.

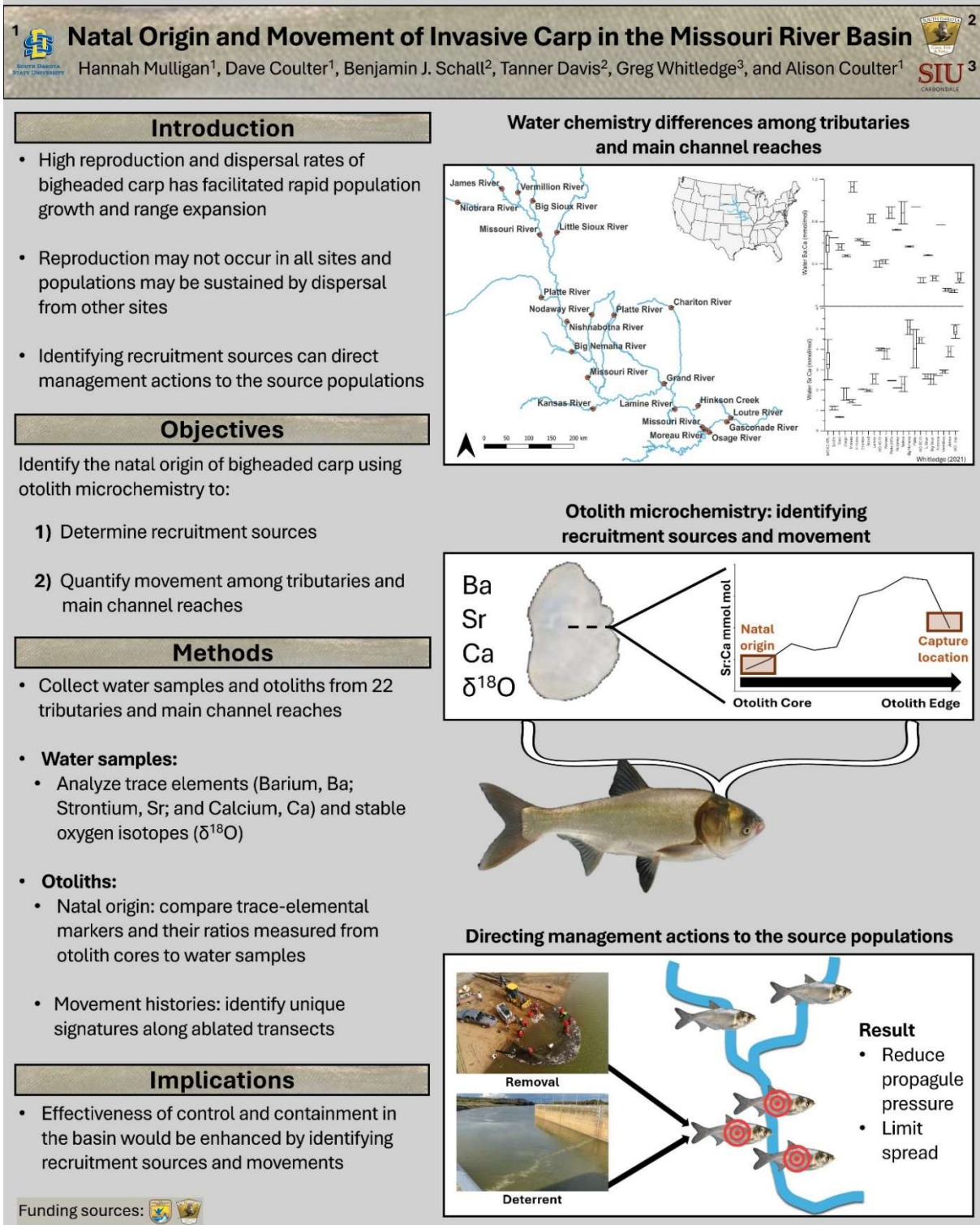


Figure 2. Poster overviewing study plan that was presented at Midwest Fish and Wildlife Conference.

State Report:**Agency:** USFWS**Project Title:** Missouri River Tributary Invasive Carp Population Assessment**Methods:***Data Collection*

In 2024, USFWS sampled nine tributaries along the lower Missouri River with an electrified dozer trawl (Hammen et al. 2019; Drews 2020). The large tributaries sampled were the James River, Vermillion River, Big Sioux River, Nishnabotna River, Kansas River, Grand River, Lamine River, and the two small tributaries were Perche Creek and Platte River, MO. Each year, two small tributaries are randomly selected to be sampled from the four following: 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 of the smaller tributaries) 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 started 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 two sample events throughout the fall. For the small tributaries, Perche Creek and Platte River MO, five transects were conducted across two sample events due to available sampleable habitat. 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 Silver Carp sampled per tributary. For the smaller tributaries, the lapilli otoliths were extracted from the first 50 Silver Carp sampled. After the first 100 or 50 otoliths were collected, additional otoliths were collected to fill length bin requirements (20 Silver Carp per 50 mm length bin) for each tributary. All otoliths are extracted from Bighead Carp due to low amount captured. Silver Carp that jumped into the boat during standard sampling (hereafter, “volunteers”) were not used in calculating relative abundances but were used in length-frequencies and growth models if their size was outside what was commonly collected.

Otoliths were stored in coin envelopes during collection then transferred to the Columbia FWCO 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 ($\geq 250\text{mm}$; 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. Relative weight was calculated for all stock-sized Silver Carp using the observed weight and a 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) to evaluate fish growth over time, differences in growth among tributaries, and to estimate annual mortality using all years of data. Any Silver Carp collected less than stock length was assumed to be age-0 and was used in the growth models. 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. The von Bertalanffy parameters, L_{inf} (cm) and K , are used to calculate mortality. Instantaneous annual mortality (M_{est}) was calculated using the updated Pauly (1980) empirical mortality estimator described in Then et al. (2015) as:

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

To have comparable information, only tributaries outlined in the updated protocols were used for demographic comparisons across years. Furthermore, due to protocol updates in otolith collection, only age estimates from 2022 to 2024 are used for age frequencies. Prior to 2022, otoliths were collected in bin intervals and not representative of the sampleable population. All analyses were done in R Studio (R Core Team 2024).

Results and Discussion

In 2024, 147 electrified dozer trawls transects were completed across all tributaries, collecting a total of 7,748 fish (Table 1). All tributaries met the target distance of sampling for each sampling event except for the Big Sioux, Vermillion, and James rivers due to water levels or barriers (Table 1). Sampling efforts collected 38 unique species, with the most prevalent species being Silver Carp, Gizzard Shad, and Emerald Shiner (Appendix A). A total of 2,975 Silver Carp (38% of total catch) were sampled across all tributaries, with 920 Silver Carp (31% of Silver Carp) assumed age-0 due to lengths being less than 120 mm (Table 1). In 2024, only three Bighead Carp were sampled, which is fewer than the number caught in all previous years of sampling.

While the assessment of relative abundance across all tributaries within a given year exhibits variability, analyzing relative abundance over multiple years within a single tributary can display trends over time.

In 2024, Silver Carp relative abundance varied across all tributaries, with the CPUE ranging anywhere from 60 to 305 of Silver Carp per hour (Table 1). Relative abundance variability is also shown within a tributary throughout time. From 2020 to 2024, the Platte, Nishnabotna, Big Sioux, Vermillion, and James rivers displayed fluctuations in their annual CPUE (Figure 1) likely due to temporal variability in environmental and hydrological conditions, catchability, location within the Missouri River basin, or the quantity of Silver Carp sampled affecting transect times. Comparatively, Perche Creek, Lamine River, and Grand River appear to have a declining trend in Silver Carp CPUE through time whereas the Kansas River has had consistent or slightly increasing annual CPUE (Figure 1). Regardless, CPUE in the tributaries have remained consistently high across all years of sampling. Further exploration into other habitats is needed to begin to explain spatial trends in the system.

In 2024, the total length of Silver Carp ranged from 15 to 880 mm with an average length of 545 mm (SE = 3.5 mm). The average length of Silver Carp has increased annually since 2020 except for 2024, likely due to a large proportion of substock sized individuals sampled ($N = 920$; Table 1). Substock Silver Carp (mean TL = 54 mm, SE TL = 1 mm) were collected in every tributary in 2024 except for the Big Sioux and James rivers, with the majority of substock individuals being sampled in the Lamine River (Table 1; Figure 2-3). Because these substock Silver Carp were sampled across a large spatial range, this indicated that a large spawning event occurred within the basin and further monitoring will help determine if those fish recruit to the population. The last large recruitment event within the basin was in 2019 and that cohort is still present in the tributaries. The growth of the 2019 cohort can be tracked through time by the increasing size distribution as seen in Perche Creek, Lamine River and the Grand River (Figure 2). However, the size distributions of Silver Carp in the Kansas, Platte, Nishnabotna, Big Sioux, Vermillion, and James rivers have remained stable throughout time with the majority of Silver Carp sampled within the 500-600 mm length bins (Figure 2-3).

The relative weight of Silver Carp in 2024 ranged from 92 to 106 and was below average for all tributaries except for the Nishnabotna, Big Sioux, and James rivers (Figure 4). From 2020 to 2024, only the Kansas, Nishnabotna, Big Sioux, and the James rivers have had the average relative weight meet or exceed the average condition benchmark of 100 at least once. The variability of relative weight within a tributary needs further exploration.

A total of 795 Silver Carp otoliths were aged in 2024 which is similar to the amount aged in the prior two years. In 2024, 920 Silver Carp that measured less than 120 mm were collected and assumed to be age-0. Due to the quantity of age-0 fish, they are not shown on the age frequency histograms so they do not disproportionately skew data but are instead noted with an asterisk (Figure 5-6). Continued sampling will show if Silver Carp spawned in 2024 will recruit to the population. In 2024, Silver Carp ranged from 3 to 12 years old when excluding age-0 fish, with the majority (27%) of Silver Carp at age-5, likely from the 2019-year class. Furthermore, the age data suggests variable recruitment of Silver Carp with strong and weak cohorts across tributaries with a notable strong cohort being from 2019 (Figure 5-6).

The 2019 cohort is present in all the tributaries and the growth of that cohort can be tracked through time (Figure 5-6). Additionally, this large year class has also been documented in other basins (MICRA 2024; MICRA 2023). Since then, there has been localized spawning events within the tributaries, but no

large recruitment event has occurred since 2019. Although the 2019-year class is prevalent in the system, older Silver Carp from other year classes have been consistently collected and can comprise the majority of the sample throughout time as seen in the Kansas, Big Sioux, and Vermillion rivers (Figure 5-6). Tributaries starting with the Kansas River and above tend to have higher proportion of older Silver Carp than the tributaries south of Kansas River (Figure 5-6). Missouri River tributaries are subject to strong cohorts in the system and more research within the basin is needed to understand what factors drive these large recruitment events and how management could exploit the population-level impact.

Tributary-specific von Bertalanffy growth models showed variation. Estimates of L_{inf} ranged from 615 to 919 mm, K estimates ranged from 0.11 to 0.47, and t_0 estimates ranged from -2.41 to -0.22 (Table 2). Low sample sizes and length variations within age classes (Figure 7) in the growth models will influence the parameters; therefore, the estimates should be interpreted with caution. The instantaneous annual mortality estimates of each tributary using the growth parameters ranged from 0.18 to 0.59 (Table 2). No spatial trends in growth parameters or mortality estimates are present, but further data collection in other habitats will improve estimates.

In summary, the 2024 sampling efforts provided data to further our understanding of patterns of Silver Carp population demographics within and across Missouri River tributaries. Tributaries at more northern latitudes appeared to have slightly larger and older Silver Carp relative to other tributaries. Age data showed large periodic recruitment events and the impact they have on the overall populations. Furthermore, all of the tributaries have high densities of Silver Carp likely due to their ideal habitat conditions and resource availability. Because of the high densities and hydrology, the tributaries have been the target of localized removals over the past few years. Further exploration into other gear types for conducting standardized sampling or habitats to be sampled as part of a population assessment is needed to further describe Silver Carp populations to inform management decisions. As incentivized harvest begins on the Missouri River through a Missouri Department of Conservation program that began in November 2024, reliable Silver Carp population assessments in additional habitats will further explain spatial trends observed in the tributaries, provide a holistic view of the basin, and guide where management can have the most impact.

Recommendations

Add stratified random sampling in other habitats as available (e.g., mainstem, side channel, backwater) within the basin to supplement demographic information and inform where management actions should occur.

Synthesize data from population assessments, movement studies, microchemistry evaluations, and other sources from across the Missouri River Basin to better understand metapopulation dynamics and guide management efforts.

Tables and Figures

Table 1. Summary of 2024 Missouri River tributary confluence sampling for Silver Carp (SVCP) using an electrified dozer trawl. Tributaries are in ascending order based off their confluence location on the Missouri River. A summary of total fish collected, total number of Silver Carp, number of substock Silver Carp ($\leq 250\text{mm}$), length range of Silver Carp, and total number of trawls completed in each tributary over two sampling events. An asterisk indicates the target number of trawls was not met in that tributary. Average catch per unit effort (CPUE) of stock or larger SVCP is reported as SVCP per hour with standard error in parentheses as well as average relative standard error (RSE) of SVCP CPUE for all tributaries.

Waterbody	RM	Total Trawls (N)	All Fish (N)	SVCP (N)	Substock SVCP (N)	CPUE (SVCP/hr)	CPUE RSE	Min -Max TL (mm)
Perche Creek	170	10	464	73	3	84 (12)	14	72 - 756
Lamine River	202	20	1,093	754	654	60 (9)	15	15 - 850
Grand River	250	20	312	137	25	67 (13)	19	35 - 755
Kansas River	367	20	864	295	34	156 (26)	17	44 - 794
Platte River MO	391	10	323	133	1	158 (26)	16	46 - 756
Nishnabotna River	542	20	1,316	478	10	305 (65)	21	49 - 839
Big Sioux River	734	14*	542	210	0	280 (56)	20	417 - 765
Vermillion River	772	17*	994	539	193	290 (68)	23	22 - 738
James River	800	15*	1,760	334	0	297 (54)	18	418 - 880
Summary		147	7,748	2,975	920	189 (35)	19	15 - 880

Table 2. Growth estimation parameters (L_{inf} , K , and t_0) from von Bertalanffy growth models and instantaneous annual mortality (M_{est}) estimates for each tributary. All Silver Carp were collected 2020-2024 and the total number of individuals aged per tributary to estimate growth and mortality are listed under SVCP.

Waterbody	SVCP	L_{inf}	K	t_0	M_{est}
Perche Creek	230	919	0.11	-2.41	0.18
Lamine Rver	541	628	0.43	-0.23	0.56
Grand River	397	643	0.35	-0.60	0.47
Kansas River	475	650	0.47	-0.25	0.59
Platte River MO	282	654	0.32	-1.01	0.44
Nishnabotna River	434	705	0.31	-0.67	0.43
Big Sioux River	437	655	0.42	-0.22	0.55
Vermillion River	463	615	0.46	-0.24	0.59
James River	408	652	0.34	-0.90	0.47

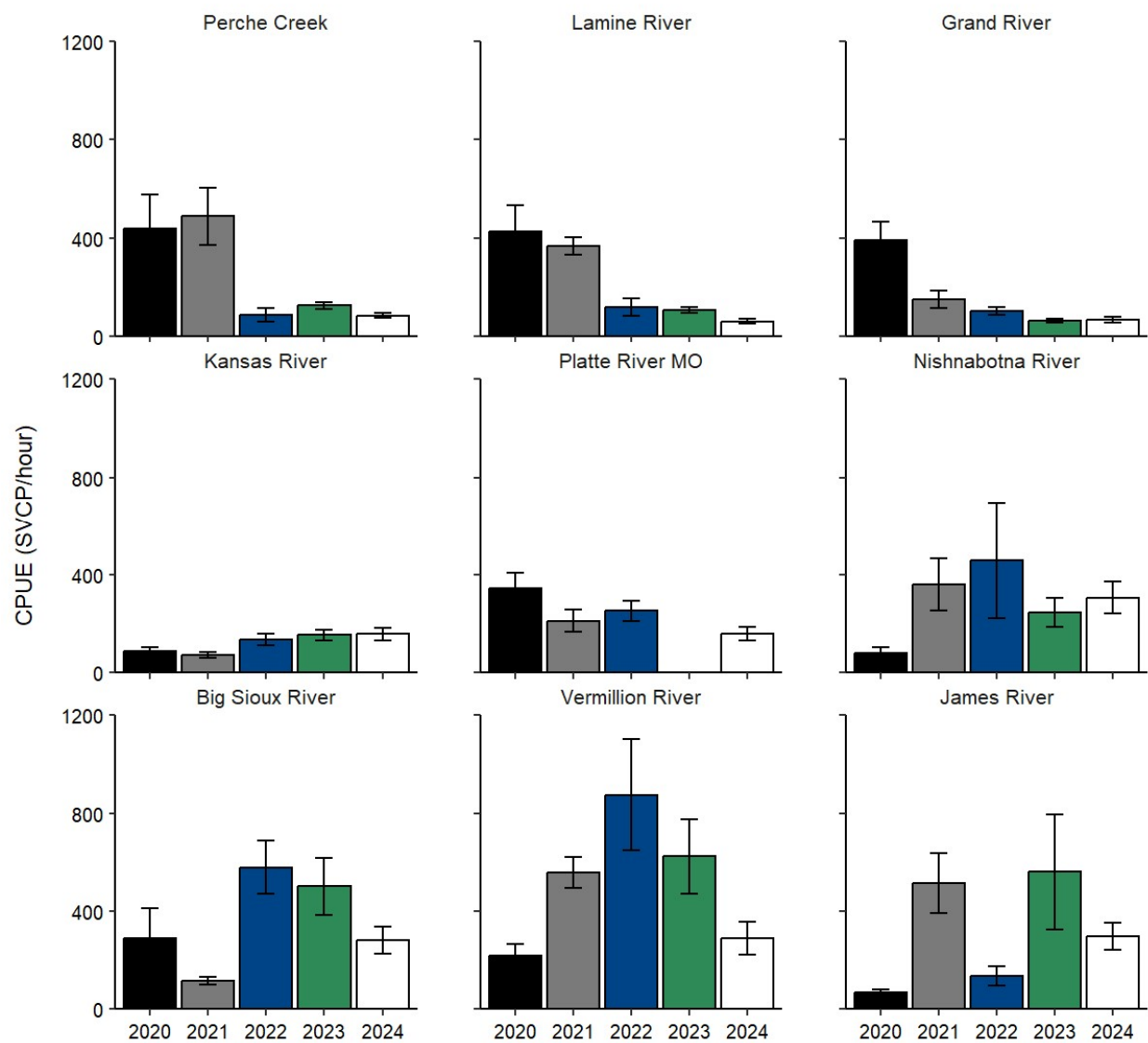


Figure 1. Average catch per unit effort (CPUE; SVCP/hour) with standard errors of Silver Carp sampled with an electrified dozer trawl in each tributary by year, 2020-2024.

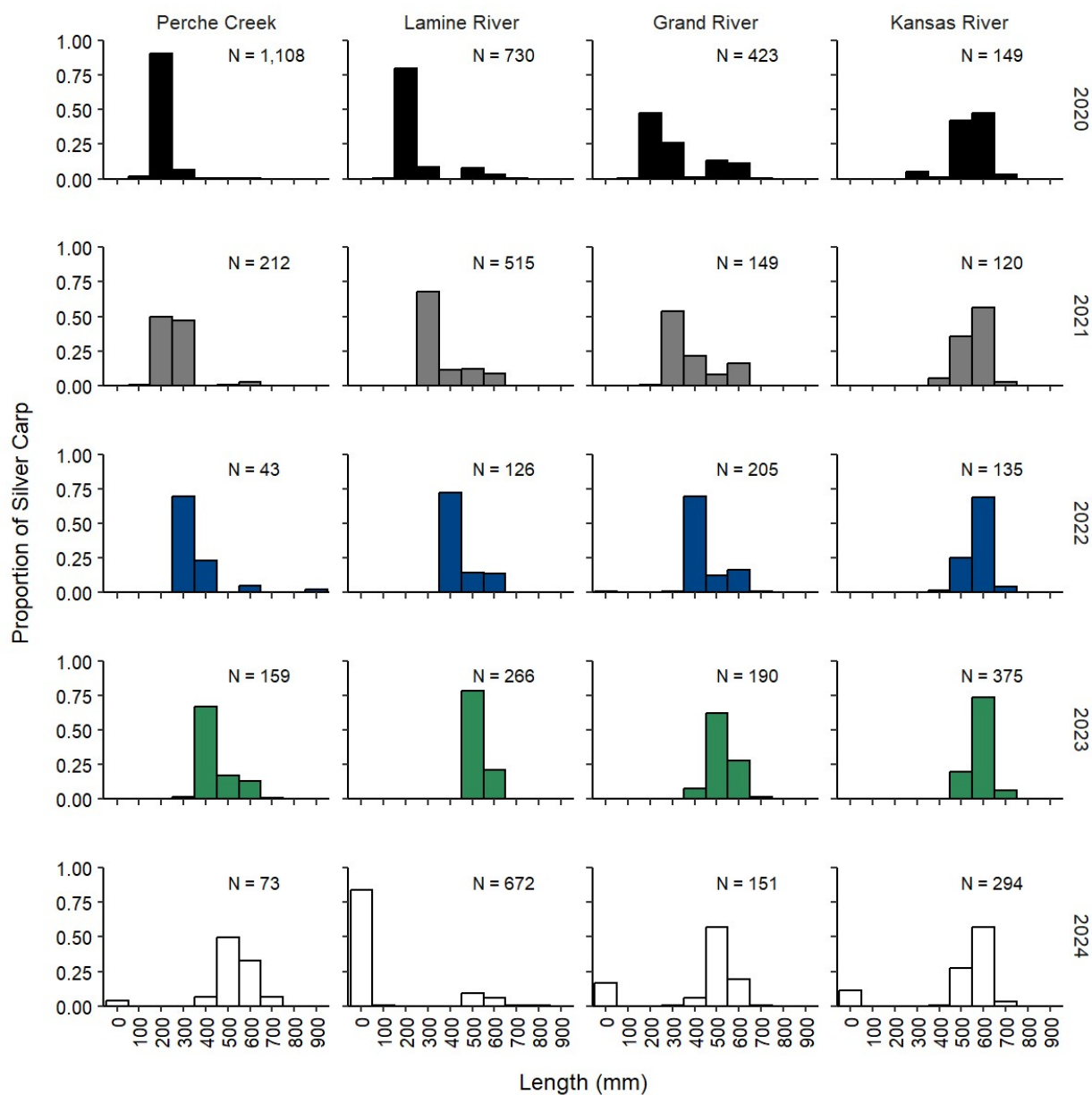


Figure 2. Relative length frequency distribution by 100 mm length bin of Silver Carp captured with an electrified dozer trawl in each tributary by year, 2020-2024. The number (N) of Silver Carp includes all lengths collected during standard protocols, SVCP volunteers, and extra samples to collect age data; therefore, the totals may not match the totals listed in Table 1 that were used to calculate CPUE.

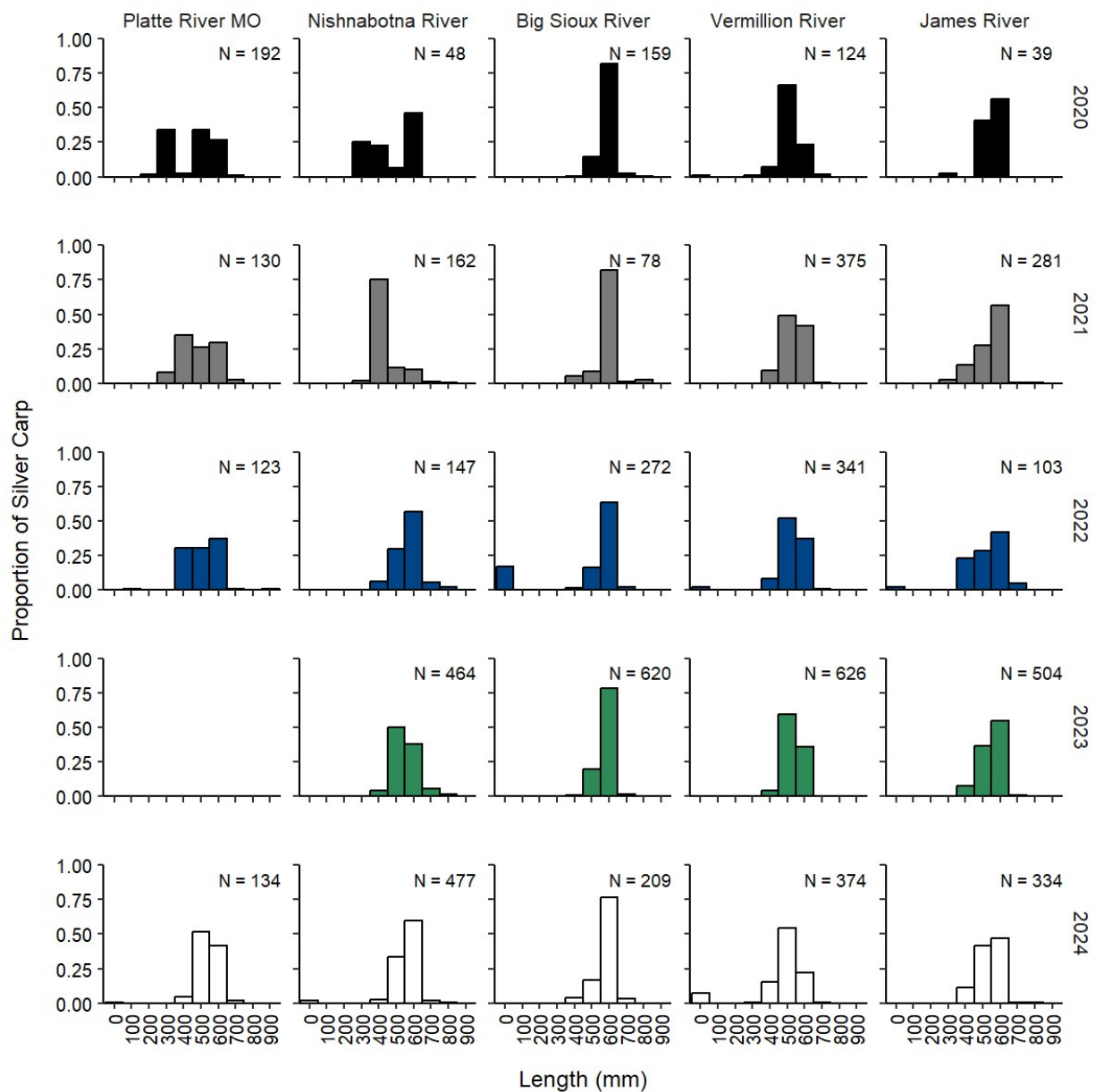


Figure 3. Relative length frequency distribution by 100 mm length bin of Silver Carp captured with an electrified dozer trawl in each tributary by year, 2020-2024. The number (N) of Silver Carp includes all lengths collected during standard protocols, SVCP volunteers, and extra samples to collect age data; therefore, the totals may not match the totals listed in Table 1 that were used to calculate CPUE.

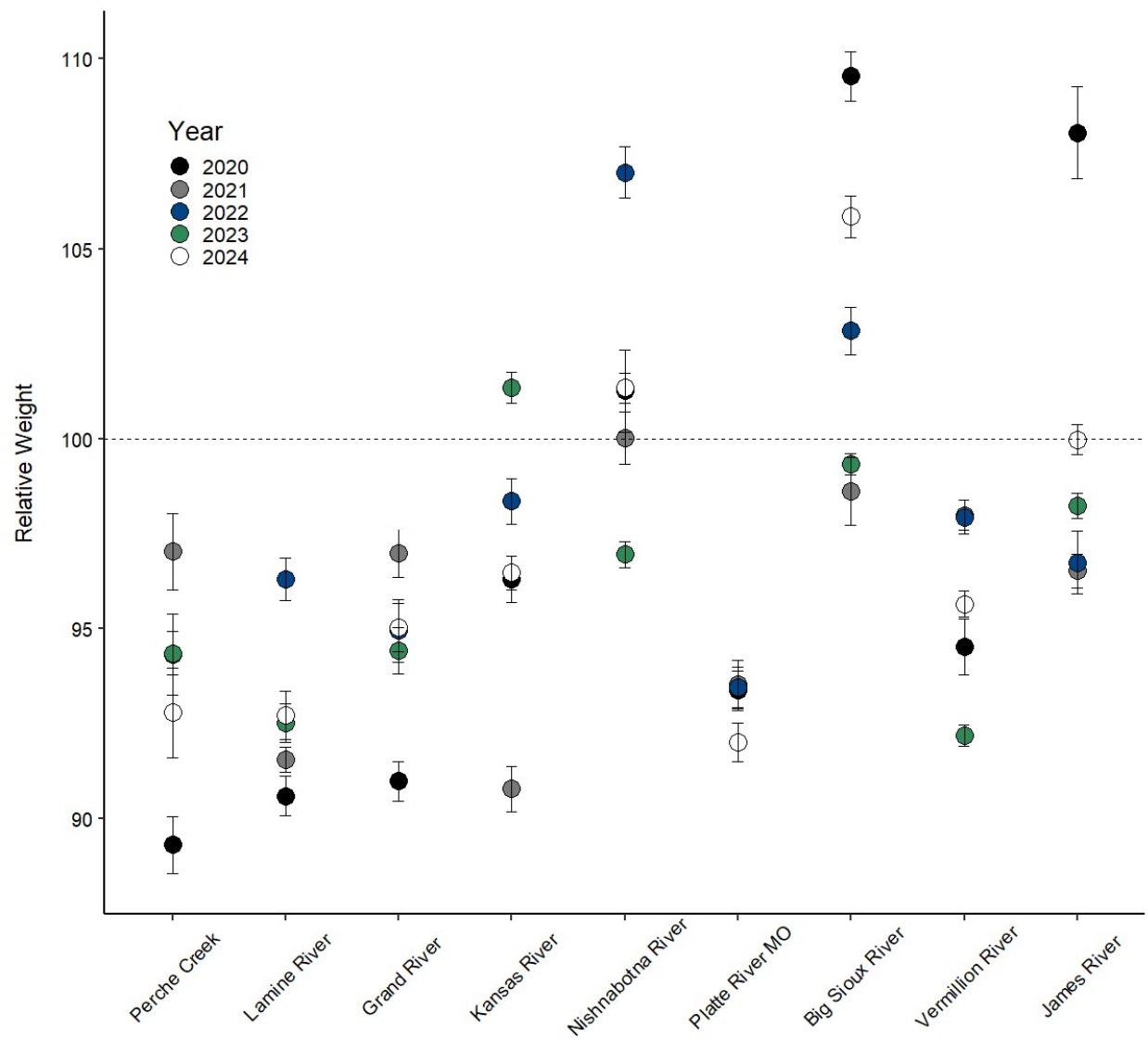


Figure 4. Relative weight (W_r) for Silver Carp (SVCP) by tributary for each year. Error bars represent one standard error and the dashed horizontal line indicates an "average" body condition based on the standard weight equation used (Lamer 2015). Tributaries are in ascending order based off confluence locations on the Missouri River.

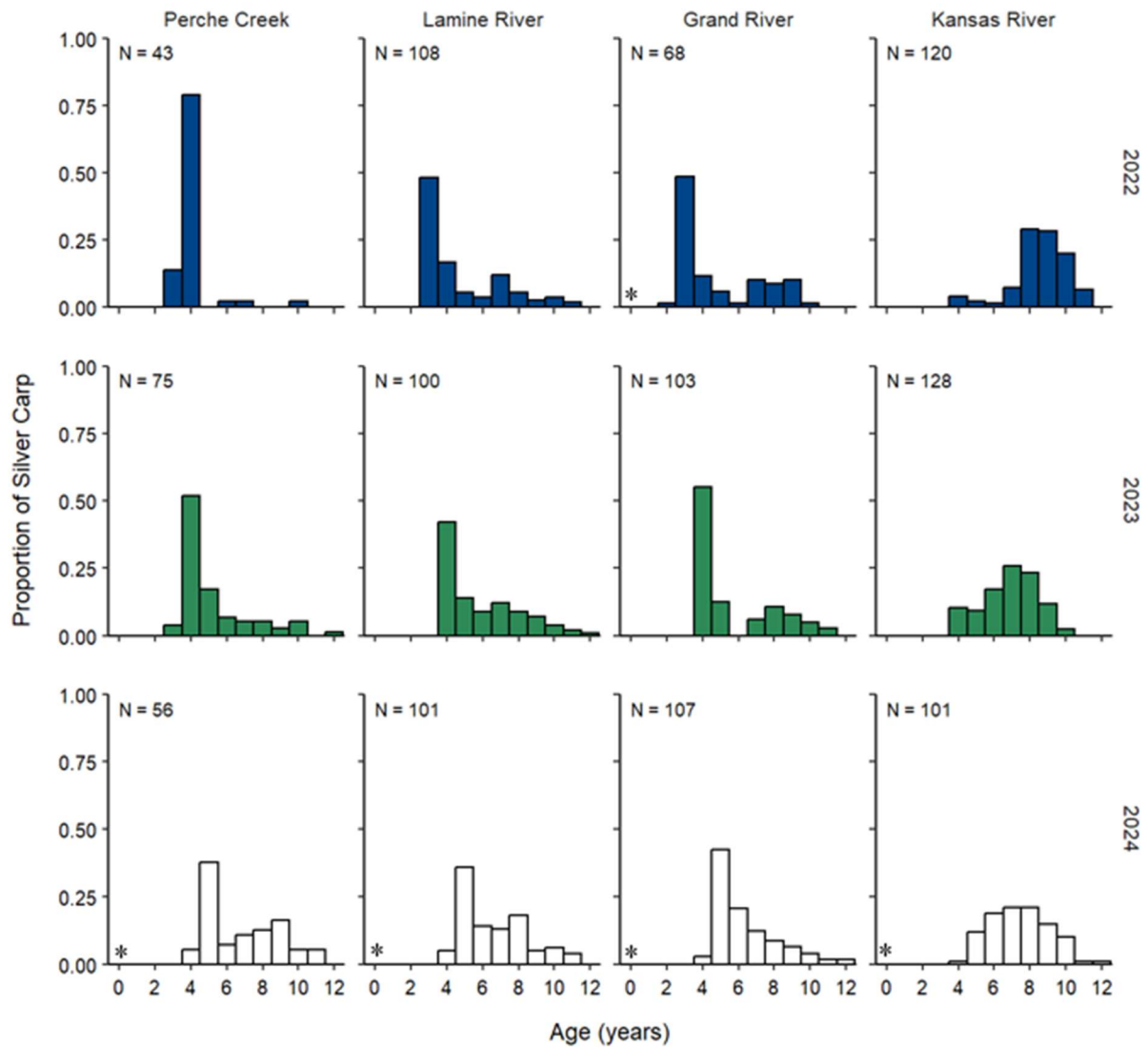


Figure 5. Relative age frequency of Silver Carp captured with an electrified dozer trawl in each tributary by year for 2022-2024. Each tributary had the first 100 Silver Carp otoliths extracted and aged. An asterisk indicates Silver Carp less than 120 mm which are assumed to be age-0. Number of age-0 Silver Carp sampled in 2024 can be found in Table 1.

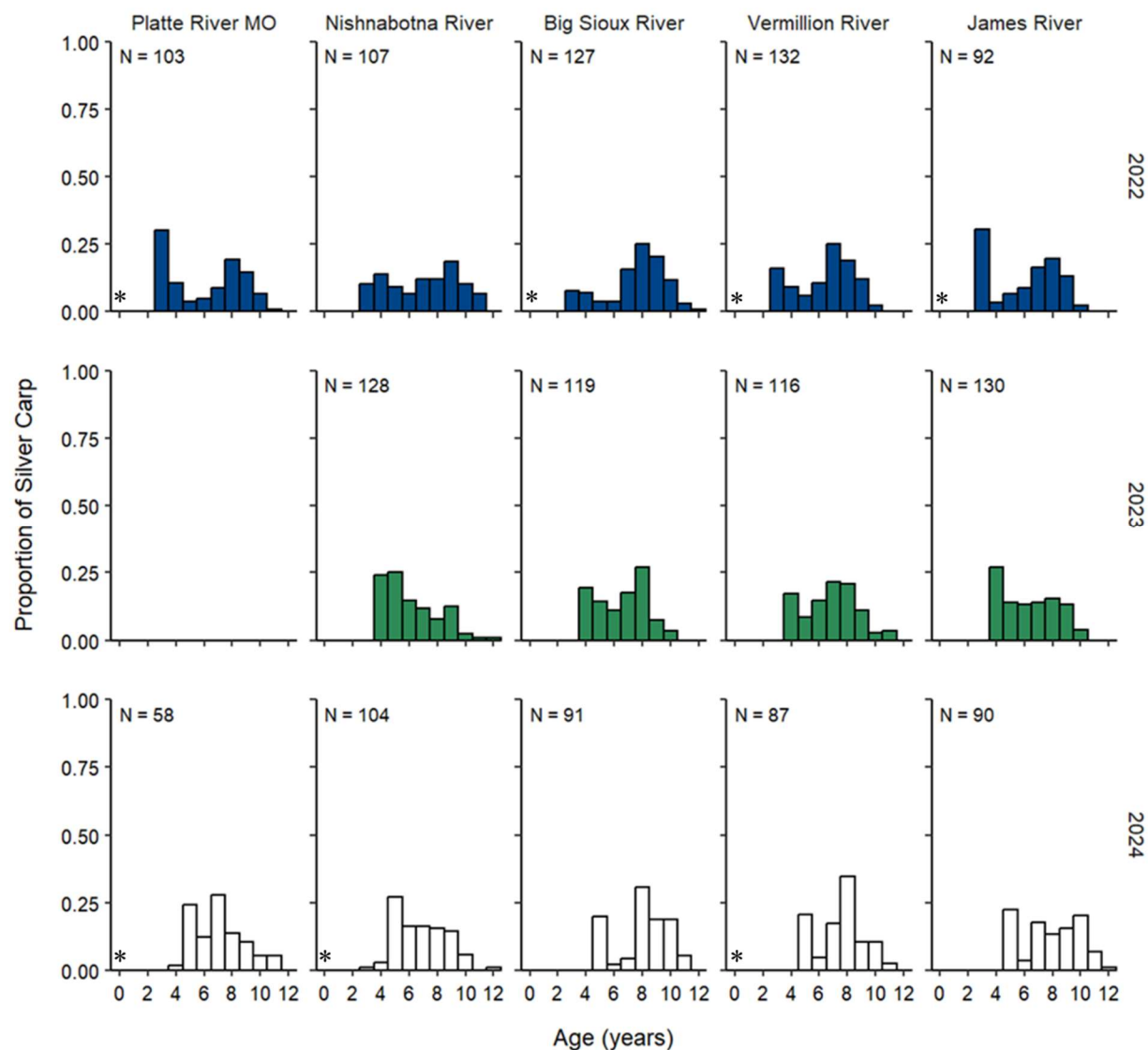


Figure 6. Relative age frequency of Silver Carp captured with an electrified dozer trawl in each tributary by year for 2022-2024. Each tributary had the first 100 Silver Carp otoliths extracted and aged. An asterisk indicates Silver Carp less than 120 mm which are assumed to be age-0. Number of age-0 Silver Carp sampled in 2024 can be found in Table 1.

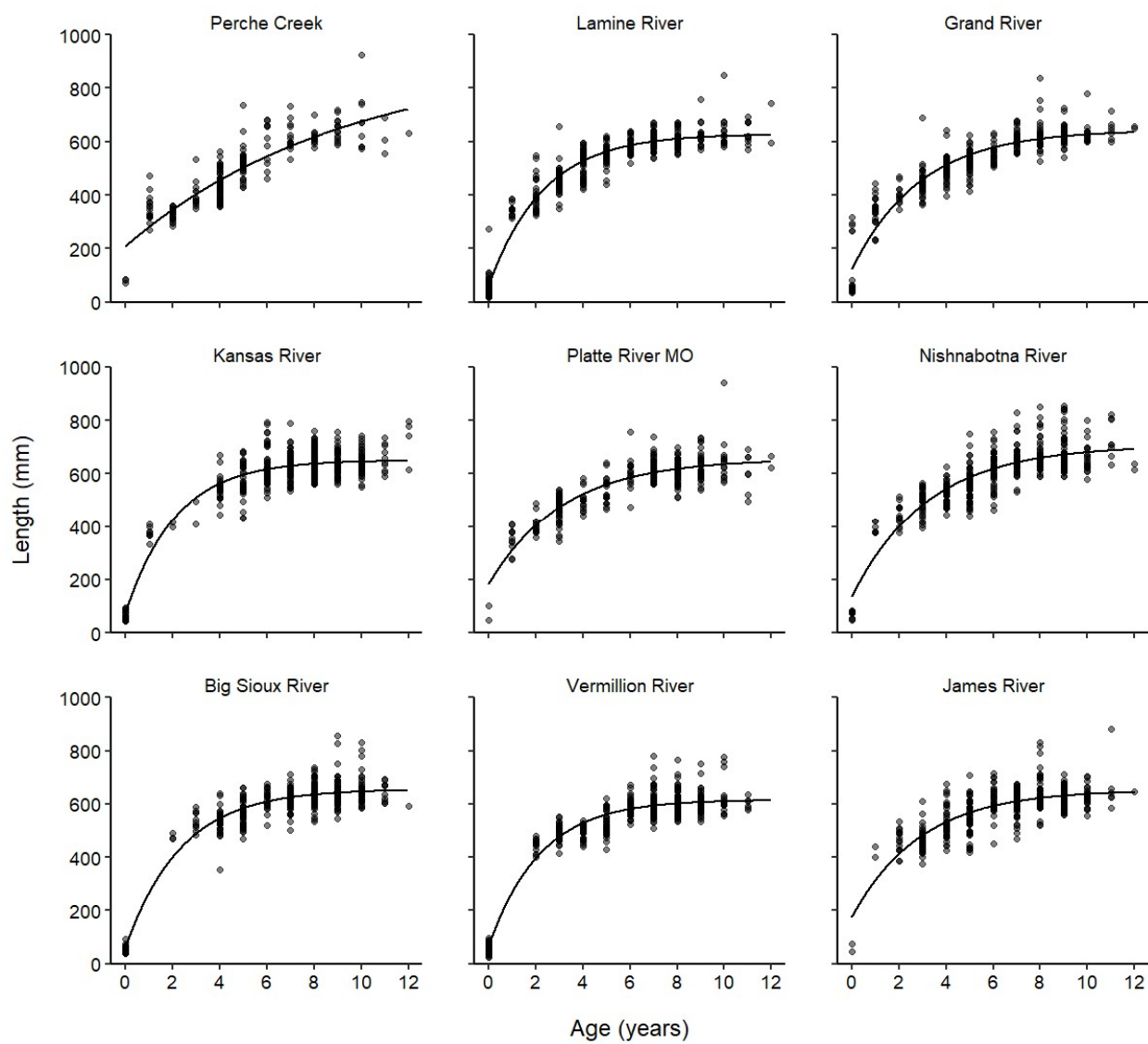


Figure 7. Length at age for all Silver Carp collected by electrified dozer trawl from 2020 to 2024.

Longnose Gar	16	13	6	24	3	29	0	2	17
Mooneye	0	0	0	0	0	1	0	0	0
Mosquito Fish	0	0	1	2	0	1	0	0	0
Orange Spot	0	1	0	0	0	0	0	0	0
Paddlefish	2	0	0	0	0	0	0	0	4
Quillback	0	0	0	0	0	0	0	0	2
Red Shiner	0	2	0	1	0	1	0	0	0
Redfin Shiner	0	0	0	0	1	0	0	0	0
River Carpsucker	1	1	0	6	0	33	3	7	0
Sauger	0	0	0	0	0	1	0	0	0
Skipjack Herring	1	2	0	0	0	0	0	0	0
Smallmouth	8	9	11	32	10	38	1	1	0
Shortnose Gar	10	13	2	6	0	42	2	4	20
Sand Shiner	0	5	0	86	6	8	0	0	0
Spottail Shiner	0	0	0	0	0	0	0	2	0
Silver Carp	73	760	151	295	134	479	210	539	334
Unidentified	0	0	1	0	0	0	0	0	0
Unidentied	0	0	0	0	0	1	0	0	0
Walleye	0	0	0	0	0	0	0	1	0
White Bass	3	0	0	2	0	0	0	0	1
White Crappie	1	0	0	0	0	0	0	0	0
Total	464	1,099	326	864	324	1,317	542	994	1,818

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