Assessing population impacts of low-use lock-and-dam structures on the Alabama River: fish hard-part microchemistry and genetics

by

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Abstract

Two techniques, hard-part microchemistry and genetics, were used in a coordinated effort to determine the impacts of low-use lock-and-dam structures on riverine fish species of varying migratory nature and capability. These approaches were selected to investigate population connectivity from different temporal perspectives: microchemistry – shorter term (over a fish's lifetime) and genetics – long-term (over multiple generations in a fish population). The first technique, fish hard-part microchemistry, has evolved into a powerful tool for fisheries managers, allowing one to quantify natal habitat, population connectivity, and individual movement patterns. To assess these objectives in a river system modified by the construction of dams, hard-part micro-elemental composition was quantified using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) for individuals of each species collected during 2017-2019. Species and structures were: Paddlefish (dentary bones), Smallmouth Buffalo (otoliths), and Largemouth Bass (otoliths). Location of capture within the study area was used to assign each fish to a population group. These locations were specific river sections that were separated by the three low-use lock-and-dam structures on the Alabama River, as well as major tributaries. Seasonal water samples were obtained from 15 sites throughout the study area during 2017-2018 to allow comparison of trace element conditions in water to those incorporated into fish hard-parts. Concentrations of Sr, Ba, Mn, Mg, and Ca were quantified. Water elemental signatures were spatially variable but temporally consistent throughout the study area, and were reflected in fish hard-part structure element-to-calcium ratios in core, age-0, edge, and whole-transect ablations. In particular, hard-part Sr:Ca ratios differed significantly among river sections for all three species. Additionally, discriminant function analyses (DFAs), determined how accurately multivariate

element signatures could classify a fish back to its river section of capture. Paddlefish yielded the highest level of accuracy (55-74%; errors nearly always assigning individuals to an adjacent river section), followed by Largemouth Bass (39-48%), and Smallmouth Buffalo, (37-47%). Differences in classification accuracy among species are likely due to a combination of factors, including differences in habitat type preferred by each species, varying life-history strategies, differences in the hard-part structures analyzed, and human influences (i.e., stocking and/or transport).

The second approach, population genetic analyses, were performed on a subset of the same fish collected for microchemistry analysis (i.e., for Paddlefish and Smallmouth Buffalo), representing fish from each river section, tributaries to the Alabama River, and neighboring watersheds. Genotyping-by-sequence techniques (GBS) identified 1,889 and 3,737 single nucleotide polymorphisms (SNPs) post filtering in Paddlefish and Smallmouth Buffalo, respectively, which were then used to estimate population diversity indices and conduct differentiation analyses. Analysis of molecular variance (AMOVA), discriminant analysis of principal components (DAPC), Bayesian clustering, and pairwise comparisons of F_{ST} values concluded that Paddlefish and Smallmouth Buffalo did not show strong evidence for genetic divergence among river sections.

When considering the combined results of these two approaches, the “potential” for population isolation trends to continue in some river sections exists based on microchemistry if no mitigation actions are taken. However, genetic analyses indicate that currently these dams have either not been present long enough, or that there is enough mixing/population connectivity occurring to prevent genetic divergence across river sections. Some limited mixing is believed to occur across lock-and-dam structures for Paddlefish and Smallmouth Buffalo, with fish movement highly restricted to passage during high water events at a crested spillway at the lowermost dam, or through lock structures.

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Introduction

The construction of dams for water supply, flood control, hydropower, navigation, agriculture and recreation accelerated rapidly in the United States during the 1930s (Neves et al. 1997; Haley and Johnston 2014). As of 1995, only 42 free flowing river systems greater than 200 km remained from the approximate 5.2 million km of river miles in the continental United States (Benke 1990; Lydeard and Mayden 1995; Neves et al. 1997). According to the U.S. Army Corp of Engineers' National Inventory of Dams (2016), there are approximately 90,000 large dams in the United States (USACE 2016).

Although the rate of new dam construction is decreasing (Neves et al. 1997), only recently have many of the hydrologic and ecological effects of dams become apparent which contribute to creating disjointed river reaches with isolated faunas (Neves et al. 1997; Graf 1999). Abiotic effects include channelization, impoundment, sedimentation, hydrologic alteration, habitat fragmentation, water quality changes, and thermal changes (Allan and Flecker 1993; Graf 1999; Warren et al. 2000; Jackson et al. 2001; Haley and Johnston 2014). Hydrologic alterations include reduced long-term downstream flows, diminished peak discharges, stabilized water levels, reduced current velocities, and a major shift from riverine to lacustrine environments (Jackson et al. 2001; Neraas and Spruell 2001).

Dams have had dramatic negative impacts on many riverine species and in many cases altered community structure and ecosystem function (Cooper et al. 2017). A study by Warren et al. (2000) attributed a 125% increase in the number of at risk southern U.S. fish populations to habitat degradation caused by habitat fragmentation, primarily from dams. Cooper et al. (2017) stated that dams have increased stream fragmentation by 801% and have significantly altered flows in the conterminous United States. Dam structures can adversely impact fish populations in multiple ways, including flow modification, (e.g., loss of stimuli for migration, loss of quantity, quality and accessibility to habitat, diminished attractive power of the river, and decreased egg survival), isolation and blocking of

movements (for migration, spawning, and dispersal for food and refuge), mortality (by passage over crested spillways, through turbines, or by the over-saturation of atmospheric gases that reach a lethal level from water passing over a spillway), reduced species diversity, and modified trophic cascades (Jackson et al. 2001; Neraas and Spruell 2001; Zigler et al. 2004; Freeman et al. 2005; Wofford et al. 2005). All these effects contribute to declines in fish populations, many of which play critical ecological and economic roles (Angermeier 1995; Burkhead et al. 1997; Hoxmeier and DeVries 1997; Lein and DeVries 1998; Warren et al. 2000; Allan 2004; Zigler et al. 2004; DeVries et al. 2009; FAO 2014; Simcox et al. 2015; Lynch et al. 2016)

The Southeastern United States has some of the highest densities of dams in the country (Graf 1999; US Army Corps of Engineers 2000; Jackson et al. 2001; Poff and Hart 2002; Downing et al. 2006). Most small-to-medium-sized rivers and nearly all large rivers in the Southeastern U.S. have been extensively dammed (Soballe 1992; Warren et al. 2000). As a result, this region has become a focus for studying the impacts of dams on fish populations in recent years. This region is also of particular interest due to its many endemic fishes, and the large proportion of its fauna that is threatened with extinction (Warren et al. 2000).

Due to the effects of dams on migratory fish populations, fish passage facilities have been developed at many dams throughout the United States to help facilitate movements past dams and restore connectivity in the riverine system. Various fishway designs include fish ladders, weirs, fish elevators, use of lock chambers, trap and haul operations, and “nature-like” bypass side channels that simulate a stream around a dam (Clay 1994; Jackson et al. 2001; Roscoe and Hinch 2010). In the last 50 years, fish passage facilities at dams have become increasingly sophisticated and efficient (Roscoe and Hinch 2010). Despite these improvements, fish passage efforts have been concentrated on the U.S. Atlantic and Pacific coasts for anadromous species such and salmonids, striped bass, and clupeids

(Jackson et al. 2001; Bernhardt et al. 2005). Relatively few fish passage approaches have been implemented in the Southeastern United States (Sudduth et al. 2007), and considerable controversy exists between resource agencies and hydropower companies about the passage and protection of river resident/potamodromous fishes (U.S. Office of Technology Assessment 1995). Specialized retrofits of existing dams to include fish passage facilities require substantial funding for planning, construction, and operation (Clay 1994). Therefore, it is critical to quantify the effects that dams have on river resident/potandromous fishes in the Southeastern United States to determine the costs and benefits of fish passage facilities.

To investigate whether lock-and-dam structures on the Alabama River are affecting fish populations, I chose to study three fish species; Paddlefish (*Polyodon spathula*), Smallmouth Buffalo (*Ictiobus bubalus*), and Largemouth Bass (*Micropterus salmoides*), that exhibit varying life history and migratory patterns. I used two approaches to quantify potential dam effects: analyses of the differences in (1) fish genetics and (2) microchemistries of fish hard-parts from locations along the river. These techniques were used to evaluate potential population impacts across differing time scales, with genetic responses presumably showing long-term impacts and hard-part microchemistry showing shorter term impacts. For example, while genetics can be useful for natal homing and stock structure or determining individual metrics such as paternity or genotype; it provides no insight into individual movements, insight that can be gained through hard-part microchemistry. Investigating populations using a combination of techniques can help to increase inferential power (Pracheil et al. 2014).

Hard-Part Microchemistry Approach

Hard-part microchemistry has become a powerful tool in fisheries science for examining migration and movement patterns, by reconstructing the environmental histories experienced by individual fish (Milton and Chenery 2003; Elsdon et al. 2008; Bock et al. 2016). Two hard-part structures

were used including otoliths (Smallmouth Buffalo and Largemouth Bass) and dentary bones (Paddlefish). Otoliths are paired structures composed of biogenic calcium carbonate in the form of aragonite, which is accreted daily in thin concentric rings. Following deposition, the aragonite rings remain unaltered, and can be referenced to specific fish ages and time periods. Trace elements are continuously incorporated into the hard-part, reflecting the ambient water chemistry at that time (Campana et al. 1997; Kennedy et al. 2002; Elsdon et al. 2008; Kerr and Campana 2014; Pracheil et al. 2014; Carlson et al. 2017). Therefore, otoliths can provide a chronology of exposure to changing water chemistry over the entire life of a fish (Campana et al. 1997; Elsdon et al. 2008; Carlson et al. 2017). Information can then be recovered at different locations within the otolith to reconstruct conditions of inhabited environments throughout the life of a fish (Campana and Thorrold 2001; Kennedy et al. 2002; Kerr and Campana 2014; Carlson et al. 2017). Hard-part microchemical analysis has proven particularly useful in determining fish movements, migration patterns, and life history characteristics of fishes (Zeigler and Whitledge 2011; Phelps et al. 2012; Bock et al. 2016), as well as for identifying natal environments and lifetime patterns of intra-river movement for fishes in large river systems (Zeigler and Whitledge 2011; Phelps et al. 2012; Pracheil et al. 2014; Bock et al. 2016; Carlson et al. 2017)

Dentary bones were used from Paddlefish for microchemistry analysis. The dentary bone is the most commonly used structure for estimating Paddlefish age because of its clear and widely spaced annuli that are retained throughout an individual's lifetime (Adams 1942; Scarnecchia et al. 1996, 2006). In contrast, Paddlefish otoliths have proven to be less useful for age estimation due to their small size, annuli crowding, and false annuli (Scarnecchia et al. 2006). Additionally, microchemistry analysis options are limited with Paddlefish otoliths because they are primarily composed of the calcium carbonate polymorphs calcite and vaterite, which differ in elemental composition when compared to otoliths comprised of aragonite, the form typically found in other teleost species (Tzeng et al. 2007; Bock et al. 2016; Carlson et al. 2017; Pracheil et al. 2017). Dentary bones, on the other hand, are calcified

structures that are part of the Paddlefish lower jaw, and contain an organic matrix and inorganic fraction that primarily consists of hydroxyapatite (Bock et al. 2016). Dentary resorption and remodeling appear to be uncommon, which makes this the structure of choice for the application of hard-part microchemistries for inferences about an individual's environmental history. A recent study by Bock et al. (2016) concluded that the Paddlefish dentary bone reflects water chemistry for commonly applied natural chemical markers, suggesting that dentary bone microchemistry could be used for reconstructing Paddlefish environmental histories in locations where spatial differences in water chemistry occur.

Genetics Approach

The genetics approach used to study the population impacts of lock-and-dam structures on fish populations in the Alabama River involved DNA marker technology which can reveal differences in mutation rates and quantify genetic variation (Liu and Cordes 2004). Many marker technologies exist, which have revolutionized the way genetics research is performed (Liu and Cordes 2004). This study utilized single nucleotide polymorphisms (SNPs), which are alternative bases at any given nucleotide position within a locus. SNPs are caused by point mutations that give rise to different alleles. A SNP can produce as many as 4 alleles, each containing one of four bases (A,T,C,or G) at the SNP site. However, most SNPs are limited to one of two alleles (either one of the two pyrimidines C/T or the two purines A/G), and are considered to be bi-allelic. SNPs are the most abundant type of genetic variation in any organism and are widely distributed within genomes (Liu and Cordes 2004; Liu et al. 2011). SNPs were selected as the DNA marker for this study because they provide a more straightforward approach for comparisons of genomic diversities and they are superior for explaining the evolutionary history of populations (Brumfield et al. 2003). Additionally, they have become a marker of choice for genetics studies due to their potential for high genotyping efficiency, automation, data quality, genome-wide

coverage and analytical simplicity (Liu et al. 2011). Finally, they can reveal hidden polymorphisms not detected by other markers (Liu and Cordes 2004).

Being able to quantify genetic variation within and among populations is crucial for understanding the evolutionary processes that promote and maintain biodiversity and for the proper management of biological resources (Moritz 2002; Poissant et al. 2005). For example, examining patterns of genetic diversity has become an integral component of many management plans concerning endangered species (Wu 2005). When dispersal pathways and migration corridors are disrupted as in the case of large lock-and-dam structures, gene flow can be reduced or eliminated. This can lead to the isolation of populations and decrease genetic diversity through the processes of genetic drift and inbreeding, potentially reducing the long-term viability of the species (Wofford et al. 2005). When few individuals are able to move between population groups as a result of a barrier, opportunities for genetic differentiation arising from local adaptation and random genetic change increase, potentially resulting in population groups with distinct genetic characteristics (Carvalho and Hauser 1998). This was the situation with the Chinese Paddlefish, *Psephurus gladius*, whose populations became fragmented and rapidly reduced across the Yangtze River as a result of the construction of Gezhouba Dam, coupled with overharvest, habitat destruction, and pollution. This work demonstrated that genetic diversity can be measurably reduced in 35-40 years (Wu 2005).

Here, my specific objectives were to: 1) use water samples to identify if the various reaches and tributaries of the Alabama River under study are characterized by spatially variable and temporally consistent trace elemental signatures, 2) use hard-part microchemistry and water chemistry analyses to determine movement patterns of individual fishes throughout the Alabama River, 3) combine hard-part microchemistry and water chemistry to predict the natal origins of these fish species groups based on microchemical signatures near the core of the hard-part cross section, 4) use hard-part microchemistry

and SNP technology to quantify potential fish population differences and the extent of mixing and connectivity of 3 fish species across potential population groups located in four sections of the Alabama River that are separated by three lock-and-dam structures, 5) use a field study to determine how elements are incorporated into dentary bones: either based on the ratio of the concentration of elements to calcium in the water or solely on parts per million counts of the elements observed, and 6) use genetic analyses to quantify if there are genetic differences among Paddlefish populations separated by dams in the Alabama River and from some of the various other watersheds that contain Paddlefish in Alabama (e.g. Tombigbee and Coosa rivers).

Study Site

The Alabama River watershed is an example of a Southeastern U.S. ecosystem that has been influenced by the presence of dams. It provides an excellent opportunity to study the impacts of dams on the riverine fishes that live there. The Alabama River is formed by the confluence of the Tallapoosa and Coosa rivers about 11 km northeast of Montgomery AL (Mettee et al. 2005a) (Figure 1). This river system drains an approximate 59,000 km^2 basin that includes portions of northeast Georgia, southern Tennessee, and east-central Alabama (Freeman et al. 2005). It flows for approximately 500 km across the coastal plain through central and southern Alabama before it joins the Tombigbee River about 48 km north of Mobile AL, forming the Mobile River, which then flows into Mobile Bay (Freeman et al. 2005; Mettee et al. 2005a; Haley and Johnston 2014) (Figure 1).

The headwaters of the Alabama River, the Coosa and Tallapoosa rivers, have wide ranging physiographic diversity, originating in the Blue Ridge, Valley and Ridge, and Upland Piedmont physiographic regions along the southern bend of Appalachia (Freeman et al. 2005). The distinctive characteristics of each of these rivers are attributed to the varied lithography and soil horizons that occur in their watersheds (Freeman et al. 2005). The Cahaba River, the most western major tributary of

the Alabama River, enters near Alabama River Kilometer Mile (RKM) 304 (Mettee et al. 2005a). It is the system's longest unregulated river, flowing for over 300 km from the valley and ridge province and onto the coastal plain (Freeman et al. 2005). The major land uses throughout much of the watershed include forest production and agriculture (Mettee et al. 2005a). Rainfall is abundant in the Alabama River watershed, averaging 127-142 cm/year, with some geographic regions within the basin typically receiving up to 150 cm/yr (Freeman et al. 2005). The highest river flows are generally experienced during February, March and April, and are lowest during September, October and November. High spring flows (exceeding 2,250 m/s) often inundate riparian and floodplain habitats for up to 50 percent of the time between March and September (Freeman et al. 2005). High spring flows often coincide with typical fish migration periods in the Alabama River system, which may help to facilitate/encourage fish to move past dams.

The United States Army Corps of Engineers (USACE) Mobile District operates three low-use lock-and-dam structures on the Alabama River and had maintained a 2.7 m deep navigation channel to ensure uninterrupted boat travel along its entire length up until 2008 (Haley and Johnston 2014; Mettee et al. 2015) (Figure 1). Commercial use of the waterway never developed as expected, and in 2008 dredging operations were confined to the lower 118 km of the river and the lock approaches at each dam. In 2014, commercial and recreational vessels were required to schedule an appointment for any lockage 30 days prior to the planned event (Dyess 2014). Overall, the navigational lock chambers at each of these dams tend to be used infrequently.

The lowermost dam, Claiborne Lock-and-Dam, (RKM 117) was completed in 1969 for the primary purposes of navigation and flood control. It has a 127 m concrete gated spillway (6 gates) and a 152 m ungated crested spillway. Laminar flow over the ungated spillway occurs when water levels reach an elevation of 10 m (USACE 2013a; Mettee et al. 2015). The next dam upstream, Millers Ferry Lock-

and-Dam, (RKM 214) was completed between 1968-1970 with the primary purposes of navigation, hydropower, and flood control. It has a 303 m gated spillway with 17 gates. The 90,000 kW hydropower structure is located 0.3 km downstream of the lock chamber at Millers Ferry (USACE 2013b; Mettee et al. 2015). The third dam upstream, Robert F. Henry Lock-and-Dam, (RKM 380) was completed in 1971 and acts primarily for navigation, hydropower and flood control. It has a 197 m gated spillway with 11 gates. The 82,000 kW powerhouse is connected to the gated spillway on its east side and is 114 m in length (USACE 2013c).

Genetics and fish hard-part microchemistry comparisons were performed across the Alabama River system below, between, and above the three different low-use lock-and-dam structures which created four river sections used in my analysis: 1) below Claiborne L&D (lower Alabama River-LAR), 2) above Claiborne L&D and below Millers Ferry L&D (Claiborne Lake-CL), 3) above Millers Ferry L&D and below Robert F. Henry L&D (Millers Ferry Reservoir-MFR), and 4) above Robert F. Henry L&D (Jones Bluff Reservoir-JBR). Fish were also collected in the Coosa (CSA), Tallapoosa (TAL), and Tombigbee (TOM) Rivers for additional comparisons (Figure 2).

Freeman et al. (2005) concluded that dams on the Alabama River have led to fish assemblage simplification and species losses as a result of flow modification. Due to this modified flow regime, the major free-flowing tributaries to the Alabama River have retained/sustained much of the riverine flow-dependent fish fauna and diversity. Now isolated in tributaries, these species are more susceptible to increased physical and chemical habitat degradation (Freeman et al. 2005). Examples of systems housing vulnerable species include two major tributaries of the Coosa River (Conasauga River, and upper Etowah River), the Coosawattee system, the Cahaba system, and a single spring located in the Coosa system, which retain 8 of the 10 federally listed species in the Alabama River system (i.e., only the two listed sturgeon species [Alabama Sturgeon (*Scaphirhynchus suttkusi*) and Gulf Sturgeon (*Acipenser*

oxyrinchus desotoi)] are not present) along with a large portion of the native fish fauna (Williams 1968; Walters 1997; Freeman et al. 2005).

Additionally, general declines in diadromous and migratory fishes have been documented (Freeman et al. 2005). As a result of these declines, studies have been conducted to understand the degree to which fish passage is occurring and to identify opportunities for improving fish passage at dam structures on the Alabama River (Mettee et al. 2006, 2015; Simcox et al. 2015). For example, prior studies have found that during normal flow conditions, the crested spillway height at Claiborne Dam and hydraulic turbulence through spill gates at Claiborne, Millers Ferry, and Robert F. Henry Dams restricts the upstream movement of most Alabama River fishes (Mettee et al. 2006, 2015). However, fish passage has been documented over the crested spillway at Claiborne Lock-and-Dam during periods when flooding inundated the crested spillway (Mettee et al. 2006; Simcox et al. 2015). Targeted conservation-lock operations, when in use during migration periods, may help facilitate fish passage in some river systems, but not be effective in others (Mettee et al. 2006; Smith and Hightower 2012; Young et al. 2012). For example, while specialized lock operations (conservation lockages) did increase the opportunity for passage and facilitated some passage events at Claiborne and Millers Ferry, relatively few fish successfully passed upstream (Simcox et al. 2015).

Study Species

Paddlefish

The Paddlefish is a long lived, highly mobile and migratory fish species that can occupy the entire water column (Boschung and Mayden 2003; Zigler et al. 2004; Simcox et al. 2015). Over the past century, reduction in the range and abundance of Paddlefish has occurred as a result of habitat alterations caused by human activities. These include the construction of large dams throughout the species' current range, which limits fish movement and reduces the amount of spawning habitat

available. Other factors contributing to reduced range and population decline include channelization and the elimination of backwater areas (critical habitats occupied at certain times of year), dewatering of streams, over harvest, and industrial pollution (Southall and Hubert 1984; Graham 1997; Hoxmeier and DeVries 1997; Mims 2001; Miller and Scarnecchia 2008; Sharov et al. 2014).

At least two genetically distinct populations of Paddlefish, the Mobile River Basin and the Mississippi River Basin, are known to currently occur in the United States. Other smaller populations from isolated watersheds are potentially distinct as well (Carlson et al. 1982; Mims 2001; DeVries et al. 2009). Mobile basin Paddlefish, in comparison to Mississippi basin Paddlefish, are generally smaller in maximum size, have shorter life spans, reach reproductive maturity at a younger age, and may reproduce more often and have higher relative fecundity (number of eggs per kg of body weight) (Epifanio et al. 1996; Hoxmeier and DeVries 1997; DeVries et al. 2009). Paddlefish in the Alabama River generally do not exceed 11-15 years old, may spawn annually, reach maturity at 5-6 years, and not do exceed 24 kg (Boschung and Mayden 2003).

Extensive movements have been documented for Paddlefish, particularly during spring spawning migrations, but also during the fall. It is not uncommon for Paddlefish to travel hundreds of kilometers during migrations (Miller and Scarnecchia 2008). A tagged individual in the Missouri River was once recorded moving 2,000 km downstream (Rosen et al. 1982) and a tagged individual in the Alabama River was recorded moving 245 km downstream (Simcox et al. 2015). Some movements past dams in both the Mississippi and Alabama river systems have been documented under certain conditions, including passage through low head navigation dams, over crested spillways and through low-use lock-and-dam structures (Southall and Hubert 1984; Jennings and Zigler 2000; Mettee et al. 2005b; Sharov et al. 2014; Simcox et al. 2015). Spawning occurs primarily on clean gravel bars, but the use of riprap has also been observed. Failure to reproduce occurs primarily when fish cannot navigate

past dams, when spawning cues are eliminated, or if fish cannot find silt-free substrate for egg deposition. Spawning is triggered by increasing water temperature and rising water level. In the Alabama River, spawning generally occurs in March when water temperatures reach 12°C (Hoxmeier and DeVries 1997; Boschung and Mayden 2003; DeVries et al. 2009; Jennings and Zigler 2009; Pracheil et al. 2009).

Paddlefish is a species of interest within the State of Alabama because of their conservation concern as a migratory species in impounded river systems, a possession/harvest moratorium, and the reopening and then recent (2018) indefinite suspension of a commercial harvest season during which they were primarily harvested for their caviar (Alabama Department of Conservation and Natural Resources 2018). Paddlefish in Alabama were vastly overfished in the 1970-1980s prompting a possession moratorium in 1988 which has led to concern regarding proper management (Pasch et al. 1980; Wood 1989; Hoxmeier and DeVries 1997). Therefore, an increased ecological understanding of the population within the Alabama River system is critical for making future management decisions for the species (Carlson and Bonislawsky 1981; DeVries et al. 2009; Phelps et al. 2012).

Smallmouth Buffalo

Smallmouth Buffalo is an abundant, benthic, deep bodied sucker species (Walburg and Nelson 1966; Mettee et al. 1996; Adams and Parsons 1998; Boschung and Mayden 2003) that can occur in rivers, but is also common in impoundments (Etnier and Starnes 1993; Boschung and Mayden 2003). Similar to other castastomids, Smallmouth Buffalo swim upstream and participate in a spring spawning migration, often selecting habitats in shallow weedy sloughs to spawn (Walburg and Nelson 1966; Adams and Parsons 1998; Boschung and Mayden 2003; Mettee et al. 2005a; Cooke et al. 2005; Lucas and Baras 2008). Additionally, Smallmouth Buffalo have been documented traveling long distances in excess of hundreds of km over extended time periods (Adams and Parsons 1998). Smallmouth Buffalo

offer contrasting life history traits, habitat use, and body size relative to Paddlefish. They are a smaller and primarily benthic species which may affect their ability to pass a dam at high flows, and they have generally less extensive migrations as compared to Paddlefish (Becker 1983; Mettee et al. 1996; Boschung and Mayden 2003; Baumgartner and Harris 2007; Simcox et al. 2015).

Largemouth Bass

Largemouth Bass also offer significantly contrasting life-history traits compared to that of both Paddlefish and Smallmouth Buffalo. First, they frequently occupy littoral habitats and second, they have relatively small home ranges and are more sedentary (Warden and Lorio 1975; Fish and Savitz 1983; Boschung and Mayden 2003; Ahrenstorff et al. 2008). Relative lack of strong migration patterns has been documented though mark-recapture and tracking studies that have indicated they rarely move more than 100 m from their point of capture in impounded systems (Lewis and Flickinger 1967; Warden and Lorio 1975; Boschung and Mayden 2003). Given that, an understanding of how dams might affect species that do not exhibit large-scale migrations is also needed. Finally, Largemouth Bass are abundant within most stretches of the Alabama River system making it possible to use them for comparison.

Methods

Water Chemistry

One water sample was collected at each site for trace elemental analysis. When sampling below a dam in the tailrace, water chemistry measurements and water sample collection was conducted several hundred m downstream of the dam structure (USEPA 1996). A Van Dorn sampler was used for water sample collection, and was held in the open position at each site at 1 m depth for approximately 30 sec to flush any residual water prior to sample collection (Farmer et al. 2013). The water sample was collected with a 50 mL sterile syringe (Henke-Sass Wolf (HSW) Norm-Ject) from the Van Dorn sampler, and filtered through a disposable 0.45 µm PTFE glass-fiber filter (Whatman GD/XP) directly into a 125

mL pre-acid washed high density polyethylene (HDPE) bottle containing 0.5 mL of 1:1 nitric acid solution for field preservation and storage (USEPA 1996; Lowe et al. 2011; Farmer et al. 2013; Walther and Nims 2015; Nelson et al. 2015; Bock et al. 2016). This process was repeated 3 times in order to fill the 125 ml bottle. Samples were placed on ice and transported back to Auburn University's Ireland Center Laboratory where they were refrigerated until transportation to the analytical laboratory for analysis (USEPA 1996). Water samples were collected quarterly (once during each season) from 15 sites during both 2017 and 2018 (Table 1, Figure 3). Sampling locations were selected to provide an even spatial distribution throughout the Alabama River and its tributaries.

Water Chemistry Analysis

Water samples were analyzed at Dauphin Island Sea Laboratory (DISL), Dauphin Island, Alabama for trace elemental analysis of calcium, strontium, barium, magnesium, and manganese. Analysis was performed using solution-based inductively-coupled plasma mass spectrometry (ICPMS), after 10-fold dilution with an internal standard solution of 2% HNO_3 with Be, In, and NIST standard to correct for matrix and drift effects on an individual sample basis. Water sample concentrations were output in ppb (equivalent to ng g^{-1}), for ease in converting to a molar ratio. A molar element: Ca ratio for water was generated using the following formulas (where X equals the element of interest) in order to compare concentrations of elements in water to concentrations in otoliths and dentary bones:

$$\frac{\text{ng g}^{-1}\text{X} \Big/ \text{MassX(g)}}{1 \Big/ \text{Mass H}_2\text{O (18g)}} = \text{nmol mol}^{-1}\text{X} \Big/ 1000 = \mu\text{mol mol}^{-1}\text{X}$$

$$\frac{\text{ng g}^{-1}\text{Ca} \Big/ \text{Mass Ca (40.078g)}}{1 \Big/ \text{Mass H}_2\text{O (18g)}} = \text{nmol mol}^{-1}\text{Ca} \Big/ 10^9 = \text{mol Ca}$$

Molar element: Ca ratios in water for Sr, Ba, Mg and Mn were reported in element μmol for every mol of calcium in water (element: Ca μmol mol^{-1} water).

Fish Collections

Paddlefish Sampling

Paddlefish fin clips and dentary bones were collected by Alabama Department of Conservation and Natural Resources (ADCNR) biologists during the regulated commercial Paddlefish season occurring on the Alabama River in 2017 (season: February 1st - March 21st), and as part of their standardized Paddlefish sampling efforts in May 2017 and February 2019. Commercial fishers used gillnets in designated stretches of the Alabama River and were intercepted by biologists for biological data collection. Location of capture designated a Paddlefish into one of four populations, separated by each dam structure, based on its capture location. These included the Lower Alabama River (LAR), Claiborne Lake (CL), Millers Ferry Reservoir (MFR) and Jones Bluff Reservoir (JBR) (Figure 2). In order to be harvested by commercial fishers, Paddlefish had to be egg-bearing females with an curved-eye-to-fork (CEFL) length greater than 863.6 mm (34 inches) when measured with a flexible tape measure along the curvature of the body (ADCNR 2013). Paddlefish dentary bones were also collected by ADCNR from fish caught in the Tallapoosa River (TAL) for comparison.

Additionally, I collected Paddlefish near water sampling locations on the Alabama River between March-May and November-January of both 2017 and 2018. The GPS location for each individual fish collected was recorded. Handling of Paddlefish was avoided after water temperatures reached 21°C to prevent additional stress (Lein and DeVries 1997; Mims 2001). These Paddlefish collections were performed using both boat-mounted, pulsed-DC electrofishing gear (Smith-Root, DC 7.5 electrofisher) and floating multifilament gillnets with a 152.4 mm minimum bar mesh (Southall and Hubert 1984; Lein and DeVries 1997; Simcox et al. 2015). Gillnets were checked at least once every two hours. Any

previously tagged or fin clipped Paddlefish was measured, weighed, and released. Fin clips were collected from all other Paddlefish, after which fish were released near the approximate location of capture.

Additional fin clips provided by ADCNR were collected either historically (2005-2015) or during spring 2017 at additional sites outside the Alabama River drainage (e.g., the Tombigbee and Coosa rivers) to provide a more comprehensive understanding of Paddlefish genetics throughout the Mobile basin.

Paddlefish Tissue Sample and Dentary Bone Collection

A fin clip of approximately 2 cm x 2 cm was collected from the left pectoral fin, preserved with 95% ethanol, and stored in a small pre-cleaned, labeled 20 ml scintillation vial. The surgical scissors used to collect samples were cleaned with 95% ethanol before and after sample collection. Other measurements recorded from Paddlefish included weight (kg [to nearest 0.01kg]), block length (uppermost part of operculum to fork of the tail) (mm), eye to fork length (EFL) – flat (measurement from front of eye straight to fork of the tail, not touching body) and curved (measurement from front of eye curved along body to the fork of the tail, in contact with body) (mm), and girth (mm). All lengths were measured with a flexible tape measure.

ADCNR biologists intercepted commercial paddlefish harvesters at boat ramps and on the water to collect dentary bones and a tissue sample. Both the right and left dentary bones were collected. Each dentary bone was removed by first cutting at the anterior end of the Paddlefish lower jaw with diagonal pliers and then making a second cut posterior to the point of greatest curvature in the dentary bone along the side of the jaw. This left an approximate 10 cm section of straight dentary bone. Any flesh adhering to the extracted piece of dentary was removed and the pair of dentary bones was then placed in a labeled envelope. All dentary bones were allowed to air dry prior to sectioning (Scarnecchia

et al. 2006; Bock et al. 2016). Any dentary bones collected during my sampling were obtained in the same manner as described above.

Smallmouth Buffalo and Largemouth Bass Sampling and Data collection

Smallmouth Buffalo and Largemouth Bass were also collected with both boat-mounted pulsed-DC electrofishing and gill nets (Walburg and Nelson 1966; Simcox et al. 2015). Both species were collected during March-December of both 2017 and 2018 throughout the Alabama River drainage (including the lower reaches of the Coosa and Tallapoosa rivers) (Figure 2). Within each major river section of the Alabama River sampled (LAR, CL, MFR, and JBR), Smallmouth Buffalo and Largemouth Bass were collected from four sub-sections evenly divided within each major river section to distribute sampling effort throughout the major river sections.

Fish were weighed (kg [to nearest 0.01kg]), measured (total length [mm]), had a fin clip removed from the right pelvic fin, and had both sagittal otoliths removed with Teflon coated forceps. Harvested otoliths were then placed in 30% H_2O_2 for 30 seconds for cleaning. Following cleaning, otoliths were rinsed in Thermo Fisher Scientific ultrapure water, dried, and stored in individual sterile pre-labeled polyethylene vials (Lowe et al. 2011; Farmer et al. 2013; Nelson et al. 2015).

Fish Hard-Part Microchemistry

Laboratory Sample Preparation

For Smallmouth Buffalo and Largemouth Bass, one otolith from each fish was selected at random to be set in an epoxy resin, and a transverse section (approximately 0.5 mm) through the core was removed using a low speed diamond blade isomet saw. Sections were then polished sequentially with 300, 600, 1200, 1800, 8000, and 14000 grit (40, 30, 12, 9, 3, 1 micron) lapping film until sections were smooth, after which they were rinsed with Thermo Fisher Scientific ultrapure water (Zeigler and Whitledge 2011; Lowe et al. 2011; Farmer et al. 2013; Nelson et al. 2015). Individual otolith sections

were then placed in a drop of Thermo Scientific ultrapure water in a covered petri dish that was floated in a bath located within an ultrasonic cleaner and sonicated for 10 minutes. Following sonicating, otolith sections were washed thoroughly once more with ultrapure water and allowed to dry (Ludsin et al. 2006; Zeigler and Whitledge 2011; Lowe et al. 2011; Farmer et al. 2013; Gover et al. 2014; Pracheil et al. 2014; Nelson et al. 2015). Following drying, each section was mounted onto standard glass slides (75 mm x 25 mm) with cyanoacrylate glue. Otolith slides were then stored in sealed pre-cleaned high-density polyethylene wide mouth 250 mL containers until microchemistry was performed and sections were aged (Zeigler and Whitledge 2011; Lowe et al. 2011; Farmer et al. 2013; Nelson et al. 2015).

A 0.5-0.65 mm transverse section was cut from the right-side dentary bone of each Paddlefish approximately 10 mm posterior to the point of greatest curvature using a low speed diamond blade isomet saw (Scarnecchia et al. 1996; Bock et al. 2016). This section was used both for age estimation and microchemistry analysis and was prepared in the same manner described above for otolith microchemistry (Smith and Whitledge 2011; Phelps et al. 2012; Bock et al. 2016).

Otolith and Dentary Bone Elemental Analysis

Otolith and dentary bone microchemical analyses were conducted at DISL. Trace element compositions were quantified using a laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) on a Agilent Technologies 7700 series ICPMS coupled to a 213 nm New Wave NWR-213 internally homogenized, optically attenuated laser system (Milton and Chenery 2003; Zeigler and Whitledge 2011; Lowe et al. 2011; Farmer et al. 2013; Kerr and Campana 2014; Nelson et al. 2015; Bock et al. 2016; Carlson et al. 2017). Each dentary bone section had a linear transect ablated from inside its first annulus to the edge of its mesial arm and each otolith section also had a linear transect ablated from the focus of its core to its proximal edge. These transects allowed me to measure the incorporation of elements over the time of each fish. A pre-ablation laser transect was performed just

before analysis along the designated laser ablation track on every fish hard-part to eliminate surface contamination (Campana et al. 1994; Hamer et al. 2003; McCulloch et al. 2005; Ruttenberg et al. 2005; Tabouret et al. 2010; Longmore et al. 2010). Pre-ablation settings were as follows: 20% energy, 5 repetition rate (Hz), 40 spot size (µm), and 100 scan speed (µm/sec). Analysis ablation settings were as follows: 25-28% energy, 10 rep rate (Hz), 25 spot size (µm), and 5 scan speed (µm/sec). The concentration (counts per second [cps]) of Ca, Sr, Ba, Mg, and Mn were quantified. Given the speed of the ablation and the number of elements tested, elemental concentrations were output every 0.512 seconds during the ablation.

Limits of Detection and Standardization

To control for drift in measurement and confirm the accuracy and precision of the analysis using LA-ICPMS, standard certified reference materials (CRMs) were periodically sampled during analyses of fish hard-parts. For otolith analyses, the standards were a glass reference standard (National Institute of Standards and Technology) (NIST-612) and Matrix Carbonate (MACS-4) (pressed pellet of oyster shell). The standards used for dentary bones were CRMs – NIST-612, MACS-3 and Microanalytical Phosphate Standard (MAPS-4) (modern bone). Two ablations of each CRM were analyzed at least every hour of equipment run time. This occurred before and after approximately every twelve (Largemouth Bass), six (Smallmouth Buffalo), and four (Paddlefish) hard-part ablations. To determine limits of detection (LOD) for the elements of interest for each otolith, the background (argon [Ar]) carrier gas was analyzed for 60 seconds prior to each ablation. LODs were calculated using Iolite v3.63 built under IGOR Pro 7 software from WaveMetrics (2017) based on the following formula used from Ludsin et al. (2006):

$$\mathrm{LOD} = \frac{3 \cdot \sigma_{\mathrm{bdg}}}{\mathrm{S} \cdot \mathrm{Y}} \cdot \sqrt{\frac{1}{\mathrm{N}_{\mathrm{bgd}}} + \frac{1}{\mathrm{N}_{\mathrm{pk}}}}$$

where σ_{bgd} equals the standard deviation (SD) of the pre-ablation background determination of elements, N_{bgd} and N_{pk} are replicate determinations used in the integration of the background and ablation signal, respectively, S is mean sensitivity (cps per unit concentration) for the NIST reference standard, and Y is the ablation yield relative to the NIST reference standard, determined from the measured count rates and known concentrations of the internal standard (Longerich et al. 1996). Based on this formula, an element's concentration had to be greater than 3 SDs above background levels to be considered greater than detection limits. I included an element in my analysis only if it was greater than the detection limit in at least 50% of my hard-part samples for a given species. If an element was below the detection limit, a zero was used as the average element concentration for that sample.

To correct for differences in the mass of ablated material, I converted raw element concentrations from cps to ppm, and adjusted element concentrations to a ratio with calcium, Ca^{43} used as an internal standard held constant at 376900 ppm for otoliths, 270000 ppm for dentary bones, and the published 81830 ppm for the NIST standard (Longerich et al. 1996; Ludsin et al. 2006; Lowe et al. 2011; Nelson et al. 2015). Element cps were transformed to ppm using Iolite v3.63 built under IGOR Pro 7 software from WaveMetrics (2017). Conversions from cps to ppm were computed by using the formula from Ludden et al. (1995); a detailed description of this formula is given in Lowe (2007).

The units used to report otolith elemental concentrations varies across the literature, with concentrations either being reported in ppm (Ludsin et al. 2006), $\mu g\ g^{-1}$ (Albuquerque et al. 2012), mmol mol^{-1}(Lowe et al. 2009; Carlson et al. 2016; Radigan et al. 2018b), or $\mu mol\ mol^{-1}$ (Sohn et al. 2005; Bock et al. 2016; Nelson et al. 2018). Given that $\mu g\ g^{-1}$ is equivalent to ppm, $\mu mol\ mol^{-1}$ can be easily derived from $\mu g\ g^{-1}$ using the following equation (X=element):

$$\frac{\mathrm{ppm}(\mu g\, g^{-1})\mathrm{X} \Big/ \mathrm{Mass\ X(g)}}{\mathrm{Internal\ Standard\ (ppm)} \Big/ \mathrm{Mass\ Ca(40.078g)}} * 10^6 = \mu\mathrm{mol\ mol}^{-1}\mathrm{X}$$

I chose to report hard-part elemental concentrations in element: Ca µmol mol^{-1} to remain consistent with other studies, and water concentrations.

Fish Age Estimation

Following LA-ICPMS analyses, an individual image of each otolith and dentary bone cross section was taken using an image analysis system (Image Pro Plus – Paddlefish and Smallmouth Buffalo; Nikon Image Analysis – Largemouth Bass) and annuli were counted to estimate age (Farmer et al. 2013). Paddlefish ages were estimated by counting annuli in dentary bones (as described in Adams 1942; Scarnecchia et al. 1996, 2006) by 2 independent readers. A double blind protocol was used in which each reader (one designated primary and the other secondary) aged each dentary section separately. If there was agreement in age (plus or minus 1 year) the final age was assigned by the primary reader. If ages between readers differed by greater than one year, sections were aged again, and then both readers consulted to agree on a final age (Scarnecchia et al. 1996, 2006). Dentary Bone annulus radii were typically measured through age 10. Largemouth Bass ages were also estimated by two independent readers by counting annuli. If there was a disagreement in fish age, both readers consulted with each other to agree on a final age for the fish. Each radius was measured on every fish. Due to difficulties in estimating the ages of Smallmouth Buffalo via otoliths (crowding of annuli, irregular annuli, old individuals, etc.) an absolute age could not be determined for each fish; however, measurements were taken for the first three annuli. These annuli were independently determined by two readers and then both readers consulted if there was disagreement where annuli radii should be measured.

Genetics

Genotyping-By-Sequencing Sample and DNA Sequencing

Genetic analyses of Paddlefish and Smallmouth Buffalo were performed in collaboration with Auburn University's Aquatic Genetics and Genomics Lab (AU-AGGL). In order to perform analyses with SNPs, AU-AGGL first needed to have each species genotyped. DNA sequencing has been the most accurate and is the most often used method for SNPs (Liu et al. 2011). Genomic DNA from all samples were extracted from fin clips using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. DNA quality was assessed by running 100 ng of each DNA sample on 1% agarose gels. DNA concentration was determined using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). DNA samples were sent to the University of Minnesota Genomics Center (UMGC) for genotyping-by-sequence (GBS) library construction and sequencing. Briefly, 100 ng of DNA was digested with 10 units BamHI-HF and NsiI-HF (New England Biolabs; NEB) enzyme combination and incubated at 37 °C for 2 h. Following digestion, samples were then ligated with 200 units of T4 ligase (NEB) and phased adaptors at 22 °C for 2 h to inactivate the T4 ligase. The ligated samples were purified with SPRI beads and then amplified for 18 cycles with 2× NEB HiFi Master Mix to add barcodes. Libraries of Paddlefish and Smallmouth Buffalo samples were purified, quantified, pooled and size selected for the 300- to 744-bp library region and diluted to 1.7 pm for sequencing. The pooled libraries were loaded across four lanes of a 150-bp single-read sequencing run on the NextSeq 550 instrument.

DNA Sequence Analyses and SNP Marker Discovery

To perform reference-based SNP calling, two rough draft genomes for Paddlefish and Smallmouth Buffalo were assembled. One DNA sample from each species was selected for whole genome sequence and sent to the UMGC for library construction and sequencing. After library construction and barcode addition, the pooled libraries were sequenced across 2 lanes of a TruSeq 2500 High Output 125-bp paired end run. A total of 392 million Illumina reads were generated for Paddlefish

and assembled into a 1.36 Gb (N50 = 3.89 kb) draft genome using MaSuRCA 3.2.4 (Zimin et al. 2013). For Smallmouth Buffalo, a total of 293 million reads were assembled into a 2.51 Gb (N50 = 2.05 kb) draft genome. Genome-wide SNPs were called using TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) 5.0 GBS pipeline V2 (Glaubitz et al. 2014) with default parameter settings. During initial SNP filtering, the minimum minor allele frequency (MAF) was set to 0.05 (overall), the minimum minor allele count (MAC) was set to 10 (overall), and minimum locus coverage (LCov) was set to 0.1 (overall). Only biallelic SNP were retained after initial filtering.

To ensure that the SNPs were polymorphic and informative for Paddlefish and Smallmouth Buffalo population genetics analyses, VCFtools (Danecek et al. 2011), TASSEL (Glaubitz et al. 2014), and GENEPOP (Rousset 2008) were used for stringent filtration of SNPs based on the following criteria: 1) individuals with more than 30% missing genotypes were removed; 2) the SNPs were called in at least 95% of individuals; 3) adjacent SNPs within 64 bp were removed to avoid loci that generated from ambiguous alignments; 4) SNPs deviating from Hardy-Weinberg equilibrium (HWE) with p-value < 0.01 were removed; 5) SNPs that showed significant linkage disequilibrium ($r^2 > 0.3$) and FDR (false discovery rate p-value < 0.01) were removed.

Paralogs Identification

Paralogous loci that arise during ancestry whole-genome duplication, autopolyploidy or allopolyploidy, or chromosomal duplication have been widely reported in species of plants, fish and amphibians (Devos et al. 2005; Lien et al. 2016; Knytl et al. 2017). Such duplication events can facilitate species evolution and adaption, but may lead to complicated genetic patterns where different ploidy levels exist in the genome (McKinney et al. 2018). Identification of paralogs remains a challenge because it is difficult to quantify the allele dosage for heterozygous individuals; for example, a heterozygote individual may display three different tetraploid genotypes at a bi-allelic locus (e.g. AAAT,

AATT, ATTT). Therefore, excluding paralogous loci is commonplace for GBS studies, regardless of their important role in species adaption and evolution (McKinney et al. 2017).

In this study, both species had been previously documented to have undergone whole genome duplication followed by genomic and chromosomal reorganization. The presumed paleotetraploid status of the North American Paddlefish was confirmed by recent duplicate gene (HOX) and phylogenetic analyses (Crow et al. 2012; Symonová et al. 2017). Meanwhile, Smallmouth Buffalo was previously reported as a tetraploid species based on the discovery of two distinct growth hormone cDNAs and polyploid karyotypes (Uyeno and Smith 1972; Ferris and Whitt 1977; Clements et al. 2004). In order to keep only bi-allelic SNPs for downstream population genetics analyses, here we followed the methods proposed by McKinney et al. (2017) to identify paralogs from GBS data using two characteristics: the relative proportion of heterozygotes in a population (H) and the deviation of allele-specific reads of each locus from a 1:1 ratio (D). SNP datasets after stringent filtering were used as input. H and D for each locus were plotted to visualize the distribution of these variables across all loci using HDplot (with minor modification, https://github.com/hzz0024/paralog-finder). Based on the distribution plots, loci with H greater than 0.5 and $|D| > 4$ were classified as paralogs and excluded. The retained loci were used for population genetic analyses.

Data Processing and Statistical Analyses

Water Chemistry

For an element to be included in the water chemistry analysis, it first had to meet the inclusion criteria (i.e., being above LOD in >50% of samples) in the otolith analysis. The goals of my water chemistry analysis were to 1) determine the spatial pattern in concentration of the elements of interest throughout the Alabama River system and its major tributaries, and 2) to determine if those patterns were consistent seasonally and between years. Linear regression was used to show how average element concentrations changed spatially throughout the river system. Means and standard errors for

element concentrations were calculated for each of the four sections of the Alabama River, as well as the Coosa and Tallapoosa rivers. One-way ANOVAs were used to compare elemental concentrations between river sections, and seasons. For all elements with significant overall differences, a Tukey HSD test was used to identify differences between river sections. To identify the best ANOVA model to use for river section comparisons, Akaike information criterion (AIC) was used to determine the parameters that should be included. Parameters included in the AIC model were river section, season and Claiborne gage height. The parameters in the model with the lowest AIC were then subsequently used in the ANOVA models for each element.

Final model ANOVAs were tested for normality of the residuals using the Shapiro Wilks test and for homogeneity of variance using Levene's test. If season or gage height for Claiborne Lock-and-Dam remained in the final model, then a Levene's test was performed on the model without gage height or season because this test cannot be run with a covariate. Although the majority of ANOVAs passed these tests, some did fail. Appropriate data transformations were performed on the failed ANOVAs; although unsuccessful in passing normality, they were still used, given that the ANOVA test is robust to violations in assumptions and has greater power than its nonparametric counterparts (Kruskal-Wallace or rank-sum approaches) (Brownie and Boos 1994; Underwood 1997; Khan and Rayner 2003; Nelson et al. 2014, 2015, 2017).

To determine consistency between sampling years, paired t-tests for each element were performed to facilitate a comparison of the samples collected in spring of 2017 and 2018, and the summer of 2017 and 2018 to determine if samples varied between years. Finally, coefficient of variation (CV) scores were calculated for each element in each river section to show consistency of variability in samples across regions and compare to CV scores of fish hard-parts.

Hard-part Edge to Water Comparison

To test for relationships between water concentration and element concentration in fish hard-parts, Paddlefish, Smallmouth Buffalo, and Largemouth Bass hard-part edge concentrations were compared to water samples collected in proximity and time to the fish, where "edge" was defined as the mean of the outer 20 µm of the hard-part (Farmer et al. 2013; Bock et al. 2016). All Paddlefish used for this analysis were collected during spring 2017. As a result, only water samples collected during this time were used for comparison. Since Smallmouth Buffalo and Largemouth Bass were collected in spring and fall of 2017, fish were separately by season of capture and were compared to water samples collected during the same time frame. All water samples collected in the correct time frame within each river section were included in estimating the mean water elemental value. Least squares linear regression was used to relate mean edge fish element concentrations to mean water concentrations.

Hard-part Microchemistry

To be included in hard-part microchemistry analyses, an element had to be above LOD in 50 percent of samples for a given species. Transect elemental data was related to the age of a fish to facilitate comparison of element concentrations at particular periods in a fishes life. Similar to techniques used by Farmer et al. (2013), comparison of transect elemental data to a period of time in a fishes life was accomplished by measuring the total distance of each ablation as well as the distance between annuli that the laser ablation traversed. Based on the total time of a sample's ablation, and the known scan speed of the ablation transect (5 µm/sec), transect ablation time increments were re-scaled to distance measurements. Distance measurements were then converted to estimated ages of fish based on measured annuli distances. When conversions were made, the estimated age at capture corresponded to the region at the hard-part edge.

Nonlinear regression was used to quantify Sr, Ba, Mg, and Mn, concentrations over the length of each fish's laser ablation transect. I used locally estimated scatterplot smoothing (LOESS) to smooth

untransformed Sr, Ba, Mg, and Mn profiles (span ranging from 0.05-0.25) (Cleveland and Devlin 1988; Cleveland and Loader 1996; Jacoby 2000; Gibb et al. 2007; Daugherty et al. 2017; Sellheim et al. 2017; Willmes et al. 2018). LOESS is a non-parametric smoothing technique that attempts to capture general patterns in stressor-response relationships while reducing noise, and making minimal assumptions about relationships among variables. The result is a line through the moving central tendency of the stressor-response relationship, allowing for visual assessment of the relationship between two variables, proving particularly useful for large data sets (Cleveland 1979; Cleveland and Devlin 1988; Gibb et al. 2007). The span controls the speed with which the influence of points decreases with distance from the point of interest (Gibb et al. 2007). Element transect profile plots for all fish were grouped by river section to visually assess whether any patterns existed. Based on variation in the concentration of elements observed within these transects, potential Paddlefish dam passage events were identified. One-way analysis of variance (ANOVA) was used to determine if elemental concentrations averaged across hard-part transect profile regions differed among river sections. Mean first 20µm (core), edge (last 20µm), first year of life, and whole otolith elemental concentrations were analyzed to test for any differences across the four sections of the Alabama River and if samples were collected from the Coosa and Tallapoosa rivers. For those elements with significant ($\alpha = 0.05$) differences among sites, Tukey HSD pairwise comparison tests were used to compare mean hard-part element concentrations among river sections to determine site differences. Normality and homogeneity of variance were tested for all ANOVAs with the Shapiro Test and Levene's test, respectively. Although the majority of ANOVAs failed these tests and appropriate data transformations did not help, ANOVA's using untransformed data were still used, given that the ANOVA test is robust to violations in assumptions and has greater power than their nonparametric counterparts (Kruskal-Wallace or rank–sum approaches). This is particularly true when sample sizes are large, as is the case here (Brownie and Boos 1994; Underwood 1997; Khan and Rayner 2003; Nelson et al. 2014, 2015, 2017).

Additionally, discriminant function analyses (DFA) were performed to see how accurately fish could be classified back to their capture locations based on the multivariate signatures observed in their laser ablation transects. DFAs were performed for all four regions of the hard-part transect profile and included all four elements analyzed. Additionally, for Paddlefish only, a slight variation in the DFA was performed in which Lower Alabama River and Claiborne Lake fish were combined (due to Claiborne Lock-and-Dam likely experiencing some fish passage over its crested spillway). This resulted in three river sections for this second DFA, with the purpose of determining if classification accuracy for Paddlefish could be further improved by treating the lower two river sections as one. Tallapoosa and Coosa river collected fish were not included in the DFAs, as they did not help improve classification accuracy.

Element transect variability trends were examined among river sections by calculating a coefficient of variation (CV) score for each fish over their entire laser ablation transect for each element analyzed. Fish were then grouped by river section and the ranges of CV values for each river section were plotted for comparison.

In Paddlefish only, Sr:Ca ablation profiles were categorized by the size of oscillations that occurred in a transect for a particular fish, whereby larger oscillations in Sr:Ca were believed to suggest greater movement potential. Each fish was grouped into one of four Sr:Ca μmol:mol oscillation size categories: less than 100, between 100 and 150, between 150 and 400, and greater than 400 (Figure 4). Fish that were grouped in the oscillation category less than 100 Sr:Ca were considered to have a consistent profile, suggesting very little movement potential. Sr:Ca oscillation patterns similar to that observed in Paddlefish were not observed in Smallmouth Buffalo and Largemouth Bass ablation transects, therefore this analysis was not warranted for these species.

Additionally, Paddlefish ablation profiles were examined to determine if an upstream or downstream dam passage event could be identified in a Paddlefish based on changes in Sr:Ca

concentration throughout the ablation transect. A passage event was therefore defined as a Sr:Ca increase greater than 250 µmol:mol (greater than 0.7 proportional increase/decrease in Sr:Ca from before/after a suspected passage event) over a distance of less than 150 µm (which corresponded to less than a year for fish with measured annuli when the suspected passage event occurred). Additionally, Sr:Ca concentration either had to remain higher than before peak levels, or dropped back down to levels equal to or less than before the passage concentration, indicating an upstream passage event (Figure 5).

Finally, all boxplots throughout this manuscript used the statistical analysis software R's default boxplot code whereby, the IQR (inter quartile range [boxplot length]) = Q_3 (quartile 3 [75th percentile]) – Q_1 (quartile 1 [25th percentile]) and the band within the box is quartile 2 (median [50th quartile]). The upper whisker = Q_3 + 1.5 * IQR and the lower whisker = Q_1 – 1.5 * IQR).

Population Genetic Analyses

Population diversity indices were evaluated by computing the observed (H_o) and expected heterozygosity (H_e) using the R package Arlequin v3.5 (Excoffier and Lischer 2010). The inbreeding coefficient (F_{is}) for each river section was calculated as well. Effective population size (N_e) for was estimated using the linkage disequilibrium method implemented in NeEstimator v2.1 (Do et al. 2014). This method is based on theory demonstrating that the amount of linkage (i.e., gametic) disequilibrium at independent loci in randomly mating, isolated populations is purely a function of the magnitude of genetic drift and can therefore be used to estimate N_e (Hill 1981). Assumptions of this method include that population size is stable, panmictic and there is no natural selection, migration or mutation (which is unrealistic).

Population connection(s) were visualized with a discriminant analysis of principal components (DAPC) using the Adegenet (Jombart and Ahmed 2011). This package provides an efficient way for genetic clustering with a few synthetic variables (discriminant functions) that could maximize

discrimination between groups while minimizing within-group variance. The DAPC was run on individuals grouped by major river sections. In each case, the optimal number of principal components was determined by alpha-score procedure with 20 repeated runs. Another Bayesian clustering algorithm based program, STRUCTURE v2.3.4 (Pritchard et al. 2000), was used for population structure analysis. The admixture model with correlated allele frequencies was applied for data analysis with a burn-in of 20,000 iterations followed by 200,000 Markov Chain Monte Carlo (MCMC) repetitions. Different numbers of assumed population genetic clusters (K = 1-10) were used to determine the best K value using the web server STRUCTURE HARVESTER (Earl and vonHoldt 2012), and were repeated 10 times for each K value.

Population differentiation analyses were performed for all pairs of major river sections using analysis of molecular variance (AMOVA) and Weir and Cockerham estimator of F_{ST} (Weir and Cockerham 1984) in Arlequin v3.5 (Excoffier and Lischer 2010). Significance level for each F_{ST} comparison was estimated using 10,000 permutations with FDR correction (initial p = 0.05). Significance level of AMOVA test was also estimated using 10,000 permutations.

Results

Water Chemistry

All five elements (Ca, Sr, Ba, Mg, and Mn) analyzed met the inclusion criteria (i.e., being above LOD in >50% of samples) in the otolith analysis and were therefore quantified in our water samples. All elements were converted into molar ratios with calcium (Ca) prior to analysis and all elements are reported in element (µmol) per Ca (mol) water. Spatial variability throughout the river system was confirmed by comparing individual sites and river sections. Seasonal consistency was also confirmed. Strontium (Sr:Ca) concentrations in water differed significantly across individual sites (ANOVA: $F_{3,74}$ = 72.02, p = <0.001) (Figure 6), but not seasons (ANOVA: $F_{3,85}$ = 0.221, p = 0.882) (Figure 7). In addition, strontium concentrations also differed among river sections (ANOVA: $F_{5,61}$ = 214.249, p = <0.001) (Figure

8). Similarly, barium (Ba:Ca) concentrations in water differed across individual sites (ANOVA: $F_{14,74}$ = 17.01, p = <0.001) (Figure 6), but not seasons (ANOVA: $F_{3,85}$ = 0.98, p = 0.406) (Table 3, Figure 7). Barium concentrations also differed among river sections (ANOVA: $F_{5,64}$ = 65.32, p = <0.001) (Figure 8). Magnesium (Mg:Ca) concentrations in water also differed across individual sites (ANOVA: $F_{14,74}$ = 34.62, p = <0.001) (Table 2, Figure 6), but not seasons (ANOVA: $F_{3,85}$ = 0.326, p = 0.806) (Figure 7). Magnesium concentrations also differed among river sections (ANOVA: $F_{5,62}$ = 68.449, p = <0.001) (Figure 8). Manganese (Mn:Ca) concentrations in water differed across individual sites (ANOVA: $F_{14,74}$ = 3.914, p = <0.001) (Figure 6), but not seasons (ANOVA: $F_{3,85}$ = 0.915, p = 0.437) (Figure 7). Manganese concentrations also differed among river sections (ANOVA: $F_{5,62}$ = 6.356, p = <0.001)(Figure 8).

Temporal consistency between water sampling years was confirmed by not detecting significant differences for individual sites for all elements, except Mg, between summer 2017 versus 2018. Strontium (Sr:Ca) concentrations did not differ significantly between spring 2017 versus 2018 (paired t(13) = -0.37096, p = 0.717) and summer 2017 versus 2018 (paired t(14) = -1.3277, p = 0.206). Barium (Ba:Ca) concentrations did not differ significantly between spring 2017 versus 2018 (paired t(13) = -1.0449, p = 0.315) and summer 2017 versus 2018 (paired t(14) = -0.91152, p = 0.378). Magnesium (Mg:Ca) concentrations differed significantly between spring 2017 versus 2018 (paired t(13) = -2.5958, p = 0.022), but did not differ significantly for summer 2017 versus 2018 (paired t(14) = -0.10107, p = 0.921). Manganese (Mg:Ca) concentrations did not differ significantly between spring 2017 versus 2018 (paired t(13) = 0.37794, p = 0.712) and summer 2017 versus 2018 (paired t(14) = 0.10476, p = 0.918). Finally, the coefficient of variation for each element in each river section was similar across river sections (Figure 9). The consistent and low variability observed among river sections provides further evidence for trace element consistency temporally.

Water-Hard-part Edge Correlation

Paddlefish

Mean Paddlefish dentary bone element concentration was significantly related to mean water element concentrations across river sections for both Sr and Mn (Figure 10). Simple linear regressions between water and dentary chemistry were positive for Sr:Ca (R^2 = 0.98, p = 0.008), and Mn:Ca (R^2 = 0.92, p = 0.044) (Figure 10). Linear regressions between water and dentary bone chemistry were not significant for Ba:Ca (R^2 = 0.01, p = 0.891) and Mg:Ca (R^2 = 0.72, p = 0.154) (Figure 10).

Smallmouth Buffalo

Mean Smallmouth Buffalo otolith element concentration was significantly related to mean water Sr concentration across river sections in fall 2017 and marginally significant for spring 2017 samples (Figure 11). Simple linear regressions between water and otolith chemistry in spring 2017 were positive for Sr:Ca (R^2 = 0.99, p = 0.077) (Figure 11). Linear regressions between water and otolith chemistry were not significant for Ba:Ca (R^2 = 0.66, p = 0.399), Mg:Ca (R^2 = 0.77, p = 0.318), and Mn:Ca (R^2 = 0.01, p = 0.952) (Figure 11). Simple linear regressions between water and otolith chemistry in fall 2017 were positive for Sr:Ca (R^2 = 0.92, p = 0.043), and Mn:Ca (R^2 = 0.87, p = 0.066) (Figure 11). Linear regressions between water and otolith chemistry were not significant for Ba:Ca (R^2 = 0.12, p = 0.653), and Mg:Ca (R^2 = 0.50, p = 0.294)(Figure 11).

Largemouth Bass

Mean Largemouth Bass otolith element concentration was significantly related to mean water Mn concentration across river sections in spring of 2017 samples; Mn:Ca (R^2 = 0.99, p = 0.013) (Figure 12). Linear regressions between water and otolith chemistry in spring 2017 were not significant for Sr:Ca (R^2 = 0.76, p = 0.323), Ba:Ca (R^2 = 0.43, p = 0.543), and Mg:Ca (R^2 = 0.11, p = 0.786) (Figure 12) Simple linear regressions between water and otolith chemistry in Fall 2017 were not significant for Sr:Ca

(R^2 = 0.38, p = 0.384), Ba:Ca (R^2 = 0.18, p = 0.581), Mg:Ca (R^2 = 0.33, p = 0.427) , and Mn:Ca (R^2 = 0.12, p = 0.652) (Figure 12).

Hard-part Microchemistry

Paddlefish

Fish Collections

Paddlefish were successfully captured from each Alabama River section during Febuary-May 2017 and the Tallapoosa River during February 2019, resulting in a final sample size of 186 dentary bones (30-56 per Alabama River section; Table 2). Fish lengths varied across river sections (ANOVA: $F_{4,181}$ = 8.225, p = <0.001) (Figure 13), but fish ages did not (ANOVA: $F_{4,181}$ = 2.032, p = 0.092) (Figure 14). Fish lengths and ages were normally distributed in some river sections but not in others, and in some cases associated transformations did not help achieve normality (Figures 15, 16). Regardless, untransformed data were still used, given that ANOVA is robust to violations of assumptions and has greater power than its nonparametric counterparts. Harvest of Paddlefish from two of the river sections (CL and MFR) consisted of all female paddlefish, while fish collected from (LAR and JBR) had a mix of male and female fish.

Detectable Elements

All elements examined with LA-ICPMS (Ca, Sr, Ba, Mg, Mn) met the criteria (> LOD in 50% of samples) to be used in dentary bone comparisons across all river sections in this study. The elements Sr, Ba, Mg, and Mn (Table 3) were used for all profile (i.e., entire transect, edge, year one, and first 20 micron) comparisons across all river sections.

Comparison Between River Sections

Paddlefish dentary bone mean whole transect Sr_{88} concentrations differed significantly among river sections (ANOVA: $F_{4,181}$ = 69.31, p = <0.001), being highest in LAR (Figure 17). Dentary bone mean edge Sr concentrations differed significantly among river sections (ANOVA: $F_{4,181}$ = 66.05, p = <0.001),

also being highest in LAR (Figure 17). Dentary bone mean age-0 Sr concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 39.24$, $p = <0.001$), being highest in LAR (Figure 17). Dentary bone mean first 20 micron Sr concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 41.16$, $p = <0.001$), being highest in LAR (Figure 17).

Paddlefish dentary bone mean whole transect Ba_{137} concentrations differed significantly among river sections (ANOVA: $F_{4,181} = 4.707$, $p = 0.001$), being highest in TAL (Figure 18). Dentary bone mean edge Ba concentrations differed significantly among sections (ANOVA: $F_{4,181} = 8.965$, $p = <0.001$), being highest in TAL (Figure 18). Dentary bone mean age-0 Ba concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 5.81$, $p = <0.001$), being highest in TAL (Figure 18). Dentary bone mean first 20 micron Ba concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 7.007$, $p = <0.001$), being highest in TAL (Figure 18).

Paddlefish dentary bone mean whole transect Mg_{24} concentrations differed significantly among river sections (ANOVA: $F_{4,181} = 12.41$, $p = <0.001$), being lowest in LAR (Figure 19). Dentary bone mean edge Mg concentrations differed significantly among river sections (ANOVA: $F_{4,181} = 14.56$, $p = <0.001$), being lowest in LAR (Figure 19). Dentary bone mean age-0 Mg concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 8.678$, $p = <0.001$), being lowest in LAR (Figure 19). Dentary bone mean first 20 micron Mg concentrations differed significantly among sections (ANOVA: $F_{4,178} = 4.585$, $p = 0.002$), being lowest in LAR (Figure 19).

Paddlefish dentary bone mean whole transect Mn_{55} concentrations differed significantly among river sections (ANOVA: $F_{4,181} = 6.144$, $p = <0.001$), being highest in JBR (Figure 20). Dentary bone mean edge Mn concentrations differed significantly among river sections (ANOVA: $F_{4,181} = 32.52$, $p = <0.001$), being lowest in MFR (Figure 20). Dentary bone mean age-0 Mn concentrations differed significantly among river sections (ANOVA: $F_{4,178} = 4.054$, $p = 0.004$), being highest in LAR (Figure 20). Dentary bone

mean first 20 micron Mn concentrations differed significantly among river sections (ANOVA: $F_{4,178}$ = 3.116, p = 0.017), being highest in LAR (Figure 20).

Discrimination Between River Sections

In the first set of discriminant function analyses (DFA) performed, I included Mn_{55}, Sr_{88}, Ba_{137}, and Mg_{24} because Paddlefish dentary bone concentrations of these elements differed among most river sections and regions of the ablation transect analyzed. In this analysis, each river section was used as a group in the function which assigned fish to the correct river collection section with a moderate degree of accuracy. MFR had the highest mean dentary transect classification percentage, followed by LAR, JBR, and CL. Total classification accuracy for the dentary mean transect was 68% (Table 4). MFR also had the highest dentary mean edge classification percentage, followed by LAR, JBR, and CL. Total classification accuracy for the dentary mean edge was 74% (Table 4). MFR had the highest dentary mean age-0 classification percentage, followed by LAR, JBR, and CL. Total classification accuracy for the dentary mean age-0 was 60% (Table 4). MFR had the highest dentary mean first 20 micron classification percentage, followed by LAR, CL, and JBR. Total classification accuracy for the dentary mean first 20 microns was 55% (Table 4). Elements that contributed to the discriminating axes were Ba_{137}, Sr_{88}, and Mn_{55} (in decreasing order of importance, Table 5). The relative similarity in concentration of these elements in adjacent river sections limits the ability to discriminate between these areas and miss-classifications almost always occurred to an adjacent river section.

In the next set of discriminant function analyses performed, I included all elements; however, in this analysis, the river sections LAR and CL were grouped into one group as opposed to two to see if classification accuracy was improved given that Paddlefish have been documented to pass the crested spillway at Claiborne Lock-and-Dam. Therefore, three river sections were used as groups in the function which assigned fish to the correct collection river section with a moderate degree of accuracy. Total classification accuracy improved to 79% for the dentary transect mean (Table 6), 84% for dentary edge

mean (Table 6), 69% for dentary age-0 mean (Table 6), and 67% for dentary first 20 micron mean (Table 6). Elements that contributed to the discriminating axes were Ba_{137}, Sr_{88}, and Mn_{55} (in decreasing order of importance, Table 7). The relative similarity in concentration of these elements in adjacent river sections limits the ability to discriminate between these areas and miss-classifications almost always occurred to an adjacent river section.

Whole Transect Variability

Paddlefish dentary bone whole transect Sr_{88} coefficient of variation (CV) scores differed significantly among river sections (ANOVA: $F_{4,181} = 12.83$, $p = <0.001$), being higher in CL and LAR than JBR and MFR (Figure 21). Dentary bone whole transect Ba_{137} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{4,181} = 1.623$, $p = 0.17$) (Figure 21). Dentary bone whole transect Mg_{24} coefficient of variation (CV) scores differed significantly among river sections (ANOVA: $F_{4,181} = 3.469$, $p = 0.009$), being lower in CL versus JBR (Figure 21). Dentary bone whole transect Mn_{55} coefficient of variation (CV) scores differed significantly among river sections (ANOVA: $F_{4,181} = 9.03$, $p = <0.001$), being lower in MFR versus CL and LAR (Figure 21). Given that patterns in Paddlefish dentary bone coefficient of variation (Figure 21) did not mimic those of the coefficient of variation for water samples (Figure 9) for the same river sections, this suggests movement potential for Paddlefish in the river sections where dentary bone CV scores are higher, in particular for Sr:Ca.

Strontium Ablation Profiles

All Paddlefish were grouped into one of four categories based on the size of Sr:Ca oscillations within each fish's ablation profile. A distinct trend emerged revealing that fish collected in the two upper river sections and the Tallapoosa River almost always had consistent Sr:Ca profiles. However, with increasing distance downstream, the two lower river sections began to show higher percentages of fish with larger Sr:Ca oscillations, suggesting increased movement potential (Table 8). Finally, based on my definition of a dam passage event via changes in a fish's strontium profile, it is suspected that 10

individual fish had evidence of a total of 12 dam passage events at Claiborne Lock-and-Dam, out of a total of 99 fish collected in Claiborne Lake and the Lower Alabama River (10.1%)(Table 9). These included both downstream and upstream passage events, which were only found to occur in fish collected from the Lower Alabama River and Claiborne Lake.

Smallmouth Buffalo

Fish Collections

Smallmouth Buffalo were successfully captured from each river section (main stem and tributaries) during February-November 2017 and July-September 2018. This resulted in a final sample size of 209 otoliths (Table 2). Fish lengths varied across Alabama River sections (ANOVA: $F_{5,203} = 4.083$, $p = 0.002$) (Figure 22). Length-frequency distributions were normally distributed between river sections (Figure 23).

Detectable Elements

All five elements examined with LA-ICPMS (Ca, Sr, Ba, Mg, Mn) met the criteria (> LOD in 50% of samples) to be used in otolith comparisons across all river sections in this study. The elements Sr, Ba, Mg, and Mn (Table 3) were used for all ablation profile comparisons across all river sections.

Comparison Between River Sections

Smallmouth Buffalo otolith mean whole transect Sr_{88} concentrations differed significantly among river sections (ANOVA: $F_{5,203} = 3.142$, $p = 0.009$), being lowest in JBR (Figure 24). Otolith mean edge Sr concentrations differed significantly among river sections (ANOVA: $F_{5,203} = 4.714$, $p = <0.001$) (Figure 24). Otolith mean age-0 Sr concentrations differed significantly among river sections (ANOVA: $F_{5,205} = 3.755$, $p = 0.003$) (Figure 24). Otolith first mean 20 micron Sr concentrations did not differ among river sections (ANOVA: $F_{5,203} = 1.941$, $p = 0.089$) (Figure 24).

Smallmouth Buffalo mean whole transect Ba_{137} concentrations did not differ among river sections (ANOVA: $F_{5,203} = 0.408$, $p = 0.843$) (Figure 25). Otolith mean edge Ba concentrations did not

differ among sections (ANOVA: $F_{5,203}$= 0.575, p = 0.719) (Figure 25). Otolith mean age-0 Ba concentrations did not differ among river sections (ANOVA: $F_{5,203}$ = 0.856 , p = 0.511) (Figure 25). Otolith mean first 20 micron Ba concentrations did not differ among river sections (ANOVA: $F_{5,203}$ = 0.641, p = 0.669) (Figure 25).

Smallmouth Buffalo otolith mean whole transect Mg_{24} concentrations differed significantly among river sections (ANOVA: $F_{5,203}$ = 3.29, p = 0.007), being lowest in TAL, JBR and MFR (Figure 26). Otolith mean edge Mg concentrations differed significantly among river sections (ANOVA: $F_{5,203}$ = 4.288, p = <0.001) (Figure 26). Otolith mean age-0 Mg concentrations did not differ among river sections (ANOVA: $F_{5,203}$ = 1.644, p = 0.15) (Figure 26). Otolith mean first 20 micron Mg concentrations differed significantly among sections (ANOVA: $F_{5,203}$ = 2.84, p = 0.017) (Figure 26).

Smallmouth Buffalo mean otolith whole transect Mn_{55} concentrations differed significantly among river sections (ANOVA: $F_{5,203}$ = 12.87, p = <0.001), being highest in CSA, TAL and JBR (Figure 27). Otolith mean edge Mn concentrations differed significantly among river sections (ANOVA: $F_{5,203}$ = 18.67, p = <0.001), being highest in CSA (Figure 27). Otolith mean age-0 Mn concentrations differed significantly among river sections (ANOVA: $F_{5,203}$ = 2.567, p = 0.0281), being the highest in TAL, CSA, and JBR (Figure 27). Otolith first mean 20 micron Mn concentrations did not differ among river sections (ANOVA: $F_{5,203}$ = 1.259, p = 0.283) (Figure 27).

Discrimination Between River Sections

Smallmouth Buffalo discriminant function (DFA) analyses used Mn_{55}, Sr_{88}, Ba_{137}, and Mg_{24}. In these analyses, each river section was used as a group in the function which assigned fish to the correct collection river section with a low degree of accuracy. JBR had the highest mean otolith transect classification percentage, followed by MFR, CL, and LAR. Total classification accuracy for the otolith mean transect was 43% (Table 10). MFR had the highest otolith mean edge classification percentage, followed by JBR, LAR, and CL. Total classification accuracy for the otolith mean edge was 47% (Table 10).

JBR had the highest mean otolith age-0 classification percentage, followed by LAR, CL, and MFR had the poorest classification. Total classification accuracy for the dentary mean age-0 was 38% (Table 10). LAR had the highest otolith mean first 20 micron classification percentage, followed by MFR, JBR, and CL. Total classification accuracy for the otolith mean first 20 microns was 37% (Table 10). Elements that contributed to the discriminating axes were Ba_{137}, Sr_{88}, and Mn_{55} (in decreasing order of importance, Table 11). The relative similarity in concentration of these elements in adjacent river sections limits the ability to discriminate between these areas.

Whole Transect Variability

Smallmouth Buffalo whole transect Sr_{88} coefficient of variation (CV) scores differed significantly among river sections (ANOVA: $F_{5,203} = 7.483$, $p = <0.001$), being highest in CSA and lowest in LAR (Figure 28). Otolith whole transect Ba_{137} coefficient of variation (CV) scores differed significantly among river sections (ANOVA: $F_{5,203} = 5.366$, $p = <0.001$) being highest in CSA and TAL (Figure 28). Otolith whole transect Mg_{24} coefficient of variation (CV) scores differed among river sections (ANOVA: $F_{5,203} = 6.223$, $p = <0.001$), being highest in LAR and CL (Figure 28). Otolith whole transect Mn_{55} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{5,203} = 1.738$, $p = 0.128$) (Figure 28). Given that patterns in Smallmouth Buffalo otolith coefficient of variation (Figure 28) mimic those of the coefficient of variation for water samples (Figure 9) for the same river sections, this suggests limited movement potential for Smallmouth Buffalo.

Largemouth Bass

Fish collections

Largemouth Bass were successfully captured from each river section (main stem and tributaries) during February-November 2017 and July-September 2018. This resulted in a final sample size of 106 otoliths (Table 2). Fish lengths varied across river sections (ANOVA: $F_{5,100} = 3.246$, $p = 0.009$) (Figure 29), but fish ages did not (ANOVA: $F_{5,100} = 0.418$, $p = 0.835$) (Figure 30). Fish lengths and ages were normally

distributed in some river sections but not in others, and in some cases associated transformations did not help achieve normality (Figures 31, 32). Regardless, untransformed data were still used, given that ANOVA is robust to violations of assumptions and has greater power than its nonparametric counterparts.

Detectable Elements

All five elements examined with LA-ICPMS (Ca, Sr, Ba, Mg, Mn) met the criteria (> LOD in 50% of samples) to be used in otolith comparisons across all river sections in this study. The elements Sr, Ba, Mg, and Mn (Table 3) were used for all ablation profile comparisons across all river sections.

Comparison Between River Sections

Largemouth Bass otolith mean whole transect Sr_{88} concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 7.104$, $p = <0.001$), being highest in TAL (Figure 33). Otolith mean edge Sr concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 5.482$, $p = <0.001$), being highest in TAL (Figure 33). Otolith mean age-0 Sr concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 6.226$, $p = <0.001$), being highest in TAL (Figure 33). Otolith mean first 20 micron Sr concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 6.975$, $p = <0.001$), being highest in TAL (Figure 33).

Largemouth Bass mean whole transect Ba_{137} concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 12.2$, $p = <0.001$), being highest in CSA and TAL (Figure 34). Otolith mean edge Ba concentrations differed significantly among sections (ANOVA: $F_{5,100} = 9.86$, $p = <0.001$) being highest in TAL (Figure 34). Otolith mean age-0 Ba concentrations differed significantly among river sections (ANOVA: $F_{5,100} = 4.691$, $p = <0.001$) being highest in CSA and TAL (Figure 34). Otolith mean first 20 micron Ba concentrations did not differ among river sections (ANOVA: $F_{5,100} = 2.01$, $p = 0.084$) (Figure 34).

Largemouth Bass otolith mean whole transect Mg_{24} concentrations did not differ among river sections (ANOVA: $F_{5,100}$ = 1.006, p = 0.418) (Figure 35). Otolith mean edge Mg concentrations did not differ among river sections (ANOVA: $F_{5,100}$ = 0.385, p = 0.858) (Figure 35). Otolith mean age-0 Mg concentrations differed significantly among river sections (ANOVA: $F_{5,100}$ =3.944, p = 0.003), being highest in CSA and JBR (Figure 35). Otolith mean first 20 micron Mg concentrations did not differ among river sections (ANOVA: $F_{5,100}$ = 1.377, p = 0.239) (Figure 35).

Largemouth Bass otolith mean whole transect Mn_{55} concentrations differed significantly among river sections (ANOVA: $F_{5,100}$ = 6.202, p = <0.001), being highest in CSA (Figure 36). Otolith mean edge Mn concentrations did not differ among river sections (ANOVA: $F_{5,100}$ = 2.243, p = 0.056) (Figure 36). Otolith mean age-0 Mn concentrations differed significantly among river sections (ANOVA: $F_{5,100}$ = 6.369, p = <0.001), being highest in CSA and TAL (Figure 36). Otolith mean first 20 micron Mn concentrations differed significantly among river sections (ANOVA: $F_{5,100}$ = 2.71, p = 0.024), being highest in CSA (Figure 36).

Discrimination Between River Sections

Largemouth Bass discriminant function (DFA) analyses used Mn_{55}, Sr_{88}, Ba_{137}, and Mg_{24}. In these analyses, each river section was used as a group in the function which assigned fish to the correct collection river section with a low degree of accuracy. JBR had the highest mean otolith transect classification percentage, followed by MFR, LAR, and CL. Total classification accuracy for the otolith mean transect was 44% (Table 12). JBR had the highest otolith mean edge classification percentage, followed by MFR and CL, and LAR. Total classification accuracy for the otolith mean edge was 39% (Table 12). LAR had the highest otolith mean age-0 classification percentage, followed by JBR, CL, and MFR. Total classification accuracy for the dentary mean age-0 was 48% (Table 12). JBR had the highest otolith mean first 20 micron classification percentage, followed by LAR, CL, and MFR. Total classification accuracy for the otolith mean first 20 microns was 44% (Table 12). Elements that contributed to the

discriminating axes were Mn_{55}, Ba_{137}, and Mg_{24} (in decreasing order of importance, Table 13). The relative similarity in concentration of these elements in adjacent river sections limits the ability to discriminate between these areas

Whole Transect Variability

Largemouth Bass otolith whole transect Sr_{88} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{5,100} = 0.73$, $p = 0.602$) (Figure 37). Otolith whole transect Ba_{137} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{5,100} = 0.28$, $p = 0.923$) (Figure 37). Otolith whole transect Mg_{24} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{5,100} = 0.815$, $p = 0.542$) (Figure 37). Otolith whole transect Mn_{55} coefficient of variation (CV) scores did not differ among river sections (ANOVA: $F_{5,100} = 0.867$, $p = 0.506$) (Figure 37). Given that patterns in Largemouth Bass otolith coefficient of variation (Figure 37) mimic those of the coefficient of variation for water samples (Figure 9) for the same river sections, this suggests limited movement potential for Largemouth Bass.

Genetics

Paddlefish and Smallmouth Buffalo

Fish Collections

Fin clips were collected or obtained from 405 Paddlefish and 303 Smallmouth Buffalo (Table 14). Of the total fish collected, 166 Paddlefish and 121 Smallmouth Buffalo were used for GBS library construction and sequencing and 159 Paddlefish and 119 Smallmouth Buffalo were used during population genetic analyses (Table 15).

SNP Discovery and Genotyping

For Paddlefish, a total of 152,964,388 high-quality reads were generated from Illumina NextSeq sequencing, with an average of 921,473 reads for each sequenced sample. A total of 99,086,273 high-quality reads were generated for Smallmouth Buffalo, with an average of 839,714 reads for each

sequenced sample. During GBS analyses, TASSEL generated 18,137 and 56,744 pre-filtered SNP loci for Paddlefish and Smallmouth Buffalo, respectively. Further individual filtering using 30% missing loci as a parameter reduced the sample size from 166 to 159 for Paddlefish, and from 121 to 118 for Smallmouth Buffalo. To ensure that SNP datasets were polymorphic and informative for population genetics analyses, further stringent filtering steps using VCFtools, TASSEL, and GENEPOP software resulted in the identification of 2,770 and 4,248 SNPs for Paddlefish and Smallmouth Buffalo, respectively.

Paralog Identification and Marker Characteristics

In order to keep only bi-allelic SNPs for downstream population genetics analyses, paralogs were identified from GBS data sets. Diploid SNP identification based on the relative proportion of heterozygotes (H) and the read deviation (D) found 1,889 (68.19%) of 2,770 markers to be diploid loci in Paddlefish and 3,737 (87.97 %) of 4,248 SNPs to be diploid SNPs in Smallmouth Buffalo. Plotting H and D revealed two distinct clouds of loci in Paddlefish (Figure 38). SNP loci within the inner dense cloud concentrated at 0.09-0.5 for H and 0 for D, suggesting the distribution of singleton loci. This cloud was ringed by a second cloud representing 31.81% of loci with clear deviation from equal ratios of sequence reads, consistent with expectations for duplicate loci. The Smallmouth Buffalo loci clustered into a cloud at 0.06-0.5 for H and 0 for D, corresponding to singleton loci distribution, with 12.03% loci outside this central range (Figure 39).

Marker Characteristics and Effective Population Size

Marker characteristics including the observed (H_o) and expected heterozygosity (H_e) were measured for fish collected from each river section (Table 16). Heterozygosity rate gradually decreased downstream from JBR to LAR. Average observed heterozygosity (H_o) ranged from 0.286 (LAR) to 0.389 (CSA) for Paddlefish, with a mean of 0.306. For Smallmouth Buffalo, the mean observed heterozygosity ranged from 0.228 (LAR) to 0.238 (JBR), with a mean value of 0.232.

Inbreeding coefficients (F_{is}) were also calculated for fish collected from each river section (Table 16). Highest levels of inbreeding were observed in LAR for both species. Observed F_{is} values in Paddlefish ranged from 0.032 (TOM) to 0.045 (LAR), with a mean of 0.038. Observed F_{is} values in Smallmouth Buffalo ranged from 0.043 (TOM) to 0.078 (LAR), with a mean of 0.063.

Assessments of effective population size (N_e) displayed moderate level of size variation among river section for each species (Table 16). Estimated N_e for Paddlefish ranged from 844 (JBR) to 1,960 (LAR). Estimated N_e for Smallmouth Buffalo ranged from 1619 (MFR) to infinite values in CL and LAR river sections.

Population Structure and Differentiation

Analyses of molecular variance (AMOVA) for Paddlefish and Smallmouth Buffalo datasets did not detect genetic differentiation among river sections, and most genetic variation was generated within individuals (Table 17). In Paddlefish, 96.16% of the total variation was explained by differences within individuals, 3.84% was due to variation among individuals within populations, and variation among populations did not contribute to the total variation. In Smallmouth Buffalo, 93.68% of the total variation was explained by differences within individuals, 6.28% was due to the variation among individuals within populations, and variation among populations only accounted 0.04% of the total variation.

To examine genetic differentiation among populations and their degree of variance in allele frequencies, pairwise F_{ST} values were measured (Table 18). F_{ST} statistics in this study were in general very low, and only one pairwise comparison was significant (between JBR and MFR in Smallmouth Buffalo). This suggests JBR and MFR are the most genetically divergent river sections for Smallmouth Buffalo. Meanwhile, no significant F_{ST} values were detected for Paddlefish between river sections.

However, F_{ST} values between CSA Paddlefish and other river sections were relatively higher than the other comparisons, most likely due to the small sample size (n=4) from the CSA.

Population structure was then examined for both species. STRUCTURE software examined the optimal number of upper genetic clusters (K) for the GBS datasets, using the 1,889 SNPs for Paddlefish and 3,737 SNPs for Smallmouth Buffalo. For both species, the optimal numbers of uppermost genetic clusters (K) were 1 (K = 1) according to the calculated probabilities of each K (Figure 40 and 41). This suggested a lack of spatial genetic clustering between fish in each river section.

Lastly, discriminant analyses of principal components (DAPC) also showed a general pattern of low genetic differentiation, similar to that of the previous analyses performed. The DAPC plot of Paddlefish, including all river sections and SNPs showed no clear separation or clusters among river sections (Figure 42), with only CSA samples slightly isolating from all other river sections. This pattern was consistent with the F_{ST} results, potentially due to the small sample size from the CSA. The DAPC analysis of Smallmouth Buffalo with all SNPs also showed no clear genetic clusters or subdivision among river sections (Figure 43).

Discussion

Overview

In this study, I examined population connectivity patterns exhibited by Paddlefish, Smallmouth Buffalo, and Largemouth Bass across four river sections and two upper tributaries of the Alabama River to determine if potential population groups in those river sections might be segregated, as well as to expand scientific knowledge about the impact of low-use lock-and-dam structures on riverine fishes. Paddlefish is commercially valuable, and is a species of concern for the State of Alabama in particular. Largemouth Bass supports important recreational fisheries. Smallmouth Buffalo is a large-bodied sucker, a group of species that has been strongly impacted by dam construction. Therefore, increased

understanding of the impacts of these dams on these species would be beneficial for their continued management.

Toward this end, I used hard-part microchemistry to infer patterns of movement and natal origin as well as to quantify population connectivity from a shorter term perspective (i.e., within the life of an individual fish) by testing for differences in micro-elemental signatures between potential population groups affected by these lock-and-dam structures. Changes in the elemental composition of hard-parts from these three species collected throughout the Alabama River and its tributaries were used to infer past patterns of river section occupancy. In addition, I used genetic analyses to assess potential differences between population groups to understand population connectivity from a longer time perspective (i.e., across multiple fish generations).

Water Chemistry

I found water samples from throughout the main stem of the Alabama River, its major tributaries, and neighboring river basins to be spatially variable, but temporally consistent in trace element concentrations, which has been defined as a critical first step for many microchemistry studies (Campana 1999; Elsdon et al. 2008; Walther and Limburg 2012; Pracheil et al. 2014). Most importantly I found trace elements in water samples were spatially variable for strontium, which increased in concentration from its origins to its terminus with the Tombigbee River. Trace element patterns found in water tend to be consistent with geology of the watershed (Newton et al. 1987; Wells et al. 2003). Consistent with what I observed, increased proximity to a coastal/estuarine environment was associated with increases in strontium concentrations (Elsdon et al. 2008; Walther and Limburg 2012; Farmer et al. 2013). The upstream-to-downstream strontium gradient observed for this system is similar to that observed in other microchemistry studies (Phelps et al. 2012; Carlson et al. 2016). Additionally, Sr:Ca ratio concentration ranges in this study were consistent with those observed in other freshwater

systems (Kraus and Secor 2004; Farmer et al. 2013; Daugherty et al. 2017). Strontium is one of the most commonly used and most informative elements in freshwater microchemistry studies (Campana 1999; Gillanders 2005; Pracheil et al. 2014) due to it replacing Ca in the calcium carbonate matrix, as well as it being stable over time (Hedges et al. 2004). The pattern observed for strontium in this study allowed it to be used as the primary indicator element for quantifying the degree of connectivity and movement in the three species examined in this study; as well as for examining early life in Paddlefish.

It is interesting to note the uniqueness of the Tallapoosa River drainage, in that its element- to-calcium ratios were extremely high relative to the rest of the water samples collected. Uniqueness in trace elements of tributaries is not uncommon (Wells et al. 2003; Zeigler and Whitledge 2011) and can prove to be very useful. The uniqueness of the Tallapoosa River water chemistry stems from the geology of this system (Freeman et al. 2005), in which these water samples contained less calcium, leading to increased trace element-to-calcium ratios. When absolute element concentrations (other than calcium) for this tributary were considered independently, they mimicked the north-to-south patterns observed for the rest of the watershed. The elevated element-to-calcium ratios in the Tallapoosa River proved to be particularly important when analyzing fish hard-parts collected from the Tallapoosa River.

Paddlefish Dentary Bone Microchemistry

My estimated Paddlefish ages were greater than what was previously believed to be the maximum age of Paddlefish in the Alabama River. Most individuals aged in this study were older than the previously observed maximum age of 11 years for the upper and lower Alabama Rivers (Hoxmeier and DeVries 1997; Lein and DeVries 1998; DeVries et al. 2009). Multiple individuals had ages estimated to be older than 20 years, with the maximum age of an individual reaching an estimated 40+ years. I expect that this large increase in maximum age was due to the management policies that were in effect

at the time the previous aging studies were conducted. Previous studies that aged Alabama River Paddlefish took place shortly after a moratorium on commercial harvesting had been enacted in order to prevent further decline in Paddlefish populations in this river system (Gengerke 1986; Hoxmeier and DeVries 1997; Lein and DeVries 1998; DeVries et al. 2009). The newly observed Paddlefish age data collected in this study show that the Alabama River Paddlefish can reach ages that are similar to those of northern latitude populations (Purkett Jr 1963; Scarnecchia et al. 1996; Paukert and Fisher 2001; Jennings and Zigler 2009). These results suggest that the lower maximum ages of Paddlefish during the 1990s from this system reflect an overfished population, dominated by younger fish. This is consistent with other studies that have documented similar effects following overexploitation (Berkeley et al. 2004a, 2004b; Hsieh et al. 2010). In heavily exploited Paddlefish populations, the observed maximum age of Paddlefish often does not exceed 11-16 years (Combs 1982; Hoffnagle and Timmons 1989; Scholten and Bettoli 2005). In comparison, fish aged in my study represent an overall population that has had the opportunity to recover over a period of several decades and, therefore, include older individuals more representative of the potential maximum age for Paddlefish in the Alabama River.

Strong relationships existed between element concentrations in ambient water and those found in Paddlefish dentary bone edge material. This has been identified as a critical observation that must occur in order for microchemistry studies to be effective in reconstructing environmental histories (Campana 1999; Pracheil et al. 2014). Significant correlations existed for Sr:Ca and Mn:Ca. Paddlefish dentary bones overall had the strongest correlations among the three species, exhibiting correlation results similar to that observed in a previous dentary bone study (Bock et al. 2016). The relationship between elements in water and those ultimately incorporated into Paddlefish dentary bones are critical for much of the rest of my study.

Dentary bone microchemistry proved particularly valuable for quantifying Paddlefish movement, connectivity, and natal origin in the study area. Paddlefish dentary bone microchemistry was first suggested as a viable alternative to Paddlefish otoliths for microchemistry analysis by Bock et al. (2016). And my results support the value of dentary bone microchemistry as a powerful tool. Previous studies have shown the capabilities of using microchemistry for distinguishing among fish populations and identifying the degree of population connectivity (Gillanders and Kingsford 1996; Campana 1999; Campana et al. 2000; Thorrold et al. 2001; Gillanders 2002). Trace element concentrations compared across river sections and DFA classifications support that some population groups are mixing, while others remain isolated, and strontium proved to be the best trace element for determining connectivity, movement and natal origin. Based on these results, fish in Millers Ferry Reservoir and Jones Bluff Reservoir are functionally isolated from Paddlefish populations in Claiborne Lake and the lower Alabama River, and any upstream movement within these systems is essentially blocked by Millers Ferry and Robert F. Henry Dams.

Conversely, the crested spillway that is periodically inundated during high flow events at Claiborne Lock-and-Dam provides an opportunity for Paddlefish populations to mix across this barrier; similarly, this is also suspected for other species passing low-head dams on the Cape Fear River, NC (Nichols and Louder 1970; Smith and Hightower 2012). A previous study by Simcox et al. (2015) used acoustic and radio telemetry to demonstrate that Paddlefish would pass the crested spillway at Claiborne Lock-and-Dam when inundated, although in limited numbers. Some Paddlefish were also observed passing this dam via the lock chambers, although this was even more limited. Other studies have similarly demonstrated that lock passages can contribute to dam passage by Paddlefish (Zigler et al. 2004; Tripp et al. 2014; Simcox et al. 2015). Significant differences observed in mean Sr:Ca over multiple parts of dentary bone ablation transects, reflecting different times during a Paddlefishes life, suggest that these river sections are relatively isolated. However, when looking at the coefficient of

variation and LDA classifications for these lower sections, the results suggest the potential for some limited mixing. The patterns of Sr:Ca concentrations from ablation transects for individual fish showed strong fluctuation in the lower river sections suggesting movement between higher and lower concentration of Sr. Evidence for mixing using microchemistry analysis has been observed in previous studies in other systems (Rooker et al. 2008; Geffen et al. 2011).

Finally, a limited number of passage events upstream and downstream of Claiborne Lock-and-Dam were suggested via the large changes in dentary bone Sr:Ca concentration over short time periods. The percentage of Paddlefish believed to have passed Claiborne Lock-and-Dam based on microchemistry profiles is consistent with the percentages observed in previous and ongoing studies (Simcox et al. 2015). Among fish variation in Sr:Ca concentrations within the same river sections were observed. In order to account for this, I defined a passage event based on exceeding a proportional increase (or decrease) from the benchmark of Sr:Ca ration observed before (or after) the passage event, rather than just exceeding an absolute Sr:Ca level. Depending upon spatial variability and temporal consistency in water chemistry of other river systems that contain Paddlefish, it is believed that this method of determining movement and particularly dam passage via strontium ablation profiles can be applied to other water bodies with some modification to reflect the uniqueness of each system. Overall, my use of dentary bone microchemistry in this study has demonstrated that the three low-use lock-and-dam structures on the Alabama River are playing a major role in constraining Paddlefish population connectivity in this system.

Many hard-part microchemistry studies have documented its value for looking at early life history and natal origins (Thorrold et al. 1998; Whitledge et al. 2007; Walther et al. 2008; Gahagan et al. 2012). Its value for analyzing Paddlefish dentary bones was also demonstrated in this study by interpreting the results of both the first 20 microns and age-0 portions of the ablation transects for the

strontium. Concentrations in these parts of the ablation profile were clearly distinguishable between river sections, similar to that of the edge and whole transect. These locations in the ablation transect are indicators of natal (first 20 micron) and juvenile (age-0) habitat use, and significant differences between each river section Sr:Ca trace element profile suggest that Paddlefish reproduction appears to be occurring within all river sections of the Alabama River. Paddlefish spawning has been observed to occur below dams and in sections of rivers separated by dams (Ruelle and Hudson 1977; Pasch et al. 1980; Wallus 1986) suggesting that the pattern being observed here occur more broadly geographically.

I identified another trend in Paddlefish collected from the Tallapoosa River (TAL). Paddlefish in the Tallapoosa and Coosa Rivers (tributaries that form the Alabama River) are believed to move between Jones Bluff Reservoir (JBR) and these tributaries (Lein and DeVries 1998; DeVries et al. 2009, Rider personal communication 2018). Water trace element signatures were significantly higher in the Tallapoosa River than in all other samples, and I wanted to determine whether a similar trend occurred in Paddlefish dentary bones collected from this system. Significant differences in fish collected from TAL versus JBR were identified for Sr:Ca in both the first 20 micron and age-0 portions of the ablation transect. Higher Sr:Ca concentrations were observed for the whole transect and edge in TAL fish as well, but these were not significantly different from fish collected from JBR. This pattern was interpreted as indicating that Paddlefish collected in the Tallapoosa River are moving freely between Jones Bluff Reservoir and the Tallapoosa River, most likely for reproduction purposes. Additionally, significant differences observed in the natal (first 20 micron) and juvenile (age-0) regions of ablation transects demonstrate spawning site fidelity as observed in other Paddlefish studies (Lein and DeVries 1998; Stancill et al. 2002; Firehammer and Scarnecchia 2006; Jennings and Zigler 2009). In other words, the Paddlefish collected in the Tallapoosa River appear to return to and reproduce in this system, whereas fish collected from Jones Bluff Reservoir do not exhibit this same pattern.

This observation also suggests that elements (particularly Strontium) are incorporated into dentary bones based on the ratio of the concentration of elements to calcium in the water rather than based solely on absolute counts of elements observed. If elements were incorporated purely based on counts of elements, there would not have been the observed increase in strontium concentrations in fish collected from the Tallapoosa River (rather, concentrations would have been lower than JBR). This is the pathway in which otoliths are believed to incorporate elements, based on the element to calcium ratio (Farrell and Campana 1996; Campana 1999). This was a unique opportunity to explore this theory in a field setting for Paddlefish dentary bones. Otoliths, in particular from Largemouth Bass, collected during this study from the Tallapoosa River, also exhibited this same pattern; however, with more dramatic differences, adding support from a field study to show that elements are incorporated into otoliths based on the element to calcium ratio in ambient water rather than based on absolute element counts. One limitation to this idea is that dentary bone Sr:Ca concentration was not highest in TAL as was water Sr:Ca concentrations when compared to the rest of the system. Edge concentrations of Sr:Ca in Paddlefish collected from the Tallapoosa River therefore would have been expected to be significantly higher than all other river sections, given that this represents the material most recently laid down in the dentary bone. However, this was not the case, and TAL Paddlefish edge concentrations were not significantly higher than the next downstream river section (JBR). I believe this may be due to the delay in elemental incorporation time (of days to weeks) observed in laboratory studies (Elsdon and Gillanders 2005; Lowe et al. 2009; Bock et al. 2016). This has been shown to be a potential limitation in microchemistry studies, where brief occupancies of habitats with different chemistry will not necessarily be documented if the time-period of residence is too short for sufficient new hard-part accretion to occur (Pracheil et al. 2014). Hard-part microchemistry has provide evidence that Paddlefish collected in the Tallapoosa may have recently moved from JBR upstream into the Tallapoosa River before being collected, coinciding with spring spawning migrations, and therefore trace elements in the dentary

bones may not have yet reflected the higher Sr:Ca concentrations of the Tallapoosa River. Additional studies will need to be performed to determine if Paddlefish are being exposed to elevated Sr:Ca concentrations for an adequate amount of time to be reflected in dentary bones. This could also be reinforced by performing telemetry studies on Tallapoosa River collected paddlefish to observe residency times for this system. A similar pattern was also observed in a few fish collected from Claiborne Lake, where edge strontium levels were exceptionally high and more consistent with that of fish collected from the lower Alabama River. These few fish may have resided in the Lower Alabama River, passed Claiborne Dam during high water events during or before the commercial Paddlefish season of 2017, and then were subsequently harvested in Claiborne Lake before the strontium levels in their dentary bones had the ability to equilibrate to levels more consistent with Claiborne Lake. It is important to recognize that a delay in element incorporation occurs, which may need to be taken into account in future Paddlefish dentary bone microchemistry studies. Overall, in the context of this study, it is believed that this lag was inconsequential.

Smallmouth Buffalo and Largemouth Bass Otolith Microchemistry

One difficulty I encountered related to estimating Smallmouth Buffalo age via their otoliths. Previously, Smallmouth Buffalo age estimates have been performed by counting annuli in scales (Martin et al. 1964; Walburg and Nelson 1966). Here I sought to determine the relationship between trace element concentrations in Smallmouth Buffalo at different time periods during their lifecycle. However, due to the complexities of Smallmouth Buffalo otoliths (crowding of annuli, irregular annuli, old individuals, etc.), it was not possible to determine an absolute age for each fish, although I was able to identify annuli for the first three years of life. Similar difficulties were encountered by Paukert (1999) when ageing Bigmouth Buffalo (*Ictiobus cyprinellus)*, a related Catostomidae species, via otoliths, including crowding of annuli near the edge and annulus accumulation at the core of the otolith.

Relationships existed between element concentrations in ambient water and those found in fish otolith edge material for my two other study species, as indicated by strong r-squared values, although most relationships were not significant. These correlations, were not as strong as those found in Paddlefish dentary bones; however, strontium in Smallmouth Buffalo and Largemouth Bass exhibited correlation results similar to that observed in other otolith microchemistry studies (Carlson et al. 2016; Radigan et al. 2018b, 2018a). The relationships between elements in water samples and what was ultimately incorporated into fish otoliths demonstrated that these elements were incorporated in relation to concentrations in ambient water.

As described earlier, I documented that Paddlefish dentary bone microchemistry was valuable in a river system that has water chemistry with sufficient spatial variation combined with temporal consistency. However, somewhat unanticipated microchemistry results were observed for my two other study species. Due to the less migratory nature of Smallmouth Buffalo and even more sedentary patterns of Largemouth Bass, I expected strong differences in otolith microchemistry among river sections and low variability in element concentrations throughout the ablation profiles. Similar to Paddlefish, strontium was the trace element that best described population connectivity patterns for both Smallmouth Buffalo and Largemouth Bass. Although some trends observed in Smallmouth Buffalo and Largemouth Bass were similar to that of Paddlefish, these patterns were not nearly as strong nor as distinct. A pattern of increasing Sr:Ca concentration in otoliths with increased distance downstream was observed, but only those samples from river sections furthest apart differed significantly. DFA classification accuracy was similar for both Smallmouth Buffalo and Largemouth Bass, and was much lower versus those for Paddlefish. Finally, CV scores for Smallmouth Buffalo and Largemouth Bass were relatively consistent between river sections for strontium and were similar to those of Paddlefish.

The patterns in Sr:Ca across river sections observed in Smallmouth Buffalo and Largemouth Bass compared to Paddlefish could be the result of a combination of factors. First, these three species exhibit different habitat use patterns. Paddlefish are pelagic (Clark-Kolaks et al. 2009) and occupy a variety of habitats including backwaters, oxbows, and main channel areas (Boschung and Mayden 2003). Contrastingly, Smallmouth Buffalo are more benthic (Gido 2002), but also occupy similar a similar variety of habitats, including but not limited to main channels, backwaters and other low water velocity habitats (Kallemeyn 1977; Edwards and Twomey 1982). Most unique in habitat use from the other two species, Largemouth Bass tend to be the most littoral, and most frequently occupy low velocity areas such as pools, backwaters, side-channels and oxbow areas in medium to large rivers (Boschung and Mayden 2003; Ahrenstorff et al. 2008). Backwaters and side channels areas may have different trace element signatures compared to that of the main channel, which would not have been detected through my main channel and tributary water sampling. Microhabitat use by different species has been proposed as to why different species incorporate elements differently or demonstrate different patterns (Swearer et al. 2003; Reis-Santos et al. 2008; Nelson and Powers 2019). This study was limited relative to the number of water samples that I could collect and analyze. Additionally, the variation observed in CV scores for Largemouth Bass and Smallmouth Buffalo could also be attributed to fluctuating trace element conditions in the habitats these fish occupy (Morris et al. 2003), which again would not have been detected by my water sampling. Additionally, different hard-part structures were used for Smallmouth Buffalo and Largemouth Bass versus Paddlefish. Furthermore, previous otolith microchemistry studies have shown that fish from the same family can incorporate elements into their otoliths differently (Gillanders and Kingsford 2003; Rooker et al. 2004; Pracheil et al. 2014). Therefore, my unanticipated results may have occurred due to the combination of different species and hard-part structures used (dentary bones vs. otoliths). If these structures incorporate elements differently, water chemistry may be sufficiently variable to be detected in one type of structure, but not in another

structure type. Therefore, Smallmouth Buffalo and Largemouth Bass may require a larger regional scale comparison to strengthen the results for these species, as suggested by the effectiveness of large-scale studies performed on other species (Gahagan et al. 2012). Another plausible explanation for some of the trends observed, in particular with Largemouth Bass, could be a result of fish potentially making their way into the Alabama River system by stocking of small impoundments connected to the system. Inconsistent micro-elemental signatures could be a result of juveniles from stocked ponds being raised elsewhere, which has been used in previous studies to identify naturally reproducing versus stocked fish (Bickford and Hannigan 2005; Gibson-Reinemer et al. 2009; Zitek et al. 2010; Rude et al. 2014; Pracheil et al. 2014). One final explanation for some of the trends observed, could be the result of human relocation of Largemouth Bass during angling tournament activities, which are common events on Alabama Reservoirs (Hendricks et al. 1995). All these factors might contribute to the patterns being observed in Smallmouth Buffalo and Largemouth Bass. Although distinctions between fish population groups in the different river sections were not observed as expected, these results are taken with caution, but suggest that these species do not freely move between river sections. This is particularly true when the results for Paddlefish, a species more capable of long distance movements, still provide strong evidence for isolation, especially in JBR and MFR.

Overall, this research supports a diverse and growing body of literature demonstrating the applicability of hard-part microchemistry for fisheries management (Campana 1999; Pracheil et al. 2014; Walther et al. 2017), now including its value for studying impounded river systems. My results for Sr:Ca microchemistry showed strong evidence of isolation of population groups from the two upper river sections, but also evidence for some limited mixing in the Alabama River's two lower river sections. Another important finding was that patterns observed for the other elements analyzed (Ba, Mg, and Mn) were not nearly as strong across the three species. These are elements that can be physiologically regulated or have ontogenetic patterns in otoliths, causing them to not necessarily reflect the

concentrations of ambient element conditions in water, whereby their use as indicators has been cautioned in freshwater systems (Geffen et al. 1998; Halden et al. 2000; Chittaro et al. 2006; Limburg et al. 2015). As such, Ba, Mg, and Mn are likely less valuable as indicators of the environmental conditions experienced by fish, leading to some of the inconsistent patterns observed.

Genetics

The development of genotyping-by-sequencing (GBS) techniques was deemed as one of the most significant scientific breakthroughs within the last decade (Andrews et al. 2016). These methods have revolutionized the field of ecological and evolutionary genomics by uncovering hundreds to thousands of markers across a genome in a single and cost-effective experiment (Davey et al. 2011). This technique proved particularly valuable for my study of Paddlefish and Smallmouth Buffalo, identifying thousands of SNPs for each species for population genetic analyses. Acipenseriform fishes share a tetraploid ancestor followed by genomic and chromosomal reorganization, as do Catostomidae species (Uyeno and Smith 1972; Schwemm et al. 2014). Therefore it was critical to identify diploid markers rather than polypoid markers because characteristics such as ambiguous homology and dosage uncertainty typically prevent the use of polyploid markers for population genetic analyses. When identifying bi-allelic SNPs via HDplot, Paddlefish showed a similar distribution of z-score (D) and proportion of heterozygotes (H) as did mountain barberry (*Berberis alpine*) (McKinney et al. 2017), a shrub also known to have a large proportion of duplicate loci (35%), which was confirmed by the same paralog identification method (Mastretta-Yanes et al. 2014; McKinney et al. 2017). Additionally, previous efforts in Paddlefish microsatellite development have shown varied proportions of singleton loci in marker discovery, ranging from 52% (13 of 25; Schwemm et al. 2014) to 75% (6 of 8; Heist et al. 2002), which overlapped the singleton loci rate (68.19%) in this study. Smallmouth Buffalo loci were primarily clustered into one cloud, corresponding to singleton loci distribution, with only a small

percentage outside its central range. This suggests that duplicate loci were present at low frequency in the Smallmouth Buffalo genome, potentially due to extensive rediploidization after whole-genome duplication (Uyeno and Smith 1972). As a result of the effectiveness of this methodology in selecting representative singleton SNPs, this study's optimized parameters for multiplex panel design and validation can benefit future studies.

This study also represents the first population genetics assessment of Paddlefish using SNP markers. Other Paddlefish population studies have been conducted using allozymes, mitochondrial DNA, or microsatellite markers (Carlson et al. 1982; Epifanio et al. 1996; Heist and Mustapha 2008; Sloss et al. 2009; Zheng et al. 2014). For example, a previous genetic survey using seven microsatellite loci observed higher genetic heterozygosity values in Paddlefish populations from Mississippi (0.68) and Poland stocks (0.59−0.60, imported from USA) compared to my study with SNPs. Given that microsatellites typically have more alleles and higher heterozygosity than SNPs (Tokarska et al. 2009), and evidence from other animal species have shown only weak or no congruence between the estimates of heterozygosity derived from the two marker types (Vignal et al. 2002), a direct comparison of heterozygosity was not conducted in this study. Additionally, inbreeding levels from Paddlefish in this study generally mirror a previous study in four geographically separate Paddlefish populations, with F_{is} ranging from 0.013 (Tallapoosa River) to 0.099 (Yellowstone/Missouri River) (Zheng et al. 2014). Overall, heterozygosity values were neither very low nor high, suggesting that fish in each river section have an intermediate level of genetic variability. Observed heterozygosities were slightly lower than expected heterozygosities which may be attributed to inbreeding. However when looking at inbreeding coefficients (F_{is}) for each river section, they were still very low and therefore inbreeding does not appear to be an issue in fish from each of the river sections.

Also, estimates of effective population sizes of these species for each river section, particularly for Paddlefish, provide natural resource managers with critical information for best management of these species. N_e is a commonly used population estimation measurement as it only requires a single population for calculation, see equation in Cervantes et al. (2011). Some factors such as the variance in reproductive success of individuals (Heist and Mustapha 2008), sample size (normally <1% of the census population), and overlapping generations may impact the accuracy of N_e, and generally lead to underestimate of population size (Marandel et al. 2019). For example, a simulation study on Thornback Ray (*Raja clavata*) found NeEstimator underestimated N_e by 31% (Marandel et al. 2019), while Waples et al. (2014) found a 10% bias for Atlantic Cod (*Gadus morhua*). Therefore, particular attention should be paid to the interpretation of N_e results. Infinite values of N_e for Smallmouth Buffalo were potentially due to the limited sample size and thus overestimation occurred (Marandel et al. 2019). Small sample sizes may also affect the accuracy of the N_e estimate for Paddlefish populations in this study, and they also may be biased due to overlapping fish generations and/or Paddlefish life history traits (i.e., iteroparity, delayed maturity, and high fecundity) (Epifanio et al. 1996; Heist and Mustapha 2008).

Population structure and differentiation analyses all converged on a similar conclusion that there was not strong evidence for population divergence among river sections for either species. When initially designing this study, it was believed that if there would be genetic differentiation among river sections, it would be observed between those that were separated by the greatest distance, and that it would be more prevalent in in a species with less migratory capabilities. Almost no genetic differentiation was observed between any river sections for either species. The only differentiation observed did occur for the less migratory of the species, Smallmouth Buffalo, although this differentiation was found to be in adjacent river sections, rather than those separated by the greatest distance.

Genetic variation among Paddlefish and Smallmouth Buffalo populations in different river sections was not detected using AMOVA, and most genetic variation was attributed to within individuals. F_{ST} statistics in general were very low for both species in this study, suggesting there is sufficient gene flow to maintain similar allele frequencies and to avoid harmful effects of local inbreeding (Lowe and Allendorf 2010). Only one pairwise comparison was significant for Smallmouth Buffalo (between MFR and JBR), providing evidence that Smallmouth Buffalo may be experiencing some genetic differentiation between these regions; as such, these dams may be impacting Smallmouth Buffalo population genetics in this system slightly more than that of Paddlefish. This difference between species may potentially be a result of three factors: 1) the life span of these individuals; and 2) early life developmental characteristics and 3) the habitats these species use for reproduction. Smallmouth Buffalo have a shorter life span than Paddlefish (Walburg and Nelson 1966), especially when comparing ages from my study. Lifespan may be a critical factor in this study because the dams on the Alabama River system have only been in place for about half a century and therefore having more generations in that period of time could potentially allow for more genetic drift. Second, Paddlefish and Smallmouth Buffalo have different preferred spawning areas. This includes shallow weedy backwater sloughs for Smallmouth Buffalo (Walburg and Nelson 1966; Edwards and Twomey 1982) and primary clean and flowing gravel bars for Paddlefish (Hoxmeier and DeVries 1997; Boschung and Mayden 2003; DeVries et al. 2009; Jennings and Zigler 2009; Pracheil et al. 2009). Additionally, Smallmouth Buffalo release adhesive eggs that attach to the substrate after which fry and juveniles remain in these backwater/low velocity areas (Wrenn and Grinstead 1971; Edwards and Twomey 1982). Paddlefish are similar in that they have adhesive eggs; however, shortly after hatching, Paddlefish begin erratically swimming, subject to passively drifting in receding/flowing waters (Purkett 1961; Wallus 1986; Jennings and Zigler 2009). These differences in early development may make it more likely for Paddlefish larvae to be moved with the current and drift past dams, facilitating some potential mixing between river sections and

reinforcing the lack of genetic differentiation. Population structure analysis confirmed similar results to differentiation, in that one genetic cluster for each species was identified from the multiple river sections. Finally, DAPC analysis also showed a general pattern of low genetic differentiation, similar to that observed in the previous analyses, where there was no clear separation or clusters among river sections for both species. A similar conclusion to this study resulted for lionfish (*Pterois volitans*) collected in the northwestern Atlantic Ocean and the Gulf of Mexico, where similar results were observed for these same analyses of population structure and differentiation (Pérez-Portela et al. 2018). Additionally, Raeymaekers et al. (2009) suggested that even if genetics did not reveal differentiation, it does not mean that processes leading to loss of diversity are not occurring, as populations may not yet have reached equilibrium since being separated by barriers.

Numerous studies on freshwater fishes isolated by artificial dams have been performed to determine the potential effects of these barriers on genetic variation and population differentiation (Yamamoto et al. 2004). Ample evidence exists showing that anthropogenic human disturbances can alter genetic variability within populations and genetic differentiation among populations (Neraas and Spruell 2001; Yamamoto et al. 2004). For example, a study performed on white-spotted charr (*Salvelinus leucomaenis*) showed reduced genetic diversity for populations above dams relative to those below dams in the system, and differences in genetic differentiation (measured as F_{ST}) were highly significant for all pairwise comparisons among populations (Yamamoto et al. 2004). In other circumstances, such as in species with low-mobility, there can be a lack of evidence for significant effects of barriers (Coleman et al. 2018). Additionally in some cases, the construction of dams can structure and increase local genetic diversity through population fragmentation and differentiation (Heggenes and Røed 2006). Typically, it has been suggested that for most fish taxa, 50-100 years is sufficient time for barrier induced divergence to occur. However, this is likely not enough time for long lived species, such as sturgeons (*Acipenseridae spp.*) to diverge, where current genetic structure can reflect historical

processes and natural impediments to gene flow (Lloyd et al. 2013; McDougall et al. 2017). Paddlefish is a similarly long-lived fish species, suggesting that the lack of genetic divergence in our study is reflecting historical genetic structure. Therefore, it is not surprising that this study in the Mobile River Basin did not reveal any genetic divergence in these long lived species. Many factors can affect whether genetic divergence will occur, including but not limited to the mobility and age of the species, the amount of habitat available between barriers, the population sizes present and the duration that barriers have been in place (Yamamoto et al. 2004; McDougall et al. 2017; Coleman et al. 2018). Finally, it is believed that the minimum effective population size necessary for viable isolated populations are estimated to be approximately 100 individuals to avoid inbreeding depression, and approximately 1,000 to maintain adaptive potential into the long-term (Frankham et al. 2014). This suggests that continued monitoring of effective population sizes in these four river sections of the Alabama River would be beneficial.

Management Implications

Results of this study have implications from a management perspective, particularly with regard to the dams in this system. Relative to Paddlefish, this study provides evidence confirming that reproduction is occurring in each river section of the Alabama River, as well as in one of its tributaries, the Tallapoosa River. In addition, genetics analyses provided effective population size estimates for fish in each river section. Finally, microchemistry demonstrates that Paddlefish populations are functionally isolated in JBR and MFR, although they do not appear to have been isolated long enough, or there may be just enough downstream drift of juveniles to prevent genetic differences from developing. Therefore, it leaves managers with a decision to make relative to what actions should be taken to mitigate population isolation. If genetic divergence is a concern in the long term, then methods should be explored to facilitate Paddlefish movements past these dams. Prolonged isolation can lead to inbreeding within populations, which could result in the potential buildup of deleterious alleles,

potentially compromising population persistence (Frankham 2005; Willi et al. 2006; Coleman et al. 2018). This provides support for long term monitoring and mitigation actions, particularly when dealing with Paddlefish, a species of concern and commercial importance. Mitigation may include the addition of fishways, fish ladders, weirs, fish elevators, modification to lock chambers, trap and haul operations, or "nature-like" bypass side channels (Clay 1994; Schmetterling 2003; Wilcox et al. 2004; Finney et al. 2006; Roscoe and Hinch 2010; McDougall et al. 2013; Coleman et al. 2018). Since fishway design/passage methods are lacking for paddlefish, this may be a beneficial investment for research.

In recent years, "nature-like" bypass side channels have been rapidly gaining in popularity as a mitigation technique, showing promise for improvements in fish passage for a broad range of species with varying swimming capabilities (Wilcox et al. 2004; Calles and Greenberg 2007; Roscoe and Hinch 2010). Due to the variety of species that could benefit from fish passage opportunities on the Alabama River, "nature-Like" bypass channels may be the best option for success in the long term. However, this mitigation technique requires rigorous site evaluations, has high costs and takes time for construction (Clay 1994; Calles and Greenberg 2007; Roscoe and Hinch 2010; Noonan et al. 2012; Williams et al. 2012). Another viable option could be to explore attraction flows, sound or other modifications to the existing locks on the Alabama River, to facilitate movement between river sections for the desired species (Simcox et al. 2015). Until a more permanent solution can be developed, other techniques that are relatively less expensive (though labor intensive) should be considered, such as trap and haul operations, which can provide benefits (demographic and genetic) quickly by the immediate addition of fish (Schmetterling 2003).

Summary

In summary, I found that Sr:Ca concentrations in fish hard-parts for my three study species was the best indicator of connectivity, movement, and natal origin for the Alabama River and its major

tributaries. I used a relatively new technique, Paddlefish dentary bone microchemistry, and proved its value in a river system with adequate spatial variability and temporal consistency in water chemistry. Entire genomes for Paddlefish and Smallmouth Buffalo were mapped; GBS techniques identified 1,889 and 3,737 SNPs in Paddlefish and Smallmouth Buffalo, respectively, and effective population sizes were identified for both species.

These two approaches were used in a coordinated effort to assess population connectivity on two timescales, 1) short- microchemistry (lifespan of a fish) and 2) long- genetics (multiple generations). Paddlefish dentary bone microchemistry revealed strong evidence of isolation in Paddlefish populations in the two upper river sections, and some evidence for limited mixing between the two lower river sections, particularly for Paddlefish. This mixing was facilitated by a crested spillway that provides a potential passage opportunity when inundated during high water events. Although there were some differences observed in microchemistry between species, a high confidence level suggest that Paddlefish microchemistry results are indicative of the isolation conditions occurring in this system due to the presence of lock-and-dam structures on the Alabama River.

The second approach, genetics analyses, revealed that Paddlefish genetics have not diverged and therefore isolation between population groups has not affected Paddlefish genetics during the approximate 50 years since the three dams were constructed on the Alabama River. However, there is some evidence that Smallmouth Buffalo populations are beginning to diverge. Therefore, when integrating results from both approaches and considering Paddlefish, a species of interest in Alabama, it appears that isolation is occurring in this system, but that there has not been a long enough period of time for this to be detected in genetics; in part because this is a long-lived species, and the amount of mixing may be adequate enough to prevent divergence due to fish passing dams through lock structures, gated spillways, over crested spillways or by downstream drift of juveniles. If managers are

concerned about potential future population divergence associated with dams on the Alabama River, long-term monitoring should be implemented, and methods to facilitate fish movement past dams should be made a research and river restoration priority.

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Tables

Table 1. Water sampling locations for 2017 and 2018, with a brief site description, lat/lon coordinates and river kilometer mile (RKM).

Site Name	Site Description	Site Coordinates	RKM
COOSA	Coosa River near Wetumpka	32.526149, -86.222694	16.1
TALLAPOOSA 1	Tallapoosa River above Fort Toulouse	32.495356, -86.245789	NA
TALLAPOOSA 2	Tallapoosa River off 229, at boat ramp 2 miles below Thurlow dam	32.509637, -85.890835	NA
ALR270	R.E. "Bob" Woodruff Lake on the Alabama River near Gunterhill Park	32.3547611, -86.4935837	434.5
ALR236	Alabama River 400 m below Robert F. Henry L&D	32.3212117, -86.7866861	379.8
ALR221	Alabama River near Benton	32.430809, -86.859480	355.7
CAHABA	Cahaba River upstream of its confluence with the Alabama River	32.322743, -87.093062	0.48
ALR181	Alabama River near Six Mile Creek	32.2714351, -87.1419330	291.3
ALR132	Alabama River 400 m below Millers Ferry L&D	32.097139, -87.399275	213.2
ALR109	Alabama River near Holley's Ferry	31.920126, -87.436442	175.3
ALR72	Alabama River 400 m below Claiborne L&D	31.611285, -87.550460	115.9
ALR47	Alabama River near Eureka	31.421455, -87.636247	75
ALR3	Alabama River upstream of confluence with the Tombigbee River	31.163166, -87.927802	4.2
TOMBIGBEE	Tombigbee River upstream of confluence with the Alabama River	31.147663, -87.965695	76.4
TENSAW	Tensaw River near Live Oak Landing	30.968324, -87.889371	NA

Table 2. Numbers of Paddlefish (dentary bones), Smallmouth Buffalo (otoliths), and Largemouth Bass (otoliths) used for hard-part microchemistry analysis for each river region. Regions are as follows: LAR-Lower Alabama River, CL=Claiborne Lake, MFR=Millers Ferry Reservoir, JBR=Jones Bluff Reservoir, CSA=Coosa River, TAL=Tallapoosa River.

Hard-part Type	Species	Region						
		LAR	CL	MFR	JBR	CSA	TAL	Totals
Otoliths	Smallmouth Buffalo	52	53	48	42	8	6	**209**
	Largemouth Bass	24	23	23	25	5	6	**106**
Dentary Bones	Paddlefish	56	43	50	30		7	**186**

Table 3. Elements used in analyses for Paddlefish (PAD), Largemouth Bass (LMB), and Smallmouth Buffalo (SBF) using LA-ICPMS. Mean limits of detection (LOD) (PPM) were calculated based on all sample runs. The coefficient of variation (CV), as determined from NIST 612 standards, is the average for all runs, and was calculated by dividing standard deviations of runs by their means. The percentage of samples greater than detection limits for an element (% > LOD) also is provided.

	Element		Sr	Ba	Mg	Mn
PAD	Element LOD		0.05	0.02	0.89	0.05
	NIST 612 CV %		14.60	16.38	15.68	14.54
	Whole Tra	%>LOD	100	100	100	100
	Age-0	%>LOD	100	100	100	100
	Edge	%>LOD	100	100	100	100
	First 20 µn	%>LOD	100	100	100	100
LMB	Element LOD		0.02	0.02	0.10	0.08
	NIST 612 CV %		14.37	16.00	14.12	13.91
	Whole Tra	%>LOD	100	100	100	92
	Age-0	%>LOD	100	100	100	92
	Edge	%>LOD	100	100	100	75
	First 20 µn	%>LOD	100	100	100	98
SBF	Element LOD		0.01	0.03	0.19	0.11
	NIST 612 CV %		14.27	15.36	14.56	13.40
	Whole Tra	%>LOD	100	100	100	100
	Age-0	%>LOD	100	100	100	100
	Edge	%>LOD	100	99.52	100	100
	First 20 µn	%>LOD	100	100	100	100

Table 4. Results of the Discriminant Function Analysis (DFA) for Paddlefish using river section as each group in the analysis. Percentage of correct classification of Paddlefish to river section is shown by the bolded diagonal and rows correspond to the actual collection river section, while columns are the river section assigned by the DFA. DFAs were performed on each part of the ablation transect.

Transect Analysis	Capture Location	LDA Classification (%) JBR	MFR	CL	LAR	Total Classification Accuracy (%)
Mean Transect	JBR	**67**	33	0	0	**68**
	MFR	2	**94**	2	2	
	CL	2	33	**37**	28	
	LAR	2	14	14	**70**	
Mean Edge	JBR	**80**	20	0	0	**74**
	MFR	0	**94**	6	0	
	CL	2	37	**33**	28	
	LAR	2	5	9	**84**	
Mean Age-0	JBR	**50**	50	0	0	**60**
	MFR	6	**78**	8	8	
	CL	2	43	**29**	26	
	LAR	0	11	17	**72**	
Mean First 20 µm	JBR	**27**	73	0	0	**55**
	MFR	4	**78**	10	8	
	CL	0	45	**29**	26	
	LAR	0	13	19	**69**	

Table 5. Discriminant variables generated by the DFA (LD-1 through LD-3) for Paddlefish with river sections as groups, and the importance of each discriminant variable in classifying fish to sites, the proportion of trace column. The value for each element in a discriminant variable column corresponds to the effect each has on that discriminator. The larger the absolute value of each element, the greater the effect.

	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		83.88	9.56	6.55
Whole transect	Elements used in DFA	Sr_{88}	0.0097	-0.0023	-0.0012
		Mg_{24}	-0.0001	-0.0004	-0.0003
		Ba_{137}	-0.0231	-0.0034	0.0438
		Mn_{55}	-0.0021	0.0155	-0.0141
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		64.57	32.65	2.78
Edge	Elements used in DFA	Sr_{88}	0.0061	0.0006	-0.0023
		Mg_{24}	0.0000	-0.0001	-0.0003
		Ba_{137}	-0.0070	-0.0185	0.0152
		Mn_{55}	-0.0047	0.0161	0.0002
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		89.07	8.37	2.56
Age 0	Elements used in DFA	Sr_{88}	0.0094	-0.0036	0.0003
		Mg_{24}	-0.0001	-0.0003	-0.0002
		Ba_{137}	-0.0577	0.0551	-0.0387
		Mn_{55}	0.0012	0.0041	-0.0091
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		92.73	6.43	0.84
Mean First 20 µm	Elements used in DFA	Sr_{88}	0.0100	-0.0030	0.0004
		Mg_{24}	-0.0001	-0.0003	-0.0002
		Ba_{137}	-0.0500	0.0705	-0.0114
		Mn_{55}	0.0013	0.0044	-0.0073

Table 6. Results of the Discriminant Function Analysis (DFA) for Paddlefish, with fish caught in the Lower Alabama River (LAR) and Claiborne Lake (CL) river sections combined as opposed to being treated separately, using river section as each group in the analysis. Percentage of correct classification of Paddlefish to river section is shown by the bolded diagonal and rows correspond to the actual collection river section, while columns are the river section assigned by the DFA. DFAs were performed on each part of the burn transect.

Transect Analysis	Capture Location	LDA Classification (%) JBR	MFR	LARCL	Classification Accuracy (%)
Mean Transect	JBR	**67**	33	0	**79**
	MFR	2	**94**	4	
	LARCL	2	23	**75**	
Mean Edge	JBR	**80**	20	0	**84**
	MFR	0	**94**	6	
	LARCL	2	18	**80**	
Mean First 20 µm	JBR	**40**	60	0	**69**
	MFR	6	**70**	24	
	LARCL	2	20	**78**	
Mean Age-0	JBR	**27**	73	0	**67**
	MFR	4	**70**	26	
	LARCL	0	22	**78**	

Table 7. Discriminant variables generated by the DFA (LD-1 through LD-2) for Paddlefish, with fish caught in the Lower Alabama River (LAR) and Claiborne Lake (CL) river sections grouped together as opposed to being treated separately, with river sections as groups, and the importance of each discriminant variable in classifying fish to sites, the proportion of trace column. The value for each element in a discriminant variable column corresponds to the effect each has on that discriminator. The larger the absolute value of each element, the greater the effect.

	Linear Discriminants		LD-1	LD-2
	Proportion of Trace (%)		89.72	10.28
Whole transect	Elements used in DFA	Sr_{88}	0.0094	-0.0004
		Mg_{24}	0.0001	-0.0001
		Ba_{137}	-0.0231	0.0424
		Mn_{55}	-0.0052	-0.0189
	Linear Discriminants		LD-1	LD-2
	Proportion of Trace (%)		68.31	31.69
Edge	Elements used in DFA	Sr_{88}	-0.0056	-0.0023
		Mg_{24}	0.0000	0.0000
		Ba_{137}	0.0020	0.0214
		Mn_{55}	0.0095	-0.0134
	Linear Discriminants		LD-1	LD-2
	Proportion of Trace (%)		95.83	4.17
Age 0	Elements used in DFA	Sr_{88}	0.0094	-0.0004
		Mg_{24}	0.0000	-0.0003
		Ba_{137}	-0.0674	-0.0239
		Mn_{55}	-0.0002	-0.0076
	Linear Discriminants		LD-1	LD-2
	Proportion of Trace (%)		98.71	1.29
First 20 µm	Elements used in DFA	Sr_{88}	-0.0098	-0.0006
		Mg_{24}	0.0000	0.0002
		Ba_{137}	0.0615	0.0183
		Mn_{55}	-0.0002	0.0078

Table 8. Percentages of Paddlefish for each river section grouped into one of four Sr:Ca (µmol:mol) oscillation size categories (<100, 100-150, 150-400, and >400). A Sr:Ca ablation profile with oscillations less than 100 µmol:mol was considered a consistent profile.

Sr:Ca (µmol:mol) Ablation Profile Types				
River Section	Oscillation Size (% of fish)			
	<100	**100-150**	**150-400**	**>400**
Tallapoosa River	86	14	0	0
Jones Bluff Reservoir	97	3	0	0
Millers Ferry Reservoir	88	12	0	0
Claiborne Lake	37	7	42	14
Lower Alabama River	9	9	75	7

Table 9. List of all the Paddlefish in my study suspected to have displayed a passage event, their Sr:Ca (µmol:mol) measurements, and proportional increases/decreases. A passage event was defined as a Sr:Ca increase greater than 250 µmol:mol (greater than 0.7 proportional increase/decrease in Sr:Ca from before/after a suspected passage event) over a distance of less than 150 µm. Additionally, Sr:Ca concentration either had to remain higher than before peak levels, or dropped back down to levels equal to or less than before the passage concentration, suggesting an upstream passage event.

FISH ID	Before Passage Event[1]		At peak		After Passage Event[2]		Peak-Before		Peak-After		Proportional Increase[1]	Proportional Decrease[2]
	Sr:Ca	Distance	Sr:Ca	Distance	Sr:Ca	Distance	Sr:Ca	Distance	Sr:Ca	Distance	Sr:Ca	Sr:Ca
LAR29	660	1434	1248	1572			588	**138**			**0.89**	
LAR36	508	1644	1038	1787			530	**143**			**1.04**	
LAR39	479	1178	1026	1318			547	**141**			**1.14**	
LAR49	400	3292	683	3384			283	**92**			**0.71**	
LAR68	521	2790	906	2921			385	**131**			**0.74**	
MAR52	362	1723	667	1812			304	**90**			**0.84**	
MAR52			612	2074	350	2196			262	**123**		**0.75**
MAR63	610	358	1271	466			662	**108**			**1.09**	
MAR63			1271	466	641	563			630	**97**		**0.98**
MAR75	469	1956	897	2020			428	**64**			**0.91**	
MAR36	508	2452	1046	2537			537	**84**			**1.06**	
MAR58	400	2368	855	2481			455	**113**			**1.14**	

1= Downstream Passage

2= Upstream Passage

Table 10. Results of the Discriminant Function Analysis (DFA) for Smallmouth Buffalo using river section as each group in the analysis. Percentage of correct classification of Smallmouth Buffalo to river section is shown by the bolded diagonal and rows correspond to the actual collection river section, while columns are the river section assigned by the DFA. DFAs were performed on each part of the ablation transect.

Transect Analysis	Capture Location	LDA Classification (%)				Total Classification Accuracy (%)
		JBR	MFR	CL	LAR	
Mean Transect	JBR	**62**	19	2	17	**43**
	MFR	6	**54**	19	21	
	CL	9	30	**43**	17	
	LAR	12	31	40	**17**	
Mean Edge	JBR	**55**	29	0	17	**47**
	MFR	6	**60**	10	23	
	CL	17	28	**26**	28	
	LAR	4	35	13	**48**	
Mean Age-0	JBR	**52**	24	2	21	**38**
	MFR	15	**31**	33	21	
	CL	8	25	**32**	36	
	LAR	10	21	29	**40**	
Mean First 20 µm	JBR	**29**	36	17	19	**37**
	MFR	10	**46**	29	15	
	CL	8	30	**23**	40	
	LAR	8	25	15	**52**	

Table 11. Discriminant variables generated by the DFA (LD-1 through LD-3) for Smallmouth Buffalo with river sections as groups, and the importance of each discriminant variable in classifying fish to sites, the proportion of trace column. The value for each element in a discriminant variable column corresponds to the effect each has on that discriminator. The larger the absolute value of each element, the greater the effect.

	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		84.63	15.21	0.16
Whole transect	Elements used in DFA	Sr_{88}	0.0260	0.0222	0.0098
		Mg_{24}	0.0006	0.0001	-0.0011
		Ba_{137}	-0.8561	-0.4956	-0.9945
		Mn_{55}	-0.0104	0.0209	0.0031
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		73.92	19.41	6.68
Edge	Elements used in DFA	Sr_{88}	0.0098	0.0282	-0.0188
		Mg_{24}	0.0005	0.0006	0.0009
		Ba_{137}	0.0350	-0.4159	0.5722
		Mn_{55}	-0.0144	0.0072	0.0021
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		82.58	17.05	0.37
Age 0	Elements used in DFA	Sr_{88}	0.0216	0.0005	-0.0040
		Mg_{24}	-0.0001	-0.0013	0.0009
		Ba_{137}	-0.5008	-0.2048	-0.3092
		Mn_{55}	-0.0015	0.0042	0.0052
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		59.96	31.83	8.21
Mean First 20 µm	Elements used in DFA	Sr_{88}	-0.0115	0.0097	-0.0024
		Mg_{24}	0.0008	0.0005	0.0005
		Ba_{137}	0.1721	-0.0918	-0.1194
		Mn_{55}	-0.0001	-0.0015	0.0026

Table 12. Results of the Discriminant Function Analysis (DFA) for Largemouth Bass using river section as each group in the analysis. Percentage of correct classification of Largemouth Bass to river section is shown by the bolded diagonal and rows correspond to the actual collection river section, while columns are the river section assigned by the DFA. DFAs were performed on each part of the ablation transect.

Transect Analysis	Capture Location	LDA Classification (%)				Total Classification Accuracy (%)
		JBR	MFR	CL	LAR	
Mean Transect	JBR	**64**	32	0	4	**44**
	MFR	22	**52**	4	22	
	CL	4	48	**17**	30	
	LAR	13	29	17	**42**	
Mean Edge	JBR	**68**	24	4	4	**39**
	MFR	30	**35**	26	9	
	CL	22	30	**35**	13	
	LAR	13	50	21	**17**	
Mean Age-0	JBR	**52**	20	0	28	**48**
	MFR	17	**35**	22	26	
	CL	4	26	**39**	30	
	LAR	8	17	8	**67**	
Mean First 20 µm	JBR	**56**	8	4	32	**44**
	MFR	17	**30**	17	35	
	CL	4	22	**35**	39	
	LAR	13	25	8	**54**	

Table 13. Discriminant variables generated by the DFA (LD-1 through LD-3) for Largemouth Bass with river sections as groups, and the importance of each discriminant variable in classifying fish to sites, the proportion of trace column. The value for each element in a discriminant variable column corresponds to the effect each has on that discriminator. The larger the absolute value of each element, the greater the effect.

	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		78.23	19.22	2.55
Whole transect	Elements used in DFA	Sr_{88}	0.0034	-0.0001	-0.0015
		Mg_{24}	0.0100	0.0166	-0.0763
		Ba_{137}	-0.0914	0.5799	0.2167
		Mn_{55}	-0.2086	0.0140	-0.1590
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		68.67	23.01	8.32
Edge	Elements used in DFA	Sr_{88}	0.0029	-0.0002	-0.0019
		Mg_{24}	-0.0171	0.0021	-0.0274
		Ba_{137}	-0.1293	0.4424	0.0735
		Mn_{55}	0.6798	0.2266	0.9537
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		70.68	28.94	0.38
Age 0	Elements used in DFA	Sr_{88}	0.0024	-0.0019	-0.0019
		Mg_{24}	-0.0556	-0.0948	-0.0385
		Ba_{137}	-0.0727	0.2594	-0.2009
		Mn_{55}	-0.1326	0.0887	-0.0596
	Linear Discriminants		LD-1	LD-2	LD-3
	Proportion of Trace (%)		76.35	19.23	4.42
Mean First 20 µm	Elements used in DFA	Sr_{88}	0.0027	-0.0013	-0.0018
		Mg_{24}	0.0011	-0.0350	0.0080
		Ba_{137}	-0.1098	0.0304	-0.1006
		Mn_{55}	-0.0271	-0.0109	-0.0048

Table 14. Total numbers of Paddlefish and Smallmouth Buffalo fin clips collected for genetic analysis for each river region. Regions are as follows: LAR-Lower Alabama River, CL=Claiborne Lake, MFR=Millers Ferry Reservoir, JBR=Jones Bluff Reservoir, CSA=Coosa River, TAL=Tallapoosa River, TEN=Tensaw River, TOM=Tombigbee River.

Species	Region							
	LAR	**CL**	**MFR**	**JBR**	**CSA**	**TAL**	**TOM**	**Totals**
Paddlefish	130	47	102	35	4		87	**405**
Smallmouth Buffalo	91	74	61	62	8	7		**303**

Table 15. Total numbers of Paddlefish and Smallmouth Buffalo fin clips used for genetic analysis for each river region. Regions are as follows: LAR-Lower Alabama River, CL=Claiborne Lake, MFR=Millers Ferry Reservoir, JBR=Jones Bluff Reservoir, CSA=Coosa River, TAL=Tallapoosa River, TEN=Tensaw River, TOM=Tombigbee River.

Species	**Region**							
	LAR	**CL**	**MFR**	**JBR**	**CSA**	**TAL**	**TOM**	**Totals**
Paddlefish	31	35	33	27	4		29	**159**
Smallmouth Buffalo	29	30	30	30				**119**

Table 16. Genetic diversity parameters of Paddlefish and Smallmouth Buffalo from each river sections. H_o indicates average observed heterozygosity. H_e indicates average expected heterozygosity. F_{is} indicates inbreeding coefficient. N_e indicates the estimate of contemporary effective population size with 95% confidence interval.

Species	River Section	n	H_o	H_e	F_{is}	N_e
Paddlefish	CSA	4	0.389	0.403	0.034	NA
	JBR	27	0.291	0.304	0.041	844 (670-1138)
	MFR	33	0.289	0.302	0.040	1516 (1124-2324)
	CL	35	0.290	0.302	0.037	1198 (954-1607)
	LAR	31	0.286	0.301	0.045	1960 (1317-3812)
	TOM	29	0.294	0.304	0.032	1673 (1144-3099)
	Mean		0.306	0.319	0.038	1438
Smallmouth Buffalo	JBR	29	0.238	0.250	0.043	1739 (1398-2299)
	MFR	30	0.232	0.249	0.060	1619 (1303-2134)
	CL	30	0.232	0.250	0.070	Infinite
	LAR	29	0.228	0.249	0.078	Infinite
	Mean		0.232	0.249	0.063	

Table 17 . Analysis of molecular variance (AMOVA) for the SNP genotypes of Paddlefish and Smallmouth Buffalo.

Species	Source of Variation	df	Sum of Squares	% Variation
Paddlefish	Among populations	5	1457.36	0
	Among individuals within populations	153	44970.90	3.84*
	Within individuals	159	43267.00	96.16*
	Total	317	89695.25	
Smallmouth Buffalo	Among populations	3	1489.05	0.04
	Among individuals within populations	114	55403.58	6.28**
	Within individuals	118	50567.00	93.68**
	Total	235	107459.63	

*=P < 0.05; **=P<0.001

Table 18. Pairwise F_{ST} values between river sections for Paddlefish (below the diagonal) and Smallmouth Buffalo (above the diagonal). A bold value indicates significance after FDR correction set at $p < 0.05$.

River Section	CSA	JBR	MFR	CL	LAR	TOM
CSA	-	-	-	-	-	-
JBR	0.00322	-	**0.00232**	0.00051	0.00149	-
MFR	0.00013	0.00056	-	0.00203	0.00206	-
CL	0.00279	0.00127	0.00064	-	0.00045	-
LAR	0.00397	-0.00066	0.00057	0.00109	-	-
TOM	-0.00017	0.0005	-0.00043	0.00125	-0.00007	-

Figures

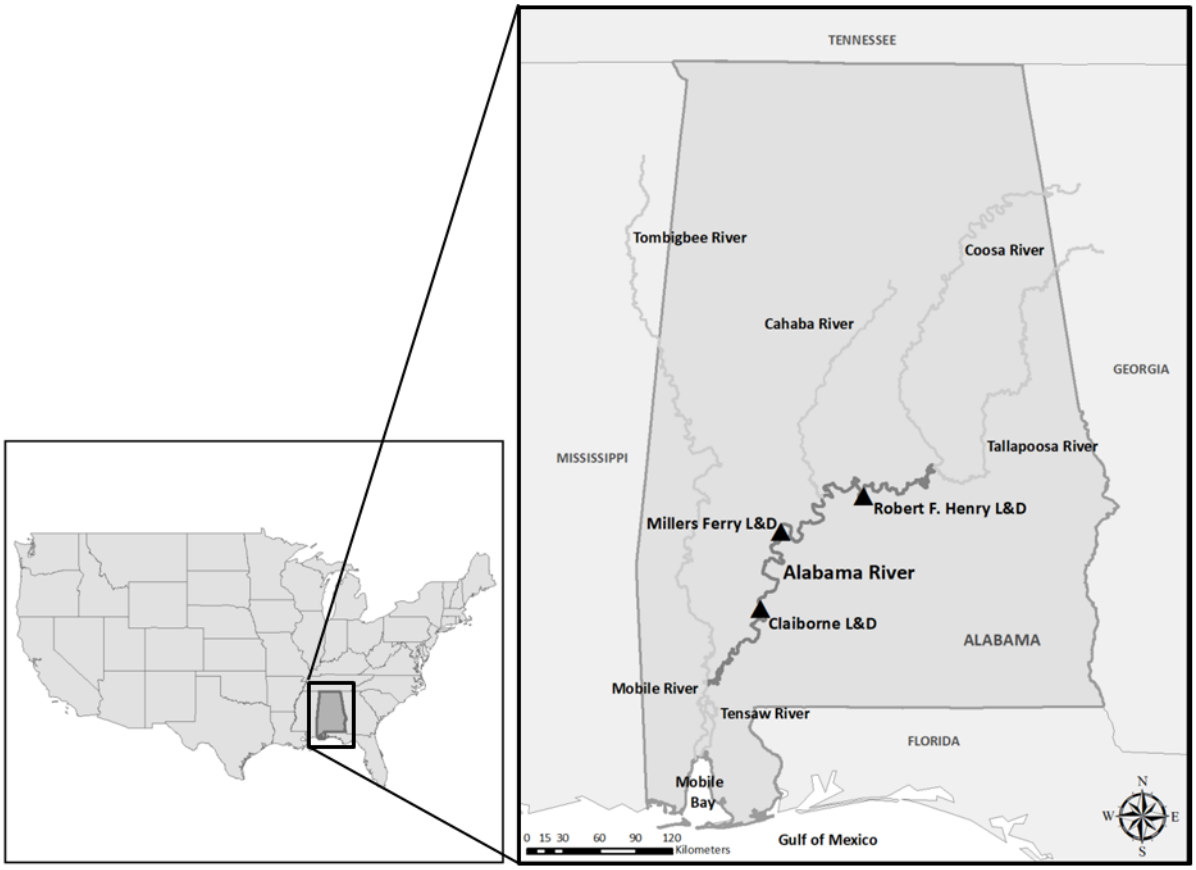
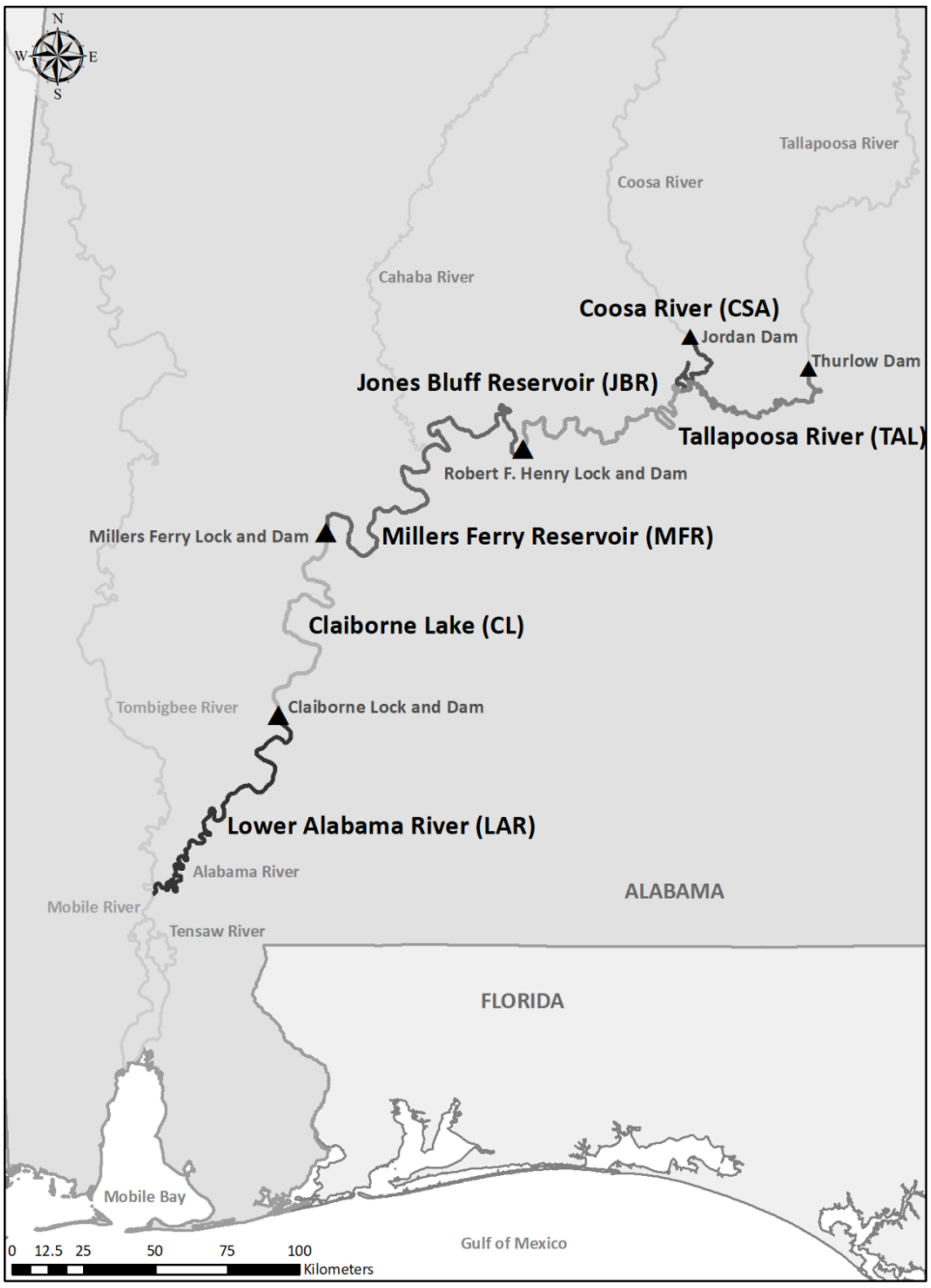


Figure 1


N
W
E
S
Tallapoosa River
Coosa River
Cahaba River
Coosa River (CSA)
Jordan Dam
Thurlow Dam
Jones Bluff Reservoir (JBR)
Tallapoosa River (TAL)
Robert F. Henry Lock and Dam
Millers Ferry Lock and Dam
Millers Ferry Reservoir (MFR)
Claiborne Lake (CL)
Tombigbee River
Claiborne Lock and Dam
Lower Alabama River (LAR)
Alabama River
ALABAMA
Mobile River
Tensaw River
FLORIDA
Mobile Bay
Gulf of Mexico
0 12.5 25 50 75 100
Kilometers
Figure 2


N
W
E
S
Coosa River
Tallapoosa River
Cahaba River
COOSA
TALLAPOOSA 2
ALR221
TALLAPOOSA 1
CAHABA
ALR270
ALR236
ALR181
ALR132
ALR109
Tombigbee River
ALR72
ALR47
ALABAMA
Alabama River
TOMBIGBEE
ALR3
Mobile River
TENSAW
Tensaw River
FLORIDA
Mobile Bay
Gulf of Mexico
0 12.5 25 50 75 100
Kilometers

Figure 3

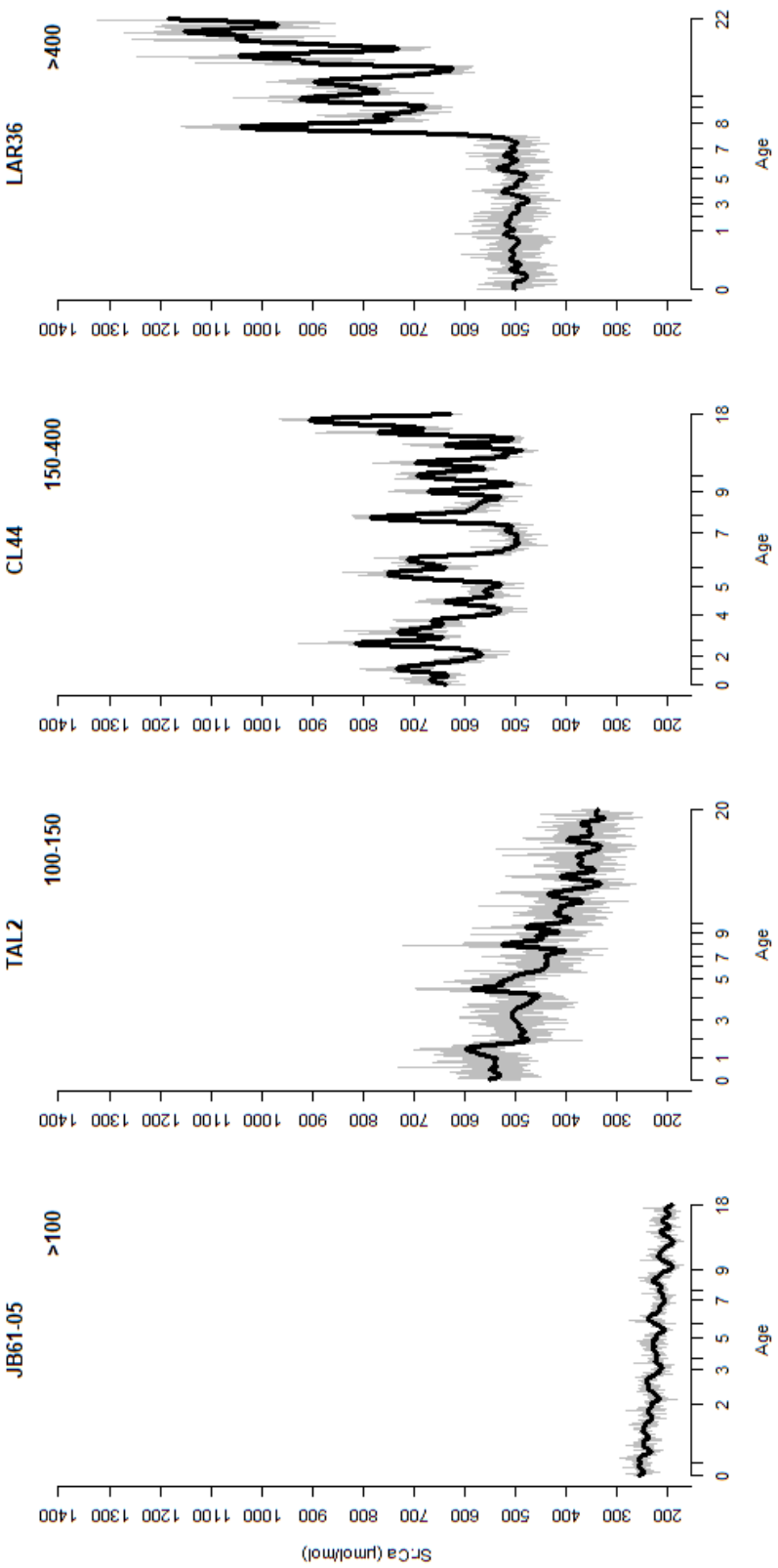

JB61-05
>100
TAL2
100-150
CL44
150-400
LAR36
>400
Sr/Ca (µmol/mol)
Age

Figure 4

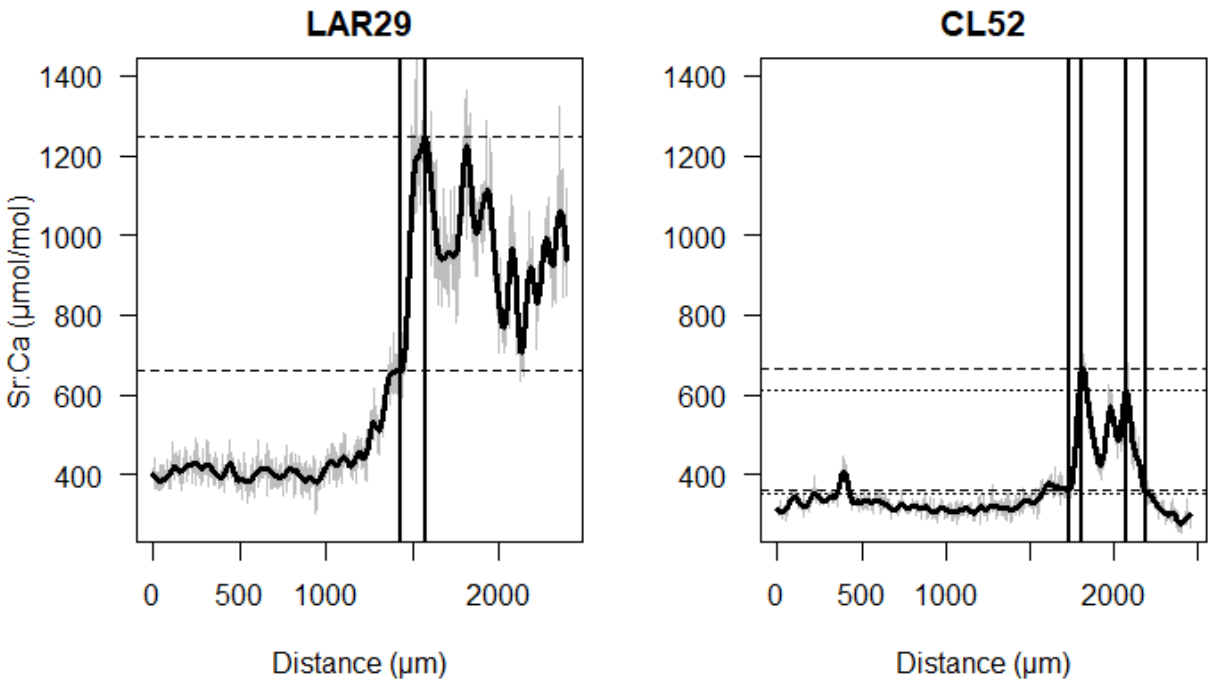

LAR29
CL52
Sr:Ca (µmol/mol)
1400
1200
1000
800
600
400
0
500
1000
2000
Distance (µm)

Figure 5

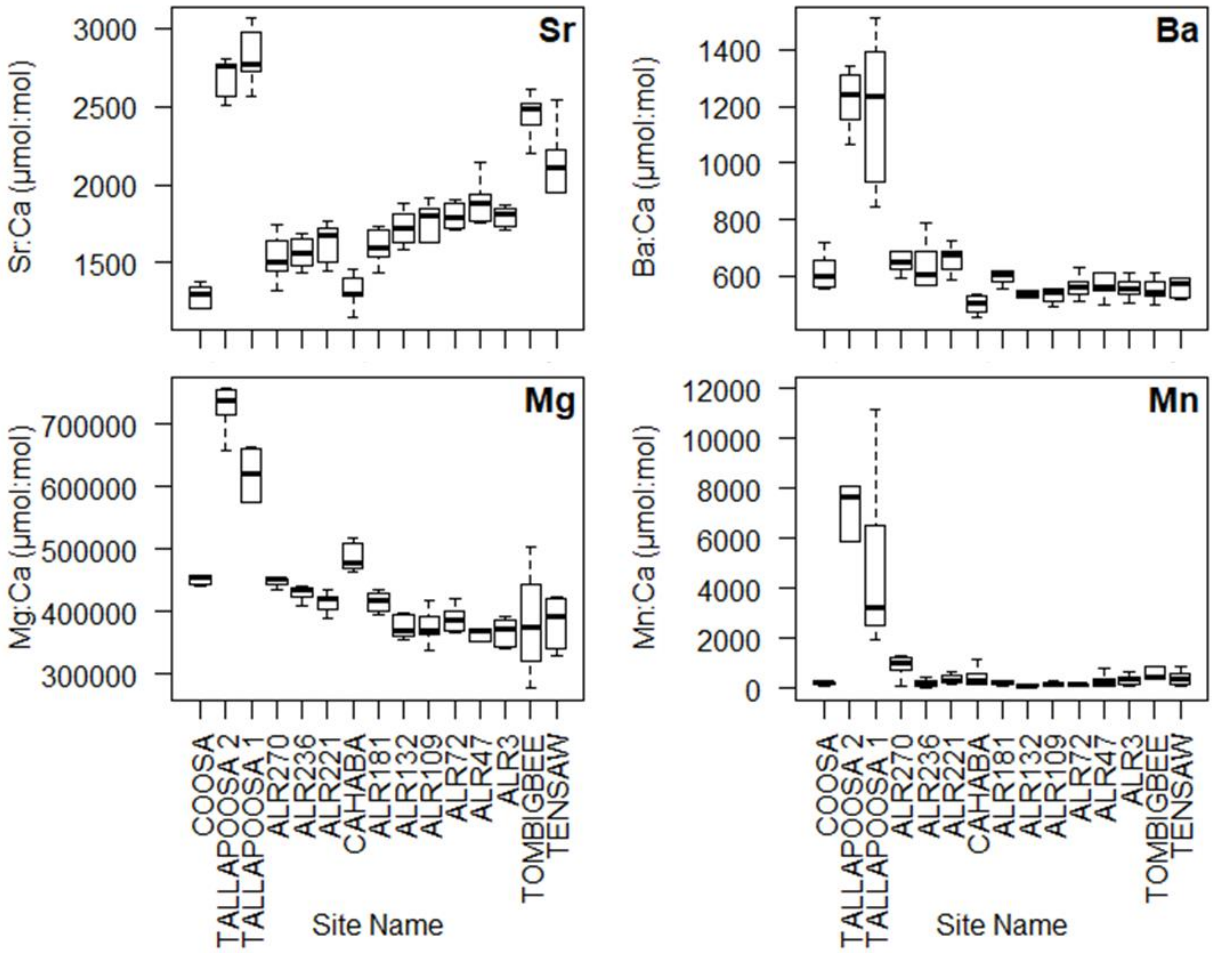

Sr
Sr:Ca (µmol:mol)
3000
2500
2000
1500
Ba
Ba:Ca (µmol:mol)
1400
1200
1000
800
600
Mg
Mg:Ca (µmol:mol)
700000
600000
500000
400000
300000
Mn
Mn:Ca (µmol:mol)
12000
10000
8000
6000
4000
2000
0
COOSA
TALLAPOOSA 2
TALLAPOOSA 1
ALR270
ALR236
ALR221
CAHABA
ALR181
ALR132
ALR109
ALR72
ALR47
ALR3
TOMBIGBEE
TENSAW
Site Name

Figure 6

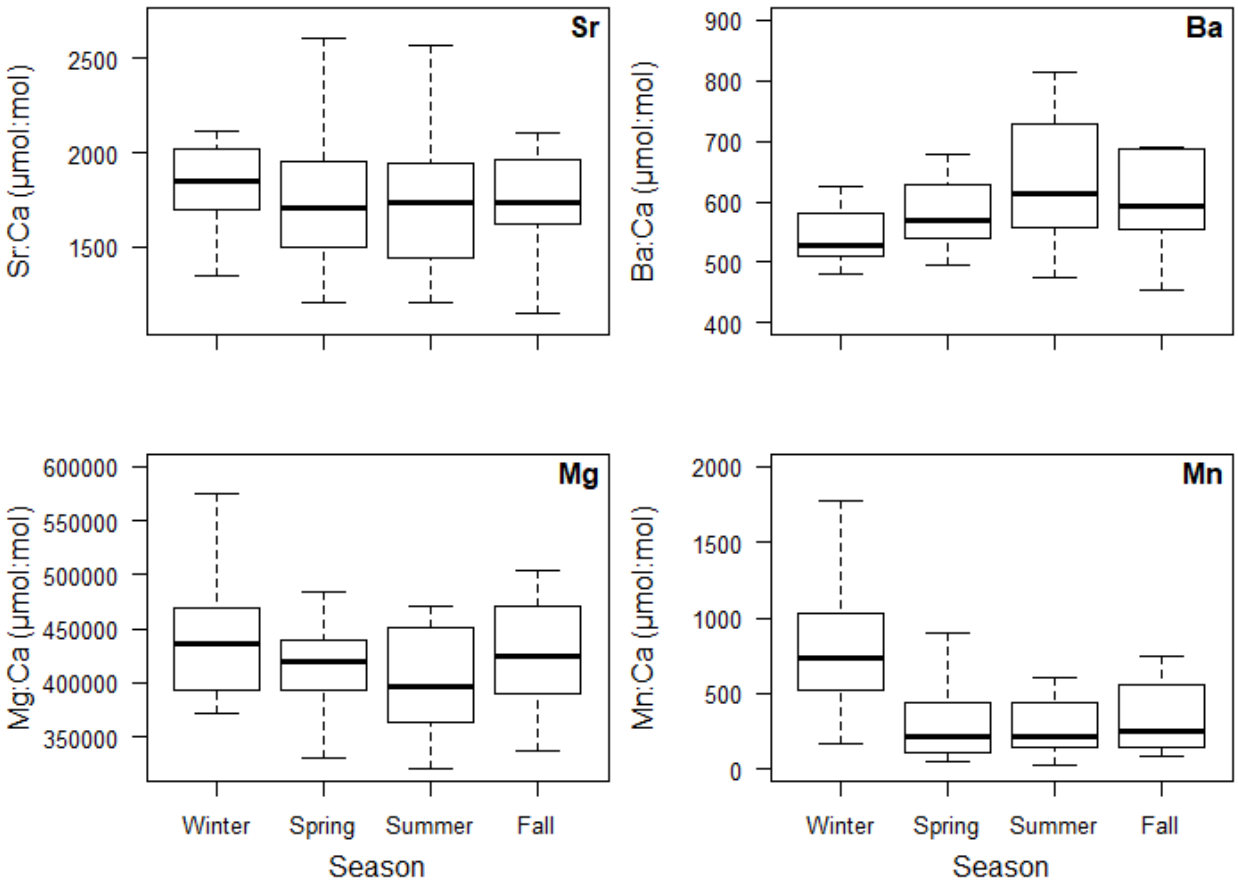

Sr
Sr:Ca (µmol:mol)
2500
2000
1500
Ba
Ba:Ca (µmol:mol)
900
800
700
600
500
400
Mg
Mg:Ca (µmol:mol)
600000
550000
500000
450000
400000
350000
Mn
Mn:Ca (µmol:mol)
2000
1500
1000
500
0
Winter
Spring
Summer
Fall
Season

Figure 7

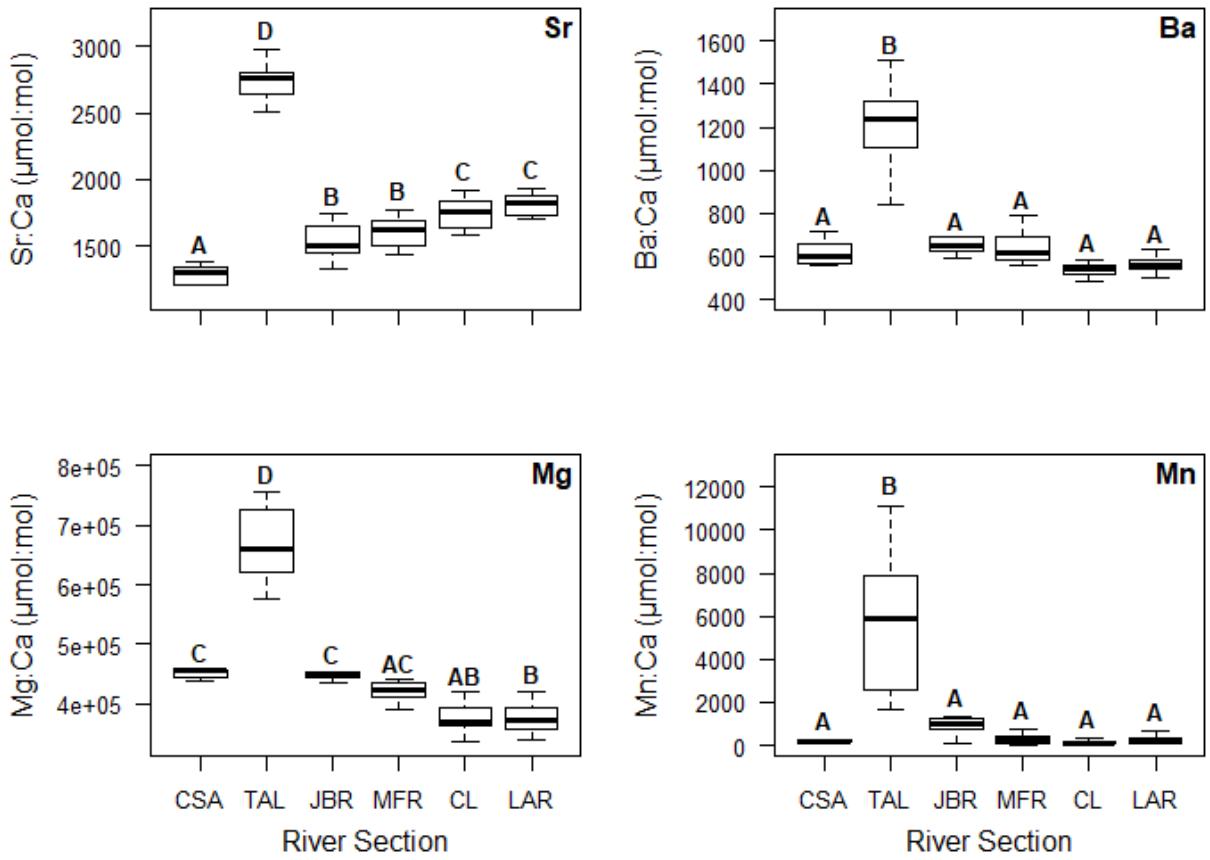

Sr
Sr:Ca (µmol:mol)
3000
2500
2000
1500
D
A
B
B
C
C
Ba
Ba:Ca (µmol:mol)
1600
1400
1200
1000
800
600
400
B
A
A
A
A
A
Mg
Mg:Ca (µmol:mol)
8e+05
7e+05
6e+05
5e+05
4e+05
D
C
C
AC
AB
B
Mn
Mn:Ca (µmol:mol)
12000
10000
8000
6000
4000
2000
0
B
A
A
A
A
CSA TAL JBR MFR CL LAR
River Section

Figure 8

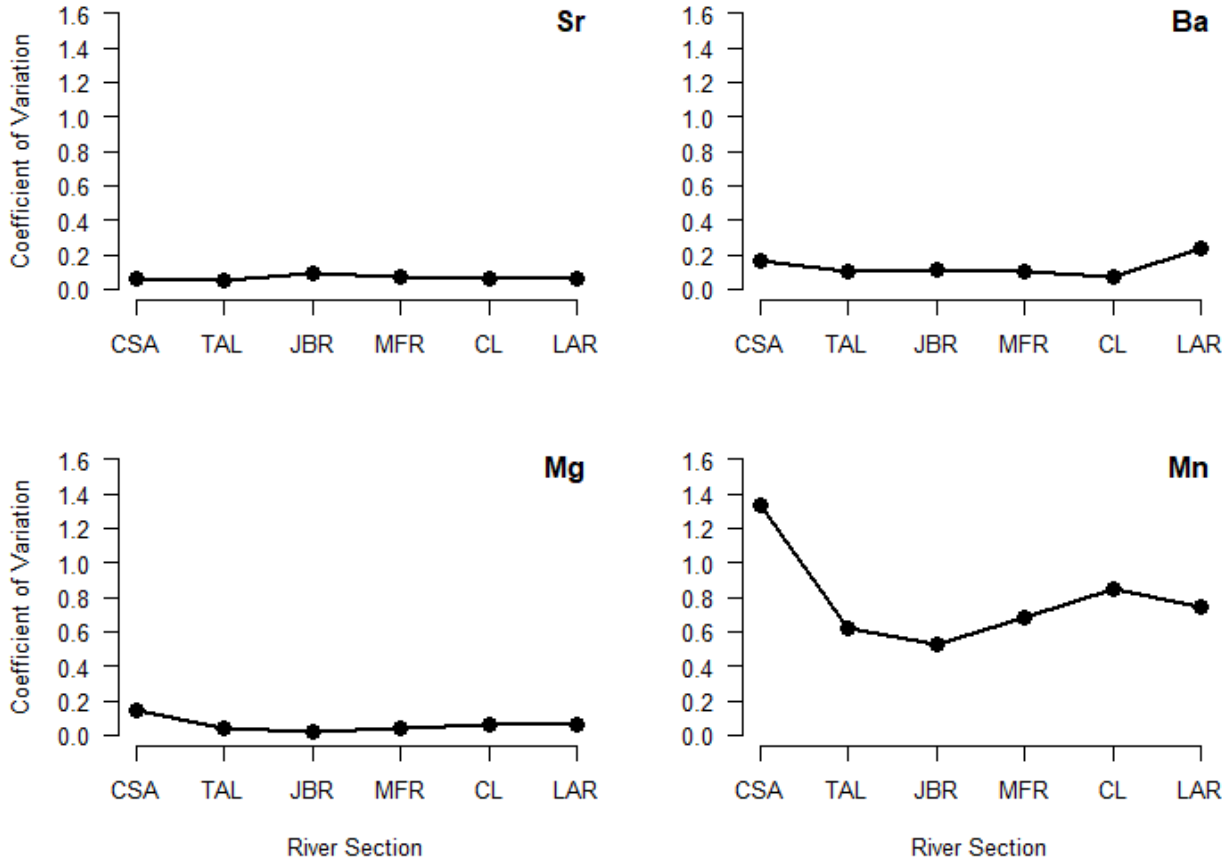

Sr
Ba
Mg
Mn
Coefficient of Variation
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CSA
TAL
JBR
MFR
CL
LAR
River Section

Figure 9

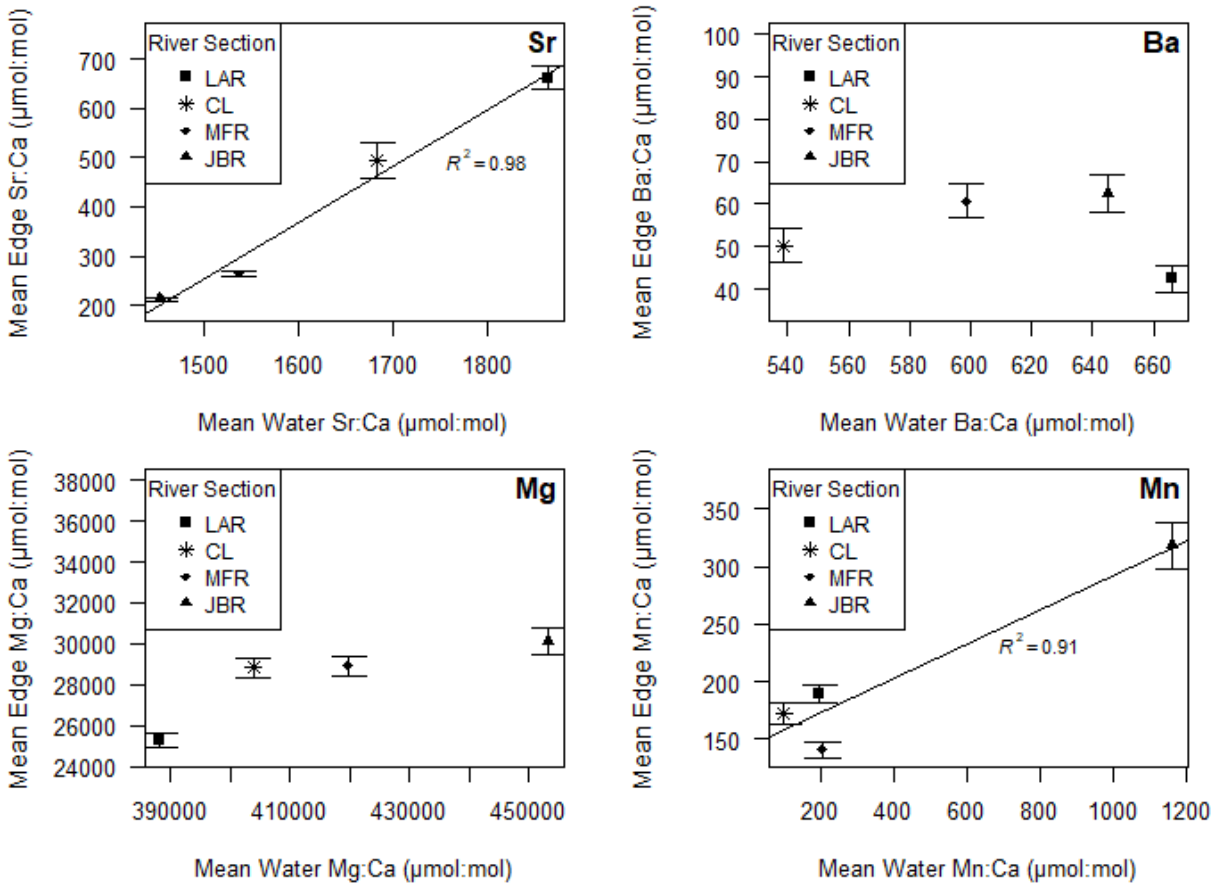

Sr
River Section
LAR
CL
MFR
JBR
$R^2 = 0.98$
Mean Edge Sr:Ca (µmol:mol)
Mean Water Sr:Ca (µmol:mol)
Ba
Mean Edge Ba:Ca (µmol:mol)
Mean Water Ba:Ca (µmol:mol)
Mg
Mean Edge Mg:Ca (µmol:mol)
Mean Water Mg:Ca (µmol:mol)
Mn
$R^2 = 0.91$
Mean Edge Mn:Ca (µmol:mol)
Mean Water Mn:Ca (µmol:mol)

Figure 10

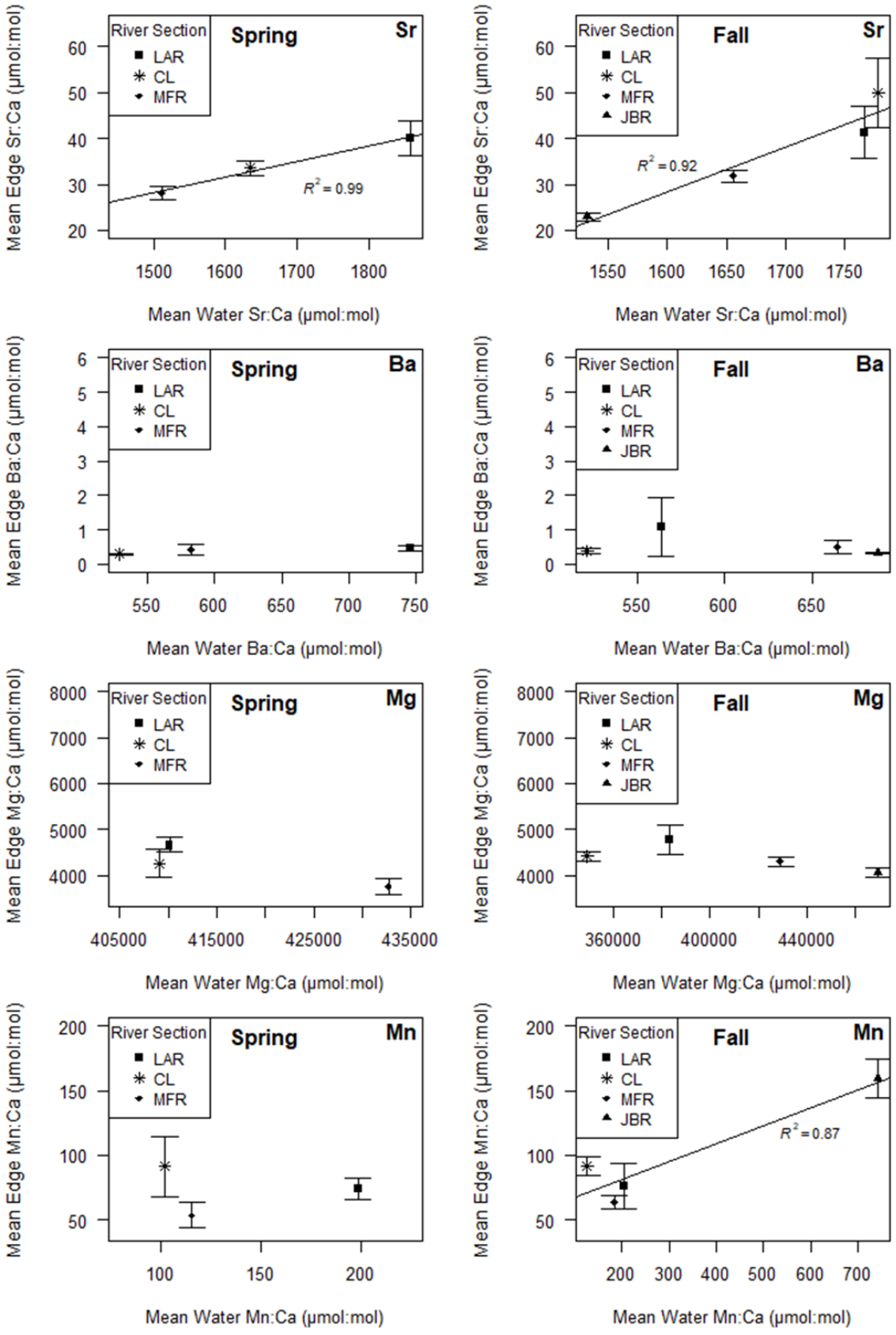

River Section
LAR
CL
MFR
JBR
Spring
Fall
Sr
Ba
Mg
Mn
$R^2 = 0.99$
$R^2 = 0.92$
$R^2 = 0.87$
Mean Edge Sr:Ca (µmol:mol)
Mean Water Sr:Ca (µmol:mol)
Mean Edge Ba:Ca (µmol:mol)
Mean Water Ba:Ca (µmol:mol)
Mean Edge Mg:Ca (µmol:mol)
Mean Water Mg:Ca (µmol:mol)
Mean Edge Mn:Ca (µmol:mol)
Mean Water Mn:Ca (µmol:mol)

Figure 11

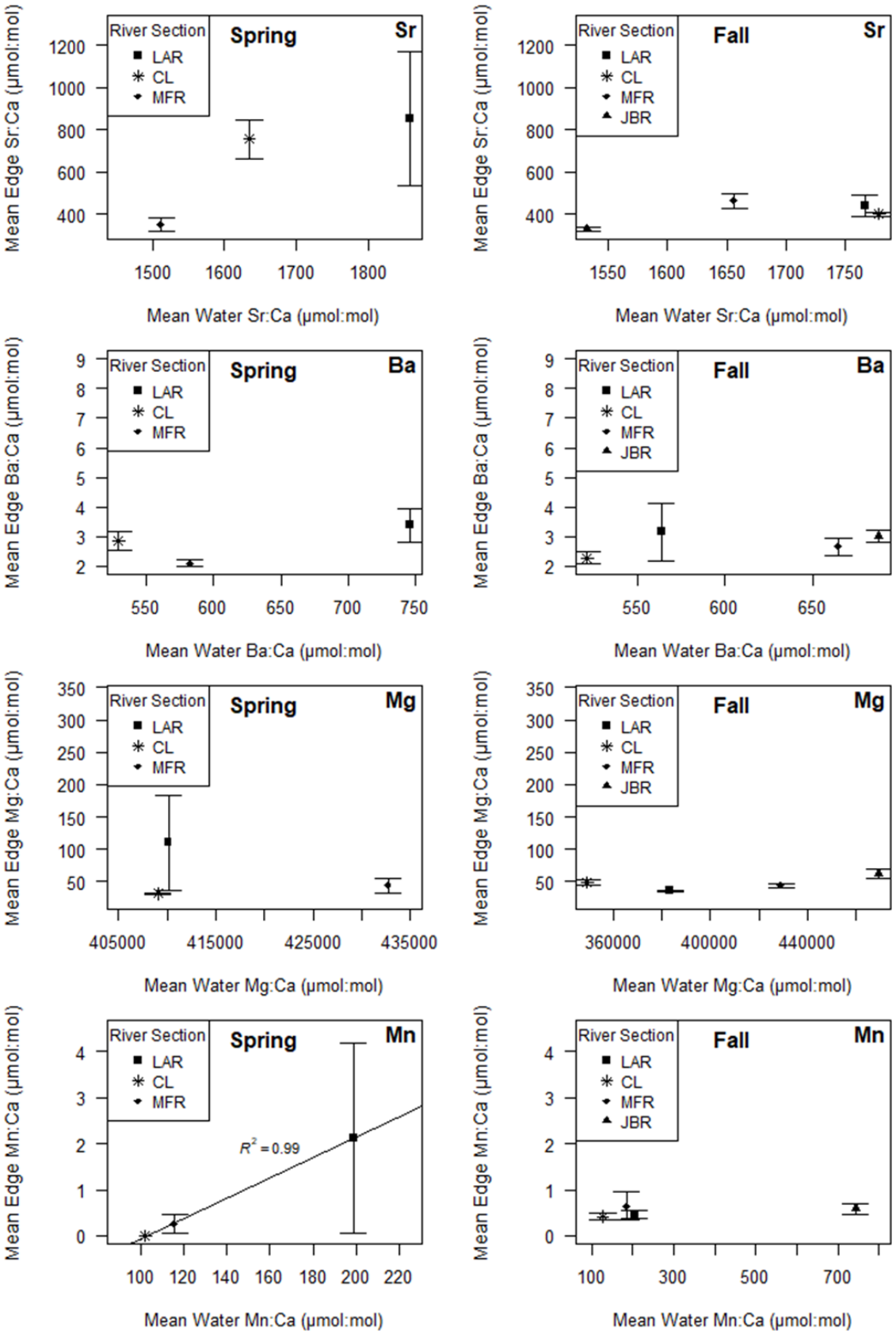

River Section
LAR
CL
MFR
JBR
Spring
Fall
Sr
Ba
Mg
Mn
Mean Edge Sr:Ca (µmol:mol)
Mean Water Sr:Ca (µmol:mol)
Mean Edge Ba:Ca (µmol:mol)
Mean Water Ba:Ca (µmol:mol)
Mean Edge Mg:Ca (µmol:mol)
Mean Water Mg:Ca (µmol:mol)
Mean Edge Mn:Ca (µmol:mol)
Mean Water Mn:Ca (µmol:mol)
$R^2 = 0.99$

Figure 12

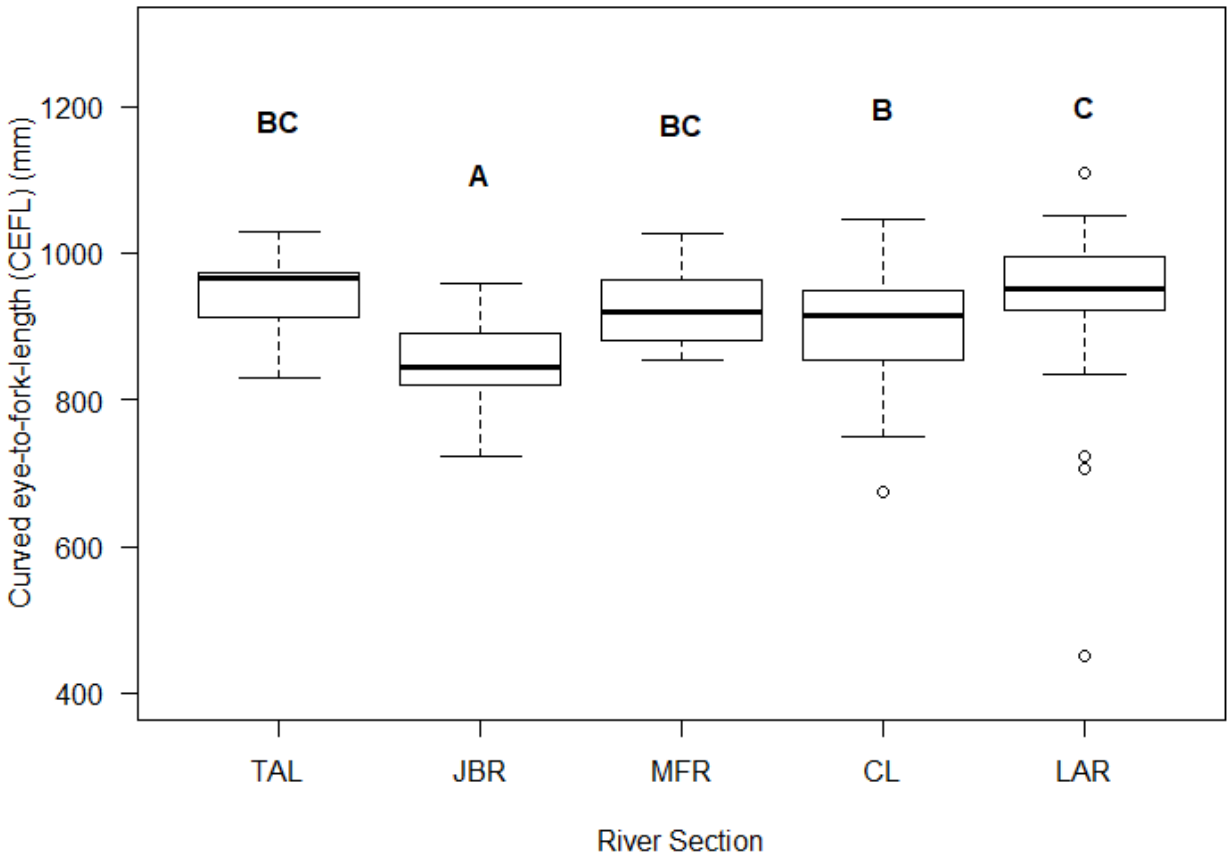

BC
A
BC
B
C
1200
1000
800
600
400
Curved eye-to-fork-length (CEFL) (mm)
TAL
JBR
MFR
CL
LAR
River Section

Figure 13

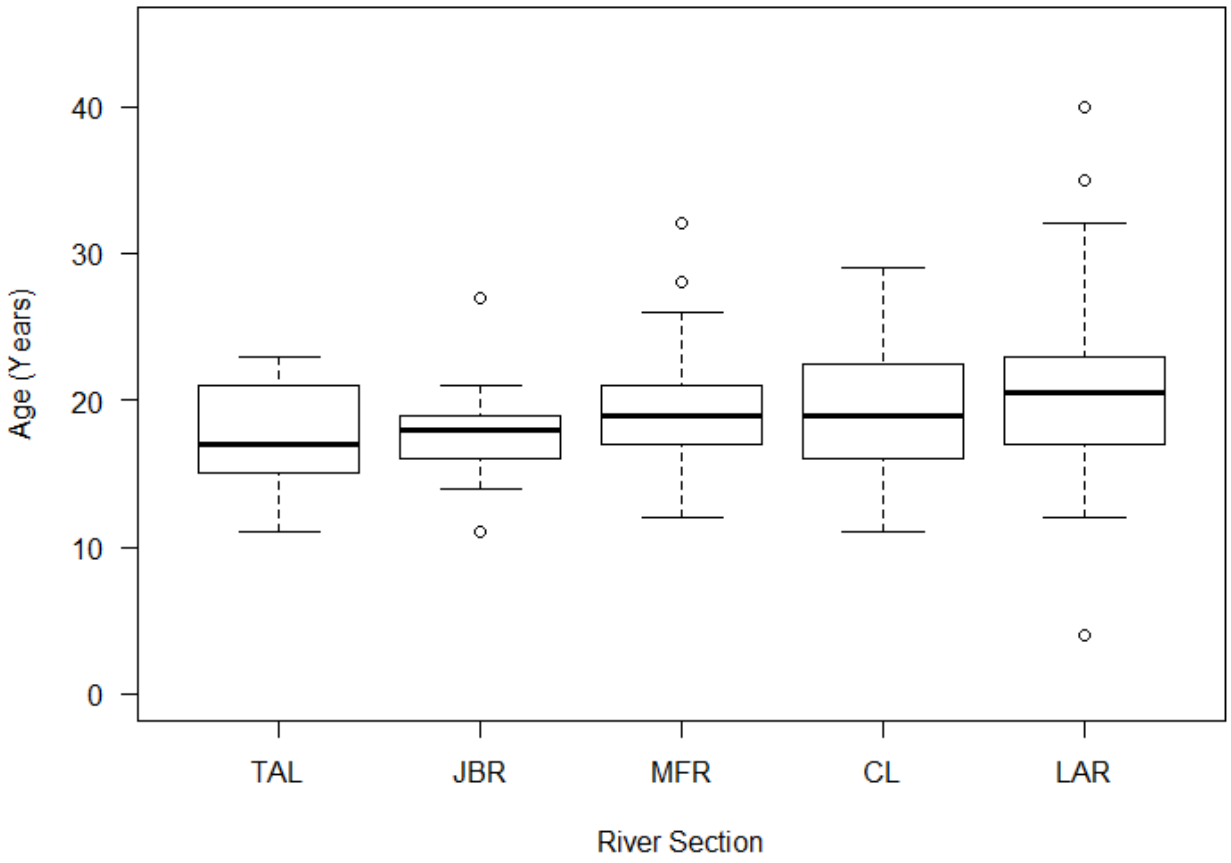


Figure 14

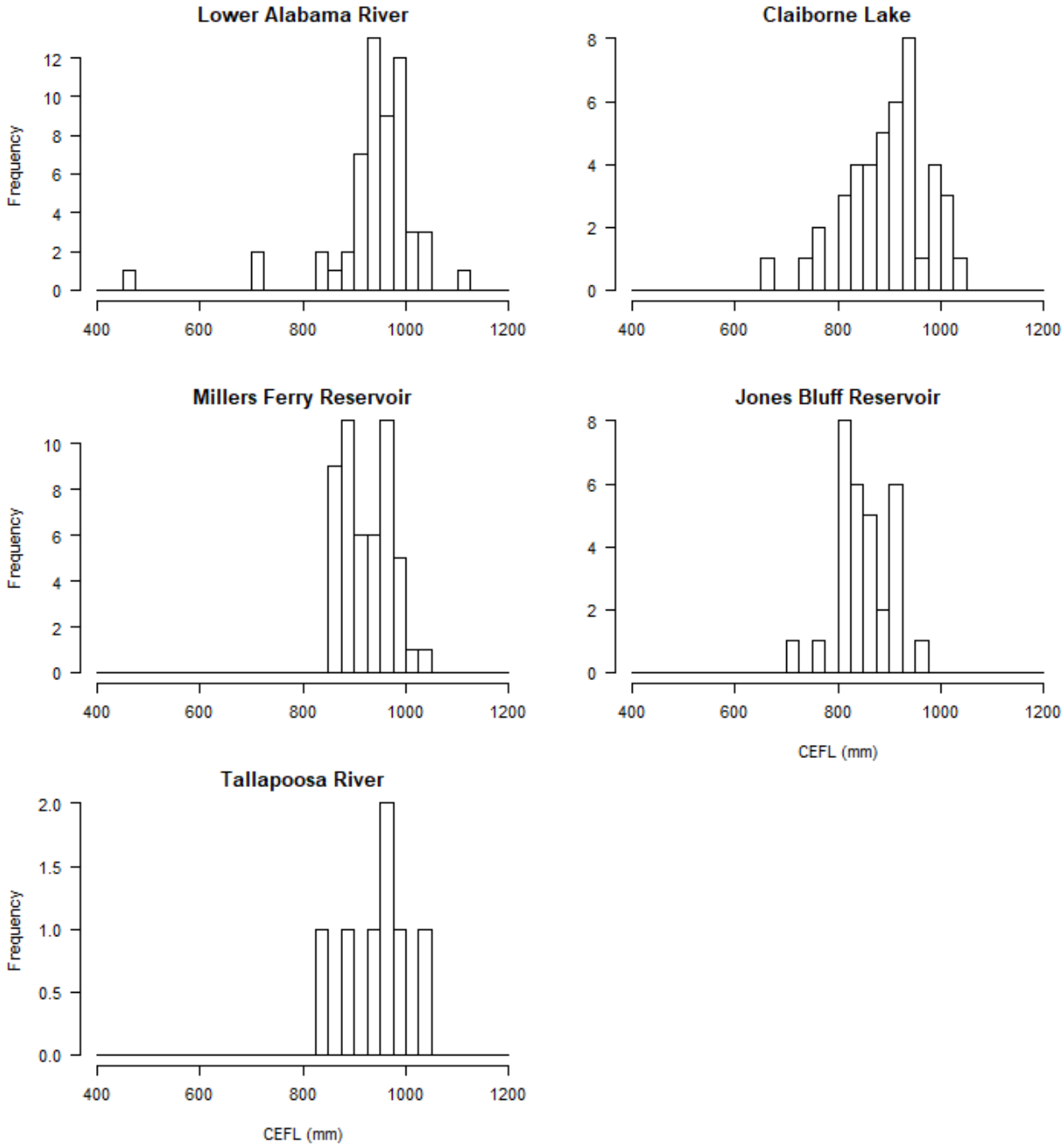

Lower Alabama River
Claiborne Lake
Millers Ferry Reservoir
Jones Bluff Reservoir
Tallapoosa River
Frequency
CEFL (mm)
0
2
4
6
8
10
12
0.0
0.5
1.0
1.5
2.0
400
600
800
1000
1200

Figure 15

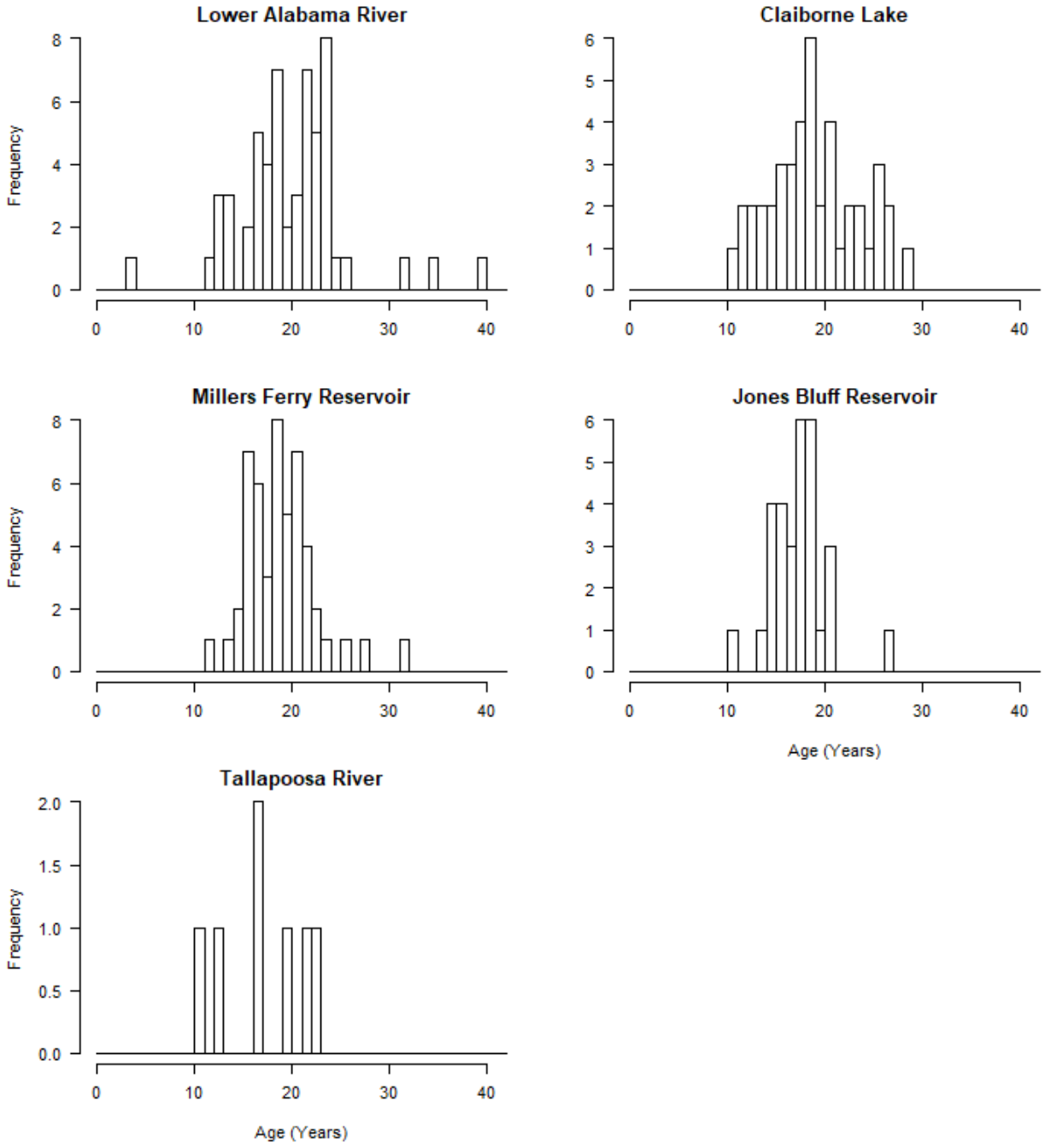

Lower Alabama River
Frequency
0
2
4
6
8
0
10
20
30
40
Claiborne Lake
0
1
2
3
4
5
6
Millers Ferry Reservoir
Jones Bluff Reservoir
Age (Years)
Tallapoosa River
0.0
0.5
1.0
1.5
2.0

Figure 16

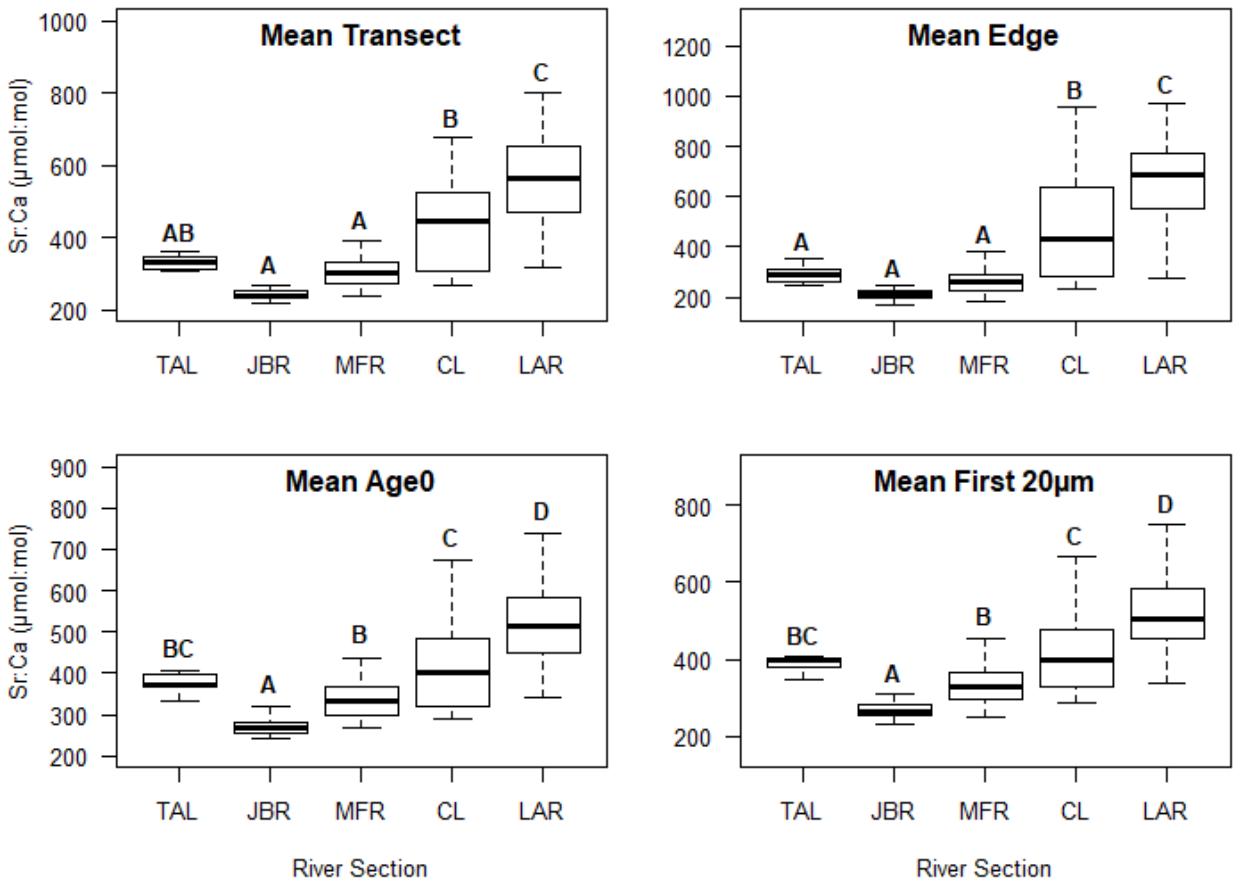

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Sr:Ca (µmol:mol)
River Section
TAL
JBR
MFR
CL
LAR
AB
A
B
C
BC
D

Figure 17

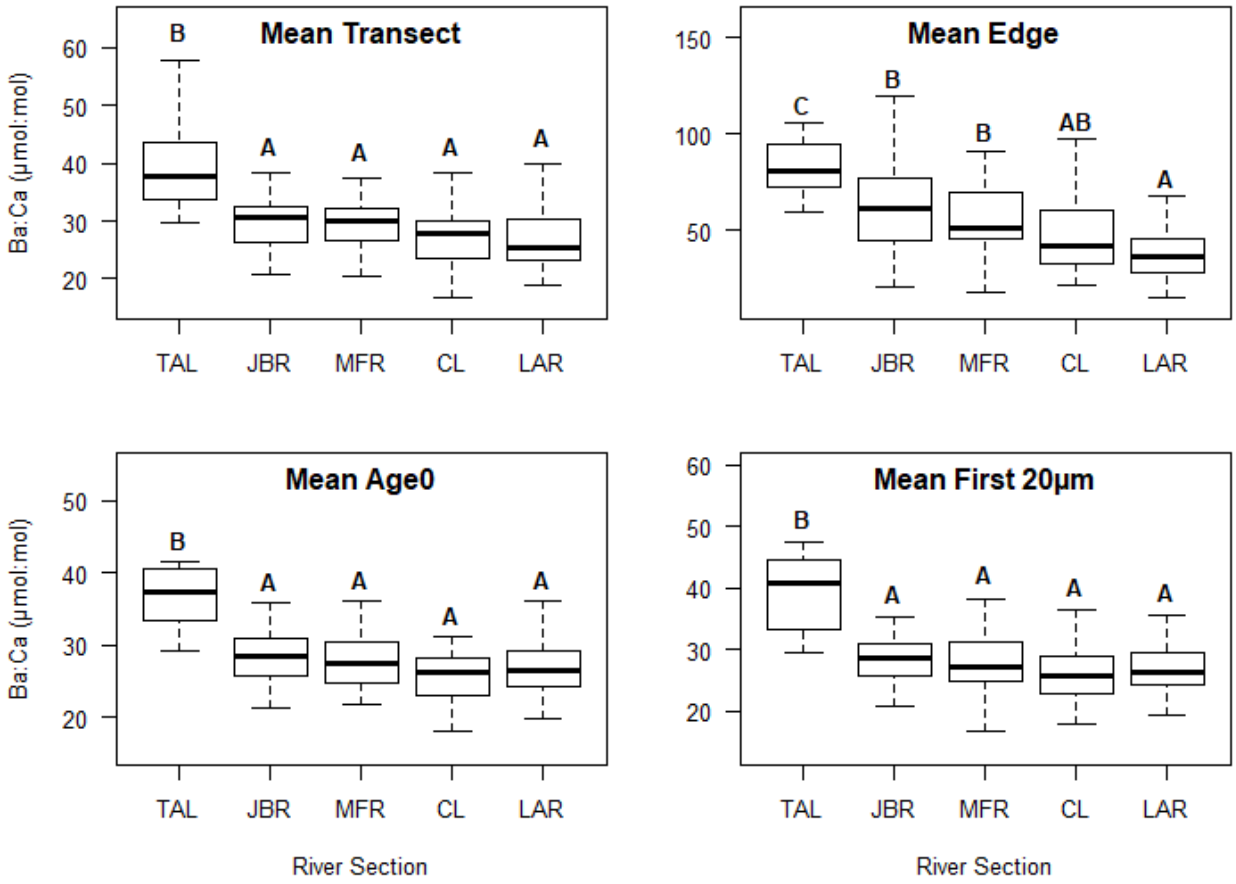

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Ba:Ca (µmol:mol)
River Section
TAL
JBR
MFR
CL
LAR
B
A
C
AB

Figure 18

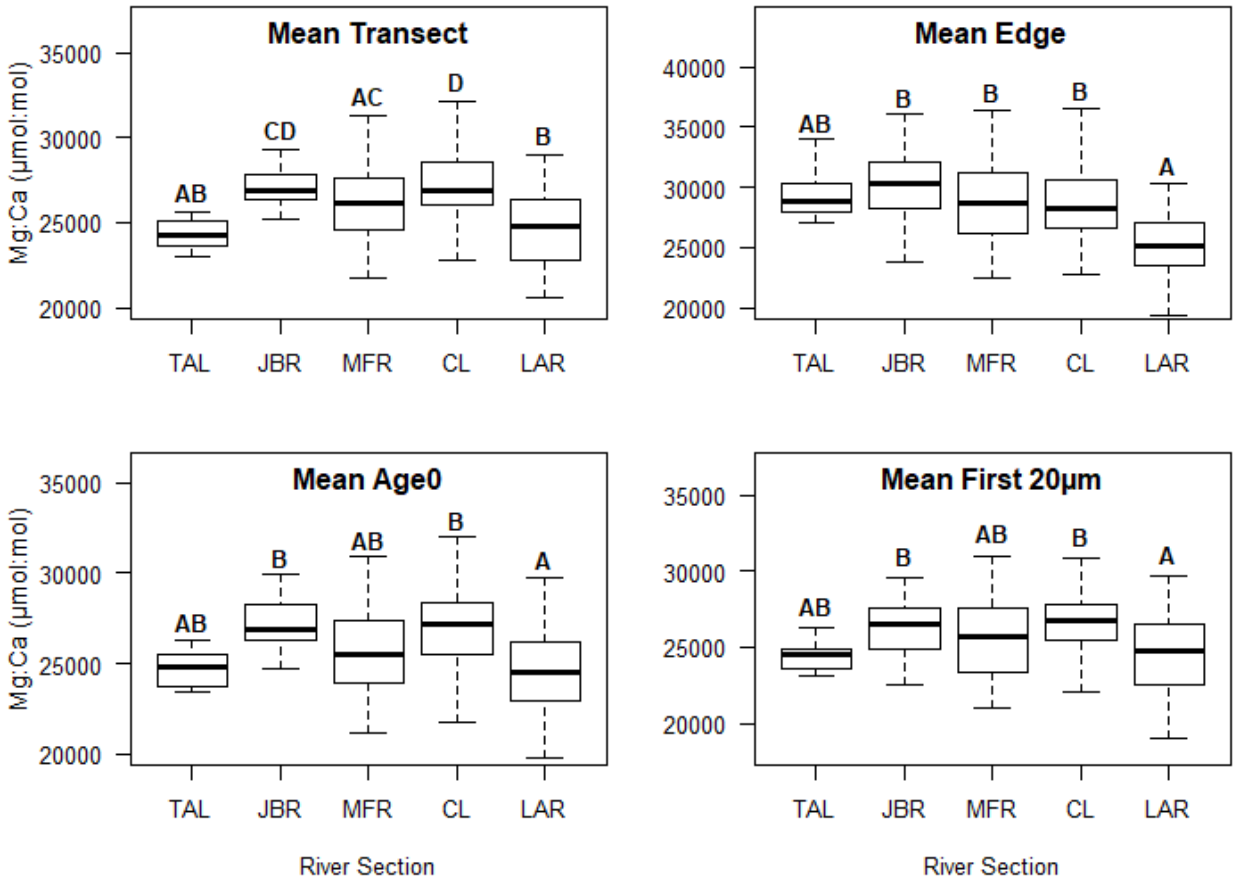

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mg:Ca (µmol:mol)
River Section
TAL
JBR
MFR
CL
LAR
AB
CD
AC
D
B
A
AB
B
B
B
A
AB
B
AB
B
A
AB
B
AB
B
A

Figure 19

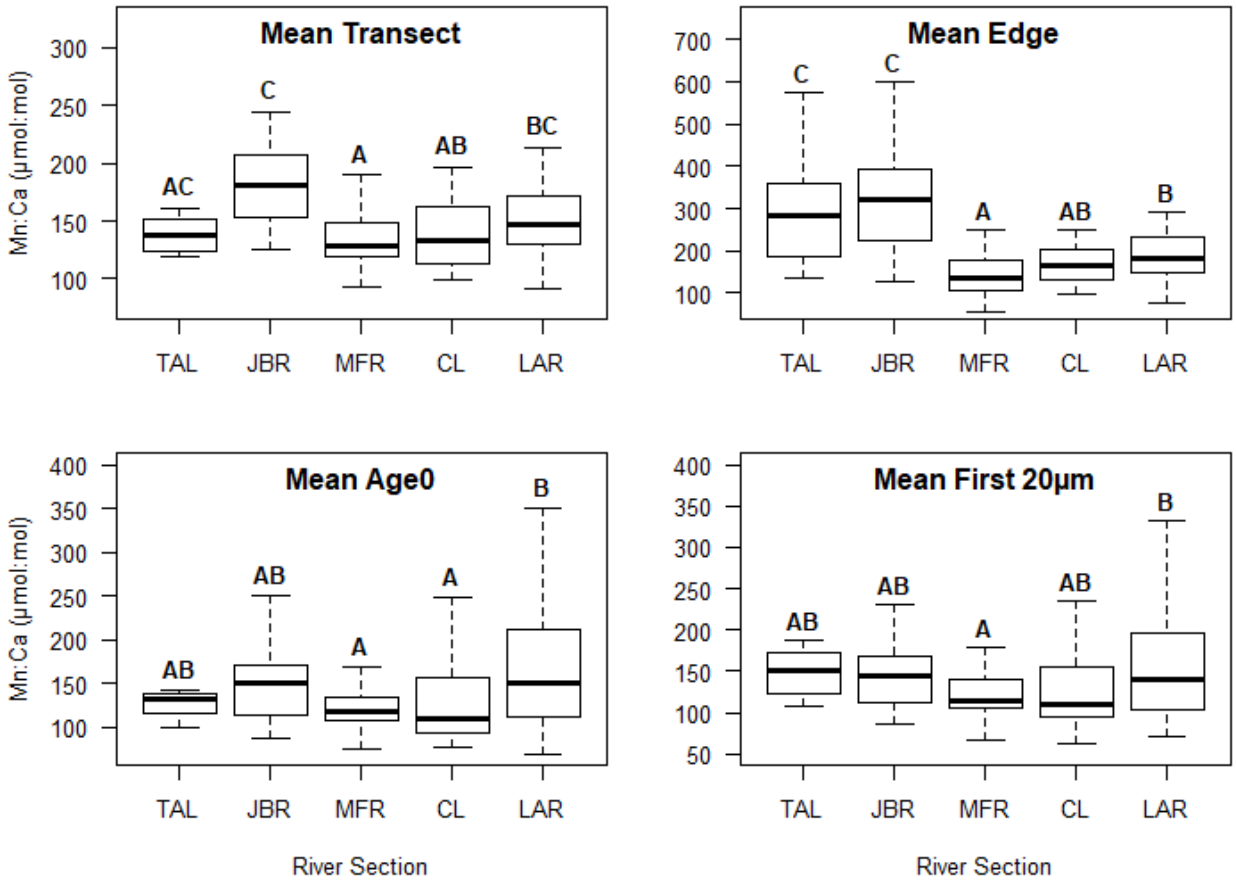

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mn:Ca (µmol:mol)
River Section
TAL
JBR
MFR
CL
LAR
AC
C
A
AB
BC
B

Figure 20

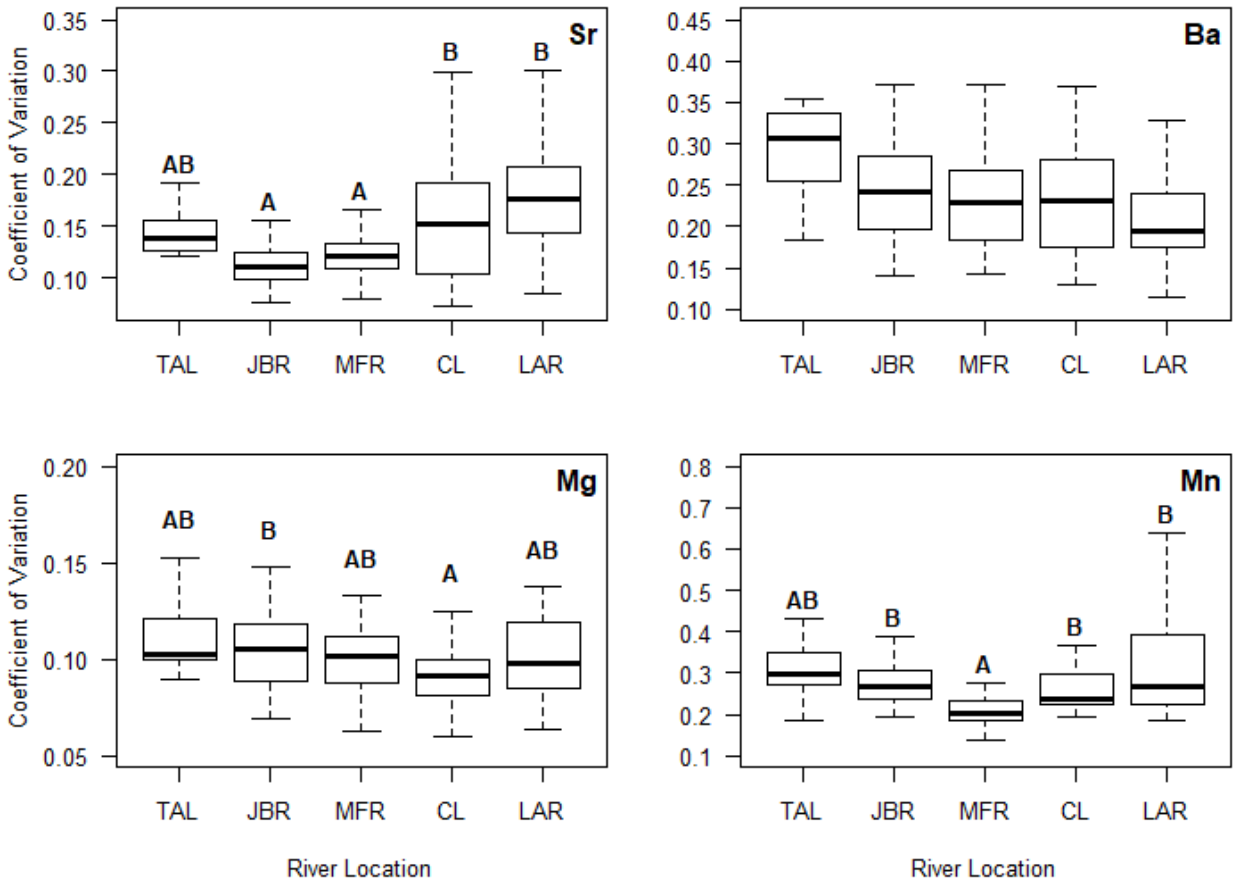

Sr
Ba
Mg
Mn
Coefficient of Variation
River Location
TAL
JBR
MFR
CL
LAR
AB
A
A
B
B
AB
B
AB
A
AB
AB
B
A
B
B

Figure 21

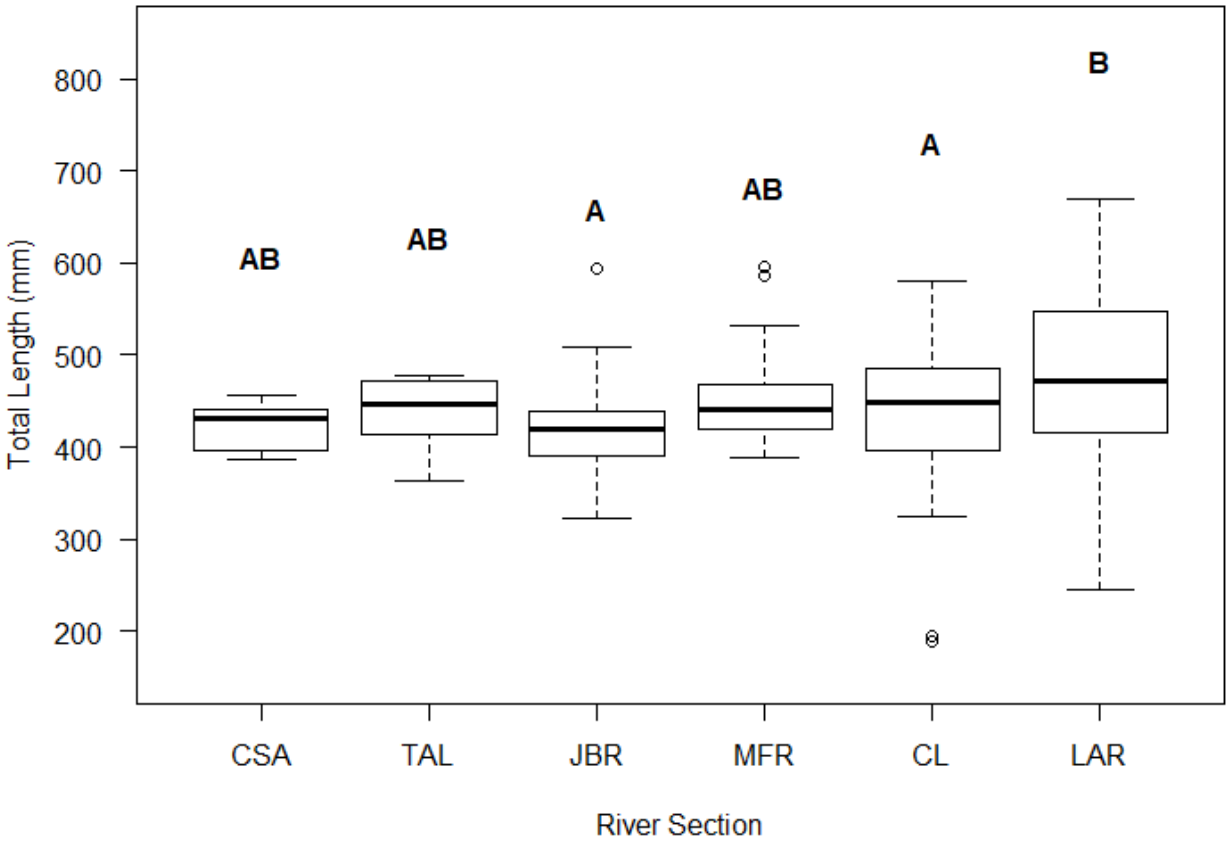

800
700
600
500
400
300
200
Total Length (mm)
AB
AB
A
AB
A
B
CSA
TAL
JBR
MFR
CL
LAR
River Section

Figure 22

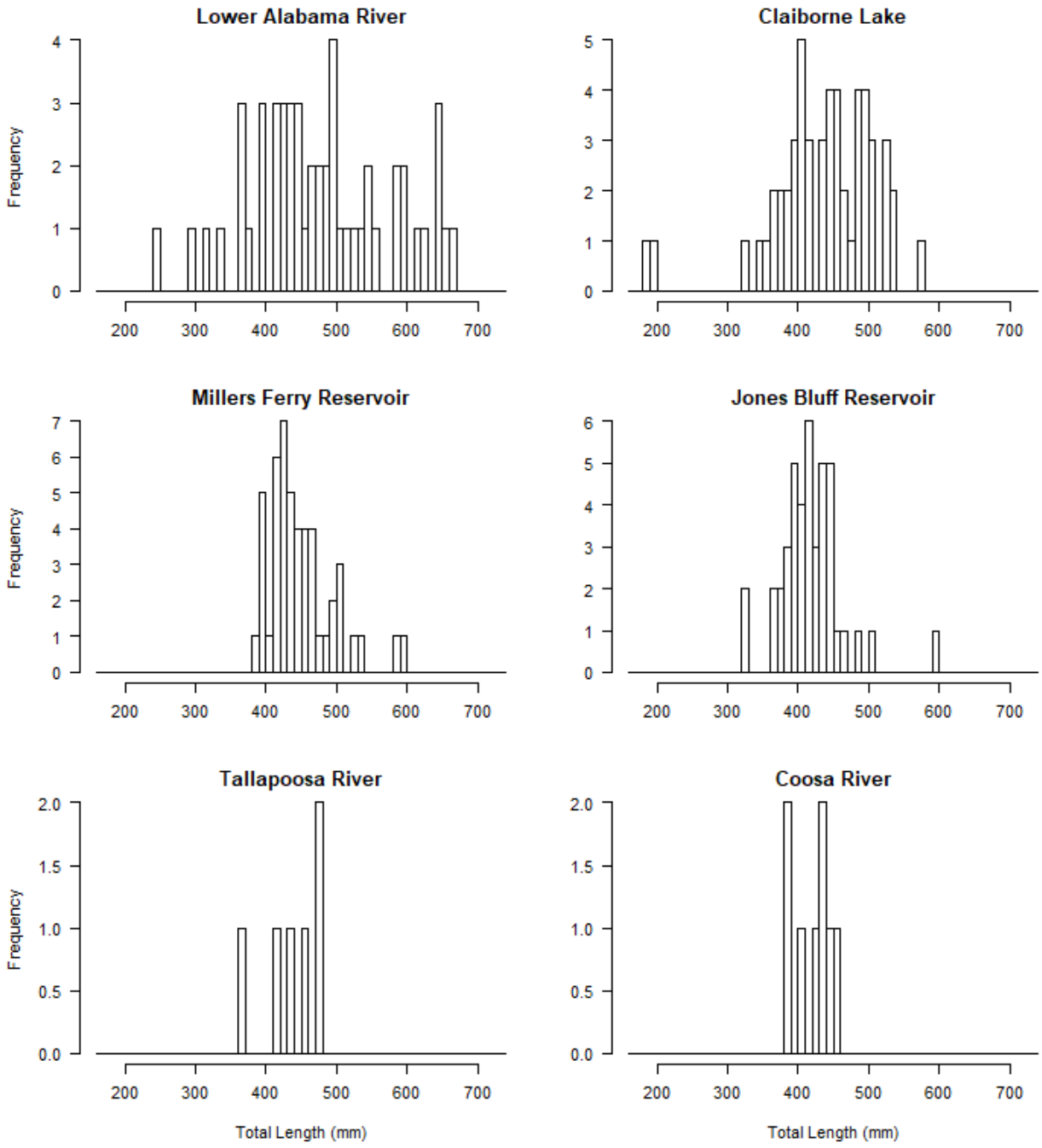

Lower Alabama River
Claiborne Lake
Millers Ferry Reservoir
Jones Bluff Reservoir
Tallapoosa River
Coosa River
Frequency
Total Length (mm)

Figure 23

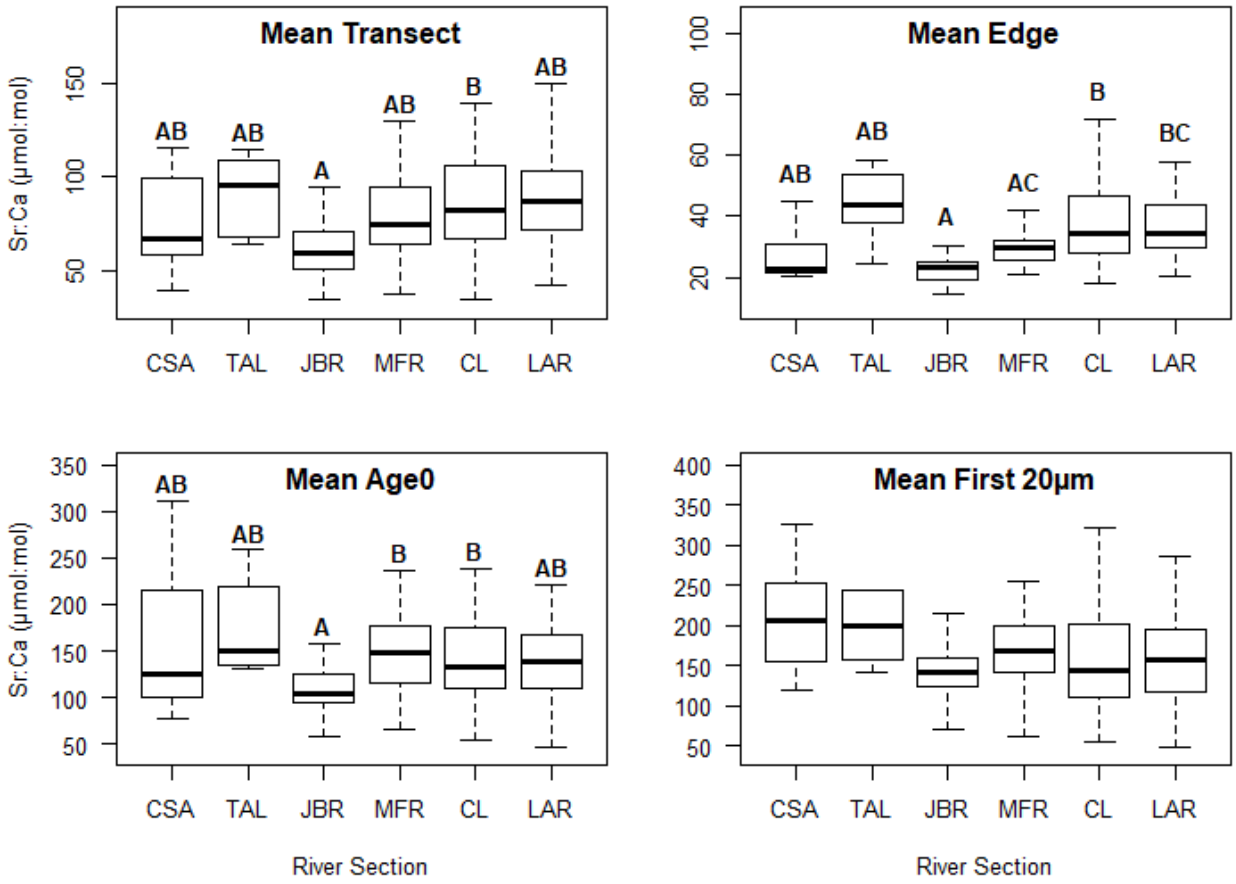

Mean Transect
Sr:Ca (µmol:mol)
50
100
150
AB
AB
A
AB
B
AB
CSA
TAL
JBR
MFR
CL
LAR
Mean Edge
20
40
60
80
100
AB
AB
A
AC
B
BC
Mean Age0
Sr:Ca (µmol:mol)
50
100
150
200
250
300
350
AB
AB
A
B
B
AB
River Section
Mean First 20µm
50
100
150
200
250
300
350
400
River Section

Figure 24

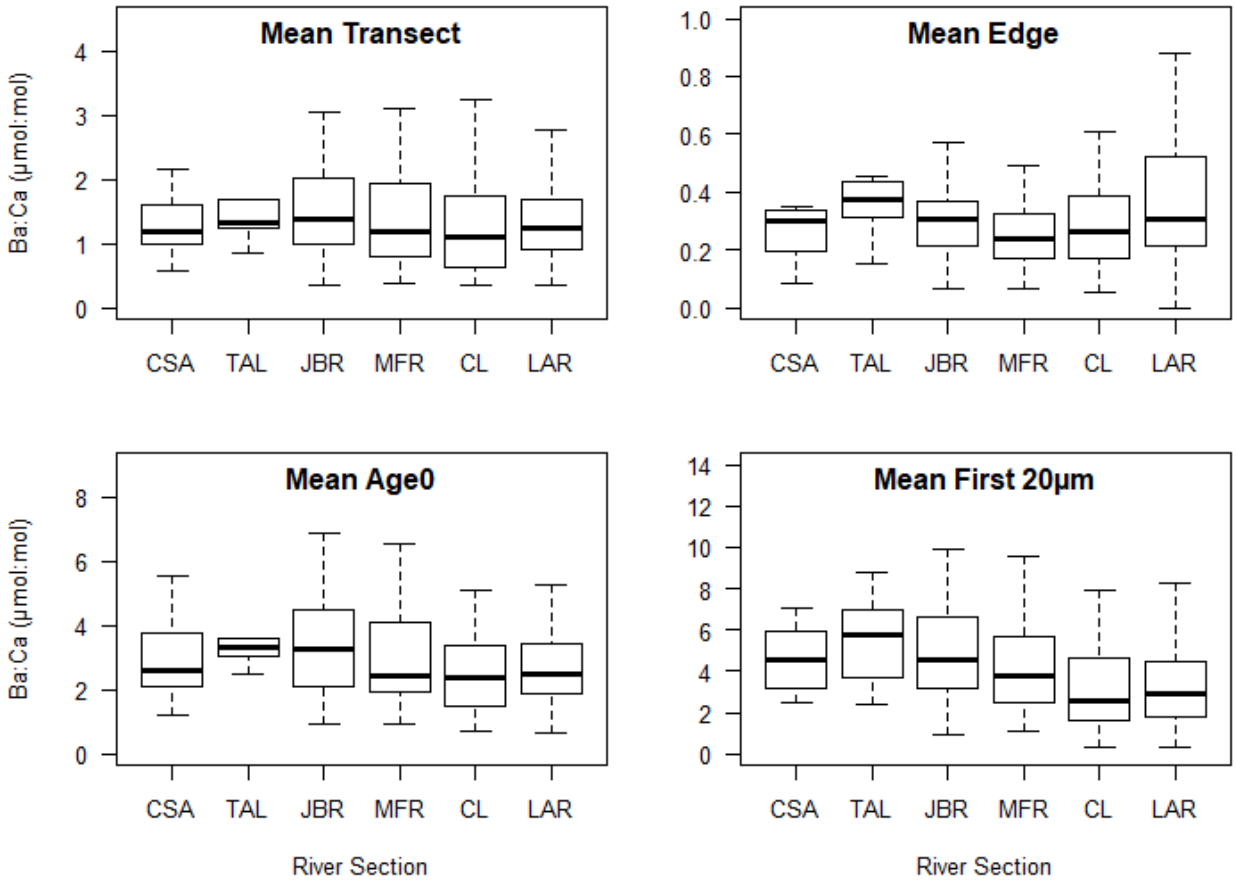

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Ba:Ca (µmol:mol)
Ba:Ca (µmol:mol)
CSA
TAL
JBR
MFR
CL
LAR
River Section
River Section

Figure 25

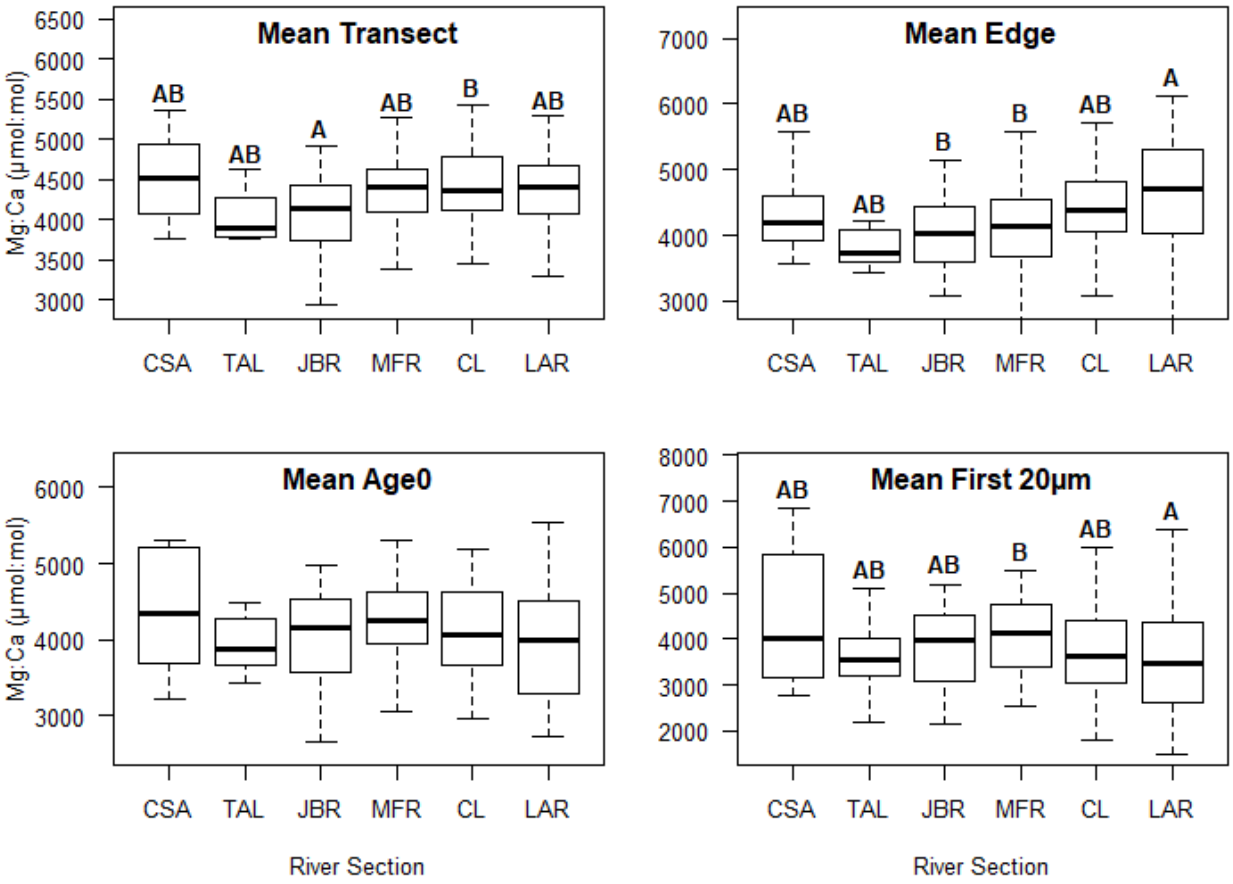

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mg:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR
AB
A
B

Figure 26

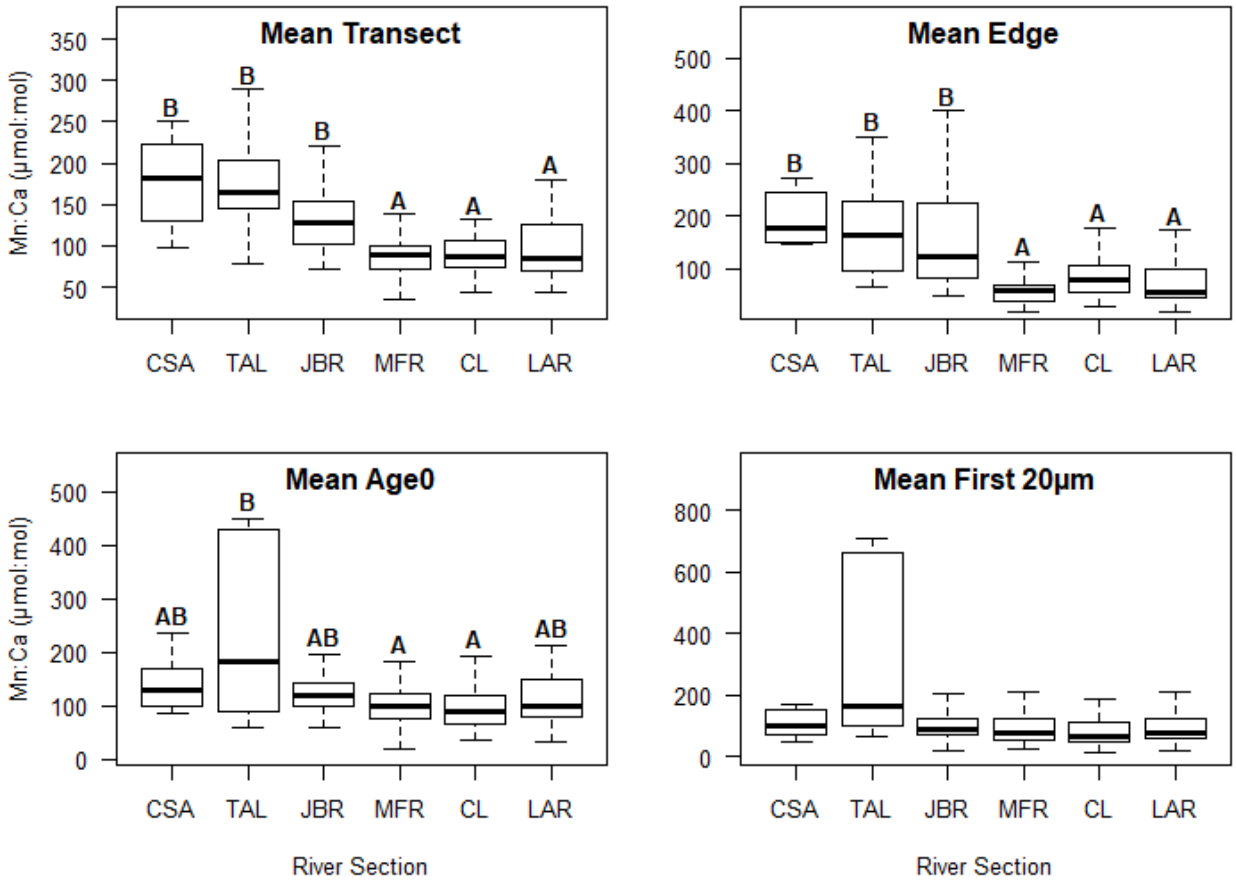

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mn:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR

Figure 27

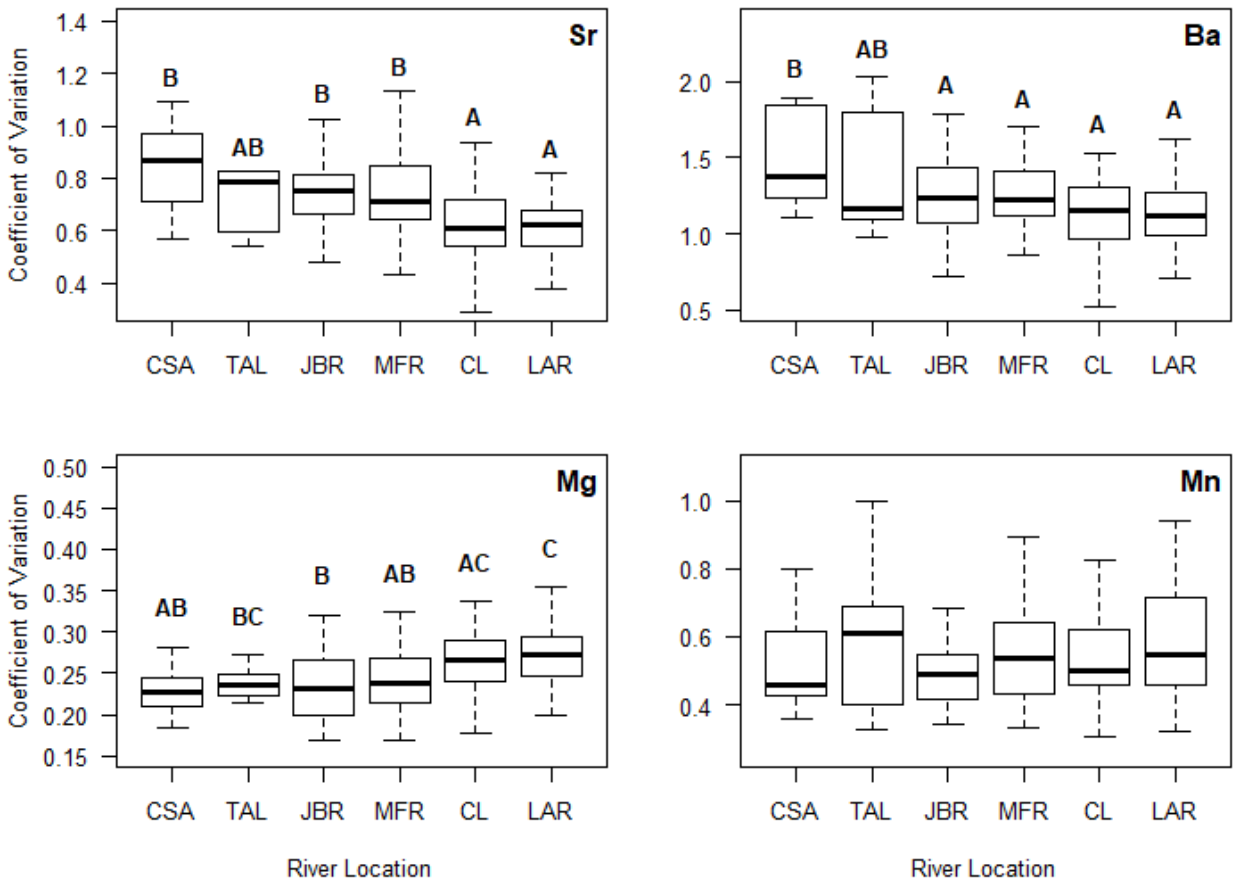

Sr
Ba
Mg
Mn
Coefficient of Variation
River Location
CSA
TAL
JBR
MFR
CL
LAR
B
AB
B
B
A
A
B
AB
A
A
A
A
AB
BC
B
AB
AC
C

Figure 28

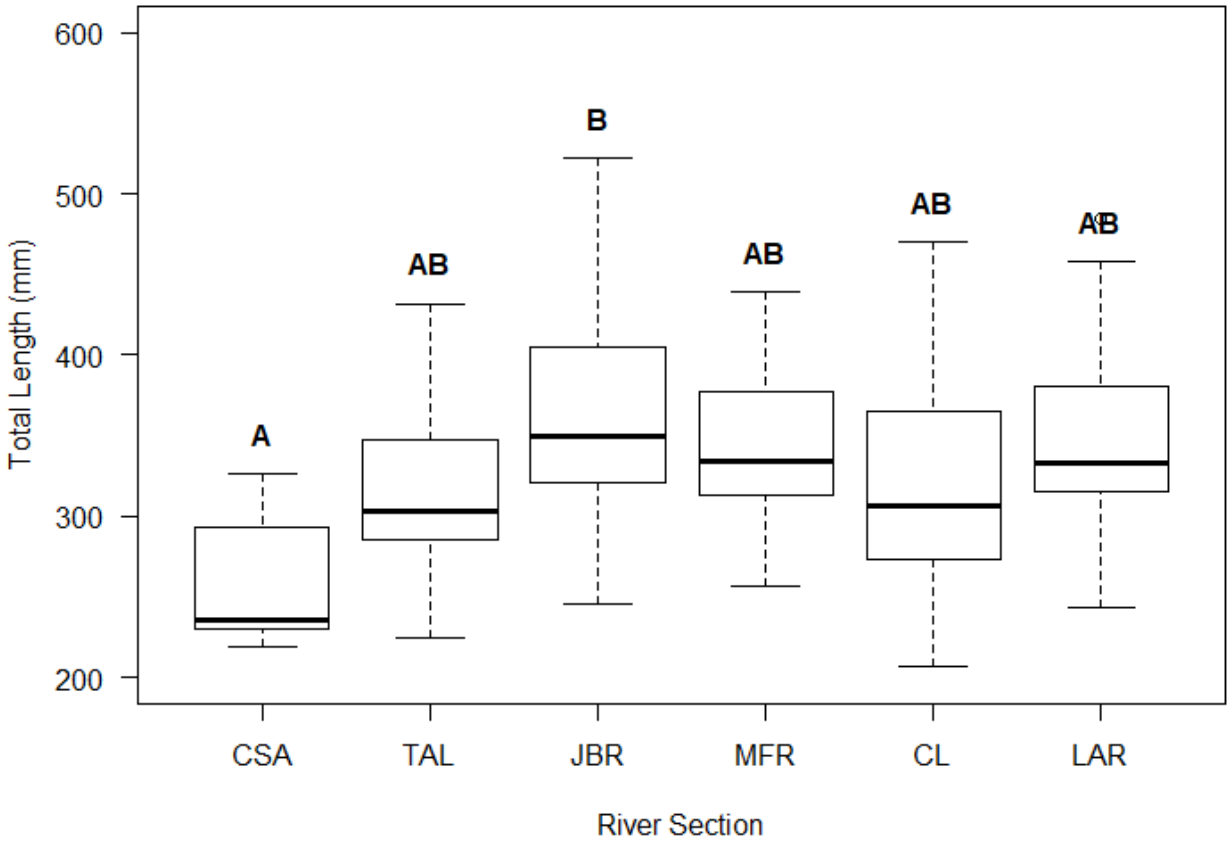

600
500
400
300
200
Total Length (mm)
A
AB
B
AB
AB
AB
CSA
TAL
JBR
MFR
CL
LAR
River Section

Figure 29

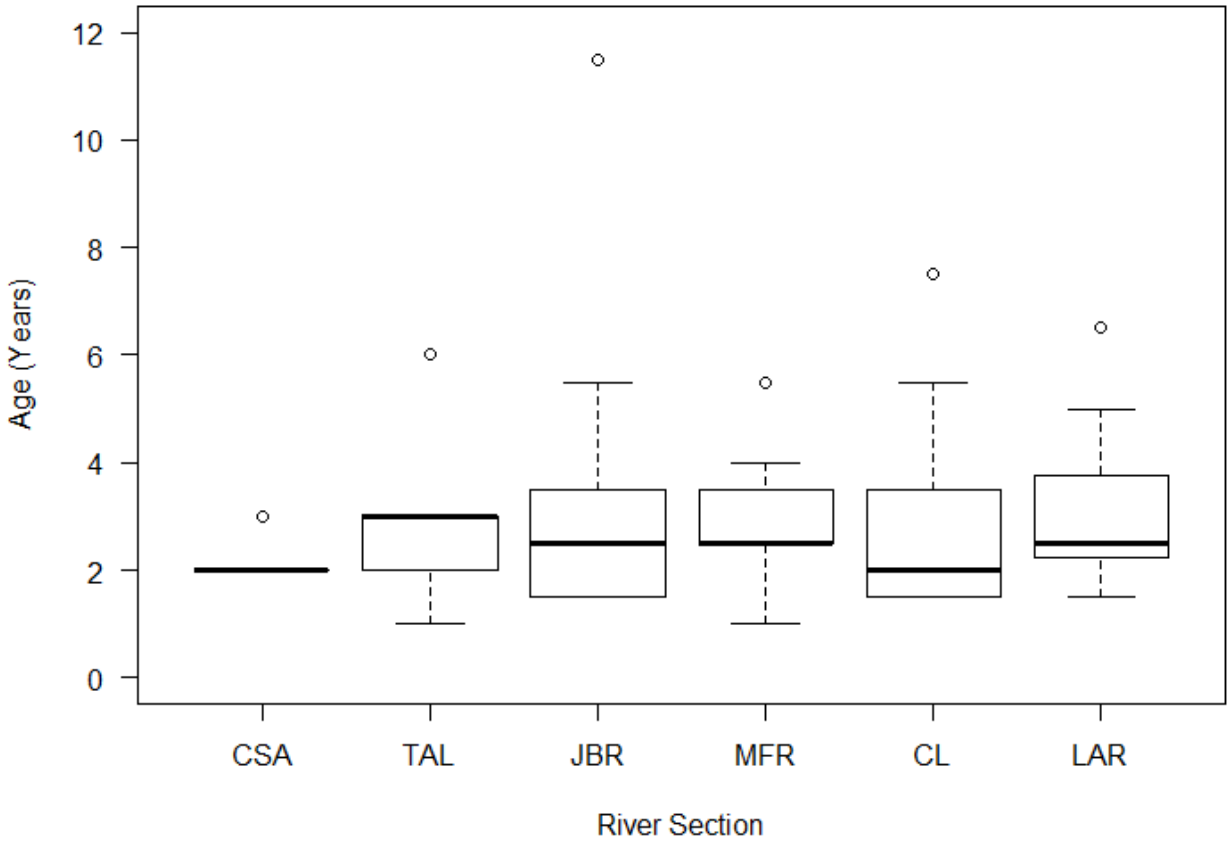


Figure 30

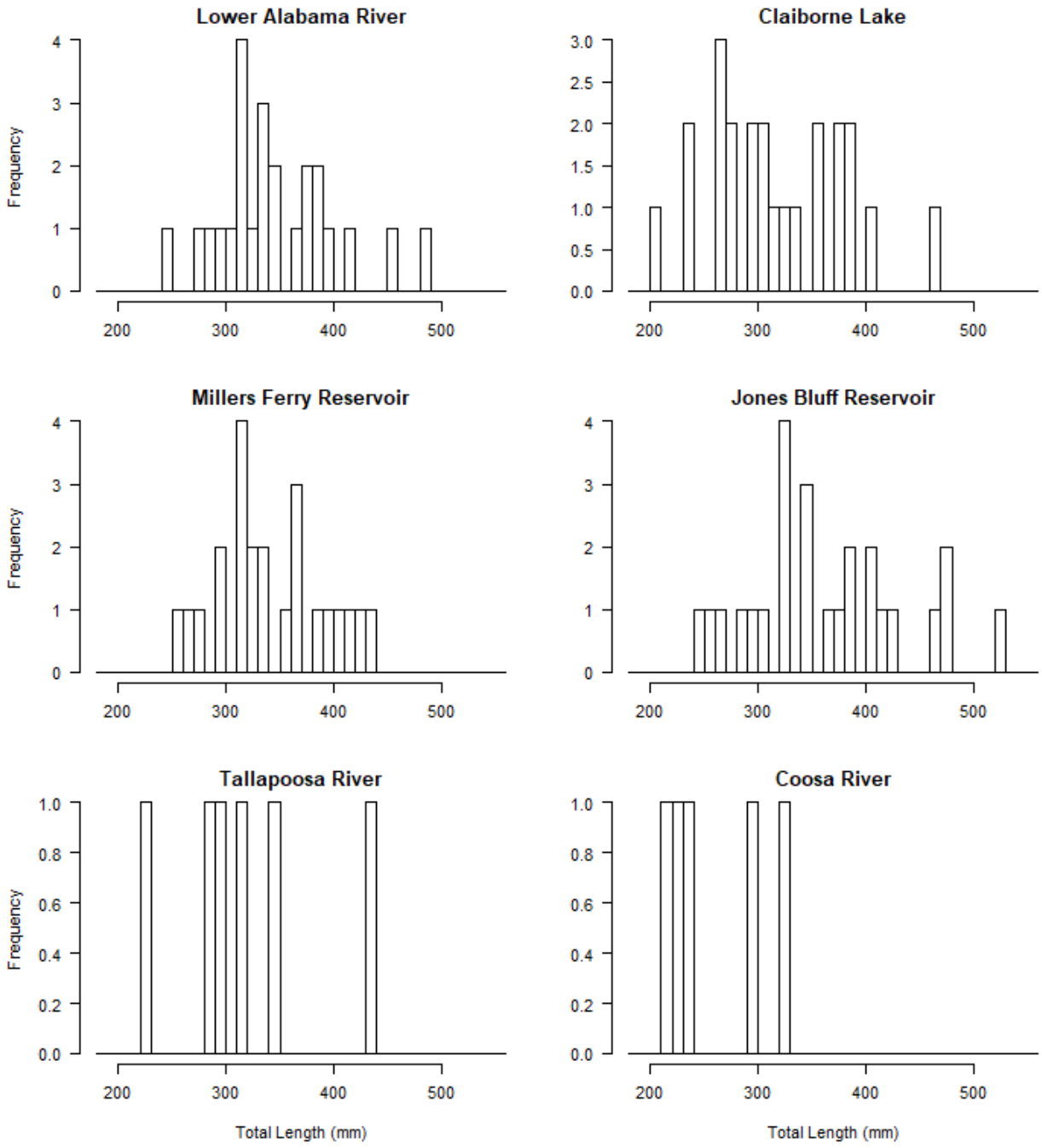

Lower Alabama River
Claiborne Lake
Millers Ferry Reservoir
Jones Bluff Reservoir
Tallapoosa River
Coosa River
Frequency
Total Length (mm)

Figure 31

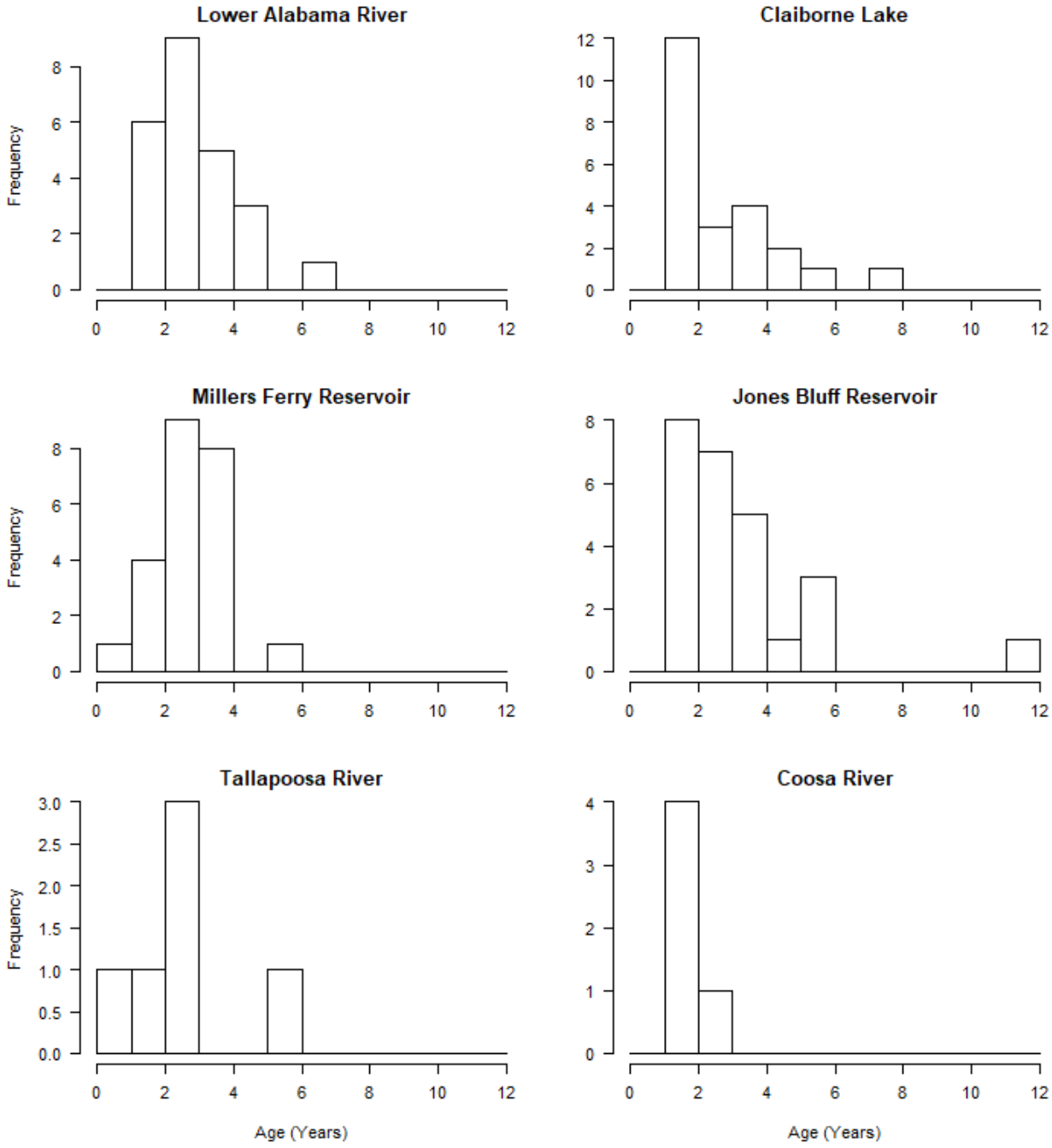

Lower Alabama River
Claiborne Lake
Millers Ferry Reservoir
Jones Bluff Reservoir
Tallapoosa River
Coosa River
Frequency
Age (Years)

Figure 32

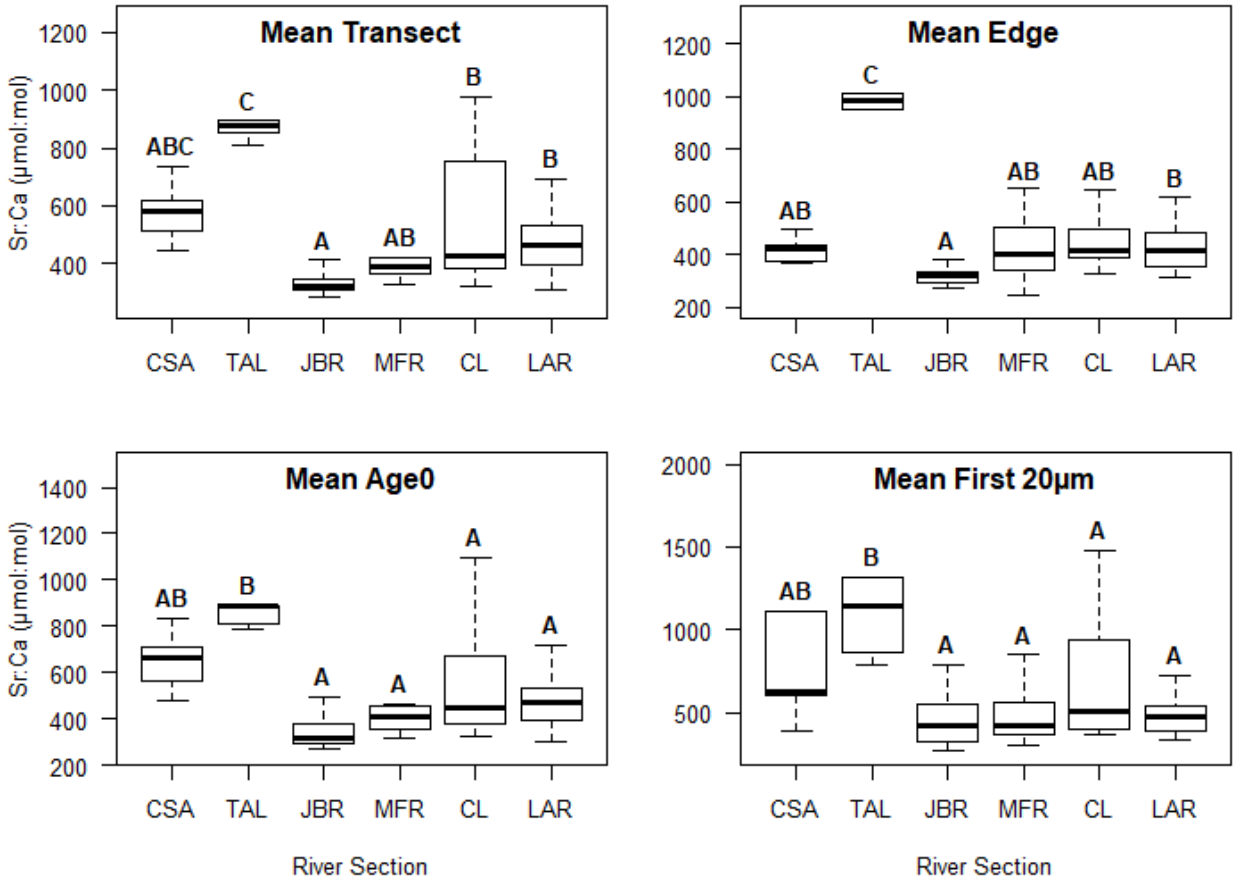

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Sr:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR
ABC
C
A
AB
B

Figure 33

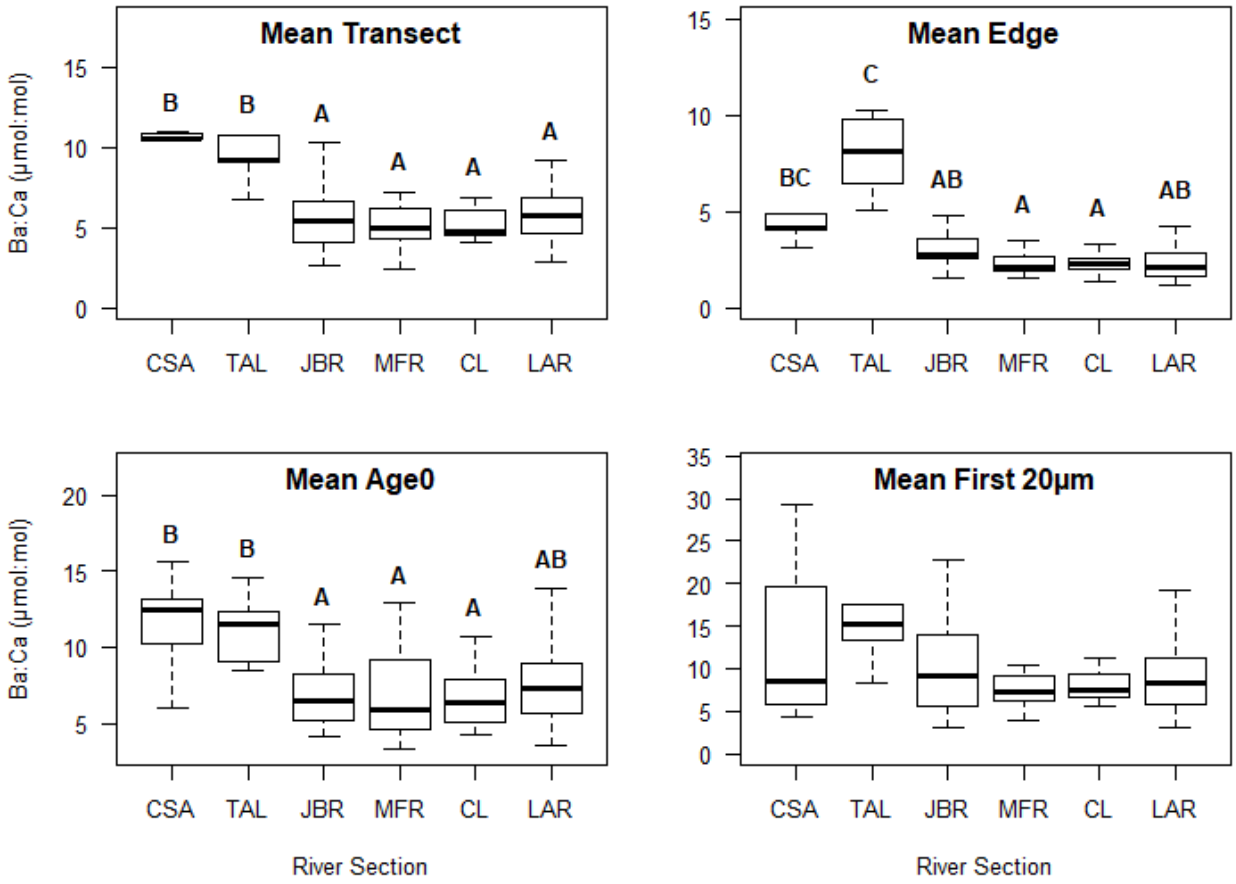

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Ba:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR
B
B
A
A
A
A
BC
C
AB
A
A
AB
B
B
A
A
A
AB

Figure 34

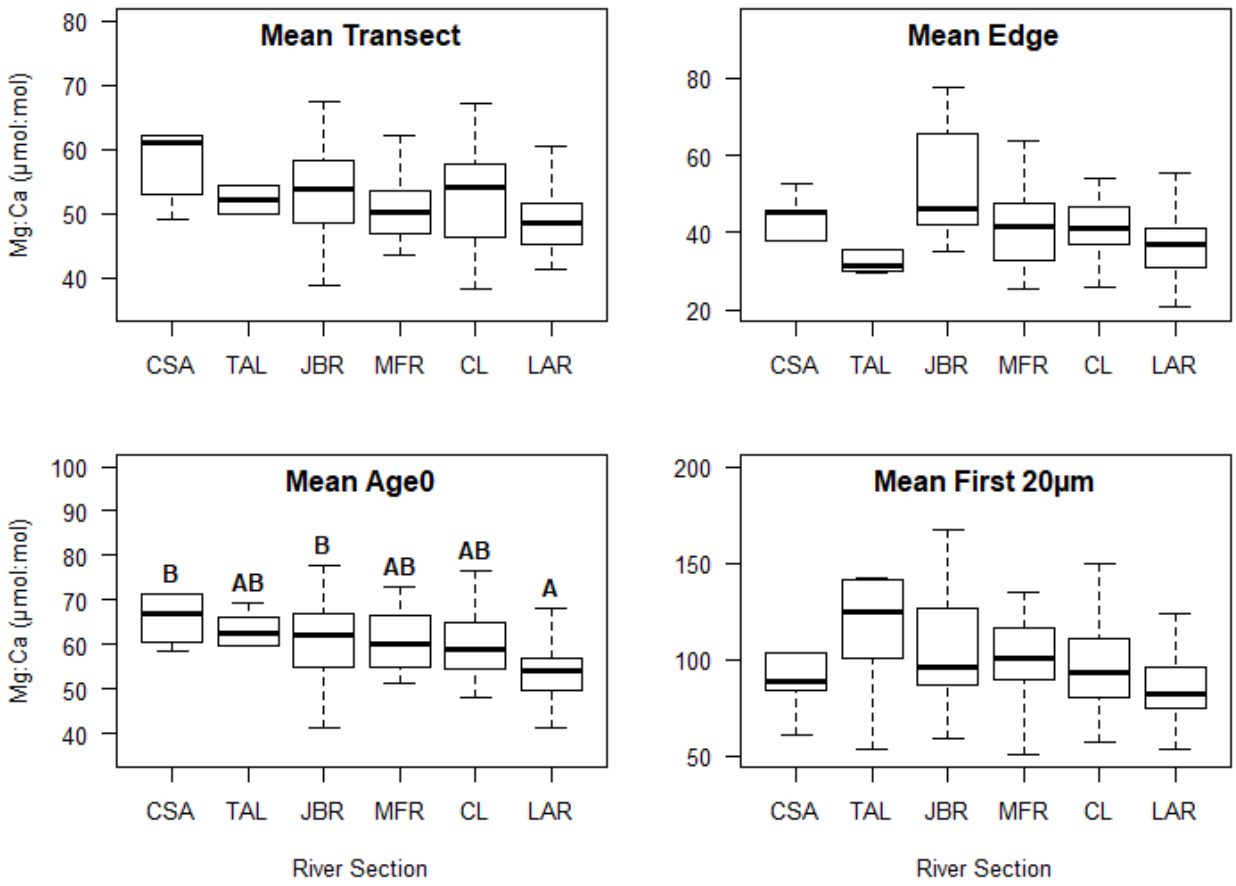

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mg:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR
B
AB
B
AB
AB
A

Figure 35

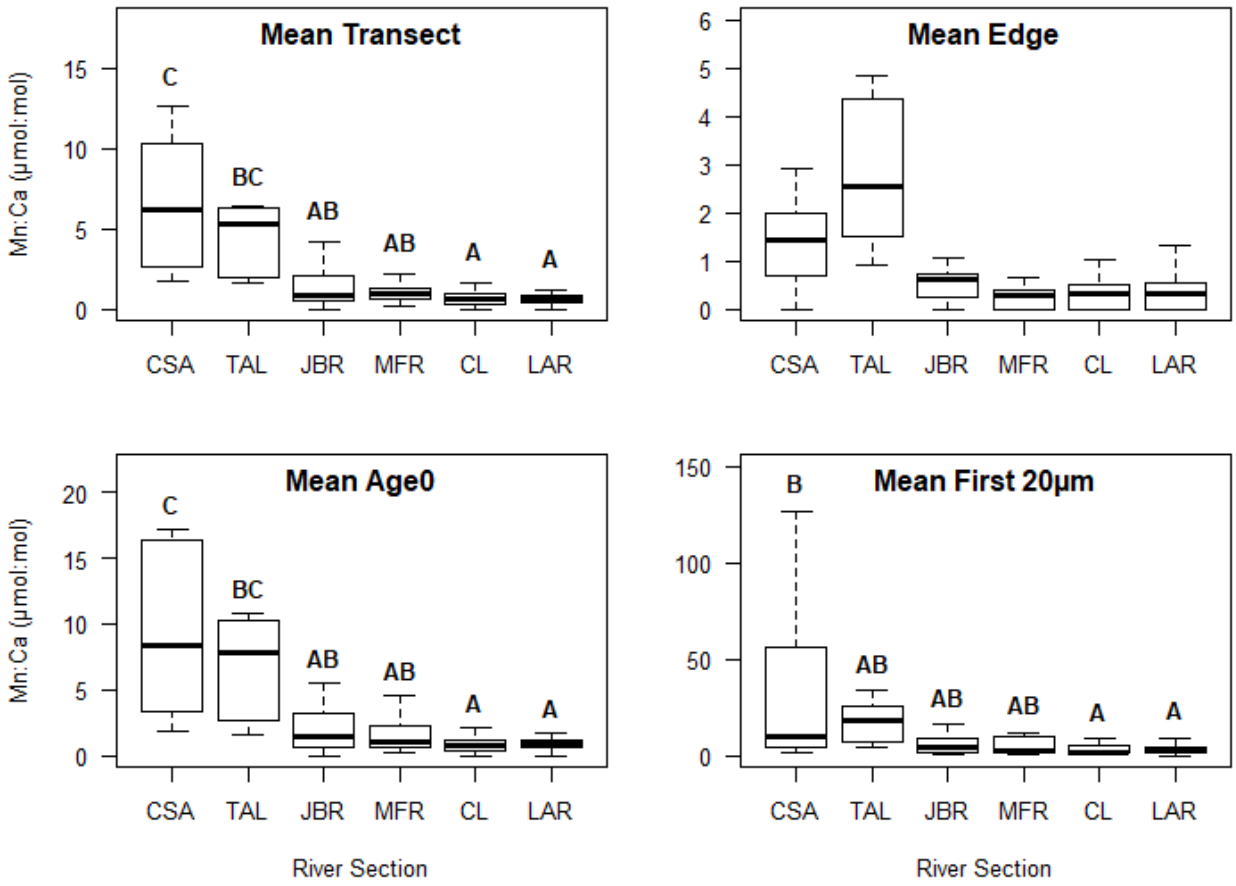

Mean Transect
Mean Edge
Mean Age0
Mean First 20µm
Mn:Ca (µmol:mol)
River Section
CSA
TAL
JBR
MFR
CL
LAR
C
BC
AB
A
B

Figure 36

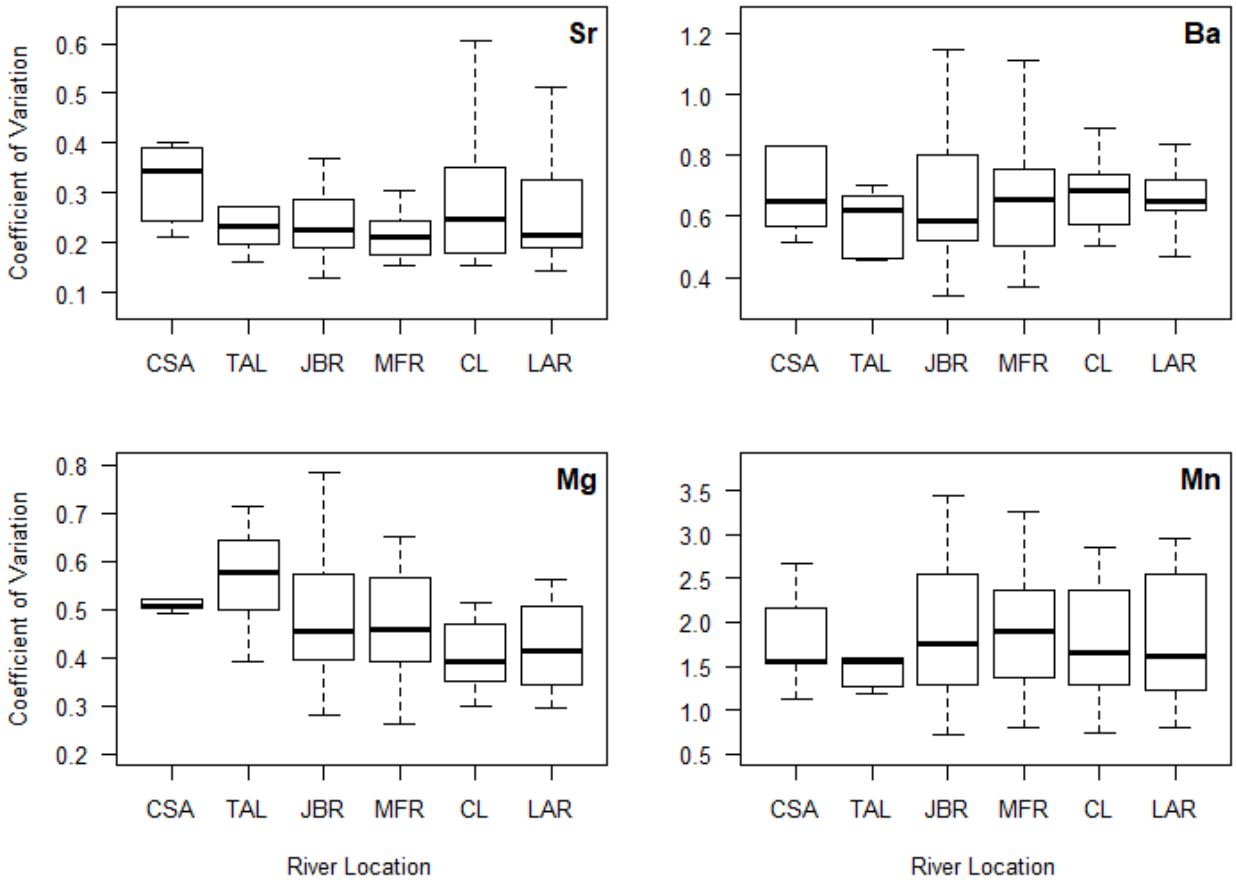

Sr
Ba
Mg
Mn
Coefficient of Variation
River Location
CSA
TAL
JBR
MFR
CL
LAR

Figure 37

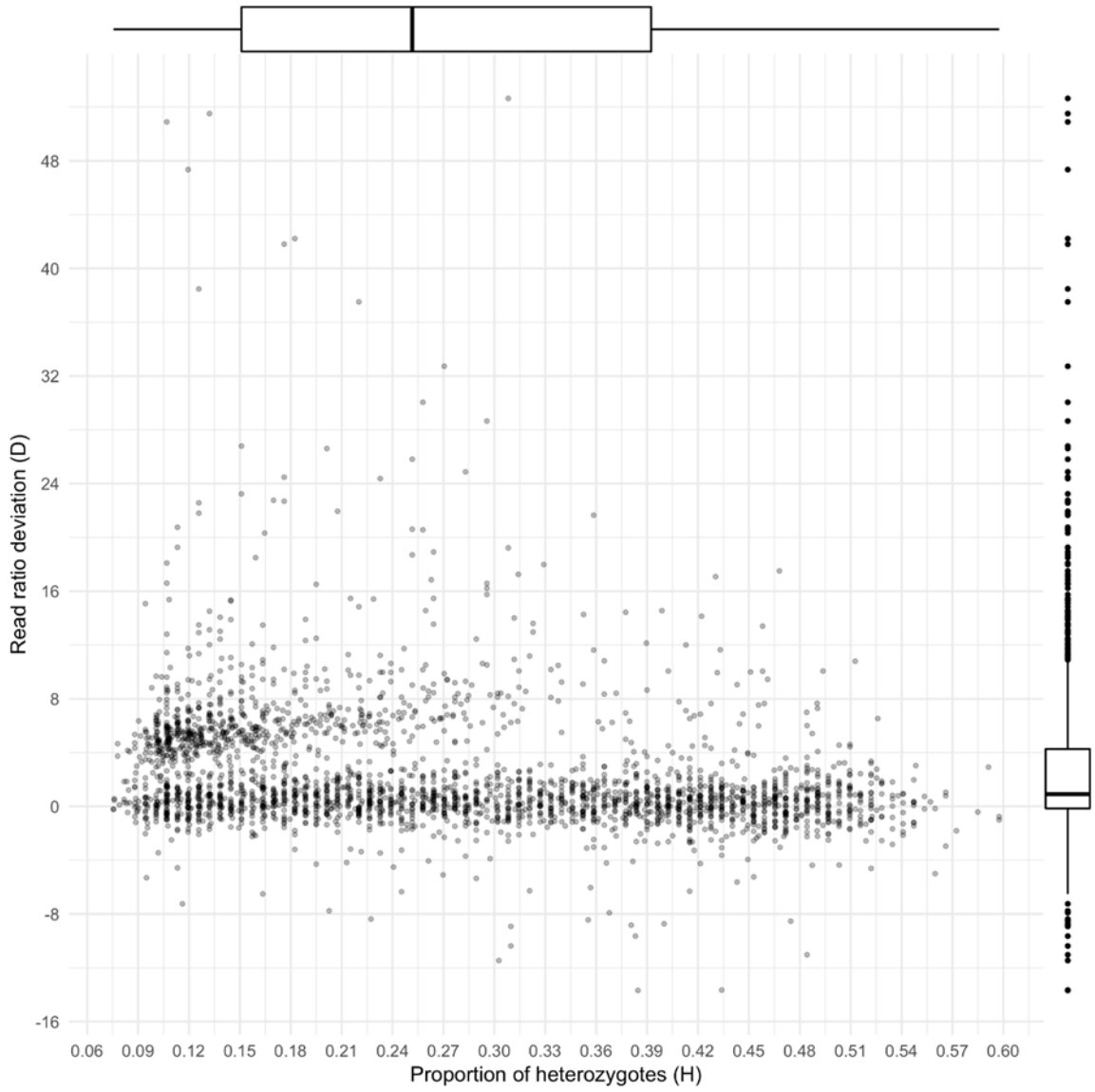

Read ratio deviation (D)
48
40
32
24
16
8
0
-8
-16
0.06 0.09 0.12 0.15 0.18 0.21 0.24 0.27 0.30 0.33 0.36 0.39 0.42 0.45 0.48 0.51 0.54 0.57 0.60
Proportion of heterozygotes (H)

Figure 38

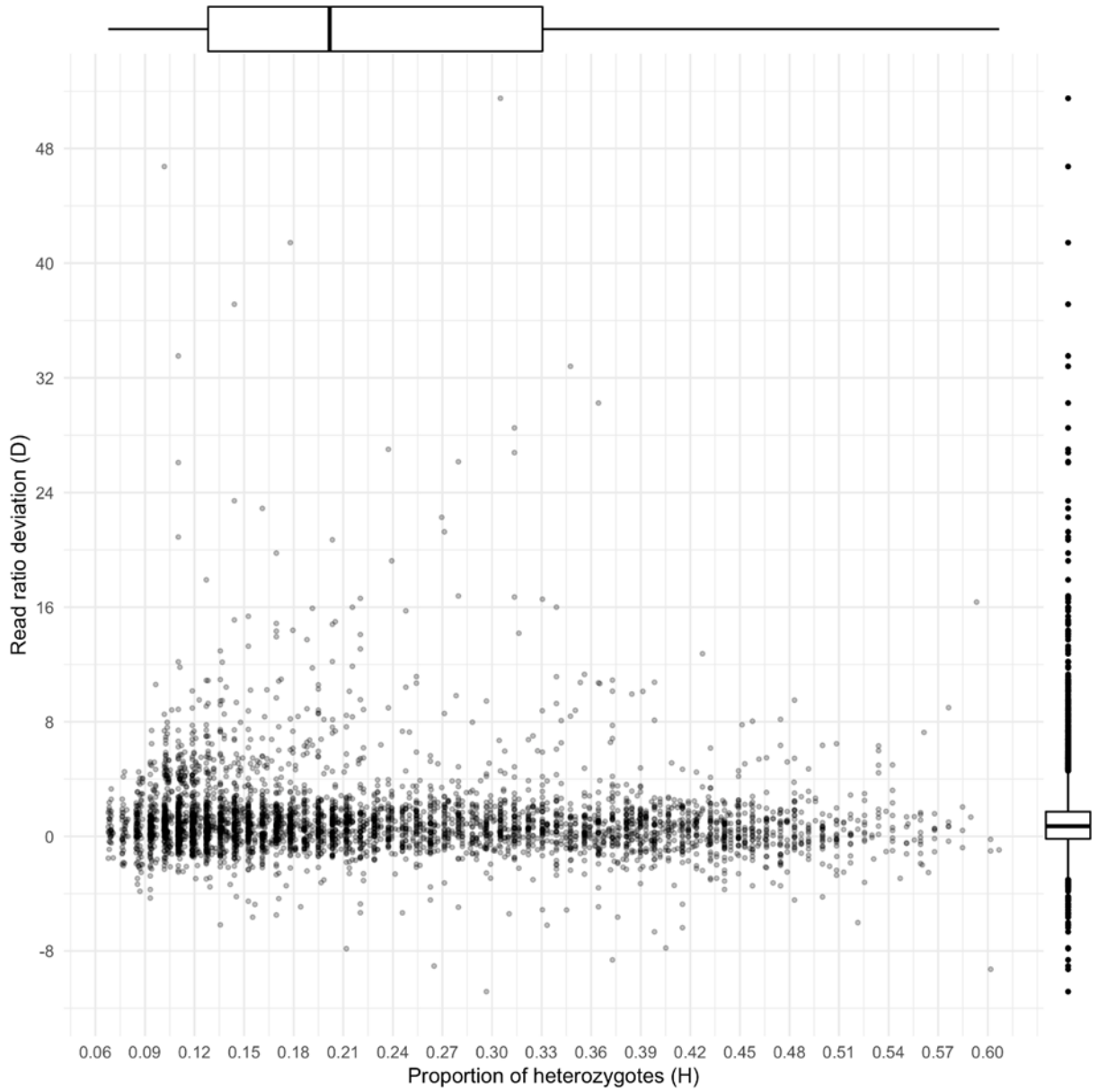
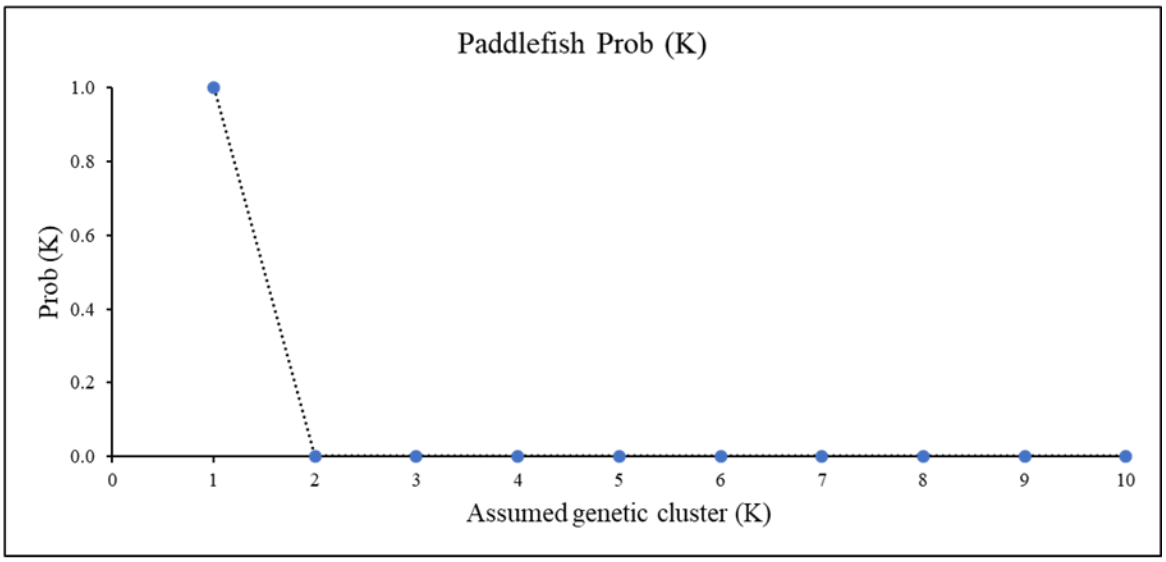
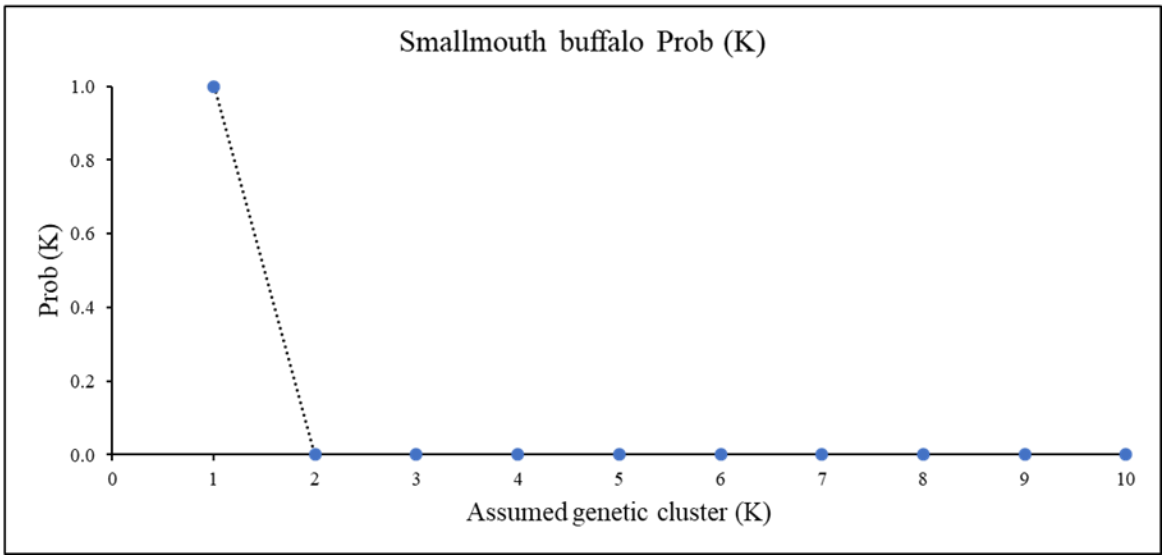
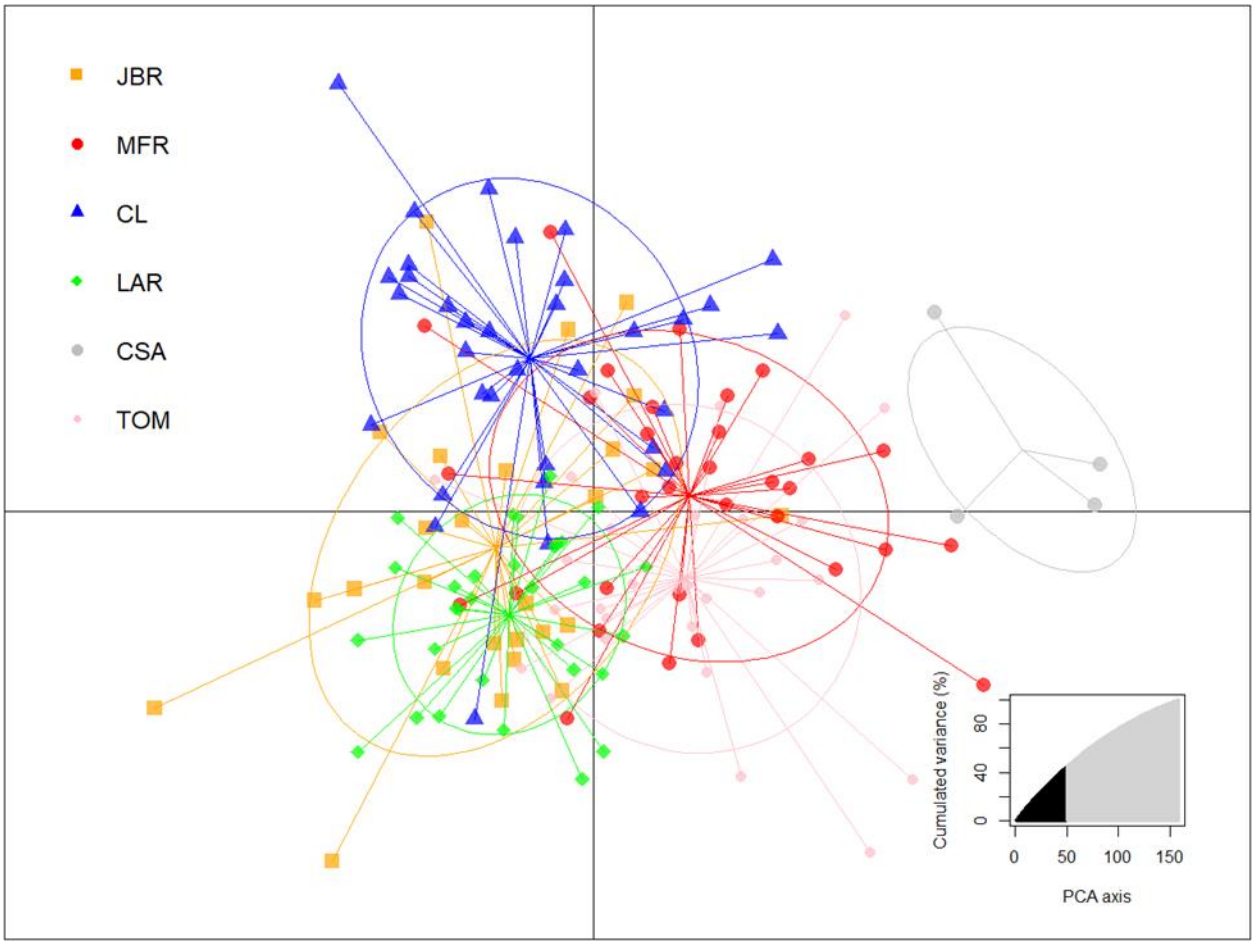

Read ratio deviation (D)
48
40
32
24
16
8
0
-8
0.06 0.09 0.12 0.15 0.18 0.21 0.24 0.27 0.30 0.33 0.36 0.39 0.42 0.45 0.48 0.51 0.54 0.57 0.60
Proportion of heterozygotes (H)

Figure 39


Paddlefish Prob (K)
Prob (K)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
8
9
10
Assumed genetic cluster (K)
Figure 40


Smallmouth buffalo Prob (K)
Prob (K)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
8
9
10
Assumed genetic cluster (K)
Figure 41


JBR
MFR
CL
LAR
CSA
TOM
Cumulated variance (%)
0
40
80
0
50
100
150
PCA axis
Figure 42

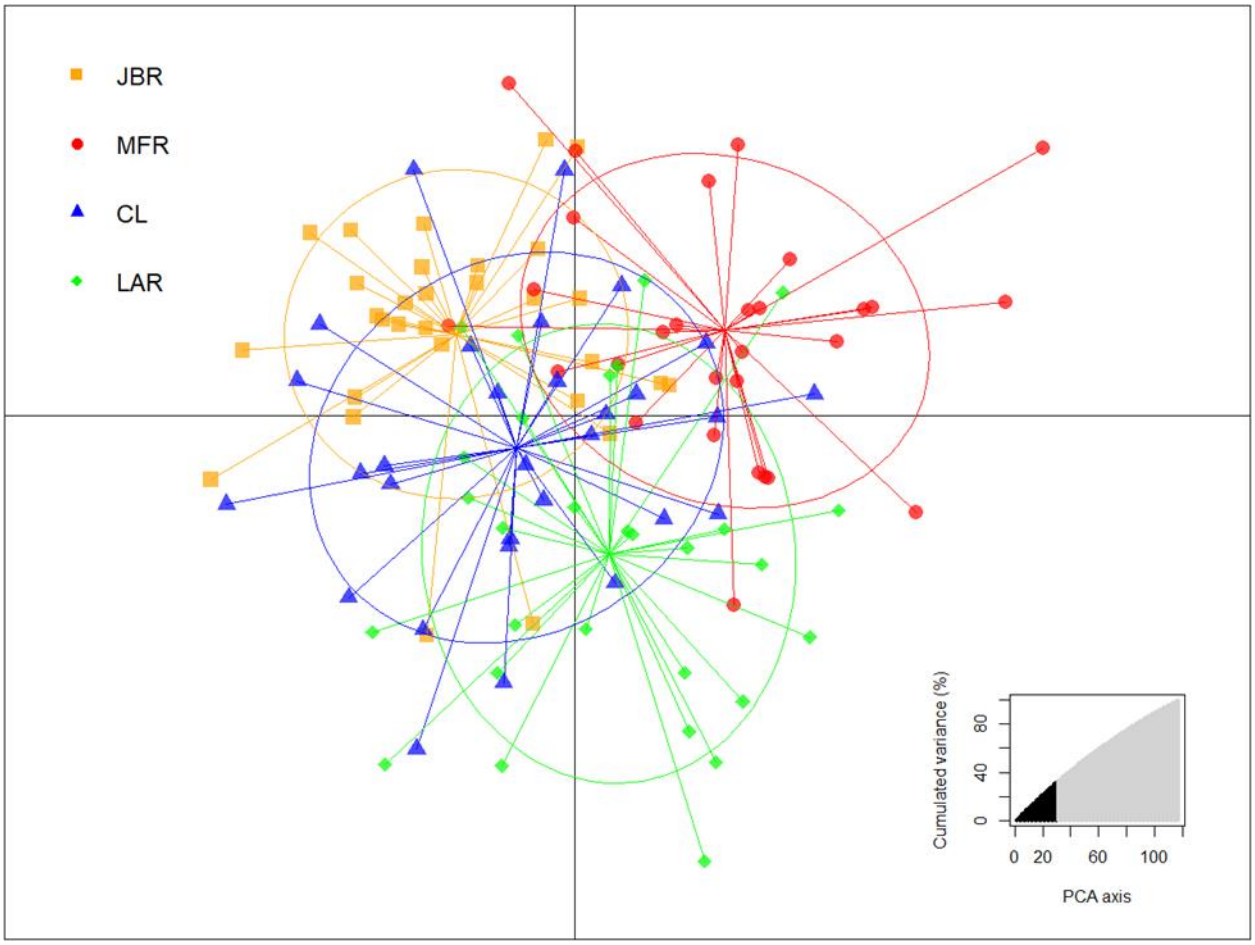


Figure 43