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Patterns in spatial use and movement of Silver Carp among tributaries and main-stem rivers: Insight from otolith microchemistry analysis

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Abstract

Invasive Silver Carp (*Hypophthalmichthys molitrix*) have established populations throughout the Missouri River basin, including in the Kansas River. Understanding the spatial extent under which these invasive fish function in large, open river systems is crucial to inform management efforts. The Kansas River may play a vital role in the life-cycle of Silver Carp in the Missouri River basin as the main-stem Missouri River has undergone a multitude of alterations, creating a channel with greater mean depths and velocities. Here, we used otolith microchemistry of Silver Carp from the Kansas River to reconstruct environmental histories as a means to assess the proportions of resident and transient individuals. Silver Carp within the Kansas River were predominantly residents (adults = 54%; juveniles = 65%) with the majority of reproduction coming from within the Kansas River itself. These results suggest removal efforts in the Kansas River may be effective means of managing this invasive fish species. Transient individuals exhibited short durations of signatures indicative of the Missouri River (mean percent of data points for adults = 10% and juveniles = 36%), suggesting movements into the Missouri River were brief. These results highlight the importance of connectivity of tributary habitat among large rivers and provides important information for invasive species management.

Introduction

Silver Carp (*Hypophthalmichthys molitrix*) are an invasive species from eastern Asia that invaded U.S.A. waterways in the early 1980's (Freeze and Henderson 1982; Conover et al. 2007; Lu et al. 2020). Since their introduction, they have expanded their range to encompass the majority of the Mississippi River Basin (Conover et al. 2007), including the lower Missouri River drainage where they have expanded as far north as North Dakota (Hayer et al. 2014). The spatial extent under which Silver Carp function in the Missouri River drainage is unclear because of the open nature and connectivity of this large river system. The lower Missouri River (below Gavins Point Dam, SD) is devoid of dams leaving approximately 1,305 river kilometers (rkm) and numerous connections to tributaries open to immigration and emigration of Silver Carp. These open corridors, coupled with their ability for long, longitudinal movements (DeGrandchamp et al. 2008; Coulter et al. 2016), has aided in range expansion into and throughout the lower Missouri River drainage.

Modifications for flood control and to maintain a navigable channel (i.e., wing dikes, levies, and bank stabilization structures) are extensive throughout the lower Missouri River. These modifications created greater mean depths and velocities while also limiting lateral connectivity with the river floodplain (Galat et al. 1998; Pegg et al. 2003; Steffensen and Mestl 2016). Consequently, limited optimal habitat remains for Silver Carp in the main-stem of the Missouri River because they tend to prefer areas with lower velocities (DeGrandchamp et al. 2008; Calkins et al 2012). Tributaries to the Missouri River, and limited habitat behind wing dikes, may provide refuge habitat for Silver Carp seeking to escape the high velocity flows of the Missouri River (Kolar et al. 2005). Additionally, tributaries may act as stepping-stones for longitudinal movement throughout the basin.

Longitudinal connectivity between populations influences biological processes (Pegg and Chick 2010) such as gene flow. Transient Silver Carp within the population facilitate this connectivity between populations. Silver Carp exhibit individual-based movement patterns where some individuals within a given population are transient and others are resident (Coulter et al. 2016; Prechtel et al. 2018). Dichotomy in individual-based movement patterns has been observed in other riverine fishes such as Common Carp *Cyprinus Carpio* (Butler and Wahl 2010), many Salmonids (Rodríguez 2002) and Guadalupe Bass *Micropterus treculii* (Perkin et al. 2010). This strategy promotes dispersal and the colonization of new areas while also ensuring that some individuals stay in suitable habitat (Coulter et al. 2016). Discerning the proportions of transient and resident individuals within Silver Carp populations can provide insight for management action (Prechtel et al. 2018). For example, populations comprised mostly of resident individuals would be ideal for removal efforts. Timing removal efforts to coincide with periods of reduced movement distances (i.e., summer months) could be effective means for removing transient individuals (Prechtel et al. 2018).

Analysis of stable isotopes and trace elemental composition of otoliths as natural tags is a useful tool for retrospectively identifying the environmental history of riverine fishes (Zeigler and Whitley 2010). Strontium (Sr) and Barium (Ba) – in ratios to Calcium (Ca) – are two common elements used in microchemistry analyses (Whitley et al. 2007; Zeigler and Whitley 2010; Crook et al. 2013; Carlson et al. 2016, Whitley et al. 2019). For example, Sr:Ca ratios were used to identify natal origins of Silver Carp captured in the Illinois River to the Illinois River itself, the Missouri River, or the middle Mississippi River (Norman and Whitley 2015). Additionally, Sr:Ca ratios were used to identify origins of Silver Carp captured in urban Chicago fishing ponds to the Illinois River (Love et al. 2019). Here, we used otolith microchemistry to reconstruct the environmental history of Silver Carp captured in the Kansas River to determine the proportion of resident and transient individuals. Additionally, we aimed to quantify tributary versus main-stem Missouri River occupancy durations of transient Silver Carp within the Kansas River. These data will provide insight into movement patterns of Silver Carp between the Missouri River and the Kansas River habitats and help to determine if removal efforts would be effective at reducing abundance of Silver Carp in the Kansas River.

Methods

Study Area

The Kansas River is a large tributary to the Missouri River (Figure 1). Its origins are at the confluence of the Smoky Hill and Republican Rivers (Quist and Guy 1999) in North-Central Kansas and flows easterly 274 kilometers (Makinster and Paukert 2008) to its confluence with the Missouri River near Kansas City, Kansas. Discharge is controlled by 18 federal reservoirs and over 13,000 small impoundments (Quist et al. 1999). The main-stem of the Kansas River has three major barriers; the Topeka Weir in Topeka, Kansas at river-kilometer (rkm) 141, Bowersock Dam in Lawrence, Kansas at rkm 83, and the Johnson County Weir in Edwardsville, Kansas at rkm 27 (Figure 1). Bowersock Dam functions as a hydropower dam and is

classified as a low-head dam (Quist and Guy 1999) that can impede upstream longitudinal movements of riverine fishes (Eitzman et al. 2007; Dean 2020), including Silver Carp (Werner 2020).

Our study area was the lower half of the Kansas River from the Topeka Weir, in Topeka, Kansas to the confluence with the Missouri River. We divided the Kansas River into three distinct segments associated with three barriers on the main-stem of the Kansas River. Segment 1 is between the confluence with the Missouri River and the Johnson County Weir (27 rkm), Segment 2 is between the Johnson County Weir and Bowersock Dam (56 rkm), and Segment 3 is between Bowersock dam and the Topeka Weir (58 rkm). Additionally, we included a 22 rkm segment of the Missouri River and a 29 rkm segment of the Wakarusa River from its confluence with the Kansas River to the Clinton Lake Dam for otolith and water data collection.

Microchemistry Data Collection

We collected water samples in autumn 2018, winter 2019, and summer 2019 to assess spatiotemporal variations in water trace element concentrations (Ciepiela and Walters 2019). We gathered water samples across the Kansas River (Topeka Weir to the confluence with the Missouri River; n = 25 samples), Wakarusa River (below Clinton Lake to the confluence with the Kansas River; n = 5 samples), and Missouri River (16 km upstream of the confluence to 7 km below the confluence with the Kansas River; n = 7 samples). We collected water samples using a syringe filtration technique from 13 sites across the three river systems (Kansas River = 9 sites, Wakarusa River = 2 sites, Missouri River = 2 sites) (Figure 1) with a 250ml polyethylene bottle. We rinsed the bottle a minimum of three times before collecting each water sample. We then rinsed a syringe filter and used it to filter 15ml of water into a cleaned and rinsed collection bottle. We stored the sample bottles in a cool, dark location until we sent them to the lab for analysis. We analyzed samples for Strontium (Sr), Barium (Ba), Magnesium (Mg), Calcium (Ca), Sodium (Na), and Manganese (Mn) at the University of Southern Mississippi's Center for Trace Analysis. Data were reported as the molar concentration for each trace element and converted to element:Ca (mmol/mol) ratios (e.g., Sr:Ca, Ba:Ca, and Mg:Ca) (Whitledge et al. 2019).

We collected juvenile and adult Silver Carp from Segments 1 and 2 from the Wakarusa River in May-August of 2018 and 2019. No Silver Carp were collected above Bowersock Dam in Segment 3 (Werner 2020). We used a combination of electrofishing gears and mini-fyke nets throughout the reach to collect Silver Carp (e.g., Werner 2020). We extracted both lapilli otoliths from a minimum of 25 Silver Carp per segment per month in 2018 and 2019. We analyzed the first 25 otoliths collected from each segment in each month in 2018. In 2019, we selected otoliths that were devoid of cracks and other imperfections. In total, we selected 300 Silver Carp otoliths for microchemistry analysis. We collected otoliths from only adult Silver Carp (>400 mm) in 2018, whereas in 2019 we collected otoliths from both juvenile Silver Carp (<400 mm) and adult Silver Carp. The majority of otoliths from juvenile Silver Carp were collected in Segment 1 (Table 1) because juveniles were rare in Segment 2 (Werner 2020). Additionally, we aimed to select otoliths for microchemistry analysis based on spatiotemporal variations on when they were collected to monitor for shifts in trace element signatures over both time and space. We extracted otoliths

by making an incision through the top of the skull into the cranial cavity and collected the otoliths using non-metallic tweezers. Then, we cleaned the otoliths of flesh and placed them in 2 ml polyethylene vials until they were prepared for ablations in the lab.

We washed the otoliths with deionized water and allowed them to dry for 24 hours. We then embedded the otoliths in epoxy (Epoxicure Epoxy Resin and Hardener, Beuhler Inc., Lake Bluffs, Illinois) and sectioned them across the transverse plane through the nucleus using a Buehler IsoMet low speed saw. We sanded otolith sections using 1,500 and 3,000 grit sandpaper and polished them to reveal annuli using 3 μ m lapping paper. We then rinsed the sectioned otoliths in deionized water, adhered them to microscope slides with double sided tape, and stored them until microchemistry analysis. We analyzed the trace element composition of the otoliths using a Thermo X-Series2 ICPMS coupled with a Teledyne-CETAC Technologies LSX-266 laser ablation system. Ablations (beam diameter = 20 μ m, scan rate = 5 μ m/sec., laser pulse rate = 5hz) began approximately 100 μ m on one side of the nucleus, ablated completely through the nucleus, to the adjacent edge of the otolith. We analyzed a standard developed by the U.S. Geological Survey (USGS) (MACS-3, CaCO₃ matrix) every 15-20 samples to adjust for instrument drift using procedures outlined by Whitley et al. (2019). Otolith microchemistry data were converted to molar concentrations for each element and reported as element:Ca (μ mol/mol) ratios (e.g., Sr:Ca, Ba:Ca, and Mg:Ca) using calcium as the internal standard and the stoichiometric concentration of calcium in aragonite Calcium Carbonate (CaCO₃) (Whitley et al. 2019).

We primarily focused on Sr:Ca ratios to classify the environmental history of Silver Carp. Strontium:Calcium ratios are commonly used in microchemistry analysis of Silver Carp otoliths (e.g., Norman and Whitley 2015, Whitley et al. 2019, Love et al. 2019). Specifically, lapilli otoliths are analyzed for their Sr:Ca ratios because they are usually comprised of the aragonite polymorph of CaCO₃ (Whitley et al. 2019). Aragonite has a higher affinity for Sr compared to other polymorphs such as vaterite and calcite (Campana 1999; Melancon et al. 2005; Pracheil et al. 2019). A combination of Sr:Ca, Ba:Ca, and Mg:Ca ratios were used as an indicator for vaterite otoliths (Mg:Ca > 400 μ mol/mol, Ba:Ca < 4 μ mol/mol, and Sr:Ca < 100 μ mol/mol) (Whitley et al. 2019). We did not use Mg in any further reconstruction of environmental history because metabolic processes more heavily regulate Mg than Sr or Ba. Magnesium contributes to the phosphorylation of enzymes and is a co-factor in adenosine triphosphate (ATP). Additionally, the hydrated and dehydrated forms of Mg have largely different radii than Ca⁺ and are likely randomly trapped in the crystal lattice (Thomas et al. 2017; Hüseyin et al. 2020). Conversely, Sr and Ba have similar radii to Ca and compete for Ca binding sites in the crystal lattice (Hüseyin et al. 2020).

Data Analysis

We used otoliths collected from Silver Carp captured from Segment 2 and the Wakarusa River in 2018 to characterize the relationship between water and otolith microchemistry signatures in the Kansas and Wakarusa Rivers. During low flow events – such as those in 2018 – the Johnson County Weir was likely a barrier to movement between Segment 1 and Segment 2 (Werner 2020; Dean 2020). Isolation of Silver

Carp in Segment 2 for an extended period (e.g., >3 months) likely facilitated trace elemental equilibrium in the signatures. Additionally, movement rates of Silver Carp are lower during low flow events (DeGrandchamp et al. 2008; Calkins et al. 2012; Coulter et al. 2016; Prechtel et al. 2018), likely limiting movement between the main-stem Kansas River and its tributaries. We characterized the relationship between water and otolith microchemistry signatures in the Missouri River using ablation data from Silver Carp captured in isolated backwaters of the Missouri River (n = 13). Missouri River Silver Carp otolith data were provided by the Center for Fisheries, Aquaculture, and Aquatic Sciences at Southern Illinois University – Carbondale. We isolated trace elemental ratio data at the edge of the selected otoliths (~30 µm) to characterize the relationship between water and otolith signatures in the Kansas, Missouri, and Wakarusa Rivers (Norman and Whitley 2015; Spurgeon et al. 2018).

We grouped water samples and otoliths by river of sample collection and assessed differences using univariate and multivariate methods. Tests of normality (i.e., Shapiro-Wilks tests) indicated water and otolith microchemistry data deviated from normality, thus we proceeded with non-parametric tests. We used a permutated multivariate analysis of variance (perMANOVA) to examine the differences in otolith and water trace elemental signatures between river systems, adjusting the P-value using the Bonferroni method for pair-wise comparisons. We then used a Kruskal-Wallis test followed by a post hoc Dunn's test to examine pair-wise differences in water and otolith trace elemental signatures between river systems, adjusting the P-value for multiple comparisons using the Holmes method.

We used a recursive partitioning modelling approach to separate trace elemental signatures in Silver Carp otoliths between river systems. We built the recursive partitioning tree using the rpart package (Thernau et al. 2019) in program R (R Core Team 2020). Recursive partitioning trees aim to split the data into homogenous groups to increase the homogeneity of the elements (i.e., trace elemental ratios) within groups (i.e., rivers) (Dinov 2018). We used a splitting criterion based on the Gini impurity index (e.g., Spurgeon et al. 2018) and selected the tree within one standard error to the tree with the lowest cross-validated error (Spurgeon et al. 2018; Thernau et al. 2019). We then used the resulting partitioning tree to predict the trace elemental threshold that distinguishes each river. We classified individual Silver Carp as “transient” if they had signatures indicative of the Missouri River or “resident” if they lacked those signatures. We excluded Silver Carp with trace elemental signatures indicative of vatarite CaCO₃ otoliths or with extremely high Sr:Ca ratios (e.g. > 5,000µmol/mol) from the analysis.

We determined the proportion of transient adult and juvenile Silver Carp in Segments 1 and 2 in both 2018 and 2019. Additionally, we determined the proportion of transient Silver Carp that were male and female to examine sex-specific movement patterns. We then isolated ablation data from the nucleus of the otolith to determine the proportion of Silver Carp in the Kansas River with natal origins from the Missouri River. We also enumerated movement events between the Missouri River and other water bodies. We classified movement events as a shift in trace element data to signatures indicative of the Missouri River at any point along the ablated transect.

We also aimed to quantify the percent of time each transient fish spent in the Missouri River versus other water bodies in the drainage to gain insight on habitat use by Silver Carp in the basin. We calculated the percent of Sr:Ca data points indicative of the Missouri River (i.e., percent of points in each spike) for each transient fish. We then plotted this value for each individual by its total length.

Results

Water Chemistry

Multivariate comparisons among rivers indicated water trace elemental signatures (i.e., the combination of Sr:Ca and Ba:Ca ratios) differ between the Kansas River and Wakarusa River ($F = 74.3$, $r^2 = 0.726$, $P = 0.003$), Kansas River and Missouri River ($F = 24.1$, $r^2 = 0.445$, $P = 0.003$), and Wakarusa River and Missouri River ($F = 41.1$, $r^2 = 0.804$, $P = 0.003$). Univariate comparisons indicate water Sr:Ca ratios ($\chi^2 = 22.5$, $P < 0.001$) and Ba:Ca ratios ($\chi^2 = 13.9$, $P < 0.001$) differ between rivers. Pair-wise comparisons revealed that water Sr:Ca ratios were higher in the Kansas River than both the Missouri River ($Z = 31.14$, $P = 0.003$) and the Wakarusa River ($Z = 4.09$, $P < 0.001$). However, water Sr:Ca ratios were similar between the Wakarusa River and the Missouri River ($Z = 1.13$, $P = 0.26$). Water Ba:Ca ratios were similar between the Kansas River and Missouri River ($Z = -1.6$, $P = 0.111$). Water Ba:Ca ratios were lower in the Wakarusa River than either the Kansas River ($Z = 3.01$, $P = 0.005$) or the Missouri River ($Z = 3.68$, $P < 0.001$) (Figure 2).

Otolith Chemistry

We used a total of 73 adult Silver Carp with a mean total length of 671 mm (sd = 46 mm) to classify the relationship between water and otolith microchemistry in the Kansas River ($n = 59$) and Wakarusa River ($n = 14$). Additionally, we used this subset of data to train and test the recursive partitioning tree for the following, larger analysis. Multivariate tests among rivers indicated otolith trace elemental signatures did not differ between the Kansas River and Wakarusa River ($F = 7.47$, $r^2 = 0.095$, $P = 0.027$), but did differ between the Kansas River and Missouri River ($F = 84.6$, $r^2 = 0.547$, $P = 0.003$), and Wakarusa River and Missouri River ($F = 75.5$, $r^2 = 0.751$, $P = 0.003$). Univariate tests indicate trace elemental signatures differed among rivers in both Sr:Ca ratios ($\chi^2 = 38.8$, $P < 0.001$) and Ba:Ca ratios ($\chi^2 = 34.5$, $P < 0.001$). Pair-wise comparisons revealed Otolith Sr:Ca ratios were the higher in the Missouri River than either the Kansas River ($Z = -5.05$, $P < 0.001$) or the Wakarusa River ($Z = 2.65$, $P = 0.008$). Additionally, otolith Sr:Ca ratios were higher in the Kansas River than the Wakarusa River ($Z = 6.06$, $P < 0.001$). Otolith Ba:Ca ratios were higher in the Missouri River than either the Kansas River ($Z = -5.86$, $P < 0.001$) or the Wakarusa River ($Z = 3.48$, $P < 0.001$). However, otolith Ba:Ca ratios did not differ between the Kansas River and Wakarusa River ($Z = -1.27$, $P = 0.127$) (Figure 3).

The recursive partitioning tree correctly classified 92% of fish collected in the Kansas River, 50% in the Missouri River, and 0% in the Wakarusa River. However, all fish collected from the Wakarusa River were classified as fish from the Kansas River (Table 2). Thus, our model could only be used to distinguish

between the Kansas River and the Missouri River (Sr:Ca > 2,082 μ mol/mol was indicative of the Missouri River) (Figure 4). Ba:Ca was the most influential variable in our model (variable importance score = 57) followed by Sr:Ca (variable importance score = 43). The inclusion of Ba:Ca in the model did not refine the model enough to be able to further distinguish between the Wakarusa River and the Kansas River and between the Wakarusa River and the Missouri River. Additionally, Ba:Ca data were more erratic with multiple unreliable data points across all individuals while the Sr:Ca data were more consistent. Therefore, we primarily used Sr:Ca ratios in environmental history reconstruction.

We reconstructed environmental histories of 276 (n = 239 adult; n = 37 juvenile) Silver Carp with approximately 46% of adults and 35% of juveniles classified as transient individuals. The proportion of transient individuals among sampling years and segment of capture for adult Silver Carp was consistently 45%-49%, except in Segment 1 during 2019 where the proportion of transients was approximately 22%. Juvenile Silver Carp were predominantly residents in 2019 with approximately 65% of individuals sampled lacking trace elemental ratios indicative of the Missouri River (Table 1). About 57% (n = 37) of transient fish we identified gender for were male and approximately 43% (n = 28) were female.

Approximately 17% (n = 19) of transient adult Silver Carp had natal origin signatures indicative of the Missouri River, 10 of which were captured in Segment 1 and the remaining 9 were captured in Segment 2. Approximately 46% (n = 6) of transient juvenile Silver Carp had natal origin signatures from the Missouri River. Five were captured in Segment 1 and one was captured in Segment 2. Overall, approximately 9% (n = 25) of all fish sampled for microchemistry analysis had natal origins predicted to be from the Missouri River (Table 3).

A single movement event into the Missouri River was most common for both adult (n = 82) and juvenile (n = 10) transient Silver Carp. Two movement events occurred less often for adults and juveniles. Approximately 22% of transient adults (n = 24) and 23% of transient juveniles (n = 3) exhibited two movement events. Three movement events were exceedingly rare in adults (n = 3) while no juveniles captured exhibited three movement events (Table 3).

Transient juvenile Silver Carp exhibited a greater percent of points above the Missouri River threshold (i.e., percent of points in each spike) than transient adult Silver Carp. The percent of points above the Missouri River threshold for adults was less than 30% for the majority of the individuals, ranging from approximately 2% to 36% of points. Juveniles were more erratic, ranging from approximately 10% to 71% of points. Overall, there was a negative relationship between the percent of points above the Missouri River threshold and total length (Figure 5).

Discussion

Examination of otolith trace elemental signatures may be a useful tool in reconstructing environmental histories and predicting natal origins of Silver Carp in the Kansas River and Missouri River systems. Our study indicated the population within the Kansas River is comprised of predominantly residential individuals, having a consistent signature indicative of the Kansas River throughout the life span of the

fish. Therefore, recruitment from within the Kansas River system is regularly occurring without connectivity to additional river systems. Segment 1 may be of particular importance for reproduction as the vast majority of juvenile Silver Carp were documented in this reach (Werner 2020). Segment 1 has an average depth less than 1.5m and is characterized by low velocity flows (Eitzmann and Paukert 2010). These habitat conditions are typically where age-0 Silver Carp are found at higher densities (e.g., Haupt and Phelps 2016) and are habitats commonly used as nursery grounds (Kolar et al. 2005, Conover et al. 2007).

Our data show that about 20% of all transient adult and juvenile Silver Carp in the Kansas River had natal origin signatures indicative of the Missouri River. These results are noteworthy because Deters et al. (2013) documented the highest egg densities within the main-stem Missouri River while tributaries (e.g., Lamine River, Bonne Femme Creek, Perch Creek, Moniteau Creek, Moreau River, and Osage River) had little egg production. However, Camacho et al. (2020) documented egg production in tributaries to the upper Mississippi River. This provides evidence that tributary habitats can be used for reproduction and is likely a function of select habitat availability. Therefore, it is likely that select tributaries throughout the Missouri River basin – such as the Kansas River – may be sources of reproduction and recruitment for Silver Carp. Identifying these sources throughout the basin would provide information as to where control efforts should be focused. Additionally, these data could reveal source-sink dynamics between the main-stem Missouri River and adjacent systems.

Transient juvenile and adult Silver Carp exhibited trace elemental signatures indicative of the Missouri River for relatively short durations (e.g., Figure 6). These results indicate that transient Silver Carp occupied the Missouri River for brief periods of time compared to other systems. Brief occupancy in the Missouri River is likely influenced by the lack of optimal habitat available for Silver Carp. Additionally, higher velocity flows in the Missouri River (e.g., Pegg et al. 2003) facilitates the lack of buildup of autotrophic biomass and reduces residence time (Hosen et al; 2019), limiting food availability for Silver Carp in this system. Areas with lower velocities, like the Kansas River and areas behind wing dikes, may provide habitat with higher resource availability as well as refuge from the swift flows within the main channel (e.g., DeGrandchamp et al. 2008; Calkins et al. 2012; Coulter et al. 2016).

A few of the transient Silver Carp we analyzed had trace elemental signatures that indicated multiple movement events through the Missouri River. These results suggest the Missouri River may function more as a movement corridor for Silver Carp as they migrate between areas of suitable habitat. Further research on movement patterns of Silver Carp throughout the Missouri River basin is needed to test this hypothesis. Movement events into the Missouri River are likely induced by a variety of factors. For example, increased movement rates as a response to a rise in river flood stage has been documented for adult Silver Carp (Peters et al. 2006; DeGrandchamp et al. 2008; Coulter et al. 2016). Increased movement rates could facilitate movement events into the Missouri River as transient individuals seek new habitats (Prechtel et al. 2018). Additionally, broad scale upstream movements occurring in the spring typically occur as Silver Carp stage for spawning events (Coulter et al. 2016). Brief forays into the Missouri River

could occur as Silver Carp seek suitable spawning habitat located in the Missouri River itself or other tributaries in the basin.

We can distinguish habitat use between the Kansas River and Missouri River because of differences in water and otolith trace elemental signatures. However, otolith trace elemental signatures of the Wakarusa River and Kansas River were too similar to distinguish use between these two water bodies, even with the incorporation of multiple trace elements (e.g., Sr:Ca and Ba:Ca). The paucity of water and otolith microchemistry data throughout the Missouri River basin limited our analysis to only between the Kansas and Missouri Rivers. Water chemistry signatures have been classified for the Platte River in Nebraska (e.g., Phelps et al. 2012; Spurgeon et al 2018) and throughout the main-stem of the Missouri River below Gavins Point Dam, SD (e.g., Phelps et al. 2012; Norman and Whitley 2015; Porreca et al. 2016; Spurgeon et al. 2018; Whitley et al. 2019). However, multitudes of other tributaries in the Missouri River drainage remain to be analyzed. Classification of these other tributaries will lead to insights in movement and recruitment sources of Silver Carp throughout the Missouri River basin.

Additional otolith trace elemental signatures need to be characterized throughout the Missouri River drainage (Hüssy et al. 2020). Our results demonstrate that the relationship between otolith and water signatures may not always be a positive linear relationship (Figures 2 and 3) and instead may resemble a logistic curve. Models built by Norman and Whitley (2015) predicted otolith Sr:Ca ratios for Silver Carp captured from the Kansas River would be centered around 3,600 $\mu\text{mol/mol}$. However, we observed consistent values of approximately 1,500 $\mu\text{mol/mol}$ (Figure 3). These differences could be explained by contamination, instrumental miscalibration, or procedural errors. However, the predicted otolith Sr:Ca values for the Wakarusa ($\sim 1,650 \mu\text{mol/mol}$) is within the 95% confidence intervals of the observed values (Figure 3), indicating these errors were negligible. Biotic and abiotic factors such as salinity, temperature, oxygen, ontogeny, food and growth, and maturation can influence how trace elements are incorporated into the crystal lattice (Campana 1999; Norman and Whitley 2015; Sturrock et al. 2015; Hüssy et al. 2020). One of these factors, or a combination of such, could have caused the negative relationship between otolith and water Sr:Ca values we observed in the Kansas River. Although these factors do not exert a strong influence on the biomineralization process of otolith formation (Hüssy et al. 2020), they could influence physiological processes governing trace element uptake and transport.

The Kansas River affords a unique opportunity for direct management and possible reduction of Silver Carp abundance because of the higher proportion of resident individuals. For example, removal efforts may be a viable option in the Kansas River, and should focus on Segment 1 because this reach is likely where reproduction is occurring (Werner 2020). Timing removal efforts to coincide with periods when Silver Carp are least active, such as during the late summer and early fall months (DeGrandchamp et al. 2008; Coulter et al. 2016), could have impacts throughout the Missouri River basin by removing a larger portion of transient individuals (Prechtel et al. 2018). Gears targeting all size groups, such as the electrified dozer trawl (Hammen et al. 2019; Werner 2020), should be used to maximize effort (Tsehaye et al. 2013), particularly during years when age-0 Silver Carp are confined to Segment 1 (e.g., Werner 2020).

Our results indicate that Silver Carp captured from the Kansas River tributary occupy waters adjacent to the mainstem Missouri River for most of their lives. Therefore, management of these invasive fish may be better suited focusing on the multitude of tributary habitats throughout the Missouri River drainage rather than the main-stem Missouri River. Future work investigating contributions of these tributary systems to the greater Missouri River population is essential to determine if management efforts focusing on tributary systems will effectively diminish the greater Missouri River population. Our analysis indicates otolith microchemistry may provide effective means to investigate this relationship.

Declarations

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Conflicts of interest/Competing interests: The authors declare no competing or conflicts of interest.

Ethics approval: Data collected for this study were in compliance with Institutional Animal Care and Use Committee ID 1271. Collection of specimens was in accordance with Scientific Collectors permit SC-104-2018 issued by the Kansas Department of Wildlife, Parks and Tourism.

Consent to participate: Not applicable

Consent for publication: All authors provide their consent for publication

Availability of data and material: All datasets analyzed during the current study are available from the corresponding author on request

Code availability: All the R code used for this study are available from the corresponding author on reasonable request.

Authors Contributions:

1. Jacob P. Werner – Contribution to conception and design, acquisition of data, and analysis and interpretation of the data. Drafting the article and final approval.
2. Quintin J. Dean – Contribution to conception and design, acquisition of data, and interpretation of the data. Revising the article critically for important intellectual content, and final approval.
3. Mark A. Pegg – Contribution to conception and design and interpretation of the data. Revising the article critically for important intellectual content, and final approval.
4. Martin J. Hamel – Contribution to conception and design and interpretation of the data. Revising the article critically for important intellectual content, and final approval.

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Tables

Table 1

Percent transient adult and juvenile Silver Carp in Segment 1 and Segment 2 of the Kansas River, and in both segments combined in 2018 and 2019. Total number of fish analyzed for otolith microchemistry are in parenthesis.

	Segment 1	Segment 2	Segments Combined
Adult Silver Carp			
2018	49% (n = 69)	49% (n = 72)	49% (n = 141)
2019	22% (n = 18)	45% (n = 80)	41% (n = 98)
Years Combined	44% (n = 87)	47% (n = 152)	
Juvenile Silver Carp			
2018	N/A (n = 0)	N/A (n = 0)	N/A (n = 0)
2019	34% (n = 32)	40% (n = 5)	35% (n = 37)
Years Combined	34% (n = 32)	40% (n = 5)	

Table 2

Classification matrix for Silver Carp collected from Segment 2 of the Kansas River and from the Wakarusa River in 2018, and from isolated backwaters of the Missouri River used to build and test the recursive partitioning tree.

	Sampled		
Predicted	Kansas River	Missouri River	Wakarusa River
Kansas River	12	2	1
Missouri River	1	2	0
Wakarusa River	0	0	0

Table 3

Number of transient adult and juvenile Silver Carp captured in the Kansas River that exhibit 1, 2, and 3, movement events into the Missouri River for each segment. Natal origins from the Missouri River is the number of transient individuals with Missouri River Sr:Ca signatures at the nucleus of the otolith.

	Number of Movement Events			Natal Origins from the Missouri River
	1	2	3	
Adult Silver Carp				
Segment 1	25	12	1	10
Segment 2	57	12	2	9
Juvenile Silver Carp				
Segment 1	9	2	0	5
Segment 2	1	1	0	1

Figures

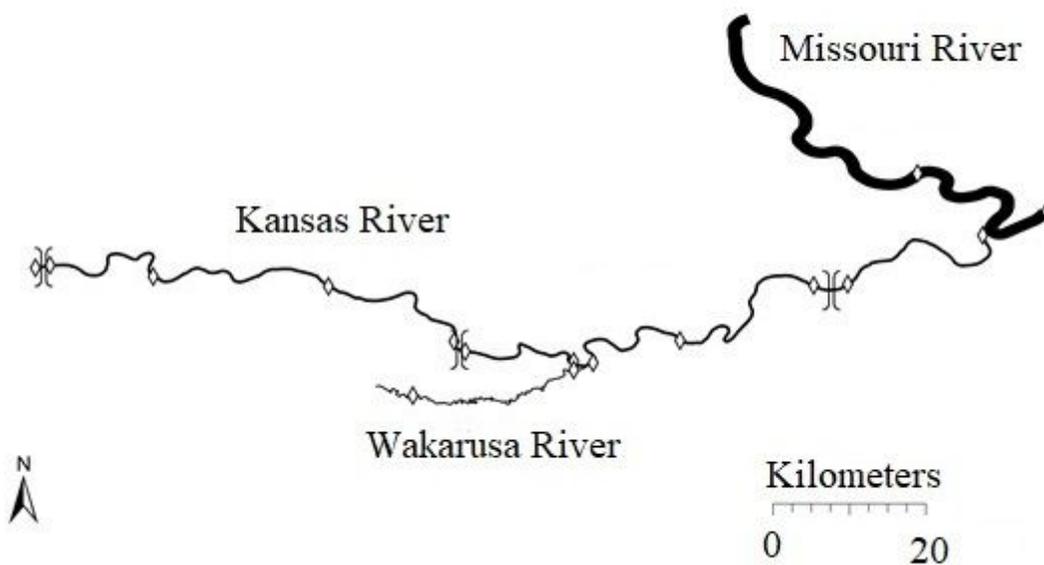


Figure 1

Kansas River study area. Vertical black lines indicate the three main barriers on the river; (right to left) the Johnson County Weir, Bowersock Dam, and the Topeka Weir. Segment 1 (27 rkm) is between the confluence with the Missouri River and the Johnson County Weir, Segment 2 (56 rkm) is between the Johnson County Weir and Bowersock Dam, and Segment 3 (58 rkm) is between Bowersock Dam and the Topeka Weir. Open diamonds indicate water chemistry sample collection sites

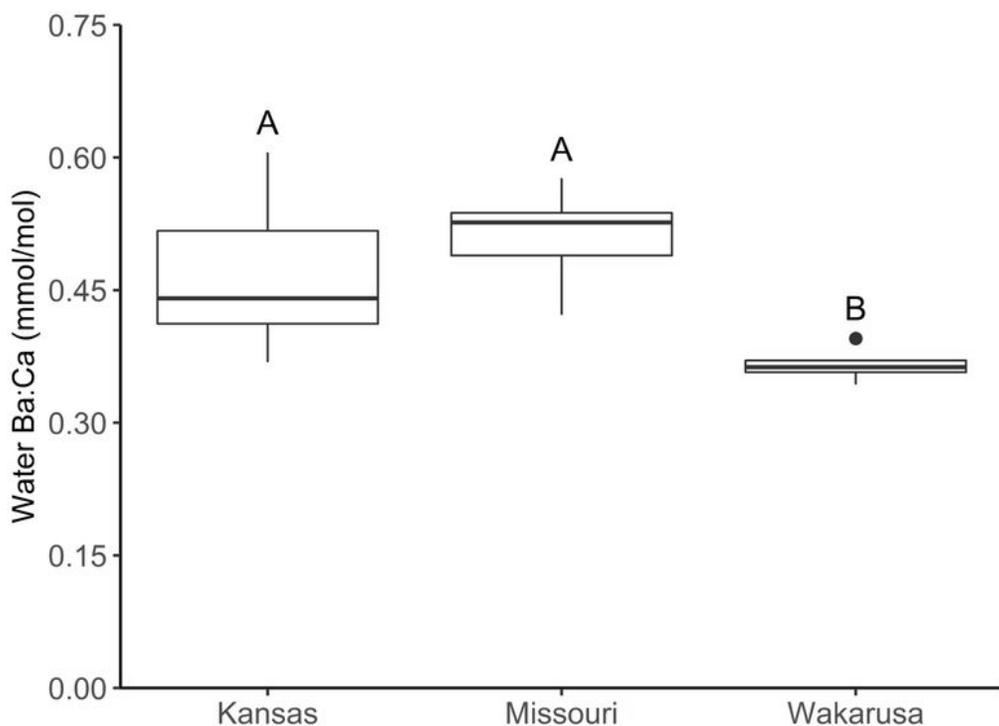
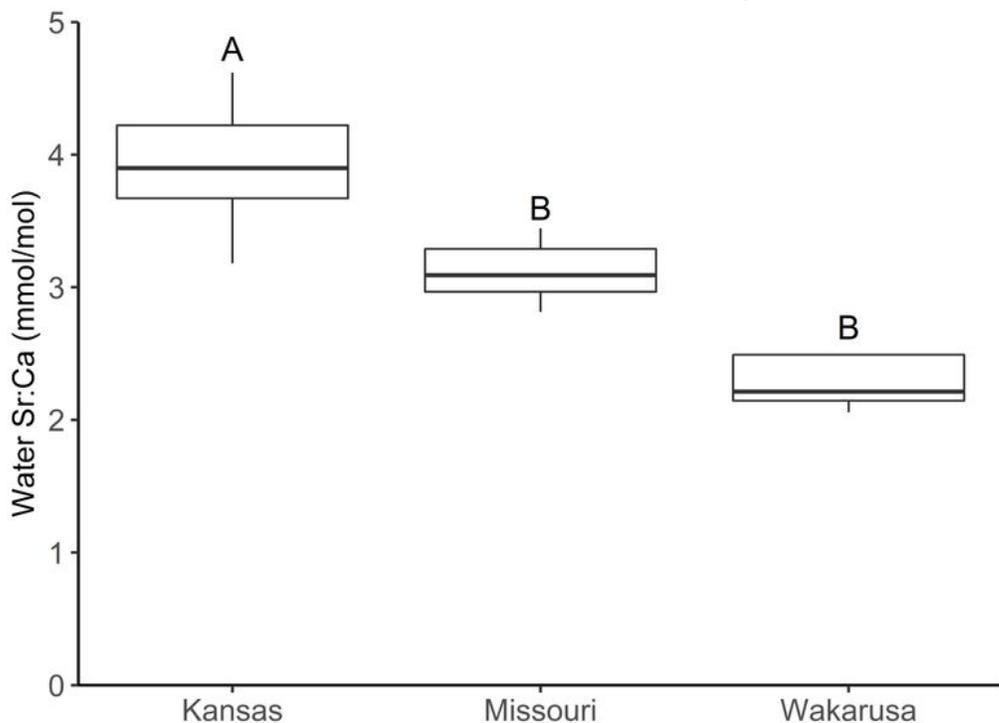


Figure 2

Comparison of water microchemistry signatures from the Kansas, Missouri, and Wakarusa Rivers. The horizontal solid line within the box represents the median value, upper and lower limits of the box is quartile ranges, and whiskers are 95% confidence intervals of the median. Points represent data outside of the 95% confidence interval. Median water Sr:Ca and Ba:Ca ratios do not differ between rivers that bear the same letter above the box plot

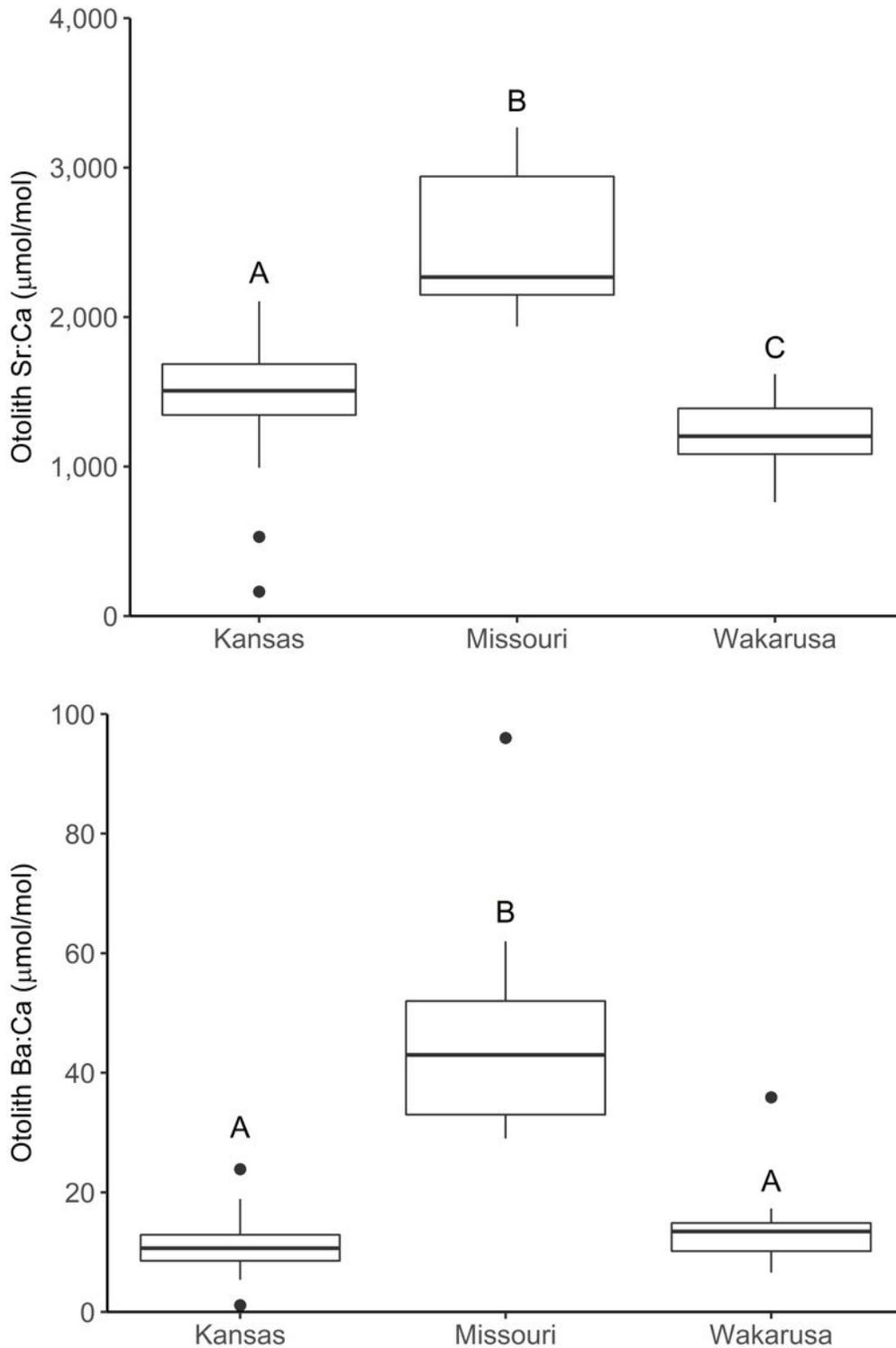


Figure 3

Comparison of otolith microchemistry signatures (~ 30µm of data isolated from the edge of the otolith) from Silver Carp captured from Segment 2 of the Kansas River in 2018, the Wakarusa River in 2018, and isolated backwaters of the Missouri River. The horizontal solid line within the box represents the median value, upper and lower limits of the box are quartile ranges, and whiskers are 95% confidence intervals of the median. Points represent data outside of the 95% confidence interval. Median otolith Sr:Ca and Ba:Ca ratios do not differ between rivers that bear the same letter above the box plot

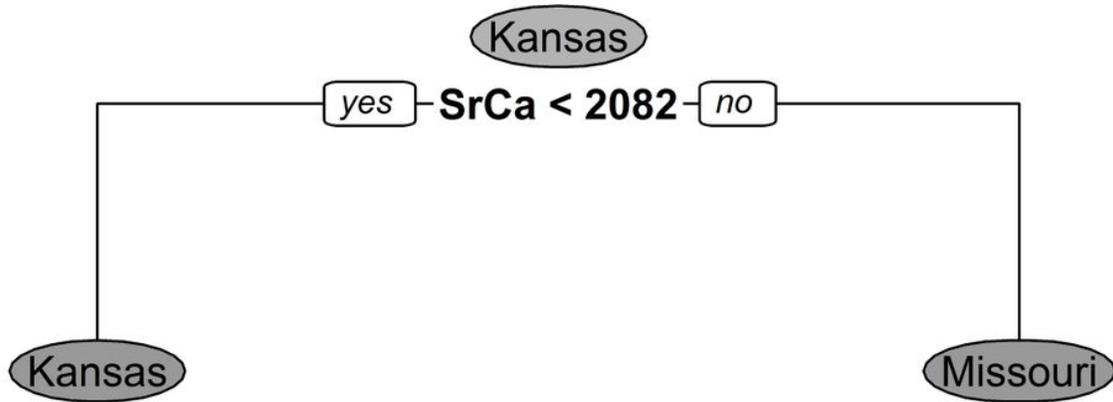


Figure 4

Recursive partitioning tree used to classify environmental history of Silver Carp between the Kansas River and the Missouri River. The tree was pruned at size = 2 with a complexity parameter (cp) of 0.14.

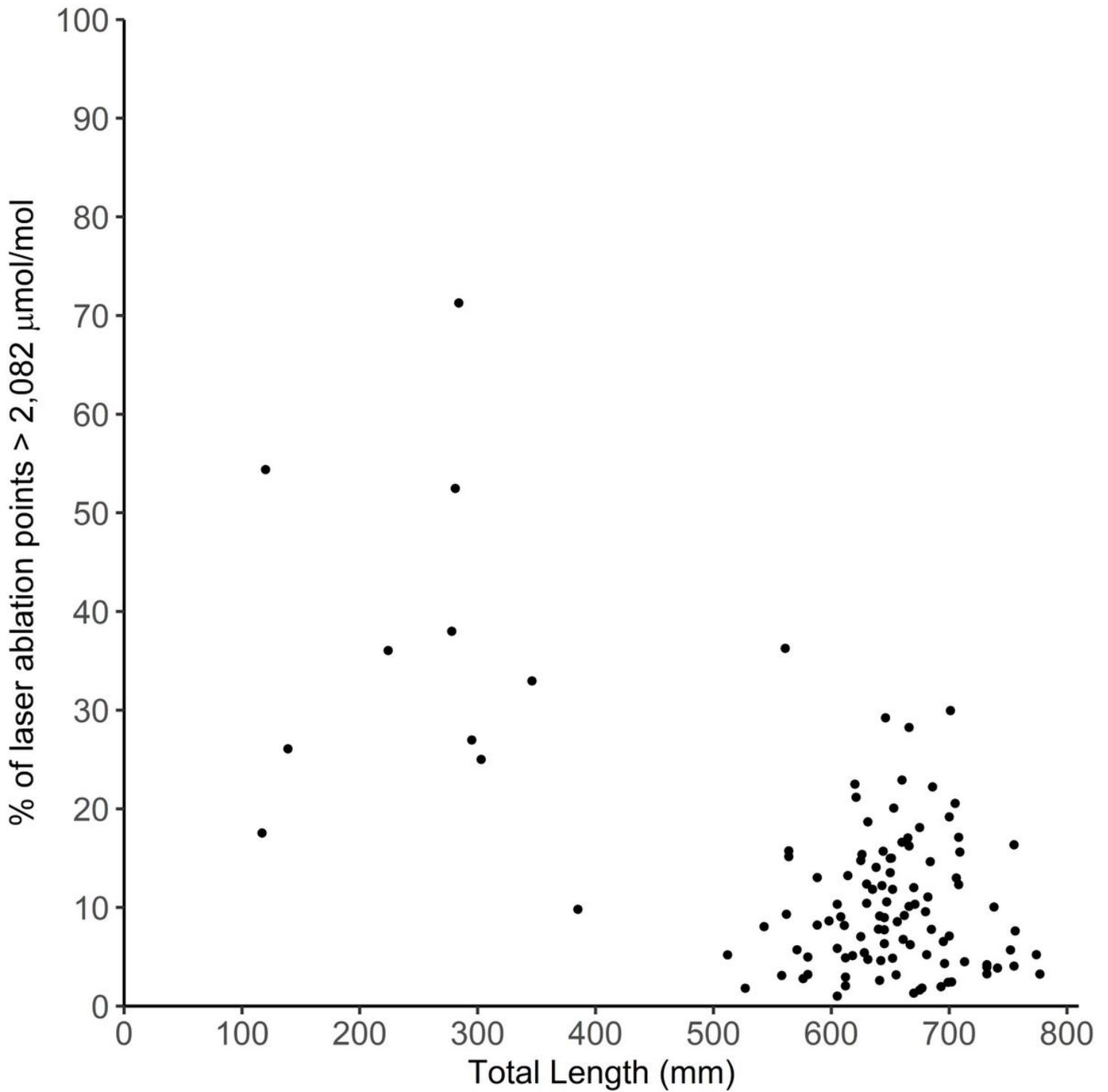


Figure 5

Percent of laser ablation points above 2,082 $\mu\text{mol/mol}$ Sr:Ca threshold for each individual transient fish by total length. Data points greater than 2,082 $\mu\text{mol/mol}$ Sr:Ca were indicative of Missouri River trace element signatures in lapilli otoliths of Silver Carp

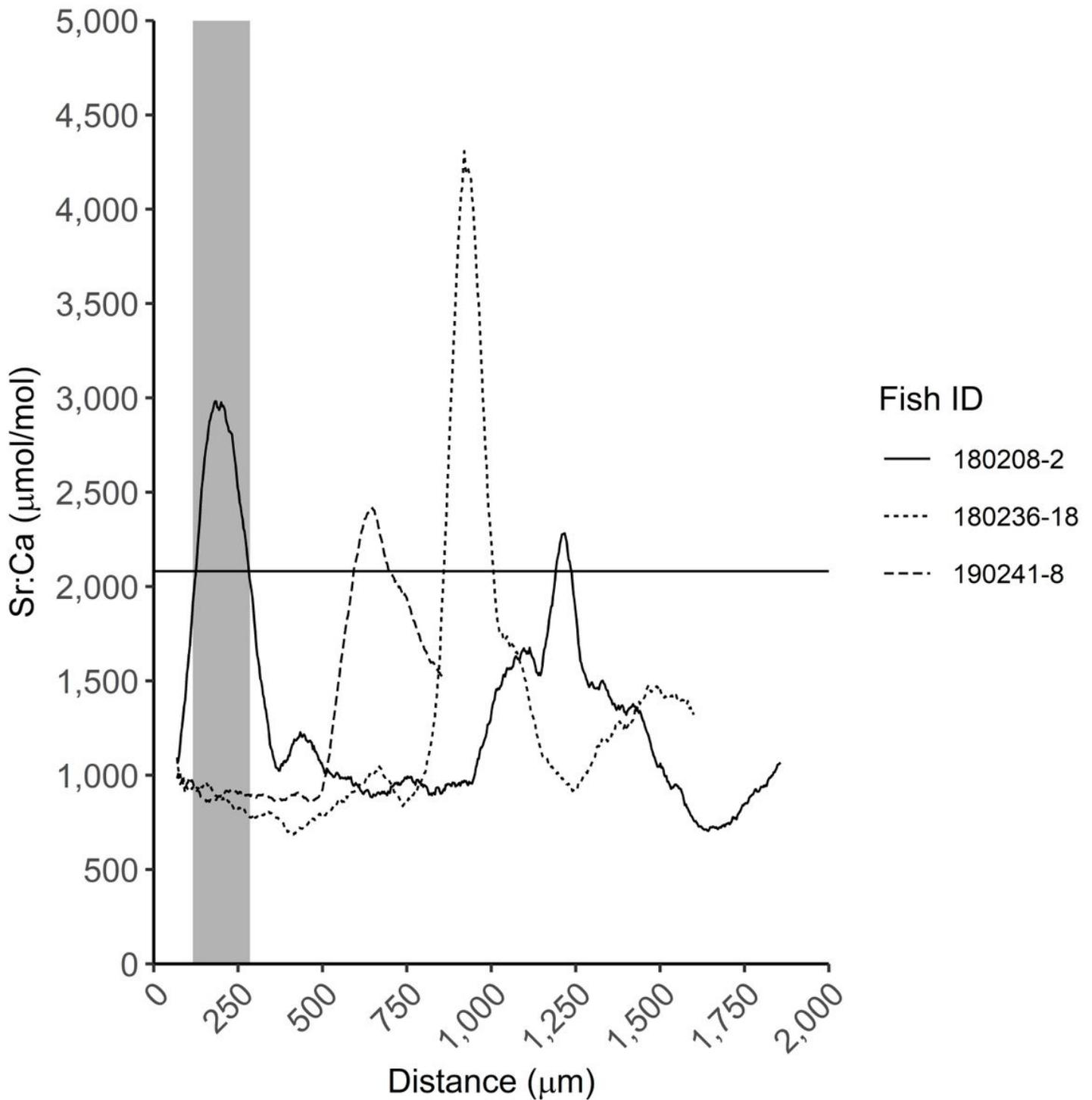


Figure 6

Example of ablation data from three Silver Carp captured from the Kansas River that exhibit trace elemental signatures indicative of the Missouri River. The horizontal line represents the threshold between the Kansas and Missouri Rivers (Sr:Ca = 2,082 µmol/mol) where signatures above the line are from the Missouri River. The shaded region is data that corresponds to the nucleus of the otolith (i.e., natal origins). Fish number 180208-2 had natal origin signatures from the Missouri River as well as another

movement event with signatures from the Missouri River later in life. Fish 180236-18 and 190241-8 both have movement events with signatures from the Missouri River