Evaluating the effects of three Alabama River dams on fish movements and population connectivity using otolith microchemistry

By

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Abstract

Dams impede fish movement and can isolate riverine populations into defined areas. The Alabama River is divided into four major sections by three lock-and-dam structures. I used otolith microchemistry to quantify movements and population connectivity among these river sections by three fish species (Freshwater Drum, White Crappie and Blue Catfish) that differ in life expectancies, spawning strategies and swimming abilities. Water sample trace-element ratios (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) from throughout the study area varied spatially but were temporally consistent. Broad patterns in water chemistry were reflected in element:calcium ratios in otolith whole-transects (i.e., across entire life), edges (reflecting time of capture), and cores (reflecting early life). Correlations between otolith-edge and season-specific water Sr:Ca ratios from the areas where fish were collected were significant for all three species, while the associations between otolith-edges and water were mostly nonsignificant for Mg:Ca, Mn:Ca, and Ba:Ca ratios. Linear discriminant analyses (LDAs) were used to determine how accurately the multivariate element signatures in otolith-edges could classify fish back to the river sections from which they were collected, resulting in mixed accuracies across species. Otolith whole-transect LDA classifications were generally similar in accuracy to otolith-edge LDAs for each species, while core region LDA classification accuracies were typically lowest, likely due to enriched element concentrations within otolith cores. I developed criteria for identifying potential dam passage events within fish lifetimes based on otolith Sr:Ca profiles, and evidence that these species move past dams appears very limited. Otolith microchemistry results generally appear to suggest that these fish tend to remain in areas near where they hatched and while movements among habitats may occur, both upstream and downstream dam passages appear rare.

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List of Abbreviations

rkm	river kilometer
L&D	lock and dam
CSA	lower Coosa River
TAL	lower Tallapoosa River
JBR	Jones Bluff Reservoir
MFR	Millers Ferry Reservoir
CLL	Claiborne Lake
LAR	lower Alabama River
FWDR	Freshwater Drum
WHCP	White Crappie
BCAT	Blue Catfish

INTRODUCTION

Dams and diversions can provide valuable benefits such as irrigation, hydroelectricity, improved river navigation, flood protection, and expanded recreational opportunities (Graf 1999). However, these benefits are often accompanied by substantial environmental damage, impairment of ecosystem services, and loss of biodiversity (Dudgeon et al. 2006; Vörösmarty et al. 2010; Poff and Schmidt 2016). Fragmentation and degradation of freshwater habitats are the main causes of freshwater biodiversity loss (Brauer and Beheregaray 2020). Dams are the most important agent by which humans have fragmented river networks worldwide (Rosenberg et al. 1997, 2000), and riverine habitats in the United States have been identified as some of the most disconnected habitats for aquatic populations in the world (Barbarossa et al. 2020).

Damming fragments river habitats for fish populations by obstructing pathways of fish movement (Jager et al. 2001). Regardless of the causes of fragmentation, it is a landscape-scale process (Fahrig 2003) that affects both structural connectivity (e.g., physical aspects of, and distance between patches), as well as functional connectivity (e.g., dispersal and gene flow between habitat patches) among habitats within a landscape (Hanski 1999; Horreo et al. 2011; Valenzuela-Aguayo et al. 2019).

While some fishes in some systems are able to overcome in-stream barriers and successfully complete both upstream and downstream passages of dams, many fishes within a river system may be unable to make any upstream movement past a dam. Fishes with greater swimming abilities are probably more likely to overcome the hydraulic forces associated with passing a barrier versus those with poorer swimming abilities (Haro et al. 2004). Swimming ability varies among species as well as across sizes within species (Katopodis and Gervais 2016).

Larger and more-capable individuals likely have increased chances of completing upstream passage events across river barriers (Katopodis et al. 2019). In addition, some fish may have the necessary swimming capabilities for completing a dam passage, but may simply not do so as a result of habitat and environmental changes (i.e., changes in flow) experienced during a passage attempt (Katopodis Ecohydraulics Ltd. 2013). Some dams may only be passable during infrequent and unpredictable high-water events (Haponski et al. 2007; Simcox et al. 2015; Hershey et al. 2021), while other dams may be entirely impassable to even the strongest swimming fishes.

In addition to the direct effects of fragmenting and altering habitats, barrier construction can also result in potentially detrimental population-level genetic effects that can accumulate over generations within isolated populations (Zarri et al. 2022). Isolated populations may be subject to increased rates of inbreeding, decreased levels of heterozygosity, reduced allelic richness, and reduced frequencies of favorable alleles (Allendorf et al. 2013; Gouskov et al. 2016), all of which can have harmful long-term population effects (Jager et al. 2001; Wofford et al. 2005).

The effects of dams on migratory fish have been relatively well documented along the U.S. Atlantic and Pacific coasts, and fish passage facilities (e.g., fish ladders, fish elevators, trap-and-haul operations) have been developed and used at many dams to help mitigate their effects on fish populations (Roscoe and Hinch 2010). However, relatively few fish passage facilities have been installed at dams in the Southeastern U.S. (Sudduth et al. 2007). There is a great deal of uncertainty regarding the ecological costs and benefits that passage facilities may have if they were implemented in this region given the unique biogeography of the

Southeastern U.S. (i.e., high levels of endemism and numerous non-migratory aquatic taxa) (McKay et al. 2013). Retrofitting existing dams with fish passage structures can be expensive to plan, construct, and operate (Clay 1994). Uncertainties in how resident and potamodromous fishes of the Southeastern U.S. may respond to differing types of passage structures indicates just how important it is to better understand how dams may be affecting fish movements (Cooper et al. 2021).

Fish movement studies have traditionally used direct methods of measuring and estimating movements (i.e., mark-recapture, radio-tracking, GPS-tracking). Indirect evidence based on approaches such as microchemical and genetic measures are being used more often, especially when direct measures are impractical (Milton and Chenery 2003; Elsdon et al. 2008). Here I use both otolith microchemistry and population genetic data to quantify indicators of movement and potential impacts of isolation for three fish species relative to three dams on the Alabama River. To evaluate within-lifetime movements, I used microchemical analyses of fish otoliths combined with elemental ratios from water samples to determine habitat residence and patterns of fish movements throughout their lifetimes. At a longer-term intergenerational scale, I used genetic data to assess if the dams of the Alabama River are affecting fish populations by restricting movements of fish among river sections. Studying fish dispersal, including the ability of the species to pass existing dam structures, is important for understanding the population dynamics of fishes in such systems, and eventually for predicting population responses to future changes in the river's environment, potentially including the restoration of habitat connectivity.

Otolith Microchemistry

Otolith microchemistry has become a significant tool for reconstructing lifetime movements of fish among habitats (Izzo et al. 2016; Walther et al. 2017; Hüssy et al. 2020). Fish otoliths are paired calcium carbonate structures that grow continuously throughout the life of a fish and assist with proprioception and orientation (Campana and Neilson 1985; Campana 1999). As fish grow, calcium carbonate layers are laid down over the surface of the otolith (Campana and Neilson 1985). Trace elements found in water are derived from the underlying geology of the watershed (Newton et al. 1987) and can be incorporated into an otolith's matrix (Campana 1999; Elsdon et al. 2008). The incorporation of elements from the water can create a signature combination of elemental concentrations in the otolith that can indicate the residency of the fish in particular habitats (Campana et al. 1995, 1999; Elsdon et al. 2008). Microanalytical techniques can quantify trace element concentrations in both otoliths and water samples to describe the lifetime habitat use and potential movements of fishes (e.g., Walther et al. 2008, 2017; Farmer et al. 2013; Swanson et al. 2020; Martinho et al. 2020; Whitledge et al. 2020; Willmes et al. 2020). By correlating ratios of elements observed within fish otoliths to ratios of the same elements within the water at locations throughout the study area, I aimed to characterize within-lifetime broad-scale movements of my study species throughout the Alabama River.

Population Genetics

While otolith microchemistry provides evidence of within-lifetime movements of fishes, genetic-based methods can quantify the degree of genetic differentiation among populations (i.e., across generations). Genetic differentiation can be related to spatial distances separating

populations to obtain estimates of gene flow, effective population sizes, rates of dispersal, and inbreeding rates (Comte and Olden 2018). Information gained from genetic analyses provides inference into longer, intergenerational effects of dams. Previous work by Wilson et al. (2004), Watts et al. (2007), Feyrer et al. (2007), and Pinsky et al. (2017), has shown that genetic-based studies can complement other types of movement-tracking studies (i.e., radio telemetry, mark-recapture, otolith microchemistry, etc.).

Genetics-based studies have become increasingly common in wildlife and fisheries sciences. The development of high-throughput DNA sequencing techniques has allowed researchers to analyze high-resolution genetic data obtained from wild organisms at reasonable costs (Brumfield et al. 2003; Allendorf et al. 2013). Several methods of high-throughput sequencing allow for the discovery and analyses of single-nucleotide polymorphisms (SNPs) distributed across and representative of a species genome (Davey et al. 2011). SNP data have been commonly used in population genetics studies and have been found to yield reliable estimates of divergence times and gene flow among populations (Morin et al. 2004, 2009).

Different movement patterns and life history characteristics across fish species results in differing effects of barriers on population connectivity (Nislow et al. 2011; Camak 2012; Gehri et al. 2021). Species that are short-lived with shorter generation times are likely to exhibit genetic differentiation among subpopulations more rapidly than long-lived species, given more opportunity for shifts in genotype (due to local selection, founder effects, genetic drift, etc.) as the populations reproduce (Lippé et al. 2006). Shorter-lived species, therefore, may be expected to demonstrate larger observed genetic effects of fragmentation due to the presence

of dams, within a given time period than would longer-lived species with longer generation times.

Goals and Study Questions

My goal here was to determine if the Alabama River dams act as barriers to the movements of three study species at both within-lifetime and intergenerational timescales. By combining otolith microchemistry with genetic-based approaches, this work will improve our knowledge of how dams are affecting fish movement both within and across generations. In order to accomplish the overall goals of this study, I addressed the following questions.

1. Does otolith microchemistry quantify fish lifetime movement patterns, and if so, are these patterns affected by the Alabama River dams.
2. Is there evidence of significant genetic differentiation among fish caught from within each of the four river sections of the Alabama River that are separated by dams?

METHODS

Study Area

The Alabama River is approximately 487 km long from its origin at the confluence of the Coosa (CSA) and Tallapoosa (TAL) rivers to where it joins the Tombigbee River to form the Mobile River. Three lock-and dam structures separate the Alabama River into 4 distinct sections. These three lock-and-dam structures were constructed and are maintained by the United States Army Corps of Engineers (USACE). Although navigation was a primary original purpose for these structures, commercial barge traffic has declined significantly over the last 20 years (Mettee et al. 2015). As with most Southeastern U.S. river systems, these structures do

not include any facility specifically designed to allow fish passage. Findings from previous studies evaluating the biological effects of the Alabama River's dams on fish communities suggest that they have had extensive and profound influences on fish community assemblages, resulting in reduced abundances of many species (Mettee et al. 2005).

R.F. Henry L&D is the uppermost dam on the Alabama River, located at river km (rkm) 379 with a static head height of 14 m, and consists of a lock chamber and 11 gated spillways attached to a hydroelectric powerhouse. Robert F. Henry L&D forms Jones Bluff Reservoir (JBR) (also known as R. E. "Bob" Woodruff Lake) which at full pool extends a maximum of 130 rkm upstream into the lower Coosa and Tallapoosa rivers (USACE 2014) (Figure 1).

Millers Ferry L&D is located at rkm 215 (Figure 1) with a static head height of 14 m, and consists of 17 gated spillways with a lock chamber, and a separate hydroelectric facility. These structures impound Millers Ferry Reservoir (MFR) (also known as William "Bill" Dannelly Reservoir) which is approximately 164 rkm in length and contains the lowermost portion of the Cahaba River, a major tributary that flows into the Alabama River near Selma AL, approximately 89 rkm upstream of Millers Ferry L&D.

Claiborne L&D is the lowermost dam on the Alabama River and is located at rkm 119 (Figure 1). Water released from Claiborne L&D flows uninterrupted toward the Mobile-Tensaw River Delta and the Gulf of Mexico. The dam consists of six gated spillways adjacent to a 154-m-wide by 10-m-high crested spillway that spans to the river's west bank. The crested spillway at Claiborne L&D is unique among the three dams on the Alabama River in that flow inundates the crested spillway when the gage height is 10 m or greater (Mettee et al. 2015; Hershey et al. 2021). Several large-bodied fish species, including Paddlefish *Polyodon spathula* and

Smallmouth Buffalo *Ictiobus bubalus*, have been documented to complete upstream passages across Claiborne Dam (Mettee et al. 2006; Simcox et al. 2015; Kratina 2019; Hershey et al. 2021), most likely over the inundated crested spillway during high-water events. Claiborne L&D impounds Claiborne Lake (CLL), which is approximately 96 rkm in length.

The spill gates at R.F. Henry L&D and Millers Ferry L&D are closed throughout most of the year to conserve water for hydroelectric generation. However, during the typical wet season from January to April, the gates at these dams are periodically opened as needed to prevent upstream flooding (Mettee et al. 2015). In addition, the Alabama River is subject to daily water-level fluctuations from hydroelectric discharges at R.F. Henry L&D and Millers Ferry L&D (Mettee et al. 2005). During periods of normal flows, Claiborne Dam's crested spillway and the hydraulic turbulence through the spill gates at all three dams likely restricts upstream movement of most fishes of the Alabama River (Mettee et al. 2015). Fish passage has been studied less at R.F. Henry L&D relative to the other two dams; however, it is suspected that this dam acts similarly to Millers Ferry L&D, preventing nearly all upstream movement by fish except limited movement via the navigational locks (Mettee et al. 2005).

In addition to significant habitat alterations resulting from dam construction on the Alabama River, these dams have fragmented the Alabama River and thus the inhabiting fish populations into four distinct river sections; 1) Jones Bluff Reservoir (JBR [upstream of R.F. Henry L&D]), 2) Millers Ferry Reservoir (MFR [between R.F. Henry L&D and Millers Ferry L&D]), 3) Claiborne Lake (CLL [between Millers Ferry L&D and Claiborne L&D]), and 4) the lower Alabama River (LAR [downstream of Claiborne L&D]).

Study Species

I chose to study three generally smaller-bodied species with shorter generation times that have some potential to disperse (c.f., larger-bodied riverine species). Little research has specifically examined how dams and fragmentation have affected populations of smaller-bodied and shorter-lived fishes in large rivers. As such, my study species are Freshwater Drum *Aplodinotus grunniens*, White Crappie *Pomoxis annularis*, and Blue Catfish *Ictalurus furcatus*. These species have varying life spans, ages at maturity, swimming abilities, and spawning strategies. They are native to the Alabama River system (Mettee et al. 1996; Boschung and Mayden 2004; Freeman et al. 2005) and little is known about their population genetics or how dams may be affecting their populations.

Freshwater Drum

Freshwater Drum (FWDR) is a generalist benthivorous species with the greatest latitudinal range of any North American freshwater fish (Mettee et al. 1996; Boschung and Mayden 2004). Freshwater Drum is not considered a sportfish and can constitute a large proportion of the fish biomass of aquatic systems (Swingle 1953). They are ecologically important in that they compete with or provide prey resources for other species via young-of-year production (Swingle 1953). The Freshwater Drum is highly fecund and unique among freshwater North American fishes in producing eggs and larvae that float at the water's surface (Page and Burr 1991) allowing downstream drift. In the presence of dams that may not allow for upstream fish passage, the downstream drift of larval Freshwater Drum eggs and larvae could result in unidirectional (downstream) transfer of genetic material.

White Crappie

White Crappie (WHCP) have an estimated maximum lifespan of 8 years, although the typical maximum age is 5–6 years (Hammers and Miranda 1991). Males and females are both capable of spawning by age 3 (Thomas and Kilambi 1981), likely resulting in the species having a short generation time relative to most other species that have been studied more extensively in the Alabama River. In a mark-recapture study of fish passage at several Upper Mississippi River dams, no White Crappie or Black Crappie *Pomoxis nigromaculatus* were found to move across a single lock and dam, either upstream or downstream (Ickes et al. 2001). Like many other fishes within the Alabama River, White Crappie are likely unable to overcome the hydraulic forces associated with passing any of the three dams on the river.

Blue Catfish

The Blue Catfish (BCAT) is native to major rivers of the Mississippi River basin, and Gulf Coast rivers of the central and southern U.S. (Graham 1999). About half of the 29 U.S. states reporting Blue Catfish as present consider them economically and recreationally important, and they are targeted both commercially and recreationally in Alabama (Graham 1999). Many catfish species have been observed to migrate long distance (Barthem et al. 1991; Oyanedel et al. 2018) and Blue Catfish are believed to be the most migratory of the ictalurid catfishes (Lagler 1961), moving upstream in the spring and downstream in the fall in response to water temperature changes (Pflieger 1997).

Water Chemistry

Sample Collections

Water samples were collected once per season from spring 2020 through summer 2021. Samples were collected while river levels were near their median heights (based on USGS gage height data [USGS 2016]). Water samples were collected from 15 different locations throughout the Alabama, Coosa, Tallapoosa, Cahaba, Tensaw, and Tombigbee rivers (Table 1; Figure 2). Samples were collected with a Van Dorn sampler held open at 1m below the river's surface for 30 seconds to flush the sampler. River water was extracted from the Van Dorn Sampler using a 50 ml sterile syringe (Henke-Sass Wolf Norm-Ject) and filtered through a disposable 0.45 µm PTFE glass-fiber filter (Whatman GD/XP) directly into a 30 ml low-density polyethylene (LDPE) bottle. This process was repeated twice, with the 30 ml LDPE bottle emptied between refills to rinse the bottle (Gunn et al. 2019). Ultrapure 67%-70% nitric acid was added (Aristar Ultra [VWR Chemicals BDH Lot: 1217120]) using a 50 mL PFA microbore dropper bottle (Savillex Inc. Eden Prairie, MN, USA) to preserve the third refill of the 30 ml LDPE bottle as the final sample (Gunn et al. 2019). Field and laboratory control samples (using ultrapure deionized water [Thermo Fisher Scientific]) were produced once per season to test for possible contamination of samples from the LDPE bottles and the nitric acid additive (U.S. EPA 1996). Samples were stored on ice and transported to the Ireland Center Laboratory where they were refrigerated until they were analyzed in the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Laboratory in the Auburn University Department of Geosciences.

Three sites (ALR221, ALR181, and TENSAW) were selected to test whether trace element concentrations differed at varying river depths. Samples were collected using the same

procedures described above, but samples were collected at 1 m below the surface (subsurface zone), within 1 m of the river substrate (benthic zone), and 1 (for ALR221) or 2 (for ALR181 & TENSAW) additional samples collected from depths that were evenly spaced in the water column between the subsurface and benthic samples (pelagic zone). Absolute collection depths for the pelagic and benthic samples varied across seasons due to water-level fluctuations at each site. The overall range of collection depths for these sites is given in Table 1.

Water Chemistry ICP-MS Analyses

Water samples were analyzed in seasonal batches using solution-based ICP-MS, for trace elemental concentrations of a suite of 26 different elements ranging in atomic mass from 9 (beryllium) to 235 (uranium). These analyses were performed via solution-based ICP-MS using a surface-water standard reference material (NIST 1640a [National Institute of Standards and Technology, Gaithersburg, MD, USA]) to correct for oxidation-matrix and ICP-MS drift effects on an individual-sample basis (Aries et al. 2000). Trace-element concentrations (in ppb by mass [equal to ng g^{-1}]) were acquired using an Agilent Technologies 7900 Quadrupole IC-PMS system and quantified and recorded using MassHunter software vB.04 (Agilent Technologies, Santa Clara, CA, USA). Water sample trace-element concentrations were quantified in ppb for simple conversion to molar ratios.

Fish Collections and Dissections

Freshwater Drum, White Crappie, and Blue Catfish were collected using a boat-mounted pulsed-DC electrofisher (Smith-Root GPP 7.5 [Smith-Root Inc., Vancouver, WA, USA]) along with the use of fyke nets, gill nets, and traditional angling gear. Fish collections began in summer 2020 and continued through spring 2021. All fish were euthanized in water containing 300 ppm

MS-222 until gill operculation ceased for 5 minutes, and placed on ice for transport back to the Ireland Center Laboratory. Start and end coordinates were recorded for all electrofishing transects and all fish caught within a transect had their collection location recorded as the midpoint of the transect. From this, capture locations were measured to the nearest rkm. Each of the four major river sections was further divided into five equal-length subsections across which fish collection efforts were focused in order to obtain representative samples throughout each river section. Fish were collected under an Institutional Animal Care and Use Committee approved protocol (PRN# 2019-3618).

In the laboratory, all fish were measured (total length in mm), weighed (total wet weight in g), had their otoliths removed (sagittae for FWDR and WHCP and lapilli for BCAT), and sex determined based upon morphological characteristics of the gonads. Caudal and pelvic fin clips were taken from each individual fish and stored in pre-cleaned 20 ml glass scintillation vials containing 95% ethanol. Surgical scissors used for collecting fin clips were wiped clean with 95% ethanol between each specimen to avoid contamination.

Otolith Microchemistry

Sample Preparation

Following dissections, one otolith was haphazardly selected from each fish and cleaned for 30 seconds in a 30% H_2O_2 solution (Macron Fine Chemicals) to remove any residual tissue material. Otoliths were then rinsed in triple-distilled ultrapure water, dried, and mounted in an epoxy resin. A 0.5 mm – 0.75 mm-thick transverse section through the core of each otolith was removed using a low-speed diamond-blade saw (South Bay Technology, San Clemente, CA, USA). Sections were mounted on glass slides using crystalbond 509 glue (Ted Pella, Redding, CA,

USA), and polished with increasingly finer grit (300, 600, 1200, 1800, 8000, and 14000 grit [40, 30, 12, 9, 3, 1 micron respectively]) lapping films and buffed with 0.5-µm Precision Alumina Powder (South Bay Technology) following established methods (Wells et al. 2003).

The age of each fish was estimated by two independent readers by counting the number of opaque otolith annuli. When readers disagreed on the estimate of the age, a third independent reader counted the annuli, and a final age estimate was determined by consensus of the three readers.

Once ages were estimated, otolith sections were removed from the glass slides, rinsed with ultrapure water (Thermo Fisher Scientific), and remounted onto petrographic slides for use in microchemistry analyses. Slides with the mounted otolith sections were then cleaned by sonicating them in ultrapure water within a petri dish floated in an ultrasonic cleaning bath (Branson 3800 [Branson Ultrasonics Inc., Danbury, CT, USA]). Slides were sonicated for 15 minutes, rinsed with ultrapure water, air dried, and stored in sealed containers until microchemical analyses were performed (Pangle et al. 2010; Zeigler and Whitledge 2011).

Otolith Microchemistry LA-ICP-MS Analyses

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analyses were performed in the ICP-MS Laboratory in Auburn University's Department of Geosciences, using an NWR-193 excimer-based laser ablation system (Elemental Scientific, Bozeman, MT, USA) coupled to an Agilent Technologies 7900 Quadrupole IC-PMS system. Prior to otolith ablations, the IC-PMS system was tuned to ensure that oxidation matrix effects were within acceptable standards (Aries et al. 2000). To quantify elemental concentrations across the life of each fish, a linear transect was ablated from the focus of the otolith core to the otolith edge. Ablation paths

were set using ActiveView2 software (Electro Scientific Industries, Portland, Oregon, USA). To remove contaminants from the ablation path, pre-ablation transects were performed with a laser spot diameter of 45 micrometers (µm), a speed of 100 µm sec^{-1}, a laser-fire rate of 25 Hz, and 10% laser energy (Tabouret et al. 2010; Longmore et al. 2010). Analysis ablations were then performed with a laser spot diameter of 25 µm, a speed of 10 µm sec^{-1}, a laser-fire rate of 20 Hz, and 35% laser energy. The concentrations of ^{24}Mg, $^{43,44}Ca$, ^{55}Mn, ^{88}Sr, and ^{137}Ba were quantified (as the counts per second [cps]) using ICP-MS MassHunter software vB.04. Given the laser fire-rate and the number of element isotopes measured, cps data were output approximately every 0.084 seconds for each element.

A synthetic calcium carbonate (USGS MACS-3 [United States Geological Survey, Reston, VA, USA]) and a glass reference standard (NIST-612 [National Institute of Standards and Technology, Gaithersburg, MD, USA]) were used as certified reference materials (CRMs) in order to control for drift in measurements and confirm the accuracy and precision of the LA-ICP-MS analyses (Schuchert et al. 2010; Jochum et al. 2012; Phung et al. 2013). Duplicate runs of these CRMs and 'blank' (laser power 0%) transects were performed before the start of each analysis session and at least once during each subsequent hour of analysis ablations (Nelson et al. 2021).

Following laser ablations, the radius to each annulus was measured from the otolith core along the ablation transect using an image analysis system (Nikon NIS-Elements Ar). These measurements allowed for an approximate age of a fish to be determined at any given point within an otolith's microchemistry dataset using the proportional distance within each year of life.

Genomic DNA Extractions and Sequencing

Genetic analyses of Freshwater Drum, White Crappie, and Blue Catfish were performed in collaboration with Auburn University's Aquatic Genetics and Genomics Lab (AU-AGGL). Genomic DNA was extracted from all fish collected from the four river sections of the Alabama River (FWDR from CSA and TAL were not included in genetic analyses) using E.Z.N.A. Tissue DNA Kits (Omega Bio-Tek, Norcross, GA, USA). Subsets of samples from each DNA extraction batch were assessed for DNA quality by performing gel electrophoresis with 1 µL of each DNA sample in 0.8% agarose gel. DNA concentrations were quantified (in ng/µL) and recorded for all samples using an Invitrogen Qubit 3 fluorometer (Life Sciences, Waltham, MA, USA). Samples with DNA concentrations below 20 ng/µL had DNA re-extracted from remaining fin clip tissues to ensure that sufficient quantities of DNA were available for sequencing. Eight samples of both Freshwater Drum and White Crappie were sent to the University of Minnesota Genomics Center (UMGC) for optimization of the sequencing processes for these two species (established protocols already existed for Blue Catfish, as genotyping studies have previously been performed on this species). All DNA samples were then sent to UMGC for genotyping-by-sequence (GBS) using restriction site-associated DNA sequencing with an Illumina NovaSeq 6000 sequencing system coupled to an S1 flow cell. Genomic data analyses are currently in progress and the associated results are therefore not included in this thesis.

Data Processing and Statistical Analyses

Water Chemistry

The limits of detection for each element were automatically calculated within the Masshunter software prior to each analysis batch, following established procedures for the

calibration of solution-based ICP-MS systems (U.S. EPA 2007). Elements below the limits of detection in >50% of the water samples were removed from the final dataset and excluded from all subsequent statistical analyses. Element concentrations were converted to molar concentrations in water, and a molar element-of-interest to calcium ratio was calculated for all remaining trace-elements included in the water sample analyses. The element:Ca ratios for water samples were derived from the micromolar (element) and molar (calcium) concentrations of these elements in water, as calculated from the following formulas:

$$\frac{\left({}^{\text{ng g}^{-1}\text{ element}} \Big/ {}_{\text{atomic mass element (g mol}^{-1})} \right) / 1000}{{}^{1} \Big/ {}_{\text{atomic mass } H_2O\ (18.015\ \text{g mol}^{-1})}} = \frac{\mu\text{mol element}}{\text{mol } H_2O}$$

$$\frac{\left({}^{\text{ng g}^{-1}\text{ Ca}} \Big/ {}_{\text{atomic mass Ca (40.078 g mol}^{-1})} \right) / 10^{9}}{{}^{1} \Big/ {}_{\text{atomic mass } H_2O\ (18.015\ \text{g mol}^{-1})}} = \frac{\text{mol Ca}}{\text{mol } H_2O}$$

The resulting ratios were all reported in element (µmol) per Ca (mol) in the water samples, and these element:Ca ratios were used in all subsequent statistical analyses for water chemistry. Following conversions from ppb concentrations to element:Ca ratios, these data were tested for outliers using Grubbs' test (Grubbs 1950; Stefansky 1972) and one outlier was removed from the TALLA2 site in the fall 2020 season for Mn:Ca (sample z-score=7.950). Linear regressions between the element:Ca ratios and Alabama River kilometer were used to show how element concentrations changed spatially throughout the Alabama River. Means and standard errors for element:Ca ratios were calculated for each of the four river sections of the Alabama River, as well as the lower Coosa, and Tallapoosa rivers (i.e., the six river sections from which fish were collected). Individual element:Ca ratios from these six river sections were

compared and tested for temporal consistency across seasons using one-way analyses of variance (ANOVAs). For each of the two seasons that were sampled in both 2020 and 2021 (i.e., spring and summer), pairwise t-tests were used to test for longer-term temporal consistency in the univariate element/Ca ratios, with the data paired by site across the two sampling years. Spatial variability among river sections was tested for using analyses of covariance (ANCOVAs) with USGS discharge data used as a potential covariate accounting for effects of water level fluctuations. Data from a USGS gage below Claiborne L&D (USGS 02428400) was included as the daily mean discharge (in cubic ft./second) on the sample collection dates. The assumptions of normality and homogeneity of variance were examined on residuals using Shapiro–Wilk tests and Levene's tests, respectively. However, given that the Levene's test cannot be run with covariates, the discharge parameter was removed from the model prior to testing for homogeneity of variance. If necessary, the appropriate data transformations were performed. The majority of the models passed these assumption tests; however, some did fail, and in these cases the ANCOVAs were still performed given that they are robust to deviations from their assumptions and have greater power than nonparametric alternatives (Brownie and Boos 1994; Underwood 1997; Khan and Rayner 2003). In all cases differences among means were considered significant at alpha ≤ 0.05. For all comparisons in which significant differences were found, mean differences between groups were determined using Tukey's post hoc tests.

To assess variability between sampling years, paired t-tests were used to compare site-specific element:Ca ratios between samples collected in spring 2020 versus spring 2021, and between summer 2020 versus summer 2021. Coefficient of variation (CV) scores were also calculated for each element in each river section to show consistency of variability in samples

across the river sections of the study area, and to compare to the CV scores of the otolith microchemistry data.

To test for differences in water chemistry ratios among samples collected at varying depths, data from each multiple-depth water sampling site were grouped into three depth zones; subsurface (1 m below surface), benthic (within 1 m of the rivers' substrate), and pelagic (all samples between the subsurface and benthic zones). Multivariate analyses of variance (MANOVAs) were used to simultaneously determine if there were significant effects of depth zone on the four water chemistry ratios at each of the multiple-depth sampling sites. Additionally, multivariate multiple regressions were performed to test for an effect of depth (in meters below the surface) on the element:Ca ratios for each multiple-depth sampling site.

Otolith Microchemistry

Iolite v4.4 software (Paton et al. 2011) was used to process LA-ICP-MS data by synchronizing laser run-time files with the counts per second (cps) data acquired by the MassHunter software. Baseline cps data measured during blank LA-ICP-MS runs were used alongside known concentrations of the elements of interest in the CRMs in order to correct for drift in the accuracy of the IC-PMS system. Limits of detection calculations, instrument drift correction, background signal deductions and conversion of raw elemental cps data into concentrations (in parts per million [ppm]) were performed using the Trace Element IS data reduction scheme in Iolite v4.4. Ca was used as an internal standard held constant at 376900 ppm (i.e., 37.69% mass) for otoliths and the MACS-3 CRM (Longerich et al. 1996; Paton et al. 2011; Nelson et al. 2019; 2021), and the published 85002 ppm for the NIST-612 CRM (Jochum et al. 2007).

The limits of detection (LOD) for each element of interest were calculated for each sample using iolite v4.4 based on the following formula from Ludsin et al. (2006) (derived from Longerich et al. 1996).

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

Where σ_{bgd} = the standard deviation of the baseline cps signal; N_{bgd} and N_{pk} = replicate determinations used in the integration of the background and ablation signal, respectively; S = mean sensitivity (cps per unit concentration) for the NIST reference standard; and Y = 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; Ludsin et al. 2006). An element's concentration needed to be greater than three standard deviations above baseline levels (after correcting for ablation yield, instrument drift, and sensitivity) in order to be above the LOD (Table 2) (Ludsin et al. 2006). Otolith microchemistry transect data were filtered for the removal of outliers using a conservative technique in which outliers were defined as individual datapoints greater than four times the 75th percentile of the nearest 50 points within an element's profile for each individual fish. This resulted in the removal of transient outliers in the continuous elemental data that were likely the result of equipment noise, while also conserving trends in the data that appeared to have ecological relevance (McMillan et al. 2017; Nelson et al. 2021).

The ppm concentrations of the elemental data were converted into a molar ratio with the internal standard (Ca) being held constant at the published value of 376900 ppm (equal to μg g^{-1}) for fish otoliths (Paton et al. 2011; Nelson et al. 2019; 2021). These conversions were

performed using the following formula, where the resulting ratio values represented the micromoles of an element per moles of calcium.

$$\frac{\left(\mu g\ g^{-1}\ \text{element} \Big/ \text{atomic mass element (g mol}^{-1}) \right)}{\left(\mu g\ g^{-1}\ \text{internal standard} \Big/ \text{atomic mass Ca (40.078 g mol}^{-1}) \cdot\ 10^{-6} \right)} = \frac{\mu\text{mol element}}{\text{mol Ca}}$$

For each sample, mean element concentrations, standard deviations, and standard errors, were calculated for each element for each of the three otolith regions (i.e., whole-transect, 20 µm edge, and 20 µm core). Mean element concentrations for each sample's otolith region that were below the LODs were assigned an average concentration value of 0 ppm (Kratina 2019). The percentage of samples with mean otolith region values above the LODs are shown for each species in Table 2. Distances (in µm) along the profiles of each otolith ablation were determined by the laser scan speed (10 µm sec^{-1}) and the elapsed time from the start of each ablation run (Hamer et al. 2015).

Otolith Microchemistry Data Smoothing

Each sample's elemental data were passed through a local polynomial regression (LOESS), using the loess package in R with a span equal to 0.05 (Kratina 2019; Nelson et al. 2021). LOESS is a non-parametric smoothing technique that captures 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 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 LOESS span controls the rate at which the influence of points decreases with distance from the point of interest (Gibb et al. 2007), and low span values

ranging from 0.025 – 0.10 are commonly used in LA-ICP-MS analyses to smooth high frequency variations that are too fine to be used for environmental interpretation (Sinclair et al. 1998; Lowe et al. 2011; Nims and Walther 2014; McMillan et al. 2017). Smoothed time series element:Ca ratio profiles were subsequently used in all analyses identifying potential dam passages by fish.

Otolith Microchemistry Comparisons Among River Sections and Otolith Regions

MANOVAs were used to test differences in the mean multivariate chemical signatures from each otolith region among river sections from which fish were collected. Significant MANOVAs were followed by ANOVAs testing for differences for each element:Ca ratio mean among river sections. ANOVA model residuals were tested for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test respectively. If necessary, the appropriate data transformations were performed and transformed data were used in the final ANOVA model only if the resulting model's residuals exhibited an improvement upon both the normality and homogeneity of variance, versus the ANOVA model using the untransformed data. Given that ANOVAs are robust to deviations from their assumptions and more powerful than their nonparametric alternatives (i.e., Kruskal-Wallace or rank-sum approaches), untransformed data were used in the ANOVAs when data transformations could not improve upon both normality and homogeneity of variance. Significant ANOVAs were followed by Tukey's post hoc tests to identify significant differences between group means. Additionally, ANOVAs were used to test for differences among otolith regions for each of the element:Ca ratios. Significant ANOVAs were followed with pairwise t-tests between otolith core versus edge regions in order to determine if otolith cores were consistently enriched in any specific

elements that might be considered for use in subsequent analyses identifying potential dam passage events.

Otolith Edge & Water Chemistry Associations

Linear regressions were used to test for significant positive relationships between the mean season-specific water chemistry ratios of individual river sections from which fish were collected and the mean element:Ca ratios measured in the 20 µm edges of fish otoliths. For each species, univariate otolith-edge element:Ca ratios were regressed on the same element ratio's mean value derived from the season and river section in which a fish was collected. Individual-element regressions exhibiting positive and significant relationships between otolith edges and water chemistry provided evidence that spatial variation in those water element:Ca ratios manifest into fish otoliths, potentially providing relevant information regarding fish movements.

Discriminant Function Analyses

Discriminant function analyses were performed to determine how accurately fish could be classified into the river sections from which they were captured based on the multivariate signatures quantified in each of the three otolith regions. Specifically, three separate linear discriminant analyses (LDAs) were performed for each species, with each LDA collectively using the mean Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios from the 1) whole-transect, 2) 20 µm edge, or 3) 20 µm core region of each fish's otolith. For Freshwater Drum, two separate sets of LDAs were performed with one using only the four river sections of the Alabama River as the classification groups, and the second set of LDAs used the full six river sections from which Freshwater Drum were collected as the classification groups (four Alabama River sections plus

lower Coosa and Tallapoosa rivers). Classification accuracies and trends in misclassifications between river sections were examined for potential evidence of movement trends between river sections for each species.

Lifetime Strontium Profile Analyses

Given that Sr:Ca was the most informative among all element ratios analyzed (see Results), individuals within a species were grouped by river section and the lifetime Sr:Ca profile (continuous lifetime Sr:Ca data) of each fish was plotted to visually assess whether general movement patterns might exist, as well as to identify individual fish that may show evidence of potential dam passages. Criteria for identifying potential dam passages were created from the observed mean-edge Sr:Ca ratios of fish collected from each of the river sections within the Alabama River basin. For each species, 95% prediction intervals (sample mean ± 1.96 · SD) were created to determine river section-specific expected ranges of otolith Sr:Ca ratios from the observed otolith edge data. Mean-edge Sr:Ca ratios from each species were tested for normality, and when necessary, these data were transformed to improve normality prior to calculating the expected Sr:Ca ranges. The untransformed intervals were then used in the criteria for identifying potential dam passages and movements among river sections for each species. Given that a fish's river section of capture was known, the following passage criteria were applied to the Sr:Ca profiles of individual fish in a reverse chronological direction (i.e., from otolith edge to core). I defined potential passage events within fish lifetimes to be any scenario where all of the following conditions were met;

1. Within an individual ablation transect, the lifetime Sr:Ca ratio profile exceeded the otolith Sr:Ca expected range (in a positive or negative direction) of the river section from which that fish was collected, for a continuous transect distance equivalent to at least ½ annuli.
2. An individual Sr:Ca profile's section exceeding the expected Sr:Ca range, could not include the 20 µm edge region of a fish's otolith (occurring when the edge region of an otolith was outside the 95% prediction interval created from edge values of all fish collected from each river section [rare but possible]).
3. When the expected Sr:Ca range of the fish's river section of collection was exceeded, those data outside the expected range had to fall within the expected Sr:Ca range of an adjacent river section to be considered as evidence of a movement between river sections or a dam passage event.

For fish in which potential dam passage events were identified, their Sr:Ca profiles were then further evaluated by these criteria to identify additional potential dam passages (or movements) within their lifetimes. Fish collected from CSA, TAL, JBR, MFR or LAR could have Sr:Ca profile variations resulting from having spent time in areas outside of the main stem of the Alabama River. For example, JBR fish could have potentially made unimpeded movements to and from CSA or TAL (the tributaries that form the Alabama River), MFR fish could have made unimpeded movements into the Cahaba River (another major tributary), and LAR fish could have potentially made unimpeded upstream movements into the LAR within their lifetimes from the Mobile-Tensaw Delta or the Tombigbee River. Based on the water chemistry in the Coosa, Tallapoosa, Cahaba, Tombigbee and Tensaw rivers (and the observed Sr:Ca ratios of FWDR from CSA and TAL), movements outside of the main stem of the Alabama River would

likely influence the lifetime Sr:Ca profiles of individual fish and possibly result in the passage criteria identifying 'false passage' events for some fish. Accordingly, in specific scenarios, I interpreted the results of the lifetime Sr:Ca passage criteria as evidence of potential dam passages, as well as simultaneous evidence of potential unimpeded movements between the Alabama River and adjacent areas outside of its main stem. From these interpretations I reported the potential maximum number of fish within each species that could be considered to show evidence of potential dam passages and I specified the number of these potential dam passages that could have alternatively resulted from my passage criteria identifying potential false dam passages (i.e., unimpeded movements among river sections).

RESULTS

Water Chemistry

For water chemistry data collected from the main stem of the Alabama River, linear regressions between each element:Ca ratio and the Alabama River km (distance from river's terminus) showed clear trends in water chemistry ratios within the river. A significant relationship was found between the Mg:Ca ratio and rkm ($R^2 = 0.162$, $p = 0.003$) (Figure 3). A marginally significant relationship was found between the Mn:Ca ratio and rkm ($R^2 = 0.067$, $p = 0.058$) (Figure 3), and a highly significant relationship was found between both the Sr:Ca ratio and rkm ($R^2 = 0.363$, $p = <0.001$), and the Ba:Ca water chemistry ratio and rkm ($R^2 = 0.408$, $p = <0.001$) (Figure 3).

The four element:Ca ratios all exhibited spatial variation among all 15 sampling sites (Figure 4) as well as among grouped river sections (Figure 5), while also exhibiting temporal

consistency across seasons (Figure 6). When considering the water chemistry data for the six river sections from which fish were collected, all element:Ca ratios differed significantly among sites and river sections, but not seasons (Table 3). CV scores of all four water chemistry ratios in each of the six river sections indicated that variation in the element:Ca ratios was generally consistent across the six river sections from which fish were collected (Figure 7).

Given that Blue Catfish and White Crappie were collected from only the four river sections of the Alabama River (i.e., JBR, MFR, CLL, & LAR), I also tested for differences in the water element:Ca ratios among only these four river sections. Three of the four element:Ca ratios differed significantly among these river sections, with only Mn:Ca being not significant among the four sections of the Alabama River.

Comparisons Between Sampling Years

Pairwise t-tests of the element:Ca ratios for individual sites between sampling years indicated that most element:Ca ratios did not differ between sampling years. However, pairwise t-tests between spring 2020 versus 2021 indicated significant differences between both the Mg:Ca ratios and Sr:Ca ratios (Table 4). Neither Mn:Ca, nor Ba:Ca concentration ratios differed between spring seasons (Table 4). Pairwise t-tests between the summer 2020 versus 2021 indicated significant differences between Sr:Ca ratios between the two summer seasons (Table 4). The Mg:Ca, Mn:Ca, and Ba:Ca ratios did not differ between summer seasons (Table 4).

Comparisons Among Sample Depths

Element:Ca water chemistry ratios did not differ across depth zones (i.e., subsurface, pelagic, and benthic zones) at any of the three multiple-depth water sampling sites (ALR221

[MANOVA: $F_{2,15} = 0.115$, p = 0.998], ALR181 [MANOVA: $F_{2,21} = 0.047$, p = 0.999]; and TENSAW [MANOVA: $F_{2,21} = 0.995$, p = 0.455]). Multivariate multiple regressions also indicated no effect of depth (in absolute meters below the surface) on any of the element:Ca ratios at any of the multiple-depth sampling sites (Table 5).

Freshwater Drum

Fish Collections

Freshwater Drum were successfully collected from all four river sections of the Alabama River as well as from the lower Coosa and Tallapoosa rivers from June 2020 through January 2021 (Table 6). A total of 243 fish were used for otolith microchemistry analyses and 200 of these fish had fin clips taken (50 per main stem Alabama River section) for population genetic analyses. Freshwater Drum lengths and ages were mostly consistent across the six river sections, except the fish from the lower Coosa River were significantly longer (ANOVA: $F_{5,237} = 7.609$; p = <0.001) (Figure 8) and older (ANOVA: $F_{5,237} = 16.89$; p = <0.001) (Figure 9) than the five other river sections.

Otolith Microchemistry

Comparisons Among River Sections and Otolith Regions

MANOVAs simultaneously comparing the four mean whole-transect element:Ca ratios of Freshwater Drum among river sections indicated significant differences in the mean whole-lifetime element signatures among the six river sections from which the Freshwater Drum were collected (MANOVA: $F_{5,237} = 8.333$, p = <0.001). Significant differences were also found among fish from these river sections for both their mean-edge microchemistry signatures (MANOVA:

$F_{5,237}$ = 9.771, p = <0.001), and mean-core microchemistry signatures (MANOVA: $F_{5,237}$ = 5.011, p = <0.001).

Considering individual elements, mean Mg:Ca ratios differed significantly among river sections in the otolith whole-transect data (ANOVA: $F_{5,237}$ = 8.214, p = <0.001), otolith edges (ANOVA: $F_{5,237}$ = 4.01, p= 0.002), and otolith cores (ANOVA: $F_{5,237}$ = 3.706, p = 0.003) (Figure 10). Similarly, mean Mn:Ca ratios differed significantly among river sections in the otolith whole-transect data (ANOVA: $F_{5,237}$ = 10.82, p = <0.001), otolith edges (ANOVA: $F_{5,237}$ = 3.427, p = 0.005), and otolith cores (ANOVA: $F_{5,237}$ = 11.33, p= <0.001) (Figure 11). Mean Sr:Ca ratios also differed significantly among river sections in the otolith whole-transect data (ANOVA: $F_{5,237}$ = 15.48, p= <0.001), otolith edges (ANOVA: $F_{5,237}$ = 23.29, p = <0.001), and otolith cores (ANOVA: $F_{5,237}$ = 8.245, p = <0.001) (Figure 12). In contrast, otolith mean Ba:Ca ratios did not differ significantly among river sections in the whole-transect data (ANOVA: $F_{5,237}$ = 1.928, p = 0.090), but did differ significantly among river sections in the otolith edges (ANOVA: $F_{5,237}$ = 14.17, p = <0.001), and cores (ANOVA: $F_{5,237}$ = 6.348, p = <0.001) (Figure 13).

In testing for differences among the mean element:Ca ratio values derived from each of the three otolith regions of Freshwater Drum, all element:Ca ratios differed significantly among otolith regions (Mg:Ca: ANOVA: $F_{2,726}$= 719.1; p = <0.001; Mn:Ca: ANOVA: $F_{2,726}$= 303.3; p = <0.001; Sr:Ca: ANOVA: $F_{2,726}$= 28.83; p = <0.001; Ba:Ca: ANOVA: $F_{2,726}$= 13.61; p = <0.001) (Figure 14). Pairwise comparisons between individual fish's core and edge element:Ca ratios clearly indicated that three of the four element:Ca ratios (Mg:Ca, Mn:Ca, Ba:Ca) were consistently greater in the Freshwater Drum otolith cores versus the otolith edges, with only the Sr:Ca ratio having the opposite pattern of being significantly greater in the otolith edges

versus the otolith cores (Figure 14) (Mg:Ca: paired t_{242} = 29.686; p = <0.001; Mn:Ca: paired t_{242} = 20.13; p = <0.001; Sr:Ca: paired t_{242} = -7.459; p = <0.001; Ba:Ca: paired t_{242} = 2.872; p = 0.004). Whole-transect element:Ca ratios were intermediate between those of otolith cores and edges (Figure 14).

Otolith Edge & Season-of-Capture Water Chemistry Associations

Freshwater Drum otolith edge Sr:Ca ratios were positively related to the season-specific water Sr:Ca ratio in the fish's river section of capture (R^2 = 0.094, p = <0.001) (Figure 15). Interestingly, otolith edge Mg:Ca ratios were negatively related to the season-specific water Mg:Ca ratios in the fish's river section of capture (R^2 = 0.031, p = 0.006) (Figure 15). Regressions for otolith edge Mn:Ca (R^2 = 0.001, p = 0.735), and Ba:Ca (R^2 = 0.007, p = 0.184) ratios versus season-specific water chemistry were not significant (Figure 15).

Discriminant Function Analyses

For Freshwater Drum, two separate sets of LDAs were performed, with the first performed using only the 200 fish collected across the four sections of the Alabama River and the four classification groups being these four river sections. The second set of LDAs used all Freshwater Drum, including those 200 fish plus the 23 and 20 fish collected from the Coosa and Tallapoosa rivers, respectively. This second set of LDAs used six classification groupings, those being the lower Coosa and Tallapoosa rivers plus the four sections of the Alabama River.

The first set of LDAs using the multivariate element:Ca means from the three otolith regions (whole transects, 20 µm edges, and 20 µm cores) of the 200 Freshwater Drum collected across the four sections of the Alabama River, resulted in overall classification accuracies of 45.5-55.5% (Table 7). Individual element:Ca coefficients associated with each linear

discriminant (Table 8) indicated that the Sr:Ca ratio played the largest role in discriminating among the four river sections of the Alabama River, regardless of the otolith region data being used in the LDA.

The second set of LDAs using the multivariate element:Ca means from the three otolith regions (whole transects, 20 µm edges, and 20 µm cores) of all 243 Freshwater Drum collected across the six sections, resulted in overall classification accuracies of 42.8-51.9% (Table 9). Individual element:Ca coefficients associated with each linear discriminant (Table 10) indicated that the Sr:Ca ratio played the largest role in discriminating among the six collection areas, regardless of the otolith region data being used in the LDAs.

Lifetime Strontium Profile Analyses

The lifetime strontium profiles of Freshwater Drum indicated that there was very little evidence that Freshwater Drum routinely moved among river sections of the Alabama River that are separated by dam structures. Lifetime Sr:Ca profiles for most Freshwater Drum tended to remain within the expected Sr:Ca ratio ranges (Figure 16) of the river sections in which individual fish were collected (e.g., Figures 17 and 18). According to the dam passage criteria I used here, 235 of the 243 total Freshwater Drum collected did not exhibit evidence of dam passages within their lifetimes. A total of only 8 individual Freshwater Drum Sr:Ca profiles showed evidence of a total of 9 potential dam passages events. Of these 9 potential dam passages, 4 were identified as potential downstream passages across dams by 4 unique fish (2 at R.F. Henry L&D [e.g., Figure 19] and 2 at Millers Ferry L&D). Given that 2 of these 4 fish were collected in MFR, their increase in the Sr:Ca ratio that met the passage criteria for a potential downstream passage across R.F. Henry L&D, may have alternatively resulted from these fish

exiting the Cahaba River to move into MFR during their lifetimes. The 2 fish collected from CLL are not suspected to have resulted from movements outside of the main stem of the Alabama River. Five of the 9 total passages were identified as potential upstream passages across dams by 4 unique fish. According to the passage criteria used, 1 of these fish (FZ370 [Figure 20]) exhibited evidence of potential upstream dam passages at both Millers Ferry L&D and R.F. Henry L&D. Given that all 4 of the fish exhibiting evidence of potential upstream dam passage events were collected in either JBR or CSA, the portions of their lifetime Sr:Ca profiles satisfying the passage criteria could have alternatively (and possibly more-likely) resulted from unimpeded movements outside of the main stem of the Alabama River (i.e., potential movements among TAL, CSA, and JBR). However, the lifetime Sr:Ca profile of 1 Freshwater Drum (FZ370) exceeded the upper limit of the expected range of TAL (see Figures 16 and 20), potentially suggesting that this fish had actually completed two upstream dam passages, having potentially moved from Claiborne Lake past both Millers Ferry L&D and R.F. Henry L&D, and upstream into the lower Coosa River where it was collected.

Applying the passage criteria to fish from the CSA and TAL river sections suggest that this species may exhibit a strong tendency to disperse from their natal areas when movements are not obstructed by dams. Using the same criteria used for identifying dam passages, of the 43 Freshwater Drum collected between CSA and TAL, 25 of these fish exhibited evidence of potential unimpeded movements among CSA, TAL and JBR within their lifetimes. Further, 19 of the 20 fish collected from TAL met the criteria such that they were suspected to have moved upstream into TAL from either CSA or JBR (e.g., Figure 21).

White Crappie

Fish Collections

White Crappie were successfully collected from the four Alabama River sections from June 2020 through October 2020. A total of 200 fish were used for otolith microchemistry analyses and 196 of these same fish were included in the genetic analyses (4 DNA samples failed QA/QC at UMGC) (Table 6). White Crappie lengths and ages were similar across three of the four river sections, with fish from Claiborne Lake being significantly shorter (ANOVA: $F_{3,196}$ = 12.09; p = <0.001) (Figure 22). Despite this, mean ages of the collected White Crappie did not differ significantly among river sections (ANOVA: $F_{3,196}$ = 2.57; p = 0.055) (Figure 23).

Otolith Microchemistry

Comparisons Among River Sections and Otolith Regions

MANOVAs simultaneously comparing the four mean-whole-transect element:Ca ratios of White Crappie among river sections indicated significant differences among river sections (MANOVA: $F_{3,196}$ = 17.691, p = <0.001). Significant differences were also found among river sections for the mean-edge microchemistry signatures (MANOVA: $F_{3,196}$ = 19.234, p = <0.001), as well as the mean-core microchemistry signatures (MANOVA: $F_{3,196}$ = 8.576, p = <0.001).

Considering individual elements, mean Mg:Ca ratios differed significantly among river sections in the whole-transect data (ANOVA: $F_{3,196}$ = 7.026, p = <0.001), otolith edges (ANOVA: $F_{3,196}$ = 2.737, p = 0.045), and otolith cores (ANOVA: $F_{3,196}$ = 3.604, p= 0.014) (Figure 24). Mean Mn:Ca ratios differed significantly among river sections in whole-transect data (ANOVA: $F_{3,196}$ = 5.497, p = 0.001), otolith edges (ANOVA: $F_{3,196}$ = 6.621, p = <0.001), and otolith cores (ANOVA: $F_{3,196}$ = 5.638, p = 0.001) (Figure 25). Mean Sr:Ca ratios differed among river sections in whole-

transect data (ANOVA: $F_{3,196}$ = 86.52, p = <0.001), otolith edges (ANOVA: $F_{3,196}$ = 147.3, p = <0.001), and otolith cores (ANOVA: $F_{3,196}$ = 27.27, p = <0.001) (Figure 26). Mean Ba:Ca ratios differed among river sections in whole-transect data (ANOVA: $F_{3,196}$ = 7.284, p = <0.001), otolith edges (ANOVA: $F_{3,196}$ = 13.47, p = <0.001), and otolith cores (ANOVA: $F_{5,237}$ = 4.021, p = 0.008) (Figure 27).

In testing for differences among the mean values derived from each of the three otolith regions of White Crappie, all element:Ca ratios differed significantly among otolith regions (Mg:Ca: ANOVA: $F_{2,597}$= 731.6; p = <0.001; Mn:Ca: ANOVA: $F_{2,597}$= 93.72; p = <0.001; Sr:Ca: ANOVA: $F_{2,597}$= 28.97; p = <0.001; Ba:Ca: ANOVA: $F_{2,597}$= 122.7; p = <0.001) (Figure 28). Pairwise comparisons between individual fish's core and edge element:Ca ratios indicated that all four of the element:Ca ratios were consistently greater in the White Crappie otolith cores versus the otolith edges (Figure 28) (Mg:Ca: paired t_{199} = 29.217; p = <0.001; Mn:Ca: paired t_{199} = 11.124; p = <0.001; Sr:Ca: paired t_{199} = 9.511; p = <0.001; Ba:Ca: paired t_{199} = 13.9; p = <0.001). Whole-transect element:Ca ratios were intermediate between those of otolith cores and edges (Figure 28).

Otolith Edge & Season-of-Capture Water Chemistry Associations

Linear regressions between White Crappie otolith-edge element:Ca ratios and the season-specific mean water element:Ca ratios of each fish's river section of capture, showed that the otolith-edge Mg:Ca (R^2 = 0.019, p = 0.050) and Sr:Ca (R^2 = 0.623, p = <0.001) ratios had significant, positive associations with their respective ratios in the water chemistry in a fish's river section of capture (Figure 29). Otolith edge Ba:Ca ratios were negatively related to the season-specific water Ba:Ca ratios in the fish's river section of capture (R^2 = 0.126, p = <0.001)

(Figure 29). The regression for otolith edge Mn:Ca ratios versus season-specific mean Mn:Ca ratios in a fish's river section of capture was not significant ($R^2 = 0.012$, $p = 0.122$) (Figure 29).

Discriminant Function Analyses

LDAs using the multivariate element:Ca means from the three otolith regions (whole transects, 20 µm edges, and 20 µm cores) of the 200 White Crappie collected across the four sections of the Alabama River resulted in overall classification accuracies of 54.0-70.5% (Table 11). Individual element:Ca coefficients associated with each linear discriminant (Table 12) indicated that the Sr:Ca ratio played the largest role in discriminating among the four collection areas, regardless of the otolith region data being used in the LDAs.

Lifetime Strontium Profile Analysis

White Crappie lifetime Sr:Ca profiles patterns indicated that dam passage events, and large movements in general, were very rare for these fish. A typical pattern in the Sr:Ca profile of White Crappie was identified as a general decrease in the Sr:Ca ratio with increased distance from the otolith's core (e.g., Figure 31). This pattern was observed almost universally among the White Crappie analyzed and it was common among fish collected from all four river sections of the Alabama River. As a result of the White Crappie otolith cores being enriched in strontium (Figure 28), the dam passage criteria I used for other species could not be applied to this species, as this would inherently resulted in substantial overestimates in the number of fish showing evidence of dam passages. Figure 31 shows how the typical pattern observed in the Sr:Ca profiles of White Crappie would have exceeded the expected Sr:Ca range (Figure 30) in a positive direction, which would have met the criteria used for identifying potential dam passages.

Despite this, several White Crappie exhibited unique lifetime Sr:Ca profile patterns, possibly indicating some limited evidence of within-lifetime movements. Specifically, 4 White Crappie collected from CLL and 1 fish from LAR had early-life Sr:Ca values sustained at exceptionally high ratios above 3500 µmol/mol. All five of these fish also exhibited large shifts in their lifetime Sr:Ca profiles to more-typical Sr:Ca ratio levels (i.e., 500 – 1500 µmol/mol) (e.g., Figure 32). Given that the majority of White Crappie exhibited very little indication of movement, and that 4 of these 5 fish were collected within CLL, I suspect that all 5 of these fish may have originated in an unknown location within CLL (possibly a backwater area) with an enriched Sr:Ca water chemistry ratio, and moved to their capture locations within their lifetimes. If this were the case, this would indicate that the 1 fish exhibiting this pattern collected from the LAR (<1 km downstream of Claiborne L&D), might have completed a downstream passage across Claiborne L&D within its lifetime (Figure 33).

Blue Catfish

Fish Collections

Blue Catfish were collected from the four river sections of the Alabama River from June 2020 through March 2021. A total of 25 Blue Catfish were collected and used for both otolith microchemistry analyses and population genetic analyses (ranging 5-8 fish per Alabama River section) (Table 6). Blue Catfish lengths and ages were generally consistent across the four river sections (lengths: ANOVA: $F_{3,21} = 3.01$; $p = 0.053$) (Figure 34) (Ages: ANOVA: $F_{3,21} = 2.31$; $p = 0.106$) (Figure 35).

Otolith Microchemistry

Comparisons Among River Sections and Otolith Regions

MANOVAs simultaneously comparing the four mean whole-transect element:Ca ratios of Blue Catfish among river sections indicated that there were no significant differences in the mean whole-transect element signatures among the four river sections of the Alabama River (MANOVA: $F_{3,21} = 1.209$, $p = 0.299$), and similarly there was no difference in their mean-core microchemistry signatures (MANOVA: $F_{3,21} = 1.721$, $p = 0.085$). In contrast, significant differences were found among river sections for the mean-edge microchemistry signatures of these fish (MANOVA: $F_{3,21} = 2.601$, $p = 0.007$).

For the mean-edge element:Ca ratios of Blue Catfish, both Mg:Ca and Sr:Ca ratios differed significantly among the fish collected from the four river sections of the Alabama River (Mg:Ca: ANOVA: $F_{3,21} = 8.826$, $p = <0.001$ [Figure 36]; Sr:Ca: ANOVA: $F_{3,21} = 4.925$, $p = 0.010$ [Figure 38]). Neither Mn:Ca nor Ba:Ca ratios differed significantly among river sections in the Mean-edge data (Mn:Ca: ANOVA: $F_{3,21} = 2.128$, $p = 0.127$ [Figure 37]; Ba:Ca: ANOVA: $F_{3,21} = 2.786$, $p = 0.066$ [Figure 39]). In both the whole-transect and core data, none of the four elements differed significantly among river sections (Figures 36–39).

Testing for differences among the mean values derived from each of the three otolith regions of Blue Catfish indicated that Mg:Ca ratios (ANOVA: $F_{2,72} = 24.45$; $p = <0.001$), Mn:Ca ratios (ANOVA: $F_{2,72} = 27.29$; $p = <0.001$), and Ba:Ca ratios (ANOVA: $F_{2,72} = 26.09$; $p = <0.001$) differed significantly among otolith regions (Figure 40). There were no significant differences in mean Sr:Ca ratios among otolith regions (ANOVA: $F_{2,72} = 0.133$; $p = 0.876$) (Figure 40). Pairwise comparisons between individual fish core and edge element:Ca ratios clearly indicated that

three of the four element:Ca ratios were consistently higher in the Blue Catfish otolith cores versus the otolith edges, with only the Sr:Ca ratio having no statistically significant difference between these two otolith regions (Figure 40) (Mg:Ca: paired t_{24} = 5.486; p = <0.001; Mn:Ca: paired t_{24} = 6.822; p = <0.001; Sr:Ca: paired t_{24} = -0.380; p = 0.707; Ba:Ca: paired t_{24} = 5.622; p = <0.001). Whole-transect element:Ca ratios were intermediate between those of otolith cores and edges (Figure 40).

Otolith Edge & Season-of-Capture Water Chemistry Associations

Linear regressions between Blue Catfish otolith-edge element:Ca ratios and the season-specific mean water element:Ca ratios of each fish's river section of capture, showed that the otolith-edge Sr:Ca (R^2 = 0.353, p = 0.002) and Ba:Ca (R^2 = 0.355, p = 0.002) ratios had significant, positive associations with the water chemistry in a fish's river section of capture (Figure 41). Otolith edge Mg:Ca ratios were negatively related to the season-specific water Mg:Ca ratios in the fish's river section of capture (R^2 = 0.212, p = 0.021) (Figure 41). The regression for otolith edge Mn:Ca ratios versus season-specific mean Mn:Ca ratios in a fish's river section of capture was not significant (R^2 = 0.049, p = 0.288) (Figure 41).

Discriminant Function Analyses

Linear discriminant analyses using the multivariate element:Ca means from the three otolith regions; whole transects, 20 µm edges, and 20 µm cores of the 25 Blue Catfish collected across the four main sections of the Alabama River resulted in overall classification accuracies of 48.0-72.0% (Table 13). Individual element:Ca coefficients associated with each linear discriminant (Table 14) indicated that the Sr:Ca ratio generally played the largest role in discriminating among the four collection areas in the LDAs using the whole transect and otolith

edge data. However, coefficients associated with the Ba:Ca ratio were considerably higher than the other three element:Ca ratios in the Blue Catfish core region LDA (Table 14), suggesting that the Ba:Ca ratio played the largest role in discriminating among river sections, based on the otolith core data.

Lifetime Strontium Profile Analyses

Lifetime Sr:Ca profiles for almost all Blue Catfish tended to remain within the expected Sr:Ca ranges (Figure 42) of the river sections in which individual fish were collected (e.g., Figure 43), with 20 of the 25 total catfish collected exhibiting no evidence of dam passage. According to the passage criteria I used, a total of 5 individual Blue Catfish Sr:Ca profiles exhibited evidence of 5 potential dam passages, all of which would have potentially occurred across Claiborne L&D in the downstream direction (e.g., Figure 44). Given that all 5 of these fish were collected in the LAR, the variations observed within their Sr:Ca profiles may have alternatively resulted from unimpeded movements into the Mobile-Tensaw River Delta, or the Tombigbee River, rather than having been the result of downstream passages across Claiborne L&D. According to the passage criteria used, 0 Blue Catfish were suspected to have performed upstream dam passages, and of the 5 Blue Catfish suspected of potentially having performed downstream dam passages, none of these fish were suspected of having performed multiple dam passages within their lifetimes.

DISCUSSION

Water Chemistry

Water chemistry ratios throughout the Alabama River basin and the lower portions of neighboring systems (i.e., the Tombigbee and Tensaw rivers) to varied spatially but were temporally consistent. This is consistent with other recent water chemistry findings from this system by Kratina (2019). These findings are important for the interpretation of fish movements, given that spatial variability and temporal consistency of the water chemistry are key components of microchemistry studies focused on quantifying the lifetime habitat use of fish (Campana et al. 1994, 1995; Elsdon et al. 2008; Gunn et al. 2019). The spatial variation in water chemistry, along with temporal consistency, facilitates my attribution of shifts in otolith chemistry to movement by fish, rather than fluctuations in element concentrations in the water.

Spatial patterns in water chemistry signatures within the Alabama River system were similar to those reported by Kratina (2019). Within the main stem of the Alabama River, Sr:Ca and Ba:Ca water chemistry ratios showed the strongest and most-significant longitudinal trends. Sr:Ca ratios in the Alabama River tended to decrease with distance upstream from estuarine and brackish areas (i.e., the Mobile-Tensaw River Delta), and Ba:Ca ratios increased with distance upstream. These findings are consistent with the water chemistry patterns typically observed in coastal river systems (Ingram and Sloan 1992; Elsdon and Gillanders 2002, 2003).

Water chemistry signatures were consistent across depths at three locations with relatively lower flow rates (i.e., sites most likely to be vertically stratified). No statistically

significant differences were found among the multivariate signatures of the subsurface, pelagic, or benthic zones at these sites, suggesting that water remains mixed throughout the water column of the river's main channel. Accordingly, subsurface (1 m depth) water samples appear to provide a good representation of the entire water column in these areas, and thus are also representative of the ambient water chemistries encountered by fish across depths of the main channel. Additional sampling of multiple depth zones in more-lentic backwater areas, especially those areas fed by tributaries, would be required to establish whether seasonal chemoclines exist in these habitats, which could influence incorporation of trace elements into the otoliths of fish as they potentially move among these habitats. Vyverman (1994) found that shallow (<20 m depth) water masses adjacent to the main river channel can exhibit chemical and thermal stratification. However, they found stratification regimes varied greatly between water masses in the same floodplain, likely depending on hydrology, basin morphometry, and landscape topography (Vyverman 1994). More research is needed to better understand how the vertical stratification of water masses could influence water chemistry ratios across depths in freshwater systems, and how chemical and thermal stratification may influence the incorporation of water chemistry ratios into freshwater fish otoliths.

Otolith Microchemistry

Mean element:Ca ratios derived from the whole-transect, edge, and core otolith regions, generally exhibited differences among river sections where fish were collected for Freshwater Drum and White Crappie. Similar patterns were also observed among river sections for most elements in Blue Catfish, although statistically significant differences among river sections were less common, likely due to the smaller sample sizes. For all three species, otolith

element:Ca ratio patterns across the river sections appeared to show the same general patterns observed in the water chemistry for the Sr:Ca and Ba:Ca ratios, while the Mg:Ca and Mn:Ca ratios exhibited patterns dissimilar to what would be expected based on the relative water chemistries of the river sections.

Of the four element:Ca ratios measured, Sr:Ca had best agreement between the water chemistry and otolith microchemistry across river sections. Both water chemistry and otolith Sr:Ca ratios in all three species (and all three otolith regions within each species) tended to be greater in downstream river sections. However, there was an interesting exception to this trend that was observed in all three species, and among most otolith regions, whereby the Sr:Ca ratios in otolith edge regions from the LAR were relatively low, and in some cases significantly lower than for fish collected from CLL. Given that Sr:Ca ratios in the otoliths would be expected to be relatively higher in the LAR based on water chemistry, it is unclear why the observed Sr:Ca ratios in the edges of otoliths of all three species from LAR were on average most similar to those of JBR and MFR. As observed in numerous otolith microchemistry studies in coastal areas (e.g., Howland et al. 2001; Avigliano and Volpedo 2013), Sr:Ca ratios in otoliths typically increase as fish are closer to coastal and estuarine environments. The disconnect I observed between water and otolith Sr:Ca ratios in the LAR could be due to fish using local habitats such as backwaters that may have lower Sr:Ca concentrations in the water versus the main channel. My water chemistry sampling did not include backwater areas, and water chemistry in these areas may differ from that in the main channel.

The relatively high element:Ca ratios observed in the Tallapoosa River water chemistry were consistent with findings by Kratina (2019), resulting from relatively low Ca concentrations

in the water. Otolith edge data from Freshwater Drum clearly showed that the high Sr:Ca ratio in the Tallapoosa River was reflected in otoliths of fish collected in this river. Interestingly, Freshwater Drum collected from the Tallapoosa River had otolith-core Sr:Ca values much lower than their edge values, suggesting that these fish may have originated from other areas (possibly JBR or CSA) and moved upstream into the Tallapoosa River (i.e., among areas not separated by dams). This was also supported by the lifetime Sr:Ca profiles of these fish, and the same may be true for Freshwater Drum collected from the lower Coosa River. The considerable overlap between the expected Sr:Ca ratios of CSA and JBR for Freshwater Drum make it difficult to identify potential movements between these areas versus fish movements into the lower Tallapoosa River. The possibility of Freshwater Drum that were collected in the lower Tallapoosa River potentially originating from downstream areas suggests that Freshwater Drum may be able to make substantial upstream movements when these movements are not impeded by dams.

Rypel (2004) found evidence supporting partial migration of Freshwater Drum, whereby females were considered to be highly motile compared to five other warmwater species (Channel Catfish *Ictalurus punctatus*, Largemouth Bass *Micropterus salmoides*, Spotted Bass *Micropterus punctulatus*, Striped Bass *Morone saxatilus*, and Black Crappie) and their respective sexes, while male freshwater drum were determined to be the most sedentary of any species or gender examined (Rypel et al. 2004). My findings support the relatively high motility of female Freshwater Drum, given that most Freshwater Drum collected from the lower Coosa and Tallapoosa rivers were female (22/23 in CSA and 14/20 in TAL), and 19 of the 25 fish found by the passage criteria to have potentially made movements among JBR, CSA, and TAL also were

female. Further, Rypel (2007) described sexual dimorphism in Freshwater Drum in which females grew faster and attained larger sizes. The skewed sex ratios I observed in Freshwater Drum collected from the lower Coosa and Tallapoosa rivers (esp. the Coosa R.) likely resulted in the differences I observed between the age and length distributions of these fish versus those in the Alabama River. The skewed sex ratios and skewed length and age distributions observed in the Tallapoosa and Coosa rivers (vs. the Alabama River) may also result from the lack of young recruits in these areas due to Freshwater Drum larvae drifting downstream towards the Alabama River.

The expected patterns of otolith element:Ca core data across river sections appeared to be somewhat obscured due to enriched concentrations of some elements in otolith cores. Comparisons among otolith regions clearly showed that all three species exhibited core enrichment in 3 or 4 of the element:Ca ratios. Despite the enrichment of some elements within otolith cores, general patterns that were observed across river sections in the otolith edges were also still apparent in the core data for some elements (e.g., Mn:Ca in Freshwater Drum, and Sr:Ca in White Crappie).

The enrichment of specific elements in otolith core regions has been described previously (e.g., Ruttenberg et al. 2005; Chittaro et al. 2006; Macdonald et al. 2020). Mechanisms by which core enrichment takes place are complex and the elements that become enriched within otolith cores can vary across species (Ruttenberg et al. 2005). It is generally accepted that otolith core enrichment results from and is influenced by numerous factors during the ontogeny of a fish (Macdonald et al. 2020). These factors may include physiological changes associated with fish age (Chittaro et al. 2006; Grammer et al. 2017), growth (Sadovy

and Severin 1994; Stanley et al. 2015), sex (Sturrock et al. 2014, 2015), metabolic activity (Høie et al. 2003; Izzo et al. 2018), diet (Ranaldi and Gagnon 2008), and genetic components (Clarke et al. 2011).

Core enrichment complicates analysis of lifetime elemental data derived from otoliths for the purposes of identifying natal origins and lifetime movements of fish (Ruttenberg et al. 2005; Chittaro et al. 2006). For White Crappie, all 4 of the element ratios I studied were enriched within the otolith cores. I found that enrichment of the Sr:Ca ratio in the White Crappie otoliths persisted beyond the 20 µm core region, with this ratio generally declining steadily from an otolith's core to edge. For this reason, the use of Sr:Ca ratio profiles was deemed unsuitable for quantifying lifetime White Crappie movements. Further research into the ontogenetic effects on early-life otolith microchemistries of my study species could potentially allow for additional inference into their lifetime movements by accounting for ontogenetically-sourced enrichment of specific elements in otoliths.

As previously observed in numerous microchemistry studies of freshwater fishes (Brown and Severin 2009), Sr:Ca ratios were most highly correlated between otolith edges and water chemistry, relative to other element:Ca ratios. This finding is consistent with previous research using Sr:Ca to reconstruct life histories of freshwater fish (Hedger et al. 2008; Snyder et al. 2022). Kratina (2019) similarly found the Sr:Ca ratio to be the most useful elemental ratio for assessing the movements of Smallmouth Buffalo and Paddlefish in this system.

The LDAs I performed using the four element:Ca ratios derived from each of the three otolith regions classified fish back to their correct river sections of capture with varying accuracies among species and otolith regions within each species. Overall, the LDA classification

accuracies that I observed here were similar to, and in some cases higher than, those Kratina (2019) observed for Paddlefish, Smallmouth Buffalo, and Largemouth Bass in this system. I expected LDAs using the otolith edge data to yield the highest classification accuracies given that the most recent otolith growth (otolith edge region) would be expected to best associate with the areas from which the fish were collected (Miller 2007). LDAs using the otolith edge data resulted in the highest classification accuracies among otolith regions for White Crappie and Blue Catfish, but the LDAs using the whole-transect data resulted in the highest classification accuracies among otolith regions for both sets of Freshwater Drum LDAs. Generally lower overall classification accuracies resulting from the otolith edge LDAs indicates that differentiating between river sections based upon the multivariate otolith microchemistry signatures of these species is difficult. However, similar classification accuracies between the otolith edge and whole-transect LDAs for Freshwater Drum and White Crappie may further suggest that these data do not show evidence of movement trends across dams. I suspect that dissimilar classification accuracies between otolith edge (72%) and whole-transect (48%) LDAs of Blue Catfish are likely the result of small sample sizes for this species. However, if additional Blue Catfish otoliths were analyzed and this pattern remained, this could indicate movement potential for this species.

Multivariate mean data derived from the 20 µm core regions of Freshwater Drum and White Crappie resulted in distinctly lower accuracies in LDA classifications relative to the whole-transect and 20 µm edge regions for these two species, most likely due to otolith core regions being enriched in certain elements, versus actual fish movement from natal areas. Enrichment of elements within the otolith core region may cause the otolith core signatures to be more

homogenous across river sections, thus resulting in these LDAs being less effective at modeling the multivariate differences between river sections (as compared to the whole-transect and edge LDAs).

The LDA of the Blue Catfish core region yielded the highest classification accuracy (60%) from otolith cores among the three study species, and the accuracy was considerably higher than the accuracy of the Blue Catfish's whole-transect LDA (48%). It is also interesting to note that the Ba:Ca ratio played a much larger role in the core region LDA versus the edge and whole-transect LDAs for this species, or versus any of the other species/otolith region LDAs. Again, these findings must be viewed with a measure of caution because of the small sample size of Blue Catfish. Analysis of additional Blue Catfish samples may potentially yield differing results.

Sr:Ca ratio values were most important in the LDAs (except for the Blue Catfish core region LDA), again supporting that strontium is the most informative element in discriminating among fish from different areas of the Alabama River basin, and thus the most useful for potentially identifying individual fish movements among these areas. No clear patterns existed in misclassifications among river sections in any of the LDAs, indicating that there is no evidence of large trends in fish movements among any of the river sections.

For Freshwater Drum and Blue Catfish, most lifetime Sr:Ca profiles remained within the expected Sr:Ca ranges of a fish's river section of capture, further suggesting that most of these fish are not making large-scale movements that include dam passages. However, large overlaps in the expected Sr:Ca ranges across river sections (for each species) resulted from fish across most river sections exhibiting generally similar distributions of otolith edge Sr:Ca ratio values,

thus limiting the ability to identify dam passage events based solely on the lifetime Sr:Ca profiles. While significant differences in mean otolith edge Sr:Ca values occurred between some river sections for each species, the high degree of similarities in the expected ranges of Sr:Ca values derived among all river sections resulted from the high levels of variation observed in otolith edge Sr:Ca values within each river section. The high within-river section variation in observed (and thus also expected) Sr:Ca ratios in fish otoliths, may have resulted from these fish using a variety of habitat types, including backwater areas, side channels and creek mouths that potentially have very different water chemistry than the main channel.

Reconstructing more-detailed environmental histories of individual fish in the Alabama River basin using otolith microchemistry would likely require more-extensive sampling of both fish and water among all types of available habitats, particularly for species that are less mobile and therefore might frequent these other locations more often relative to large riverine swimmers such as Paddlefish. Collecting water samples at finer spatial scales and within backwater areas and small tributaries, could reveal insights into the variability among water chemistries that could be encountered by fish making small movements between the main channel and adjacent habitats. Variation in water calcium concentrations can also have an important influence on otolith element:Ca ratios (Hamer et al. 2015), and the calcium concentrations within these backwater areas could differ significantly versus the main channel (as was seen in the Tallapoosa River). Water chemistry signatures in backwater areas may also vary seasonally (not seen in the main channel) due to main-channel flooding events that periodically inundate these areas, thus altering the relative inputs of creeks and small tributaries into these backwaters. Variability in water chemistry of backwater areas would

certainly influence otolith microchemistry signatures of fish using these areas and may reduce the overall accuracy of discriminations among fish from each river sections and limit the ability to identify dam passage events using patterns of otolith microchemistry.

Management Implications for Fish Passage

My otolith microchemistry results generally suggest that dam passages across the three lock-and-dam structures of the Alabama River are likely limited for these species. The data I present here indicate some evidence of potential downstream dam passages by Freshwater Drum, White Crappie, and Blue Catfish, but no clear evidence of any upstream passage for White Crappie and Blue Catfish, and only limited evidence for upstream dam passages by Freshwater Drum. However, the criteria I used to identify potential dam passages by individual fish was a conservative approach that may be underestimating the number of fish moving among river sections within their lifetimes. The inclusion of genetic analyses and the potential future use of direct methods of tracking fish movements (e.g., tagging and tracking) would provide further insight into the dam passage rates and movement trends of the relatively smaller-bodied and shorter-lived fish species of the Alabama River.

Overall, the otolith microchemistry data suggest that relatively smaller-bodied species in the Alabama River may derive some benefit from additional passage opportunities at these three dams. Options for increasing passage opportunities at the Alabama River dams range from relatively low-cost targeted nonnavigational lock operations (Simcox et al. 2015), to the installation of bypass channels and fish passage structures (e.g., fish ladders), or trap-and-haul operations (Roscoe and Hinch 2010). In recent years, the construction of bypass channels designed to mimic natural habitats, have shown encouraging signs of allowing for the successful

upstream and downstream passages of fish with a wide range of swimming abilities (e.g., Calles and Greenberg. 2007, Nyqvist et al. 2018; Tupen 2020). However, the design and construction of bypass channels of this type are generally very costly, and these projects require considerable amounts of time for site evaluations prior to implementation (Clay 1994; Calles and Greenberg 2007; Roscoe and Hinch 2010). Discussions of potential restoration options on the lower Alabama River continue, with feasibility studies having been initiated to consider a diversity of passage scenarios. Additional research into the effects of the Alabama River dams on fish populations is necessary to best inform any future management decisions regarding fish passage.

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TABLES

Table 1. Water sample collection site information, including names, river section, depths, general descriptions, site coordinates and river kilometers. Ranges of overall sampling depths are shown for the three multiple-depth water sampling sites.

Site Name	River Section	Sample Depth(s)	Site Description	Site Coordinates	River km
COOSA	CSA	1 m	Coosa R. downstream of Wetumpka, AL	32.526149, -86.222694	Coosa R. rkm 6.5
TALLA2	TAL	1 m	Tallapoosa R. 2.9 km downstream of Thurlow Dam	32.509637, -85.890835	Tallapoosa R. rkm 74.0
TALLA1	TAL	1 m	Tallapoosa R. upstream of Fort Toulouse-Jackson Park	32.495356, -86.245789	Tallapoosa R. rkm 2.4
ALR270	JBR	1 m	Alabama R. downstream of Gunter Hill Park	32.354761, -86.493584	Alabama R. rkm 431.9
ALR236	MFR	1 m	Alabama R. 400 m downstream of Robert F. Henry L&D	32.321212, -86.786686	Alabama R. rkm 378.4
ALR221	MFR	1 - 5 m	Alabama R. downstream of Gardiner Island	32.430809, -86.859480	Alabama R. rkm 354.4
CAHABA		1 m	Cahaba R. upstream of its confluence with the Alabama R.	32.322743, -87.093062	Cahaba R. rkm 0.5
ALR181	MFR	1 - 13 m	Alabama R. upstream of Whites Bluff	32.271435, -87.141933	Alabama R. rkm 291.5
ALR132	CLL	1 m	Alabama R. 400 m downstream of Millers Ferry L&D spillways	32.097139, -87.399275	Alabama R. rkm 214.5
ALR109	CLL	1 m	Alabama R. downstream of Gulletts Bluff Park	31.920126, -87.436442	Alabama R. rkm 177.1
ALR72	LAR	1 m	Alabama R. 400 m downstream of Claiborne L&D	31.611285, -87.550460	Alabama R. rkm 118.4
ALR47	LAR	1 m	Alabama R. upstream of Eureka Landing	31.421455, -87.636247	Alabama R. rkm 77.5
ALR3	LAR	1 m	Alabama River upstream of confluence with the Tombigbee R.	31.163166, -87.927802	Alabama R. rkm 4.0
TOMBIG		1 m	Tombigbee R. upstream of confluence with the Alabama R.	31.147663, -87.965695	Tombigbee R. rkm 4.1
TENSAW		1 - 10 m	Tensaw R. upstream of Live Oak Landing	30.968324, -87.889371	Tensaw R. rkm 45.0

Table 2. Mean limits of detection (LOD) of the four elements of interest used in microchemistry analyses of Freshwater Drum (FWDR), White Crappie (WHCP), and Blue Catfish (BCAT), and the percentage of otolith-region mean values greater than the limits of detection. Mean coefficients of variation (CV) for each element measured from the two certified reference materials used in LA-ICP-MS analyses (MACS-3 and NIST-612) are also shown for each species.

Species			**^{24}Mg**	**^{55}Mn**	**^{88}Sr**	**^{137}Ba**
FWDR n=243	Mean LOD of samples (µg/g)		0.019	0.078	0.011	0.013
	% samples > LOD	Whole Transect	100	100	100	100
		Edge (20 µm)	100	98.8	100	100
		Core (20 µm)	100	100	100	100
	Ref. materials	MACS-3 CV (%)	23.21	21.96	22.75	30.16
		NIST-612 CV (%)	14.57	15.03	11.64	16.27
WHCP n=200	Mean LOD of samples (µg/g)		0.010	0.075	0.006	0.009
	% samples > LOD	Whole Transect	100	100	100	100
		Edge (20 µm)	100	100	100	100
		Core (20 µm)	100	100	100	100
	Ref. materials	MACS-3 CV (%)	17.92	18.21	18.53	23.36
		NIST-612 CV (%)	9.42	10.92	10.17	11.57
BCAT n=25	Mean LOD of samples (µg/g)		0.008	0.057	0.004	0.008
	% samples > LOD	Whole Transect	100	96.0	100	100
		Edge (20 µm)	100	96.0	100	100
		Core (20 µm)	100	100	100	100
	Ref. materials	MACS-3 CV (%)	15.83	17.41	17.40	25.77
		NIST-612 CV (%)	11.82	14.31	13.25	12.50

Table 3. Water chemistry multiple comparison tests and results. From water chemistry data collected among the six river sections from which fish were collected.

Element Ratio	**Water Chemistry Comparisons**		
	(element:Ca~Site)	(element:Ca ~ River Section*discharge)	(element:Ca~Season)
Mg:Ca	$F_{11,60}$ = 18.710, p = <0.001	River Section: $F_{5,54}$ = 24.296, p= <0.001 Discharge: $F_{1,54}$ = 20.413, p= <0.001 Interaction: $F_{5,54}$ = 1.102, p= 0.370	$F_{3,68}$ = 1.680, p = 0.540
Mn:Ca	$F_{11,59}$ = 10.740, p = <0.001	River Section: $F_{5,53}$ = 22.348, p= <0.001 Discharge: $F_{1,53}$ = 7.835, p= 0.007 Interaction: $F_{5,53}$ = 1.673, p= 0.157	$F_{3,67}$ = 2.374, p = 0.234
Sr:Ca	$F_{11,60}$ = 72.550, p = <0.001	River Section: $F_{5,54}$ = 106.05, p= <0.001 Discharge: $F_{1,54}$ = 0.332, p= 0.567 Interaction: $F_{5,54}$ = 0.884, p= 0.498	$F_{3,68}$ = 0.405, p = 0.750
Ba:Ca	$F_{11,60}$ = 35.400, p = <0.001	River Section: $F_{5,54}$ = 78.590, p= <0.001 Discharge: $F_{1,54}$ = 5.823, p= 0.019 Interaction: $F_{5,54}$ = 1.874, p= 0.114	$F_{3,68}$ = 0.855, p = 0.469

Table 4. Paired T-tests of all sites within the six river sections from which fish were collected, paired by season between the two sampling years.

Element Ratio	Water Chemistry Paired T-tests	
	spring: 2020 vs. 2021	summer: 2020 vs. 2021
Mg:Ca	t_{11} = 6.527, p = <0.001	t_{11} = 1.651, p = 0.127
Mn:Ca	t_{11} = 0.805, p = 0.438	t_{11} = 0.957, p = 0.359
Sr:Ca	t_{11} = -2.244, p = 0.046	t_{11} = 2.212, p = 0.049
Ba:Ca	t_{11} = 1.579, p = 0.143	t_{11} = -0.676, p = 0.513

Table 5. Statistics resulting from multiple regression analyses testing for the effect of depth (in meters below the surface) on each of the element:Ca ratios, at each of the multiple-depth water sampling sites. Depth did not have significant effects on any water chemistry ratios at any of the three multiple-depth sampling sites.

Element Ratio	Multiple-Depth Water Sampling Site		
	ALR221	ALR181	TENSAW
Mg:Ca	$F_{1,16} = 1.436$, $p = 0.248$	$F_{1,22} = 0.092$, $p = 0.764$	$F_{1,22} = 1.224$, $p = 0.281$
Mn:Ca	$F_{1,16} = 0.148$, $p = 0.705$	$F_{1,22} = 0.074$, $p = 0.788$	$F_{1,22} = 0.999$, $p = 0.328$
Sr:Ca	$F_{1,16} = 0.380$, $p = 0.546$	$F_{1,22} = 0.033$, $p = 0.857$	$F_{1,22} = 1.940$, $p = 0.178$
Ba:Ca	$F_{1,16} = 1.246$, $p = 0.281$	$F_{1,22} = 0.005$, $p = 0.943$	$F_{1,22} = 1.405$, $p = 0.249$

Table 6. Sample totals by river section for Freshwater Drum (FWDR), White Crappie (WHCP), and Blue Catfish (BCAT) collected from six river sections within the Alabama River basin.

	River Section						
Species	**CSA**	**TAL**	**JBR**	**MFR**	**CLL**	**LAR**	**Total**
FWDR	23	20	50	50	50	50	243
WHCP	-	-	50	50	50	50	200
BCAT	-	-	8	5	5	7	25

Table 7. Linear discriminant analysis classifications of Freshwater Drum to the four river sections of the Alabama River, using the mean element:Ca ratios of the whole-transect, edge, and core otolith regions.

Otolith Region	Section collected from:	Classified as: JBR	MFR	CLL	LAR	Section Accuracy (%)	Overall Accuracy (%)
Whole Transect	JBR	35	7	3	5	70.0	55.5
	MFR	9	22	8	11	44.0	
	CLL	11	8	25	6	50.0	
	LAR	6	9	6	29	58.0	
Edge (20 µm)	JBR	16	14	2	18	32.0	50.5
	MFR	10	26	7	7	52.0	
	CLL	3	11	29	7	58.0	
	LAR	9	6	5	30	60.0	
Core (20 µm)	JBR	38	9	2	1	76.0	45.5
	MFR	12	17	10	11	34.0	
	CLL	17	11	11	11	22.0	
	LAR	11	7	7	25	50.0	

Table 8. Discriminant function analyses linear discriminants with proportions of trace and variable coefficients for the classification analyses of Freshwater Drum using otolith mean whole-transect, mean edge, and mean core multivariate element signatures. LDAs were performed with the four river sections of the Alabama River as the grouping classifications.

Whole Transect	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		46.77	39.87	13.36
	element:Ca coefficients	^{24}Mg	0.9529	0.2287	0.6554
		^{55}Mn	-1.4438	0.7849	-0.0821
		^{88}Sr	2.7592	3.8901	0.9171
		^{137}Ba	0.7167	-2.2435	3.4847
Edge (20 µm)	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		64.60	26.87	8.53
	element:Ca coefficients	^{24}Mg	0.6073	-0.4751	0.3240
		^{55}Mn	-0.0021	-0.0158	0.0855
		^{88}Sr	3.1386	0.7882	-0.6706
		^{137}Ba	0.2339	-2.4747	-0.7497
Core (20 µm)	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		86.43	8.53	5.04
	element:Ca coefficients	^{24}Mg	-0.0032	-0.0047	-0.0062
		^{55}Mn	-0.8531	0.8559	-0.6793
		^{88}Sr	3.1257	2.0822	-3.5127
		^{137}Ba	0.0774	1.3343	2.3077

Table 9. Linear discriminant analysis classifications of Freshwater Drum to all six river sections from which they were collected, using the mean element:Ca ratios of the whole-transect, edge, and core otolith regions.

Otolith Region	Section collected from:	Classified as: CSA	TAL	JBR	MFR	CLL	LAR	Section Accuracy (%)	Overall Accuracy (%)
Whole Transect	CSA	13	1	2	2	0	5	56.5	51.9
	TAL	0	7	8	1	1	3	35.0	
	JBR	0	2	33	6	4	5	66.0	
	MFR	1	2	7	21	9	10	42.0	
	CLL	1	0	10	8	25	6	50.0	
	LAR	1	0	5	11	6	27	54.0	
Edge (20 µm)	CSA	17	0	2	1	0	3	73.9	49.0
	TAL	0	2	4	8	4	2	10.0	
	JBR	2	0	16	13	2	17	32.0	
	MFR	1	0	11	25	8	5	50.0	
	CLL	0	0	2	11	30	7	60.0	
	LAR	2	0	9	6	4	29	58.0	
Core (20 µm)	CSA	7	1	8	4	3	0	30.4	42.8
	TAL	4	3	10	0	2	1	15.0	
	JBR	2	0	37	7	3	1	74.0	
	MFR	2	0	12	17	8	11	34.0	
	CLL	1	0	16	7	14	12	28.0	
	LAR	1	1	10	6	6	26	52.0	

Table 10. Discriminant function analyses linear discriminants with proportions of trace and variable coefficients for the classification analyses of Freshwater Drum using otolith mean whole-transect, mean edge, and mean core multivariate element signatures. LDAs were performed with all six river sections from which Freshwater Drum were collected as the grouping classifications.

	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**	**LD-4**
	Proportion of Trace (%)		53.39	32.15	11.18	3.27
Whole Transect	element:Ca coefficients	^{24}Mg	0.4886	-1.3428	1.3693	-1.7834
		^{55}Mn	0.6687	1.6052	-0.2125	0.5109
		^{88}Sr	4.1804	-2.1748	-1.0630	2.1674
		^{137}Ba	-1.7627	-0.8260	3.1381	1.7316
	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**	**LD-4**
	Proportion of Trace (%)		70.81	17.73	8.71	2.75
Edge (20 µm)	element:Ca coefficients	^{24}Mg	0.5751	0.1801	-1.3281	-0.9639
		^{55}Mn	-0.0028	0.0097	-0.0463	0.0936
		^{88}Sr	3.0471	1.2882	1.2197	-0.0219
		^{137}Ba	-1.2638	2.2737	-0.0362	-0.1610
	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**	**LD-4**
	Proportion of Trace (%)		65.62	28.94	3.97	1.47
Core (20 µm)	element:Ca coefficients	^{24}Mg	-0.0008	-0.0026	-0.0123	-0.0076
		^{55}Mn	-0.5395	-0.6960	0.2367	1.4481
		^{88}Sr	4.1665	-0.4730	-1.3946	3.1900
		^{137}Ba	0.5780	-1.2999	1.8037	-2.4292

Table 11. Linear discriminant analysis classifications of White Crappie to river sections, using the mean element:Ca ratios of the whole-transect, edge, and core otolith regions.

Otolith Region	Section collected from:	Classified as: JBR	MFR	CLL	LAR	Section Accuracy (%)	Classification Accuracy (%)
Whole Transect	JBR	31	9	1	9	62.0	69.5
	MFR	1	37	3	9	74.0	
	CLL	0	8	40	2	80.0	
	LAR	15	2	2	31	62.0	
Edge (20 µm)	JBR	34	6	2	8	68.0	70.5
	MFR	4	34	5	7	68.0	
	CLL	0	5	45	0	90.0	
	LAR	10	12	0	28	56.0	
Core (20 µm)	JBR	26	9	3	12	52.0	54.0
	MFR	7	31	7	5	62.0	
	CLL	2	11	32	5	64.0	
	LAR	20	5	6	19	38.0	

Table 12. Discriminant function analyses linear discriminants with proportions of trace and variable coefficients for the classification analyses of White Crappie using otolith mean whole-transect, mean edge, and mean core multivariate element signatures. LDAs were performed with the four river sections of the Alabama River as the grouping classifications.

Whole Transect	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		88.23	6.22	5.54
	element:Ca coefficients	^{24}Mg	0.2177	0.7449	-6.1383
		^{55}Mn	-0.2521	1.1550	-0.1442
		^{88}Sr	5.5918	0.0317	-0.1553
		^{137}Ba	-1.1494	1.7682	1.1854
Edge (20 µm)	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		92.71	4.70	2.59
	element:Ca coefficients	^{24}Mg	-0.2338	1.0992	-2.3140
		^{55}Mn	0.0934	1.4099	-0.5227
		^{88}Sr	5.6711	-0.2362	-1.4554
		^{137}Ba	-0.6278	0.8376	1.7126
Core (20 µm)	Linear Discriminant		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		88.66	9.99	1.35
	element:Ca coefficients	^{24}Mg	1.1115	-2.4301	-2.6263
		^{55}Mn	-0.7391	0.5866	-0.3635
		^{88}Sr	3.1275	0.5978	0.1577
		^{137}Ba	-0.0016	0.8056	-0.7761

Table 13. Linear discriminant analysis classifications of Blue Catfish to river sections, using the mean element:Ca ratios of the whole-transect, edge, and core otolith regions.

Otolith Region	Section collected from:	Classified as: JBR	MFR	CLL	LAR	Section Accuracy (%)	Overall Accuracy (%)
Whole Transect	JBR	5	1	0	2	62.5	48.0
	MFR	1	2	0	2	40.0	
	CLL	3	0	1	1	20.0	
	LAR	1	1	1	4	57.1	
Edge (20 µm)	JBR	6	0	0	2	75.0	72.0
	MFR	1	0	2	2	0.0	
	CLL	0	0	5	0	100.0	
	LAR	0	0	0	7	100.0	
Core (20 µm)	JBR	6	0	1	1	75.0	60.0
	MFR	3	2	0	0	40.0	
	CLL	1	0	3	1	60.0	
	LAR	0	2	1	4	57.1	

Table 14. Discriminant function analyses linear discriminants with proportions of trace and variable coefficients for the classification analyses of Blue Catfish using otolith mean whole-transect, mean edge, and mean core multivariate element signatures. LDAs were performed with the four river sections of the Alabama River as the grouping classifications.

Whole Transect	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		50.69	37.29	12.02
	element:Ca coefficients	^{24}Mg	-0.3189	3.5397	3.7572
		^{55}Mn	0.2440	0.2193	-0.3463
		^{88}Sr	2.3764	-3.4874	3.3198
		^{137}Ba	-0.6359	-0.7907	-0.1320
Edge (20 µm)	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		87.98	11.18	0.85
	element:Ca coefficients	^{24}Mg	-2.9826	1.1224	-1.7349
		^{55}Mn	-0.6832	-0.5085	1.9456
		^{88}Sr	-2.8506	-2.7929	-1.5624
		^{137}Ba	0.5929	-2.1356	-1.1223
Core (20 µm)	Linear Discriminants		**LD-1**	**LD-2**	**LD-3**
	Proportion of Trace (%)		61.23	30.58	8.19
	element:Ca coefficients	^{24}Mg	-0.0347	0.0142	-0.0081
		^{55}Mn	0.2636	-0.0585	0.1759
		^{88}Sr	-0.0003	-0.0050	-0.0030
		^{137}Ba	2.6511	1.2412	-0.4895

FIGURES

Maps

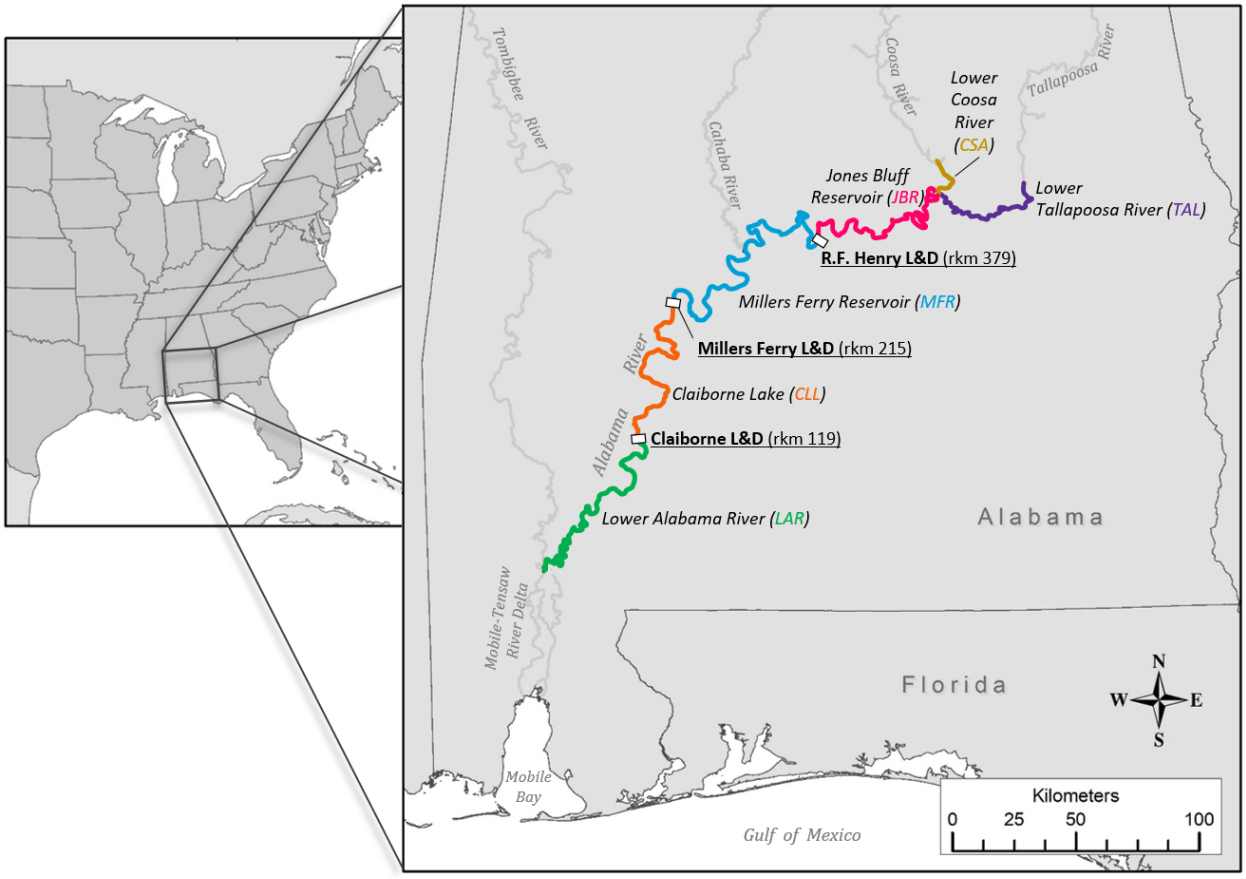

Figure 1. The study area with lock-and-dam locations (underlined) and river sections (with abbreviations).

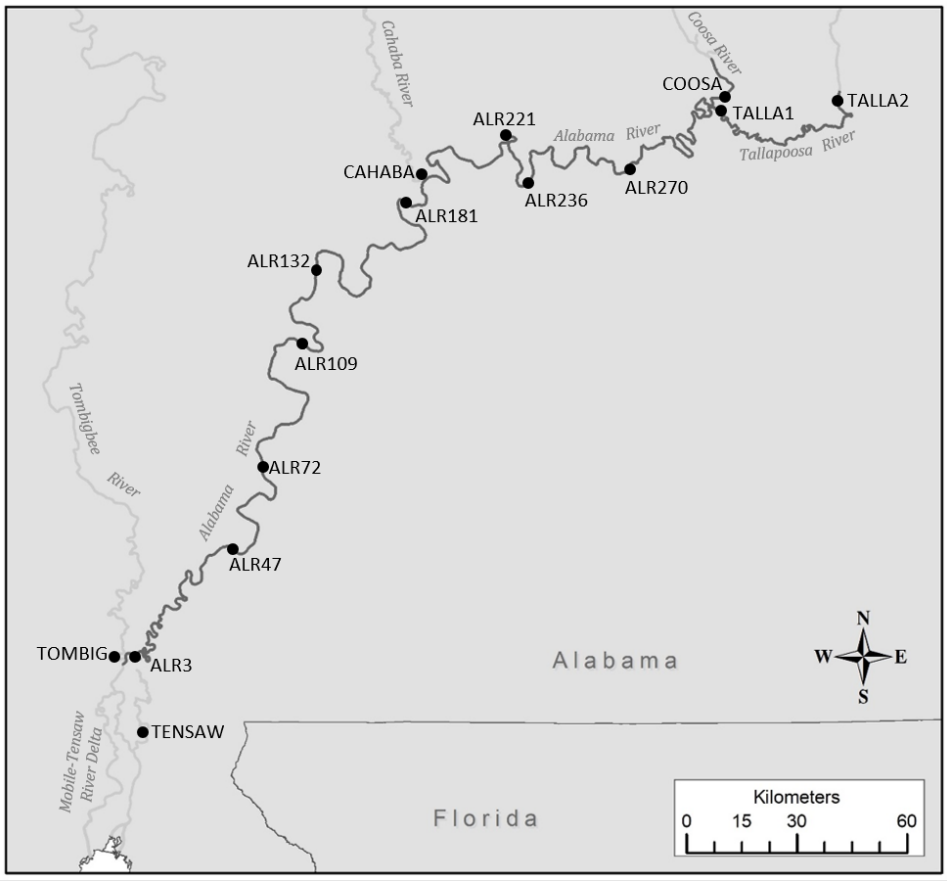

Figure 2. Locations of the 15 water sampling sites.

Water Chemistry

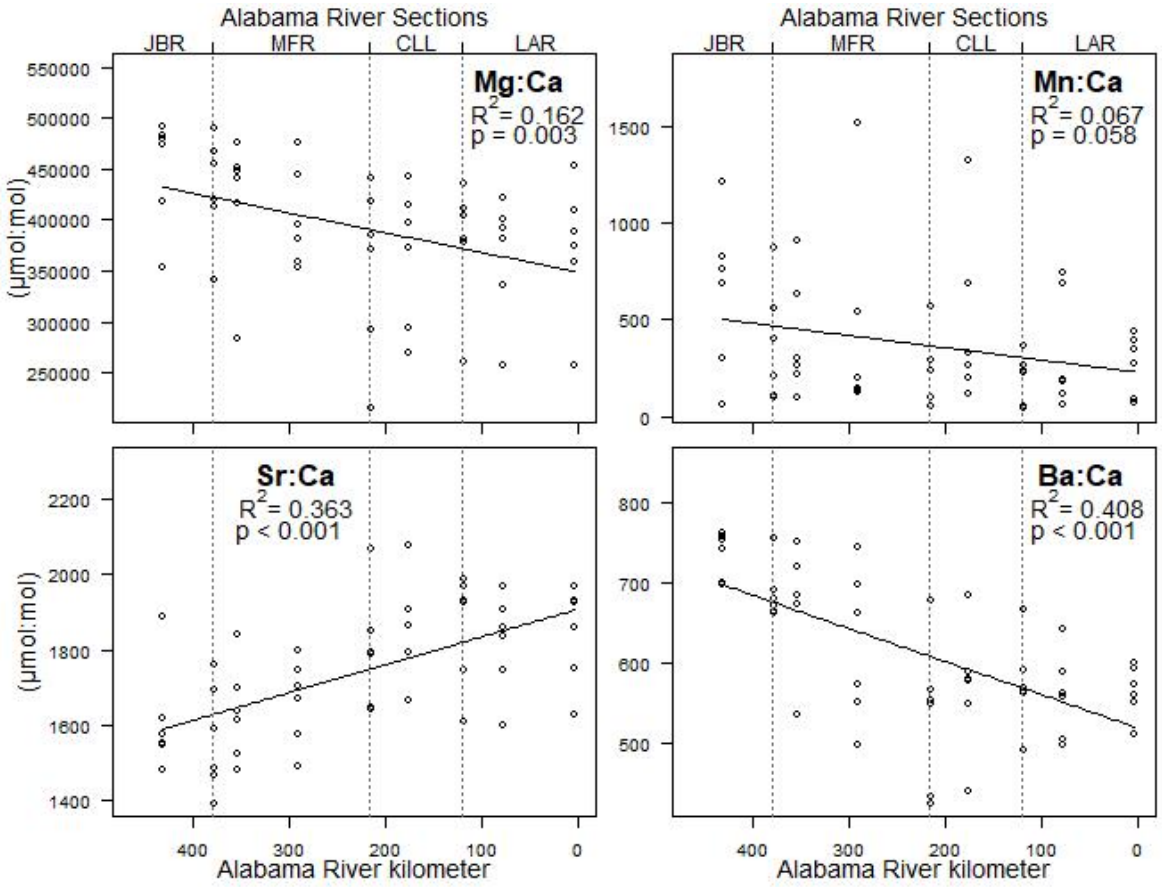

Figure 3. Water chemistry ratios across the sampled 428 rkm range of the main stem of the Alabama River. Vertical dashed lines indicate dam locations.

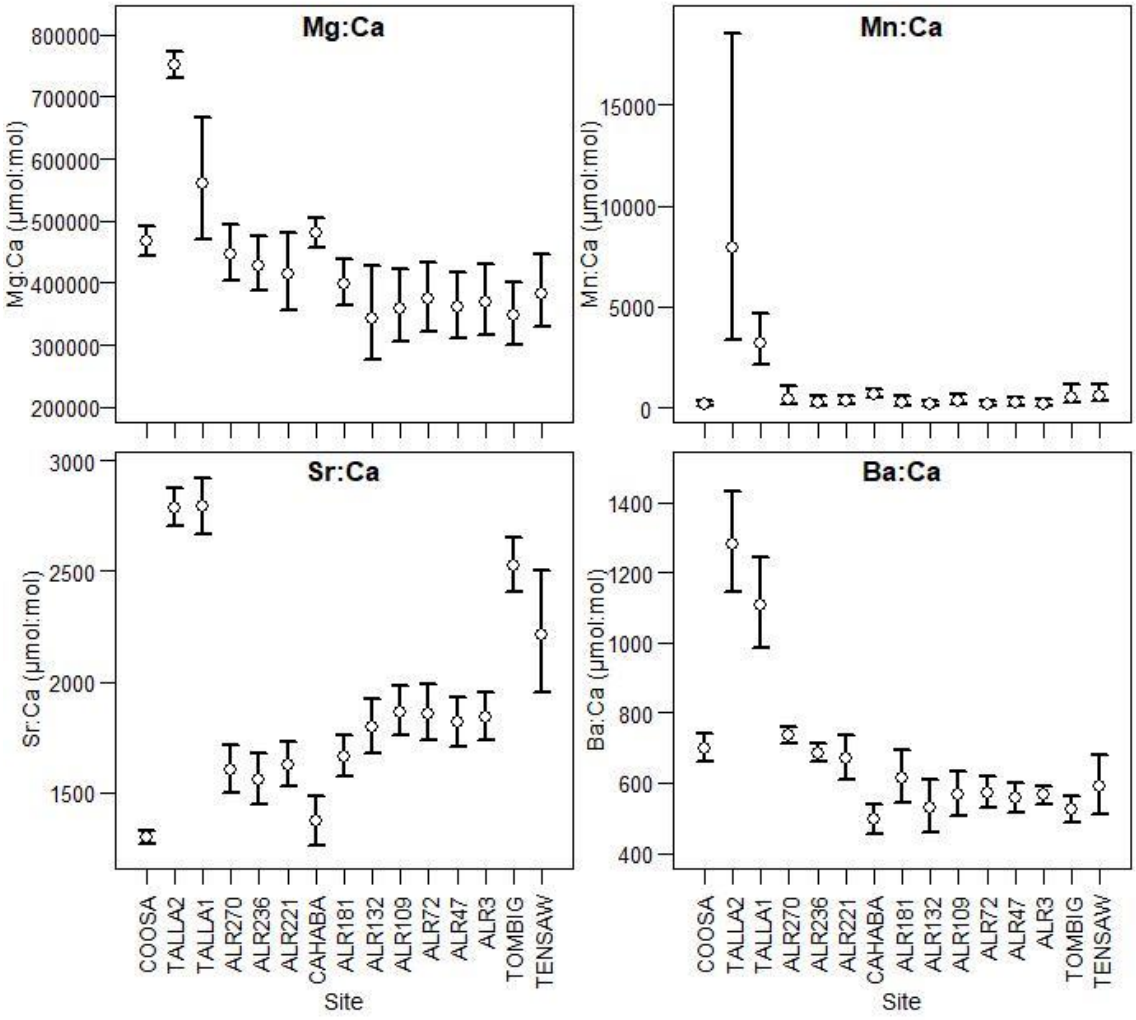

Figure 4. Mean water chemistry ratios (with 95% confidence intervals) across all 15 water sampling sites.

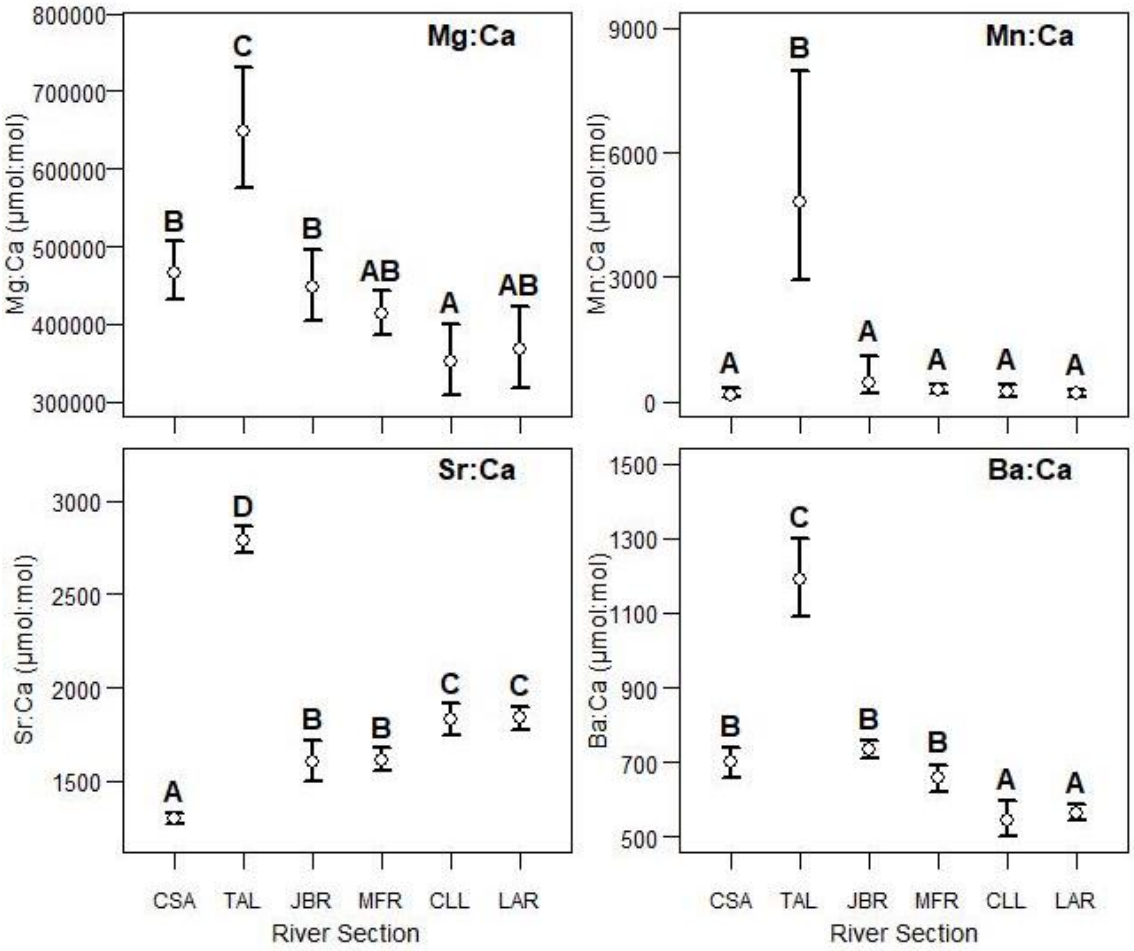

Figure 5. Mean water chemistry ratios (with 95% confidence intervals) across the six river sections from which fish were collected. Letters indicate significant differences between river sections for each element:Ca ratio.

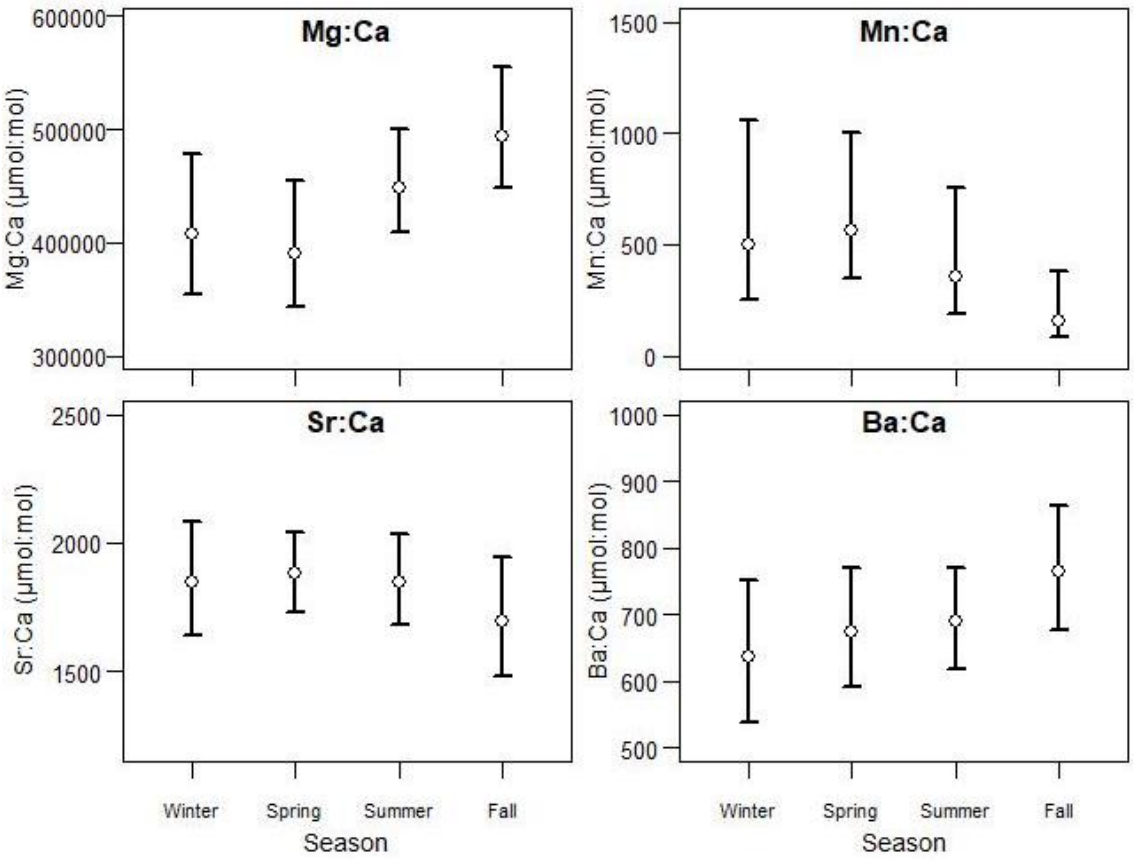

Figure 6. Mean water chemistry ratios (with 95% confidence intervals) across seasons. No significant differences were observed among seasons.

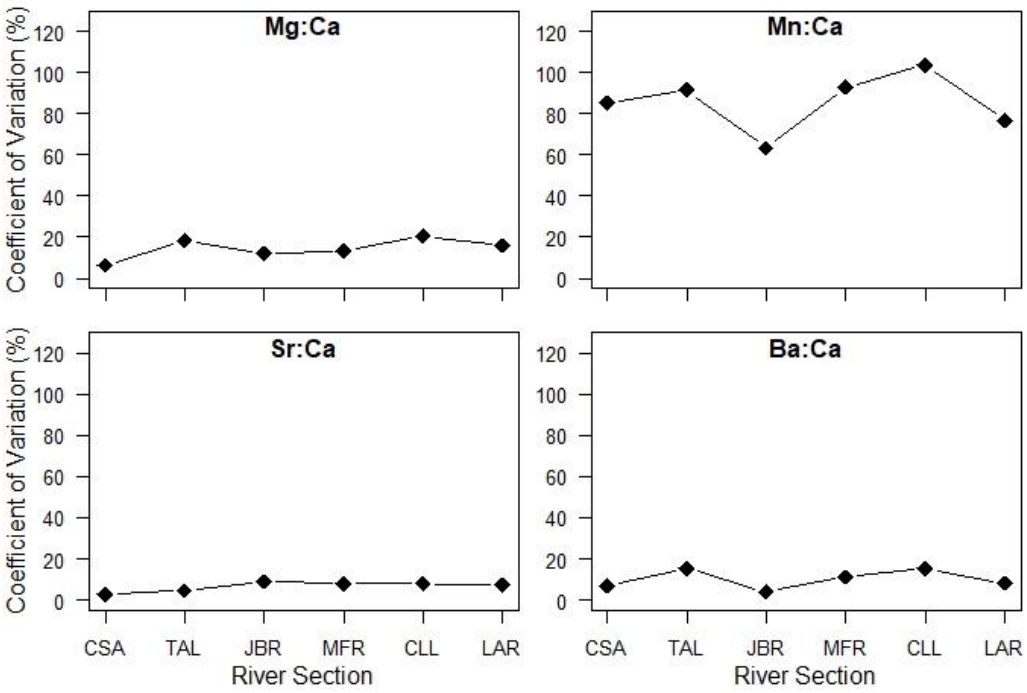

Figure 7. Coefficient of variation (CV) scores of water chemistry ratios across the six river sections from which fish were collected.

FRESHWATER DRUM

FWDR Lengths & Ages

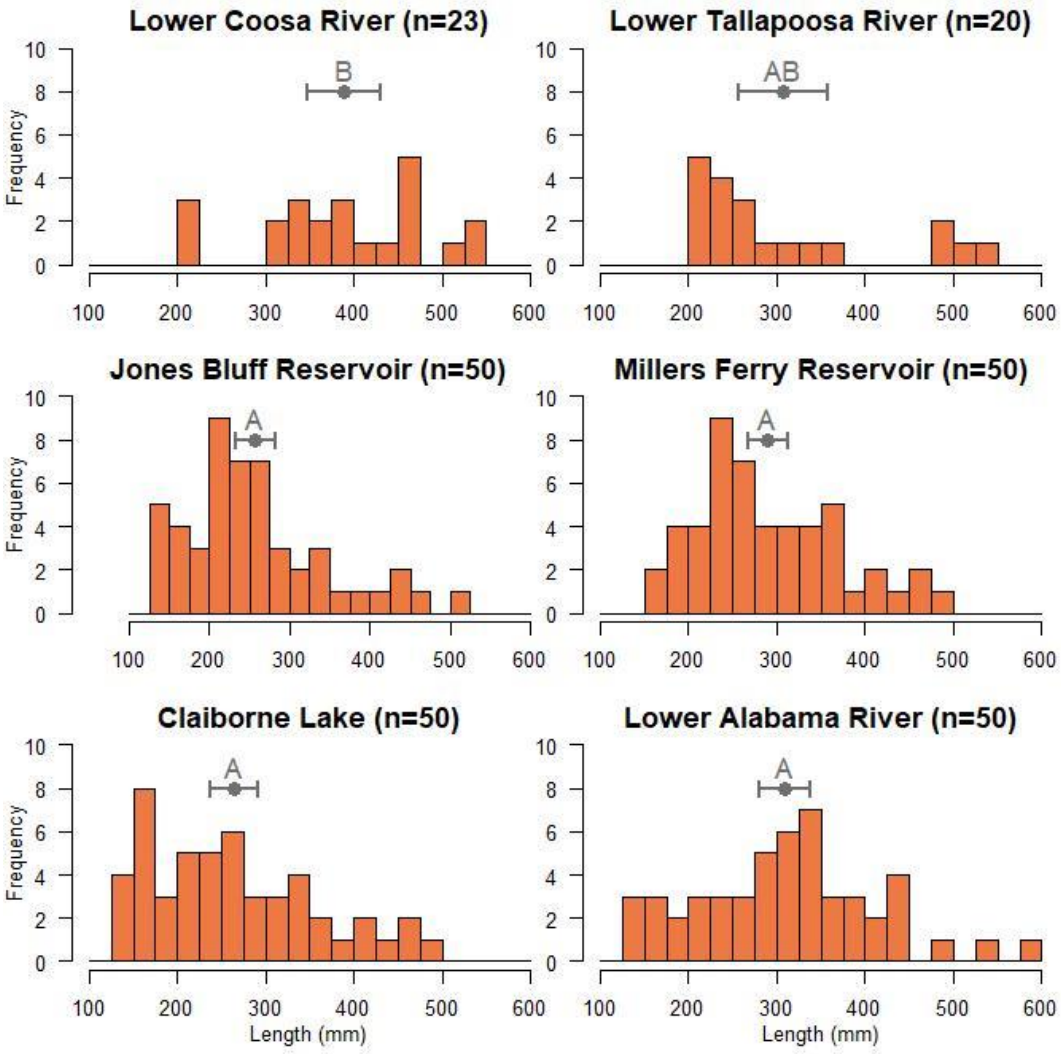

Figure 8. Length-frequency distributions of Freshwater Drum across the six river sections from which these fish were collected. Means with 95% confidence intervals are shown and letters indicate significant differences among river sections.

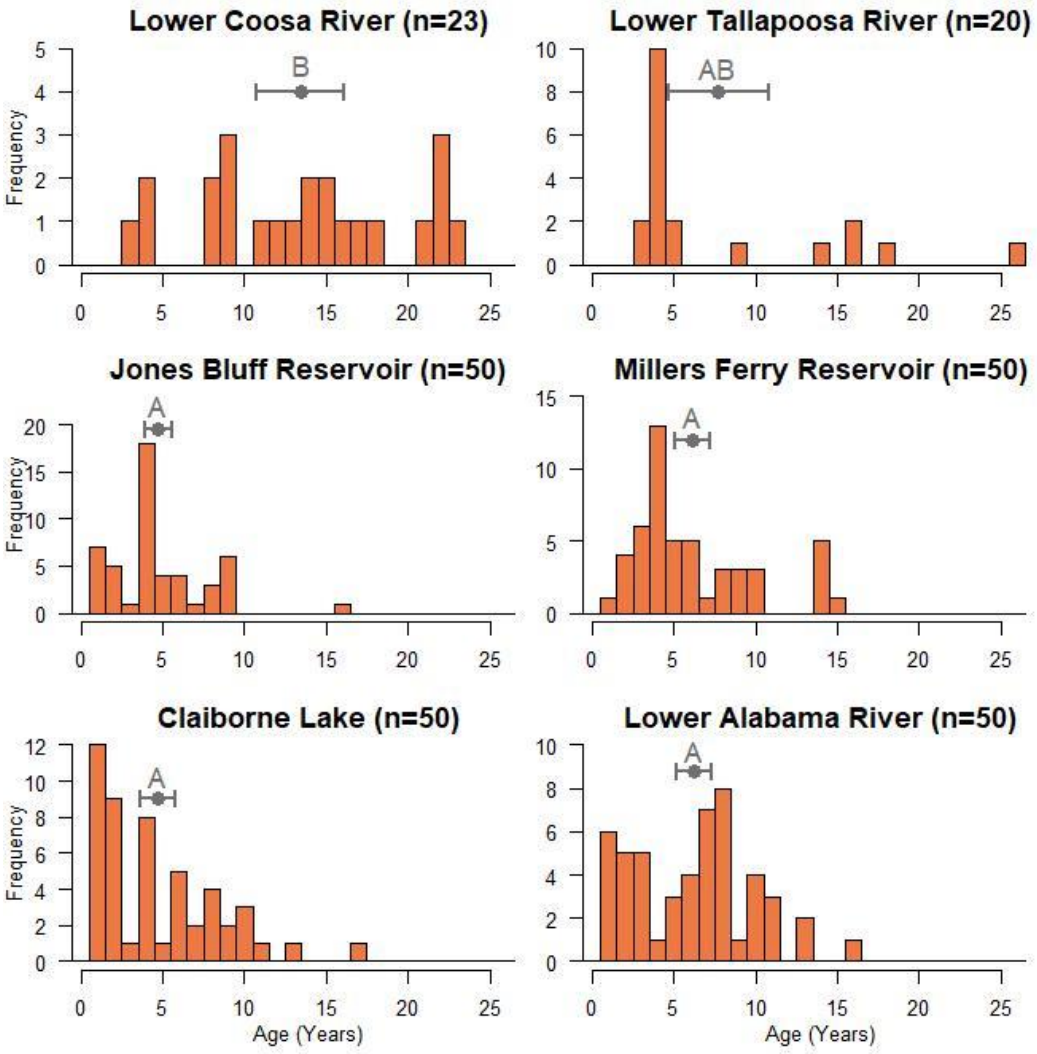

Figure 9. Age-frequency distributions of Freshwater Drum across the six river sections from which these fish were collected. Means with 95% confidence intervals are shown and letters indicate significant differences between river sections.

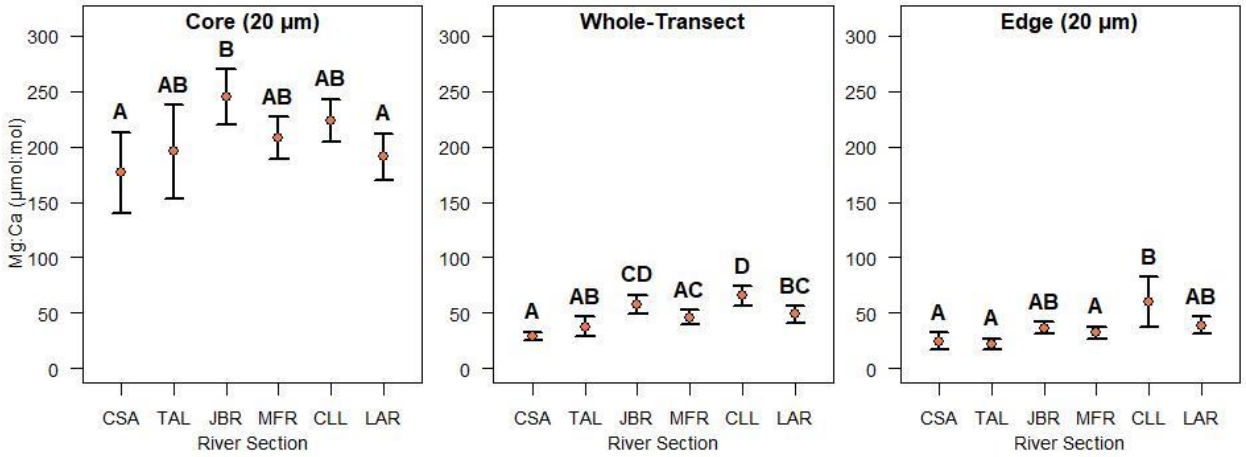

Figure 10. Freshwater Drum mean Mg:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the six river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

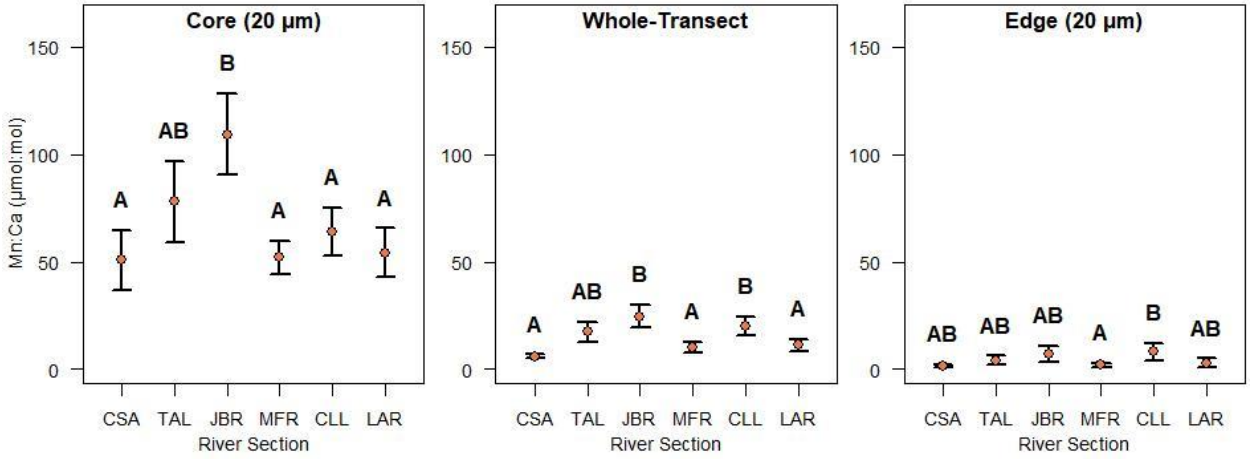

Figure 11. Freshwater Drum mean Mn:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the six river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

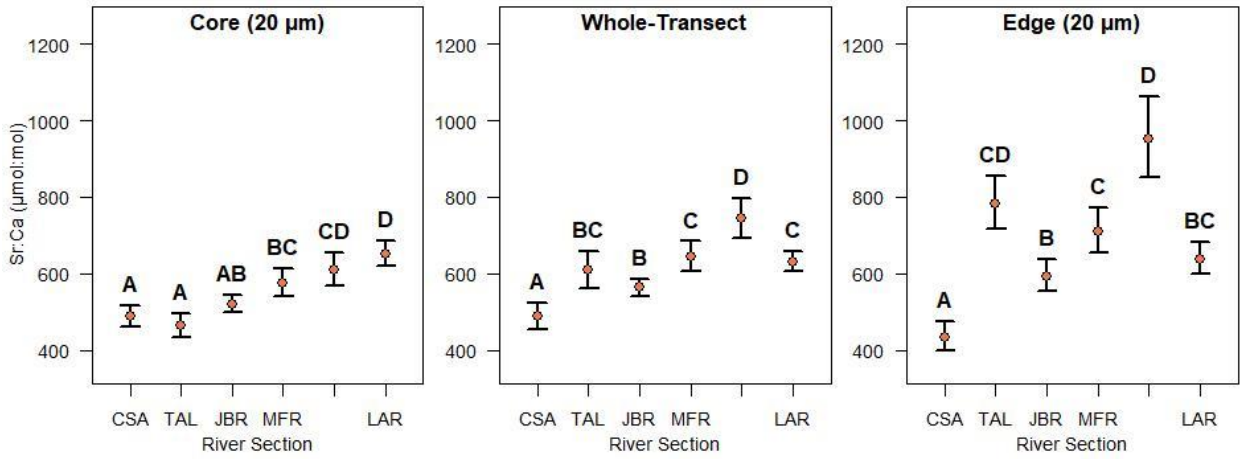

Figure 12. Freshwater Drum mean Sr:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the six river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

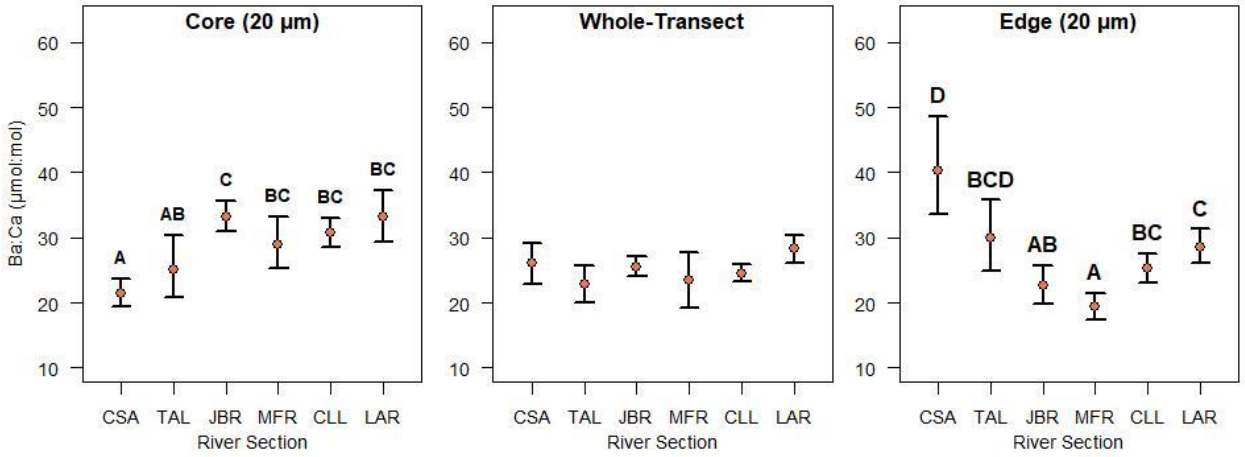

Figure 13. Freshwater Drum mean Ba:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the six river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

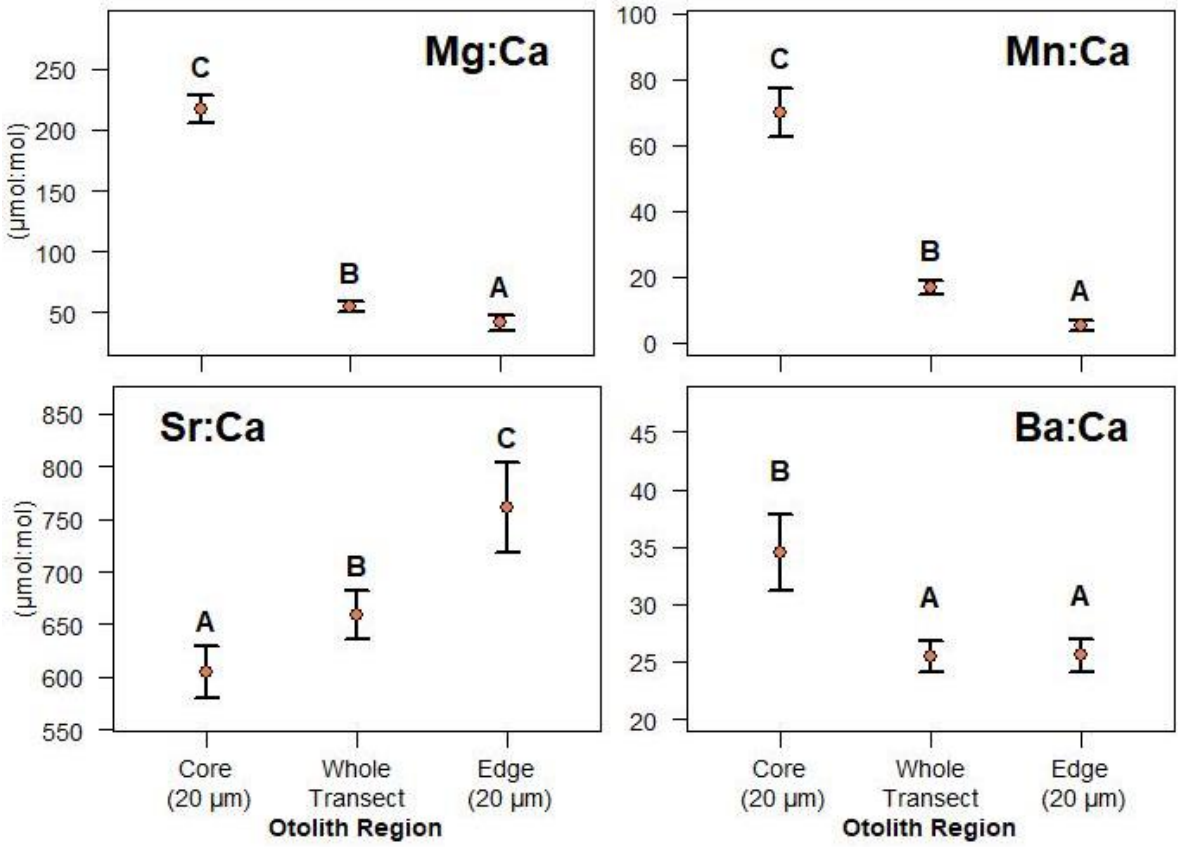

Figure 14. Freshwater Drum mean element:Ca ratios (with 95% confidence intervals) across the three otolith regions. Letters indicate significant differences between otolith regions for each element:Ca ratio.

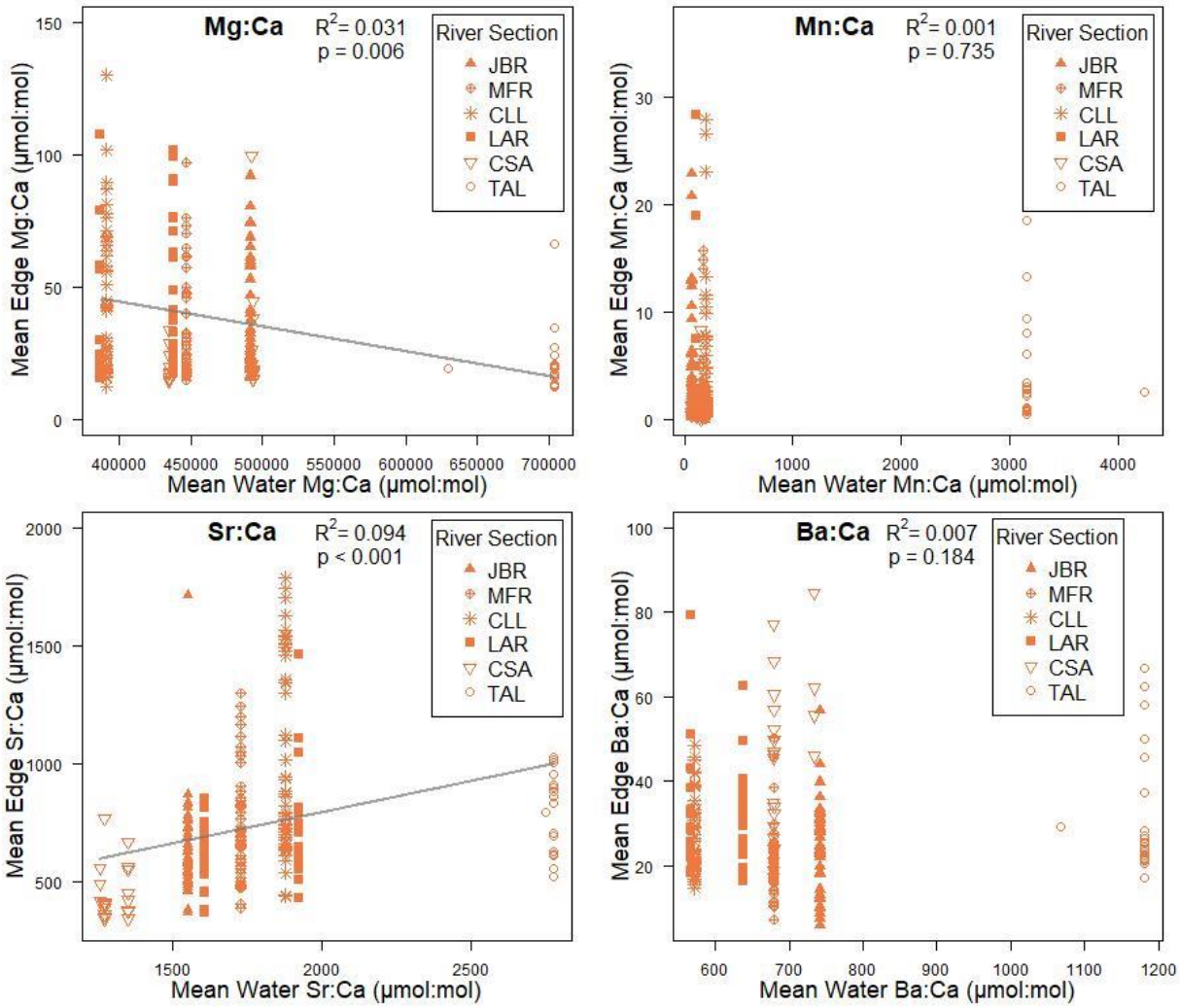

Figure 15. Freshwater Drum mean otolith-edge element:Ca ratios versus mean water element:Ca ratios from the river sections and seasons in which fish were collected. Solid regression lines indicate a significant positive relationship, dashed regression lines indicate a significant negative relationship.

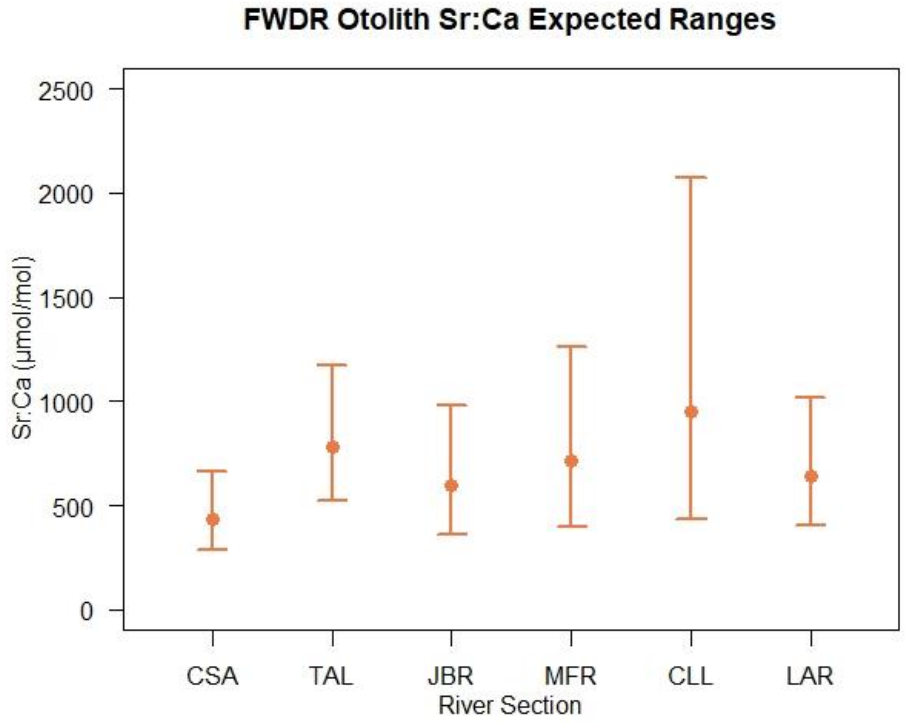

Figure 16. Expected ranges of Freshwater Drum otolith Sr:Ca ratios across the six river sections these fish were collected from. These intervals were calculated as the 95% prediction intervals of the Sr:Ca ratios in otolith edges of fish collected from each river section, and used in the criteria for identifying potential dam passages and potential movements of fish. Mean Sr:Ca values derived from Freshwater Drum collected from each river section are shown as dots and bars represent the 95% prediction interval for Sr:Ca values in each river section.

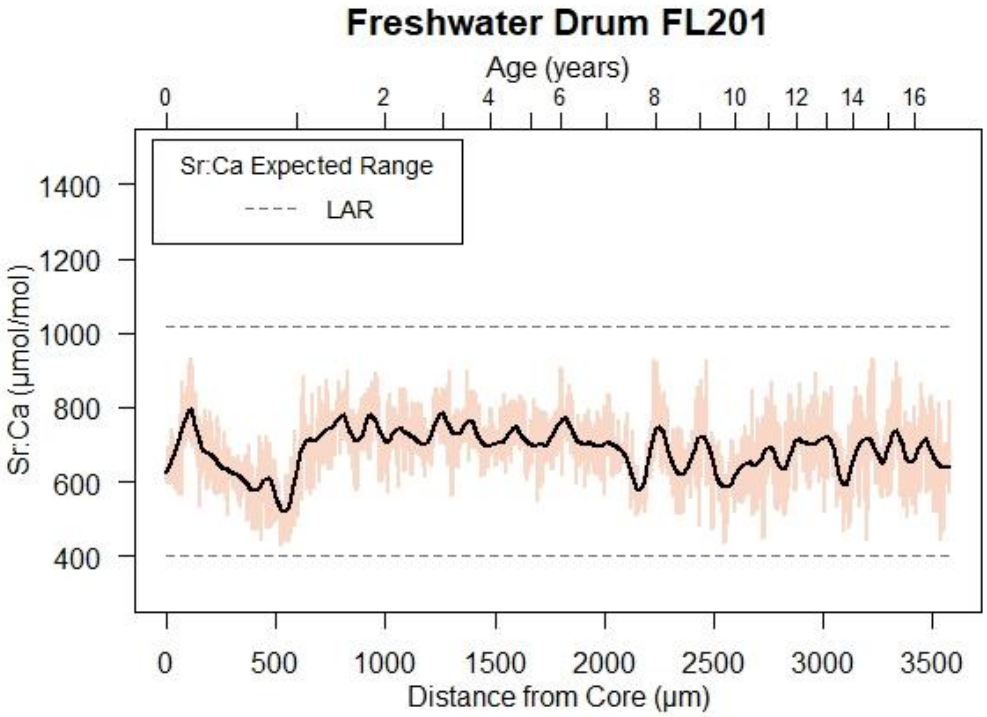

Figure 17. An example of a typical Sr:Ca profile pattern observed among Freshwater Drum collected from the Alabama River. This individual fish (FL201) was collected from the lower Alabama River (LAR) at rkm 117, and is suspected to have remained within LAR for the entirety of its life.

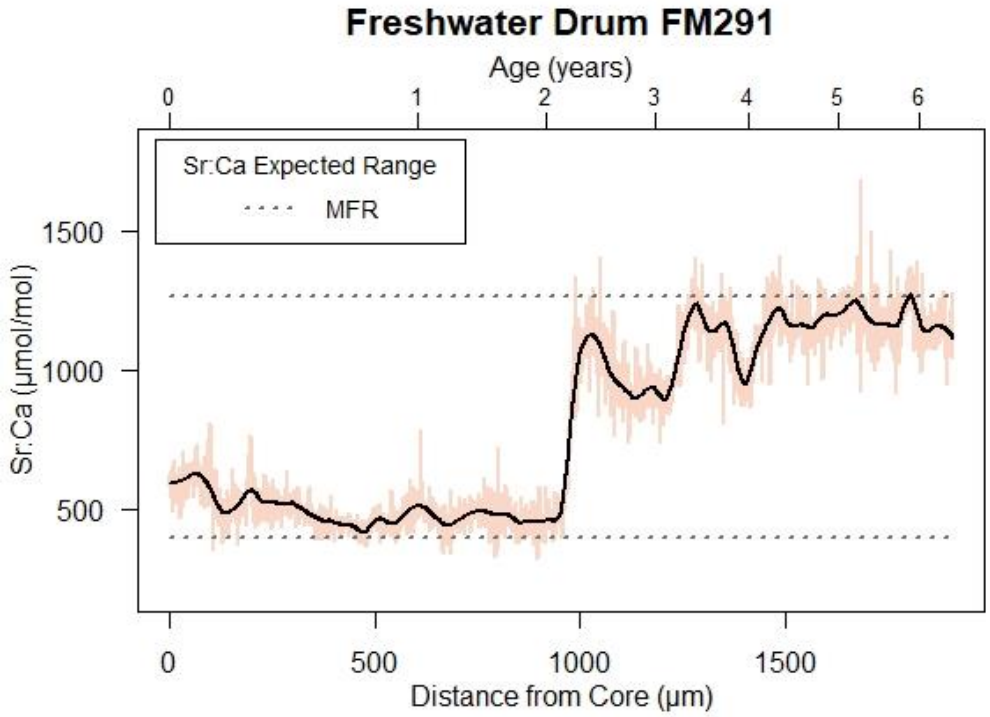

Figure 18. A commonly observed Sr:Ca profile pattern among Freshwater Drum collected from the Alabama River. This fish (FL201) was collected from Millers Ferry Reservoir (MFR) at rkm 312 and shows evidence of movement at approximately age 2 (~1000 µm from core) . However, this movement is not suspected of being a dam passage and the fish is suspected to have remained within MFR for the entirety of its life.

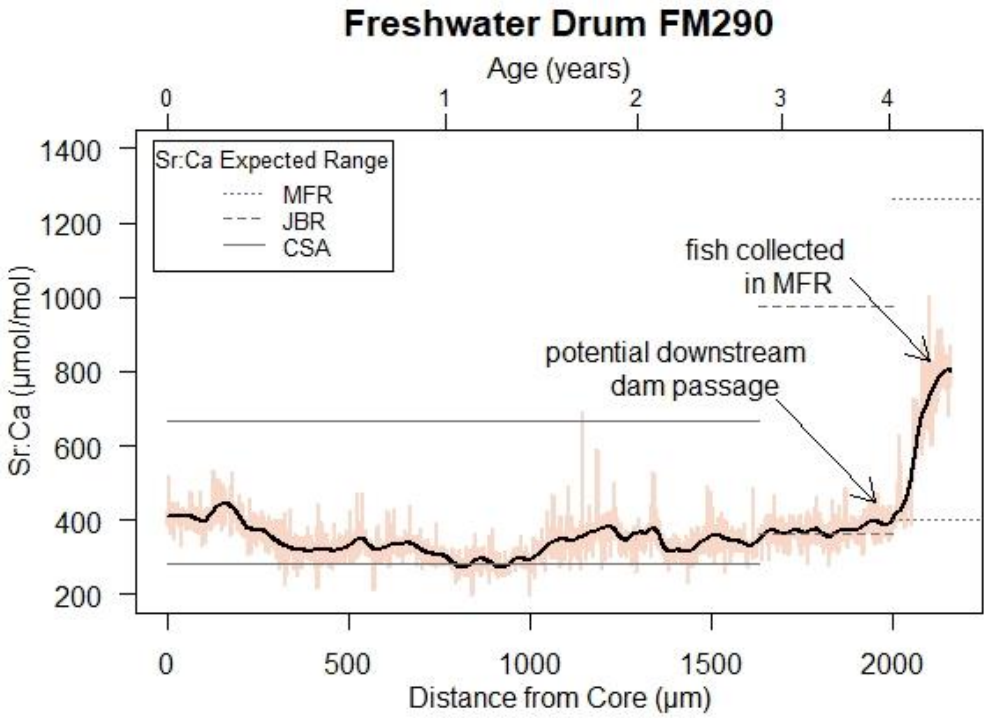

Figure 19. Lifetime Sr:Ca profile of a 4 yr.-old Freshwater Drum (FM290) collected from Millers Ferry Reservoir (MFR) at rkm 312. The Sr:Ca profile shows a potential downstream movement from the lower Coosa River (CSA) into Jones Bluff Reservoir (JBR), and a potential downstream passage across R.F. Henry L&D into Millers Ferry Reservoir (MFR) occurring around age 4. A large portion of this fish's lifetime Sr:Ca profile is shown as being outside the range of expected Sr:Ca values for Freshwater Drum collected from MFR. A potential alternative scenario is that this fish could have made an unimpeded movement from the Cahaba River into MFR around age 4.

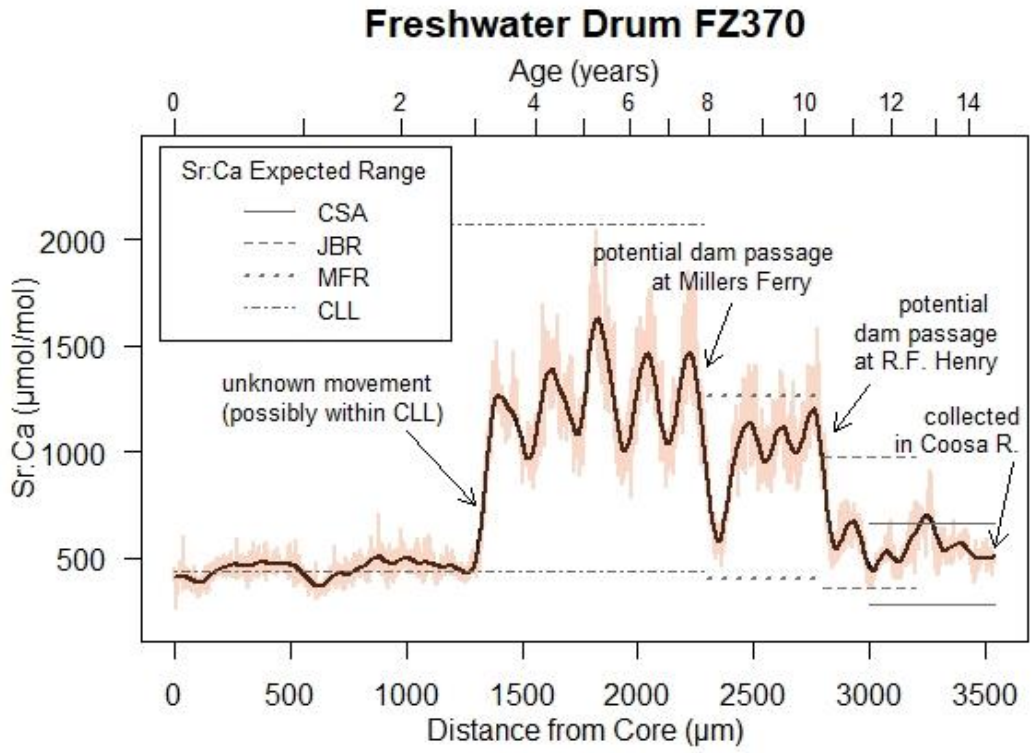

Figure 20. Lifetime Sr:Ca profile of a 14 yr.-old female Freshwater Drum. This was the only fish identified by the passage criteria has having potentially made two dam passages within its lifetime.

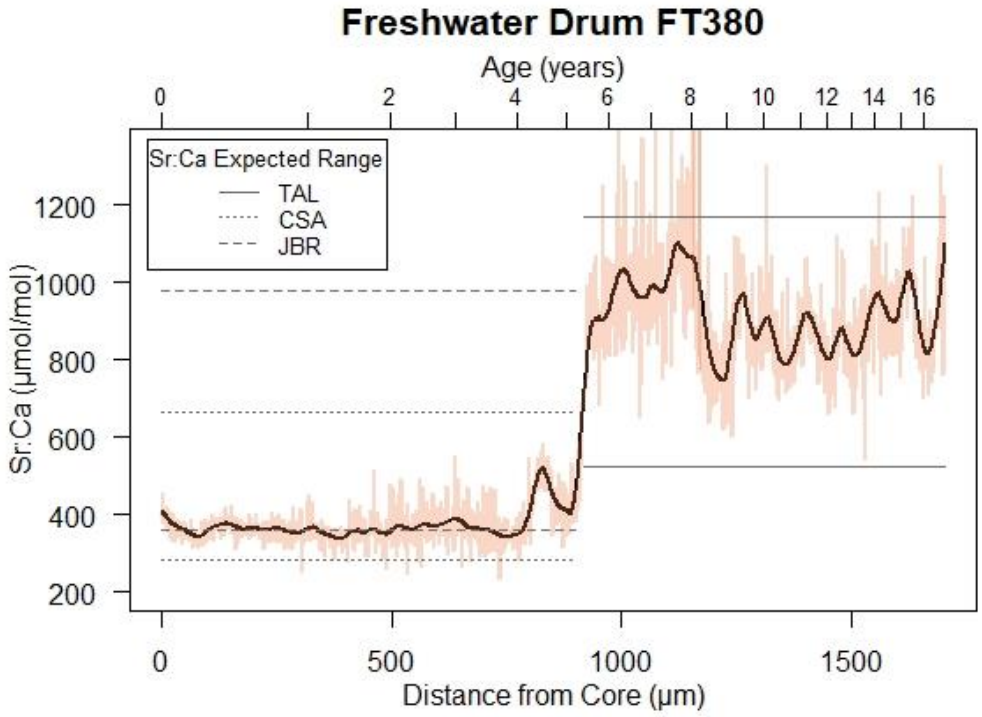

Figure 21. Typical lifetime Sr:Ca profile of a Freshwater Drum collected from the lower Tallapoosa River (TAL). This profile shows a 16 yr.-old fish suspected to have moved upstream into the Tallapoosa River from Jones Bluff Reservoir (JBR) or from the lower Coosa River (CSA), at approximately age 5.

WHITE CRAPPIE

WHCP Lengths & Ages

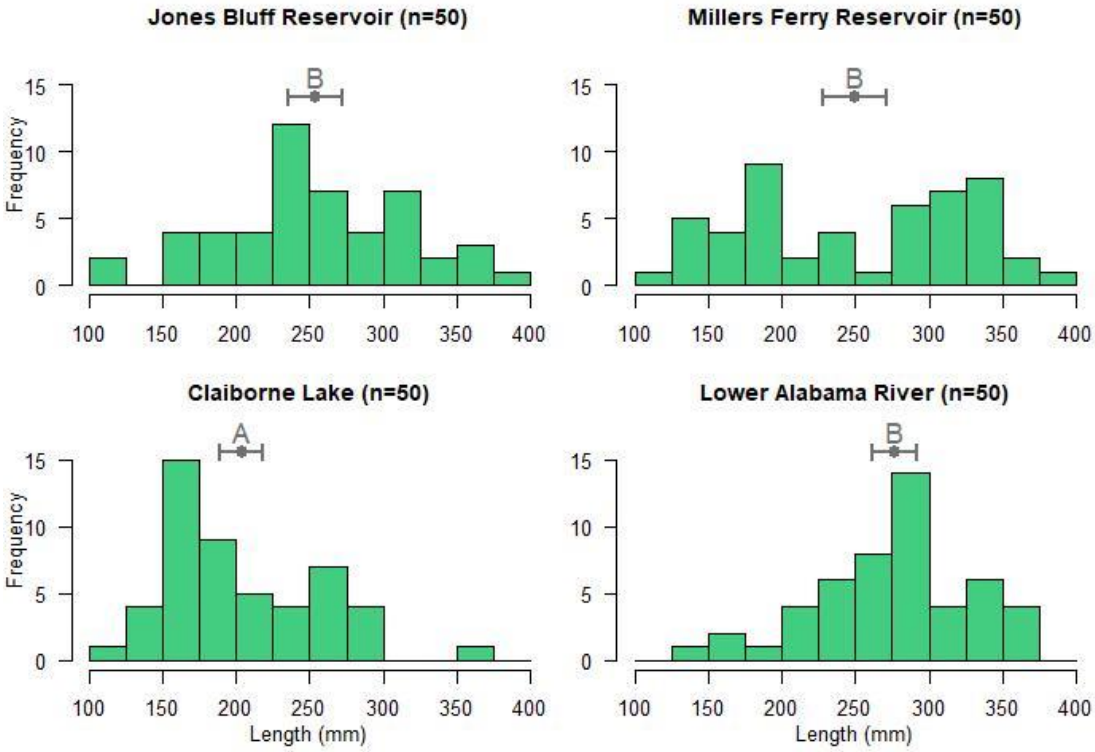

Figure 22. Length frequency distributions of White Crappie across the four river sections of the Alabama River. Means with 95% confidence intervals are shown and letters indicate significant differences among river sections.

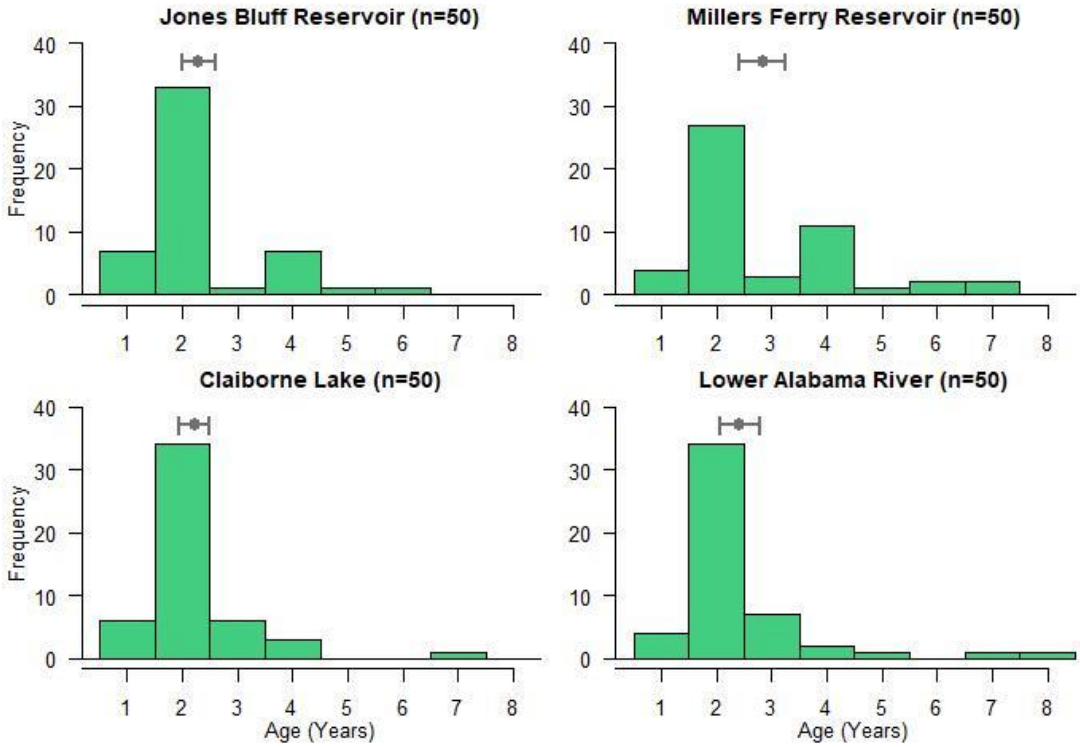

Figure 23. Age frequency distributions of White Crappie across the four river sections of the Alabama River. Mean ages with 95% confidence intervals are also shown. No significant differences in age were found among the four river sections.

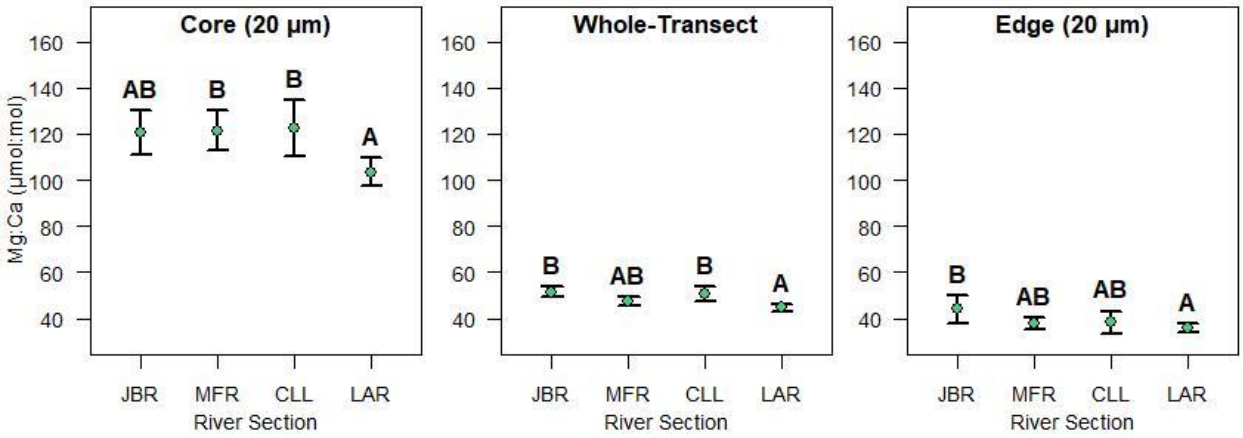

Figure 24. White Crappie mean Mg:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

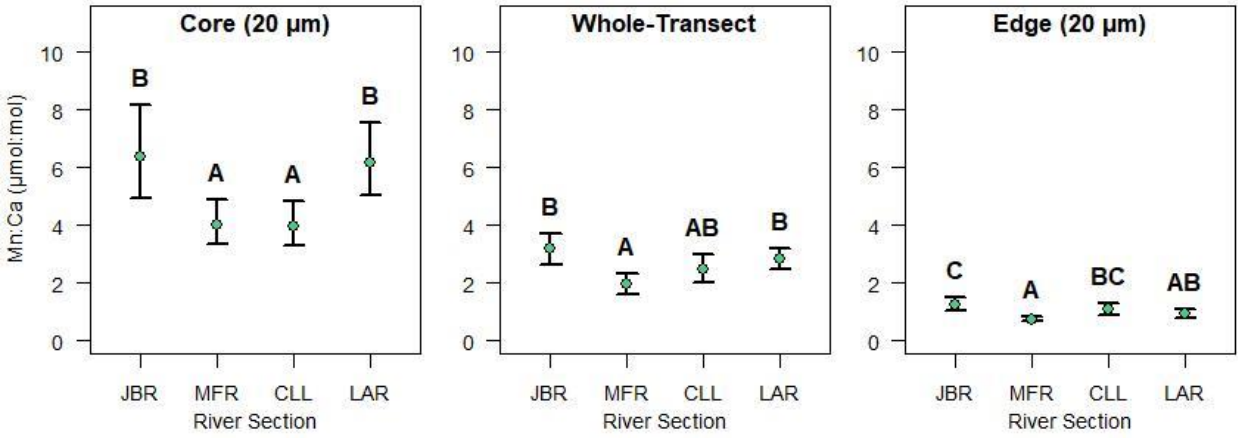

Figure 25. White Crappie mean Mn:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

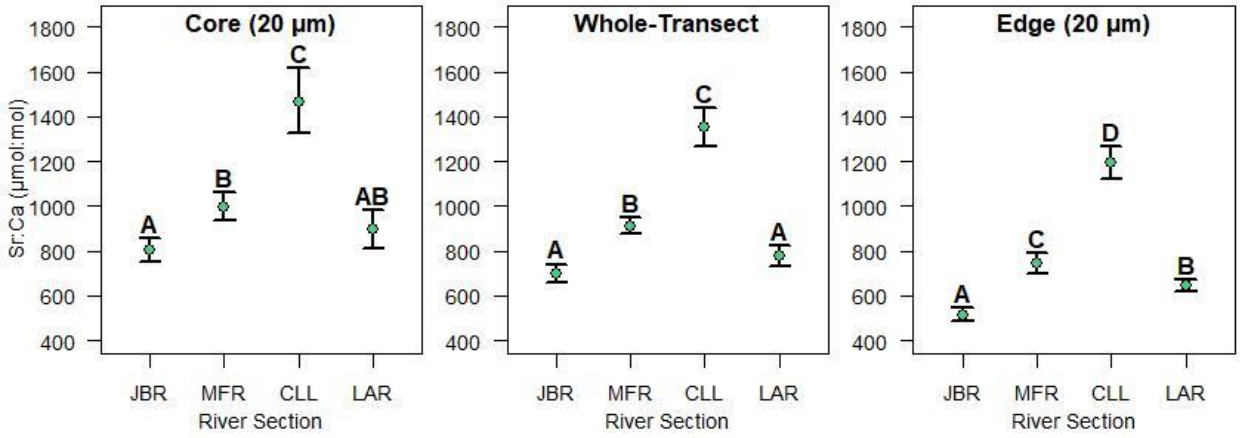

Figure 26. White Crappie mean Sr:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

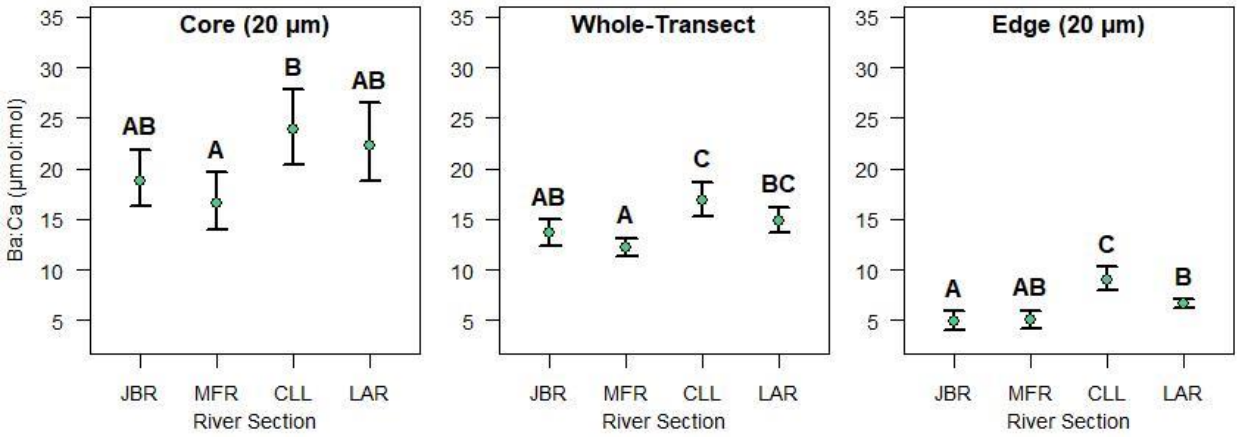

Figure 27. White Crappie mean Ba:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

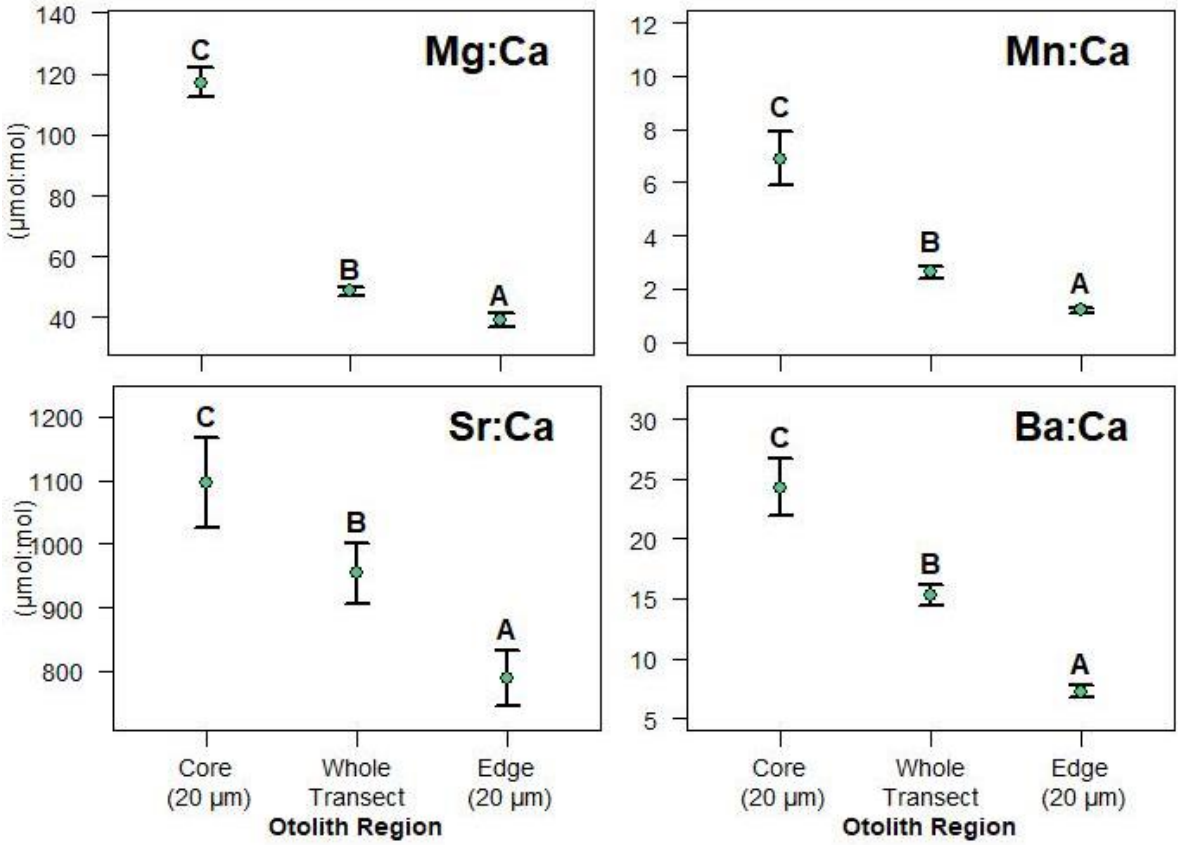

Figure 28. White Crappie mean element:Ca ratios (with 95% confidence intervals) across the three otolith regions. Letters indicate significant differences between otolith regions for each element:Ca ratio.

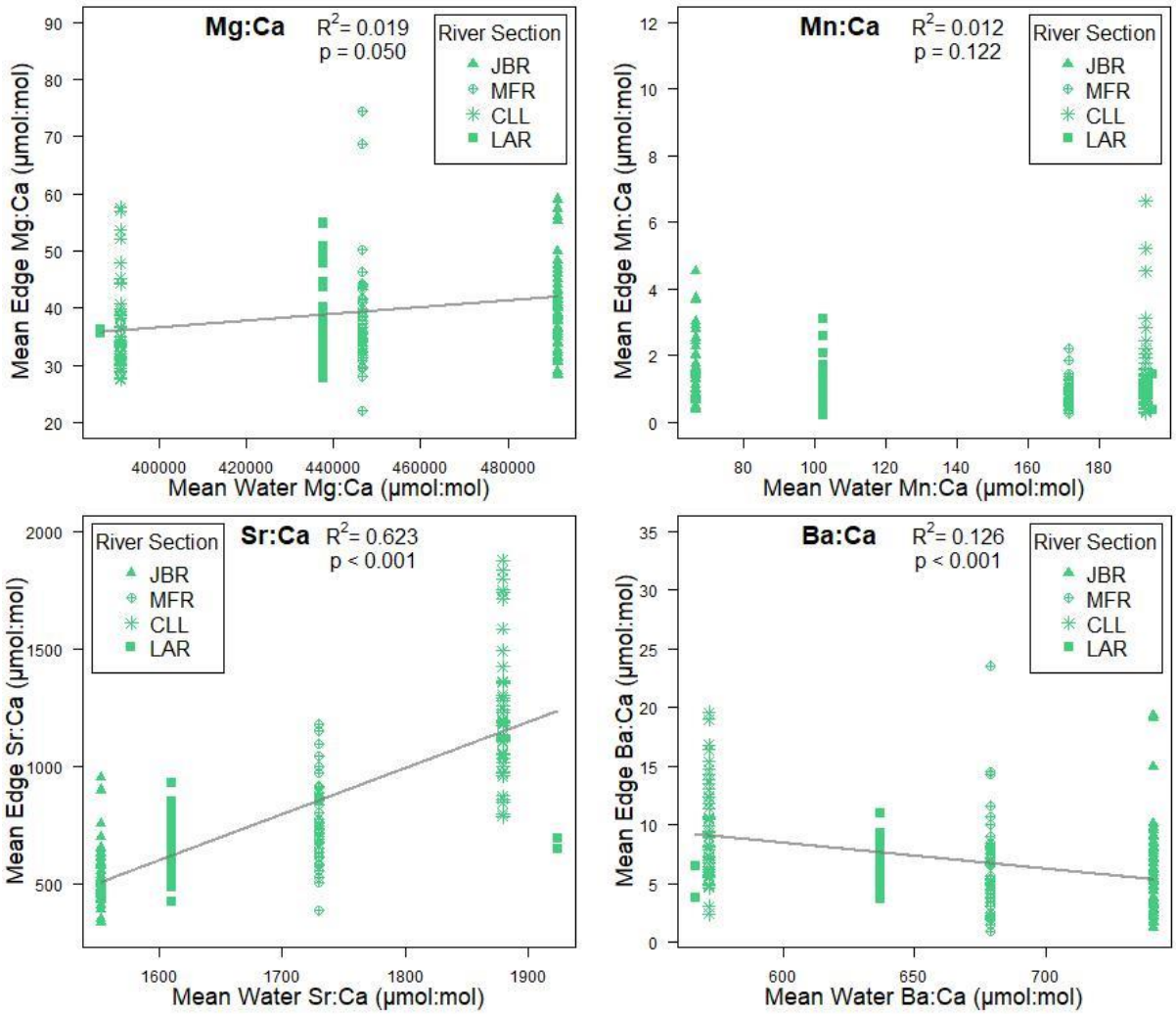

Figure 29. White Crappie mean otolith-edge element:Ca ratios versus mean water element:Ca ratios from the river sections and seasons in which fish were collected. Solid regression lines indicate a significant positive relationship, dashed regression lines indicate a significant negative relationship.

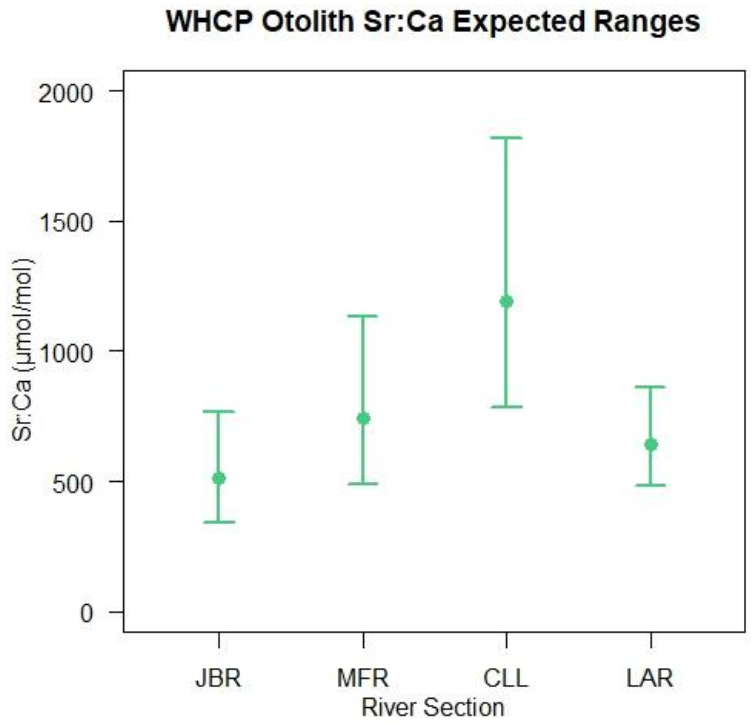

Figure 30. Expected ranges of White Crappie otolith edge Sr:Ca ratios across the four river sections these fish were collected from. These intervals were calculated as the 95% prediction intervals of the Sr:Ca ratios in otolith edges of fish collected from each river section. These intervals could not be used reliably for identifying potential dam passages of White Crappie due to Sr:Ca levels being enriched in otolith cores of these fish. Mean Sr:Ca values derived from White Crappie collected from each river section are shown as dots and bars represent the 95% prediction interval for Sr:Ca values in each river section.

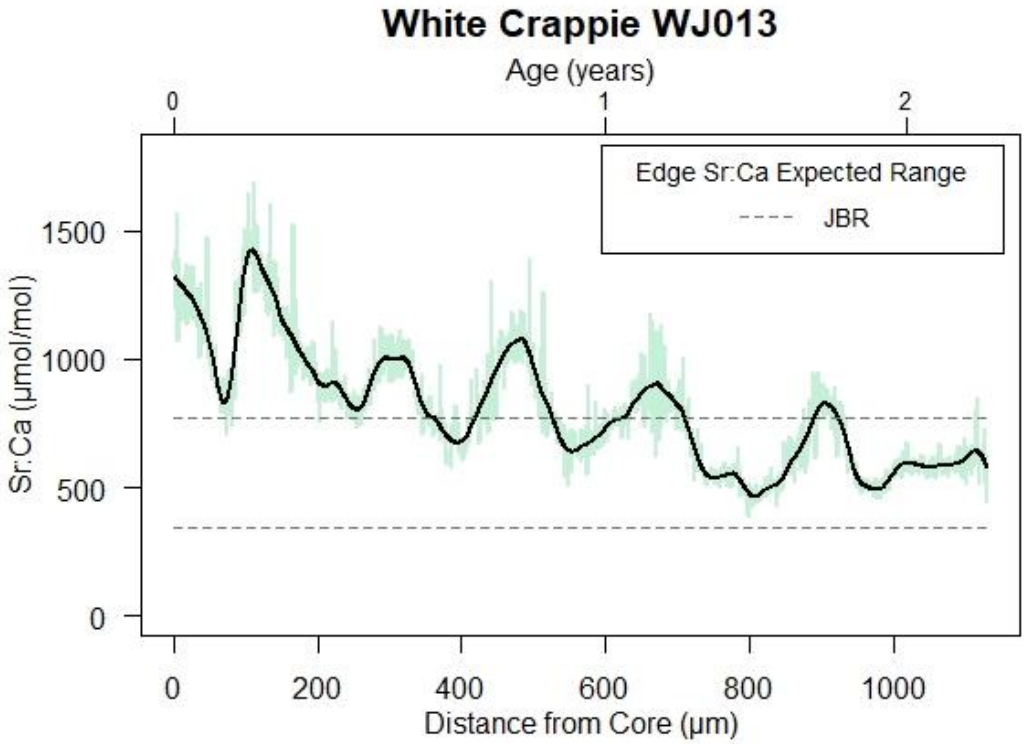

Figure 31. An example of a typical Sr:Ca profile pattern observed among White Crappie collected from the Alabama River. Sr:Ca generally decreased from otolith core to edge, making the identification of potential passage events unclear based on expected ranges of Sr:Ca ratios derived from otolith edges. This individual fish (WJ013) was collected from Jones Bluff Reservoir (JBR) at rkm 381, and is suspected to have remained within JBR for the entirety of its life.

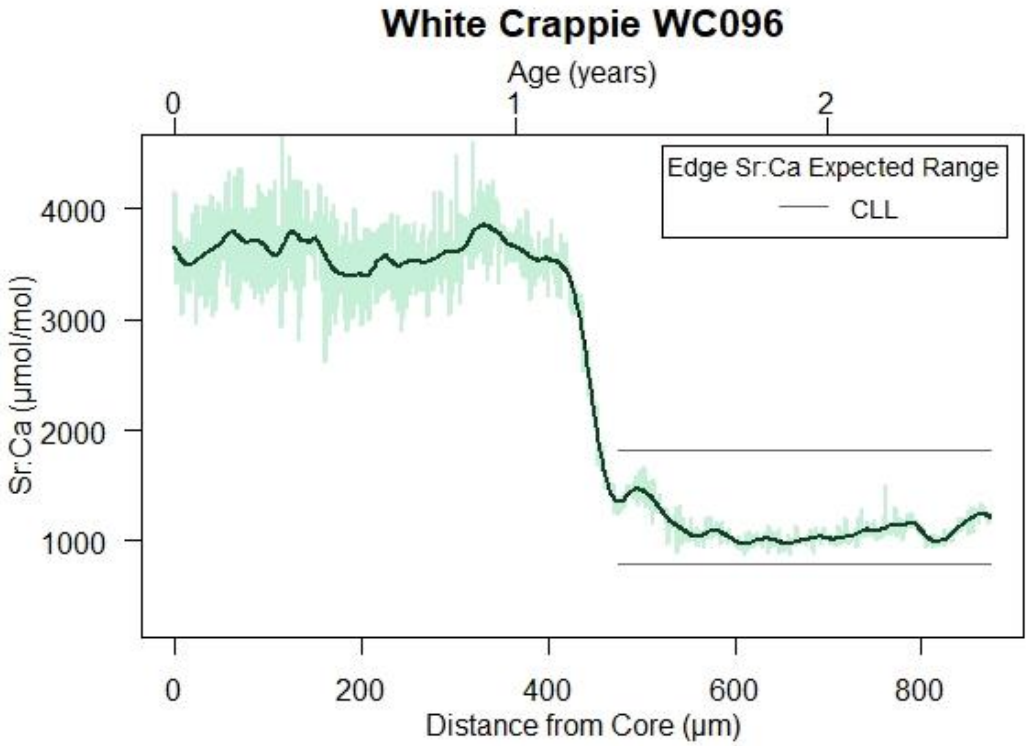

Figure 32. Lifetime Sr:Ca profile of a 2 yr.-old White Crappie (WC096) captured within Claiborne Lake (CLL) at rkm 124. This fish is suspected to have made a distinct move between habitats, as shown by the large shift in the Sr:Ca ratio, however a dam passage is not suspected and the natal location (Sr:Ca > 3500 µmol/mol) of the fish is unknown.

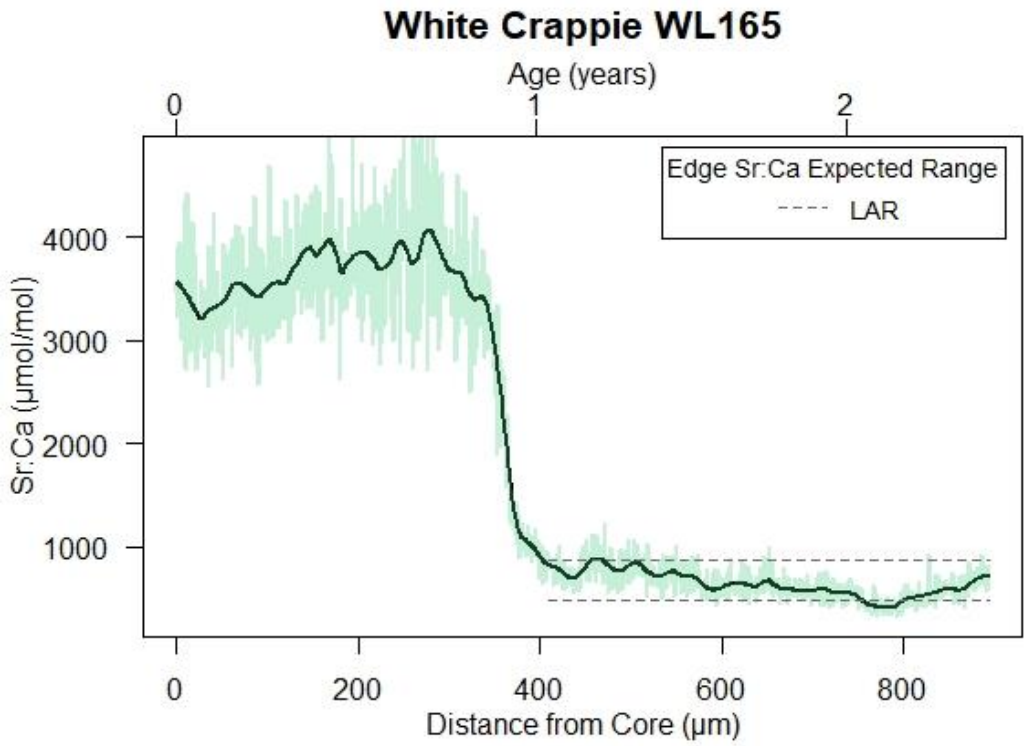

Figure 33. Example of a suspected downstream passage event (Sr:Ca decline) for an individual White Crappie (WL165) collected from the lower Alabama River (LAR) at rkm 118. Sr:Ca ratios >3500 µmol:mol were only observed in other White Crappie collected from Claiborne Lake, suggesting this fish may have completed a potential downstream passage across Claiborne Lock & Dam.

BLUE CATFISH

BCAT Lengths & Ages

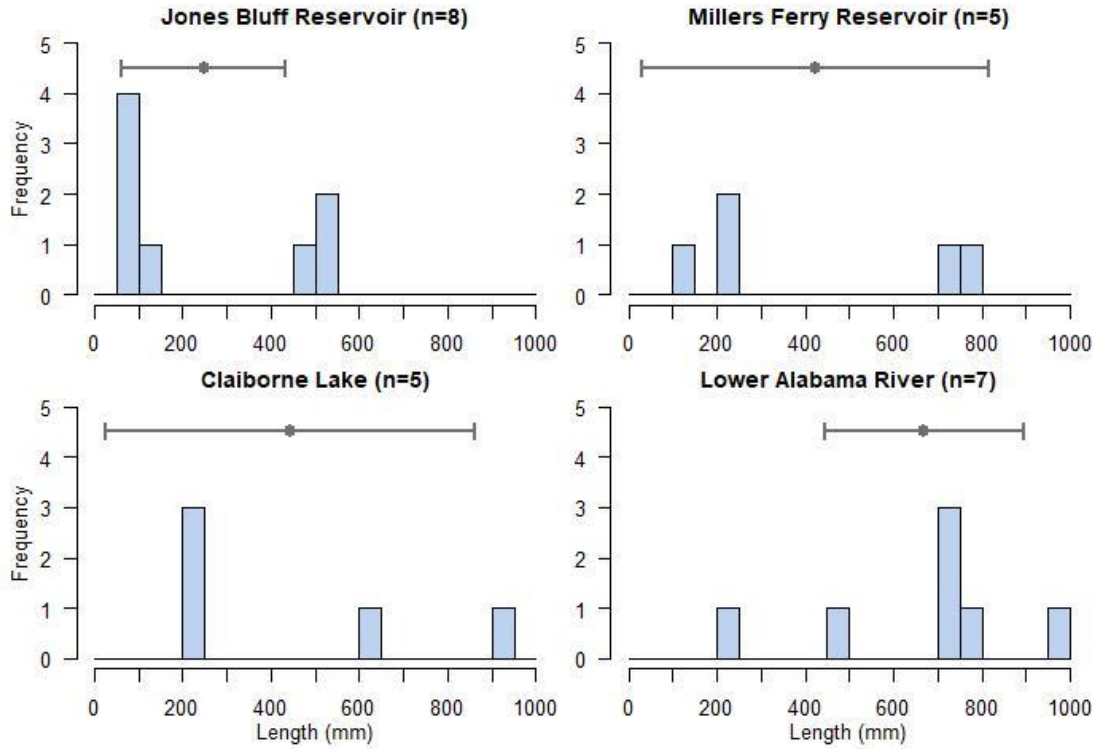

Figure 34. Length frequency distributions of Blue Catfish across the four river sections of the Alabama River. Group means and 95% confidence intervals are also shown. No significant differences in total length were found among the four river sections.

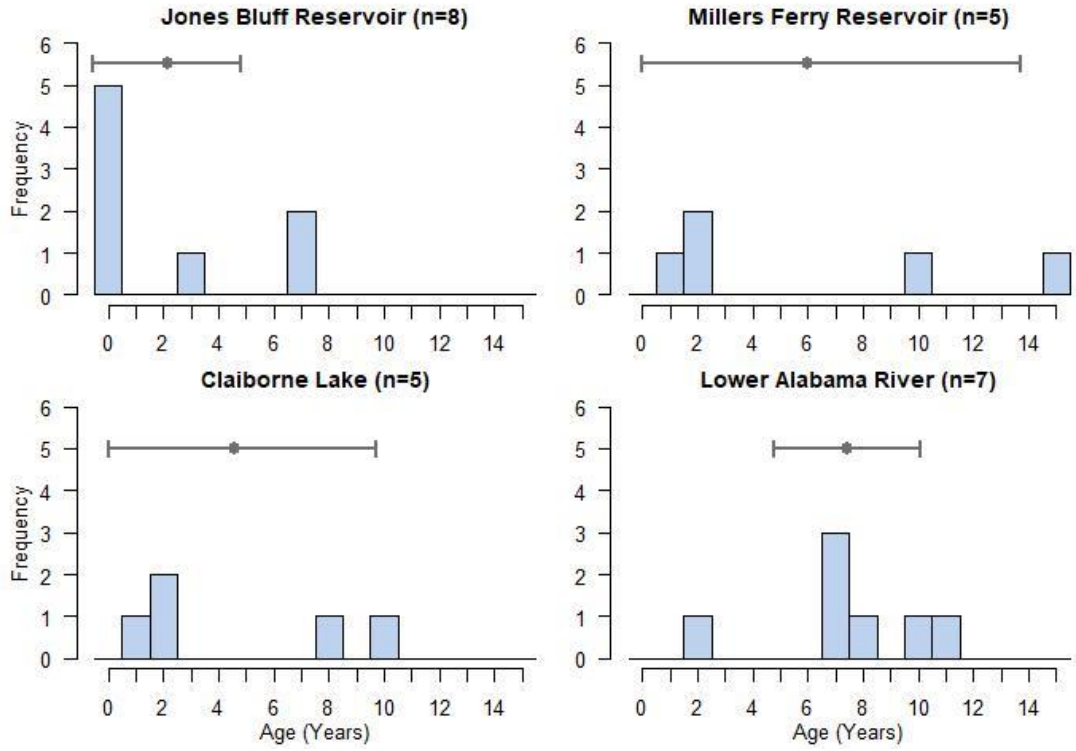

Figure 35. Age frequency distributions of Blue Catfish across the four river sections of the Alabama River. Group means and 95% confidence intervals are also shown. No significant differences in age were found among the four river sections.

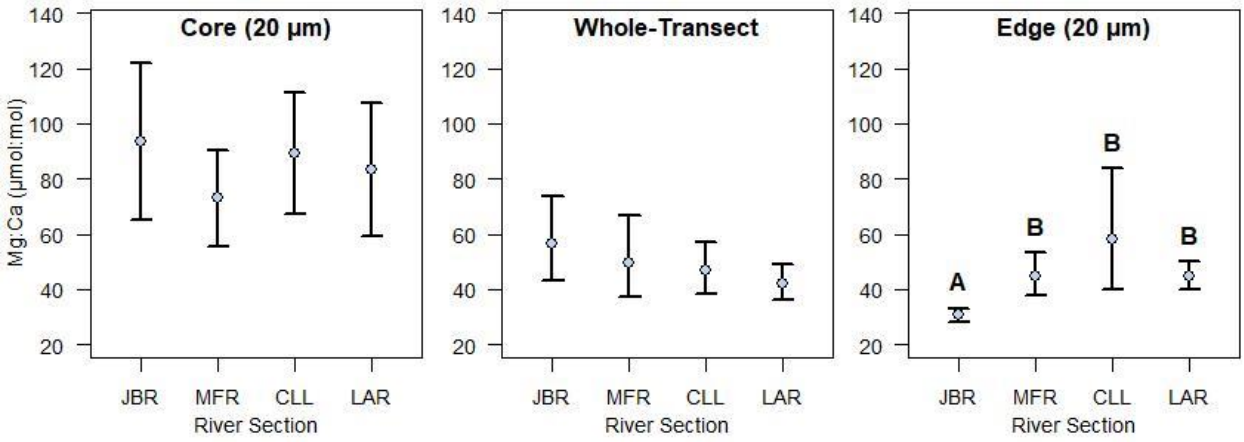

Figure 36. Blue Catfish mean Mg:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

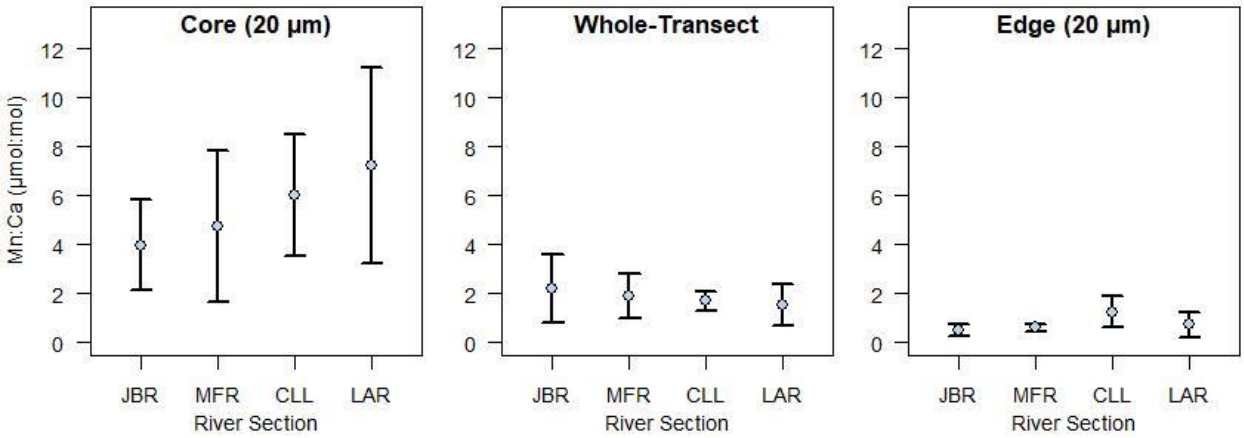

Figure 37. Blue Catfish mean Mn:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected.

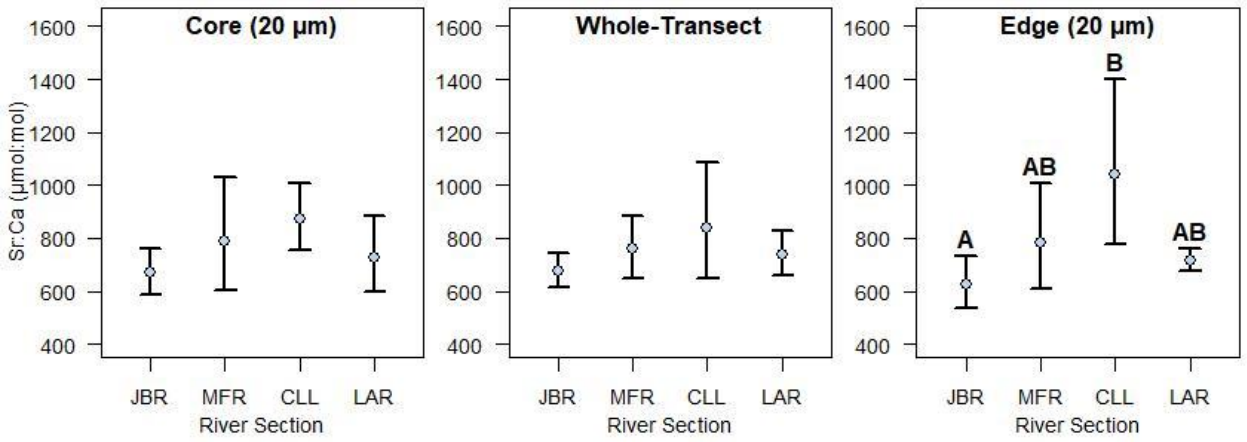

Figure 38. Blue Catfish mean Sr:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected. Letters indicate significant differences between river sections within each otolith region.

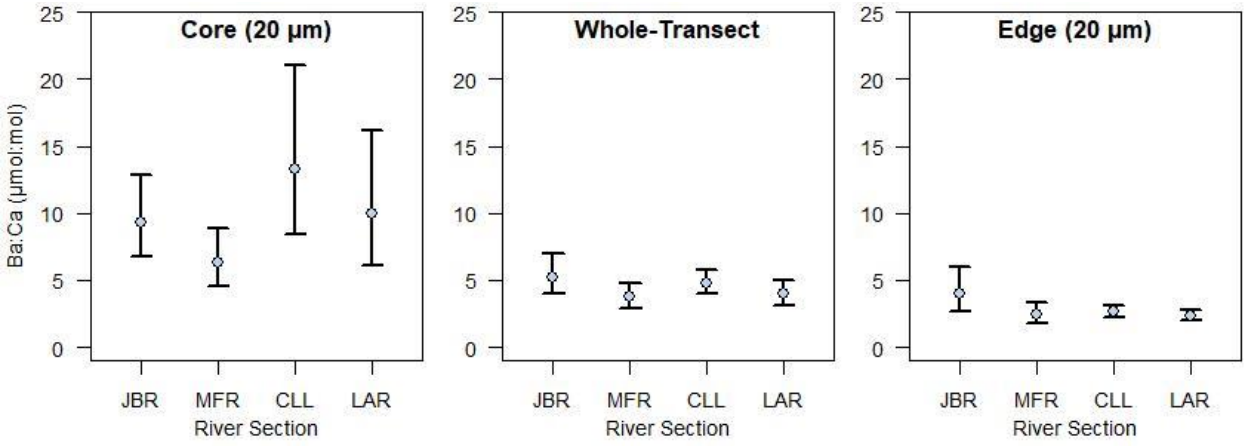

Figure 39. Blue Catfish mean Ba:Ca ratios (with 95% confidence intervals) derived from each of the three otolith regions across the four river sections from which these fish were collected.

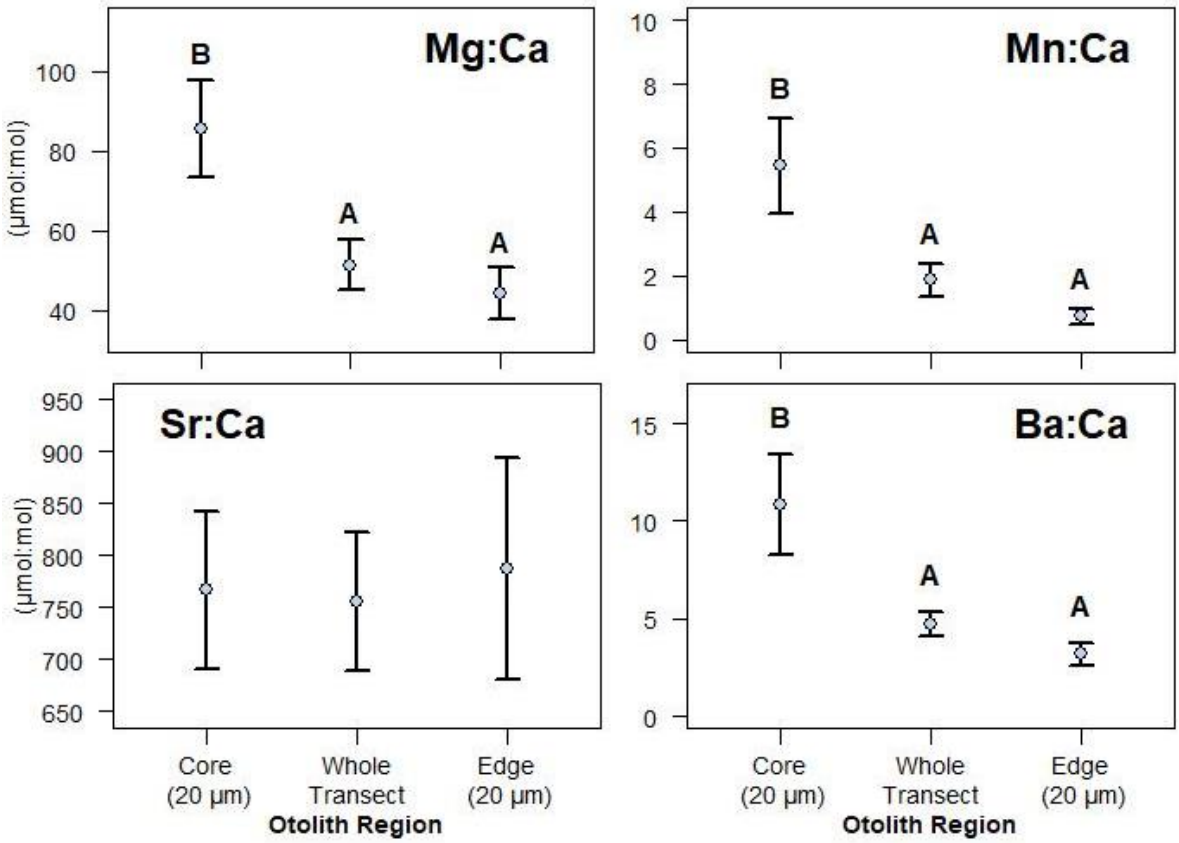

Figure 40. Blue Catfish mean element:Ca ratios (with 95% confidence intervals) across the three otolith regions. Letters indicate significant differences between otolith regions for each element:Ca ratio.

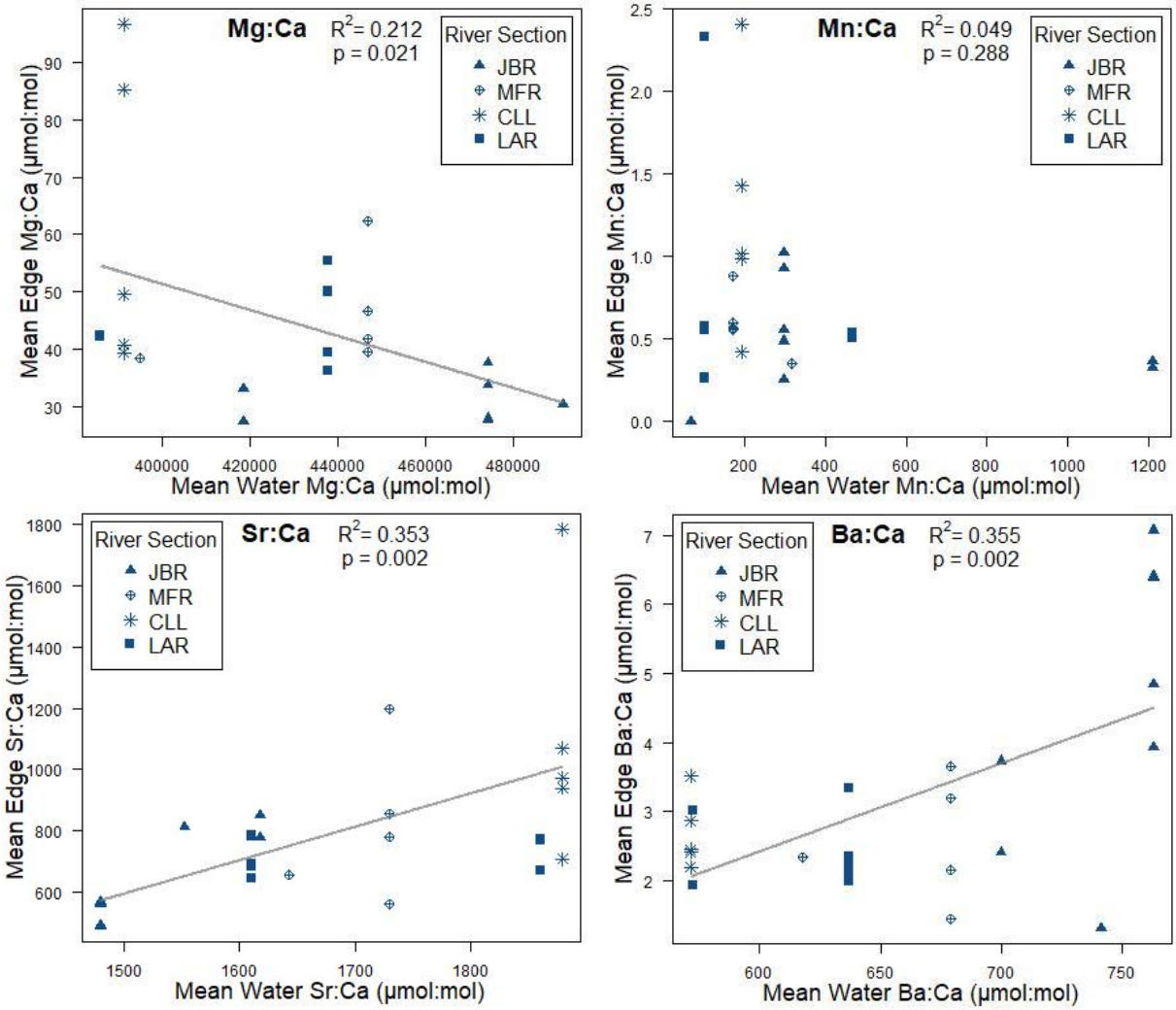

Figure 41. Blue Catfish mean otolith-edge element:Ca ratios versus mean water element:Ca ratios from the river sections and seasons in which fish were collected. Solid regression lines indicate a significant positive relationship, dashed regression lines indicate a significant negative relationship.

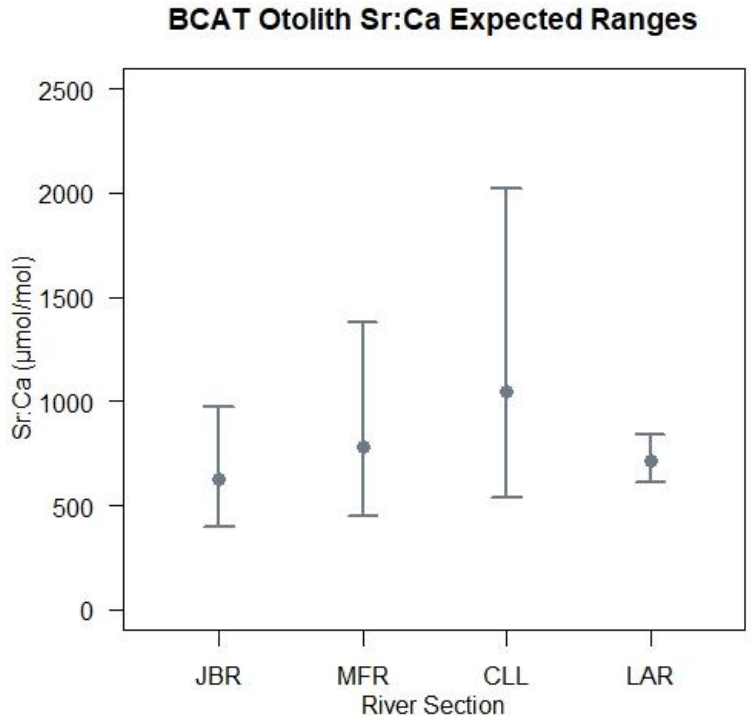

Figure 42. Expected ranges of Blue Catfish otolith Sr:Ca ratios across the four river sections these fish were collected from. These intervals were calculated as the 95% prediction intervals of the Sr:Ca ratios in otolith edges of fish collected from each river section, and used in the criteria for identifying potential dam passages and potential movements of fish. Mean Sr:Ca values derived from Blue Catfish collected from each river section are shown as dots and bars represent the 95% prediction intervals for Sr:Ca values in each river section.

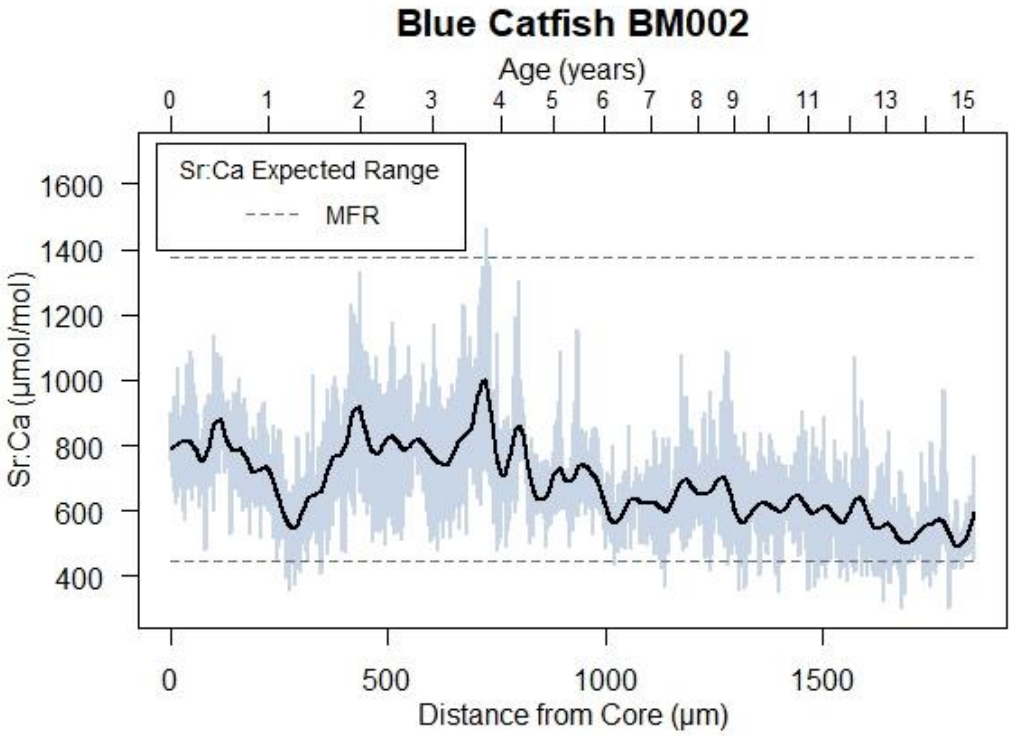

Figure 43. An example of a typical Sr:Ca profile pattern observed among Blue Catfish collected from the Alabama River. This fish (BM002) was collected from Millers Ferry Reservoir (MFR) at rkm 378, and is suspected to have remained within MFR for the entirety of its life.

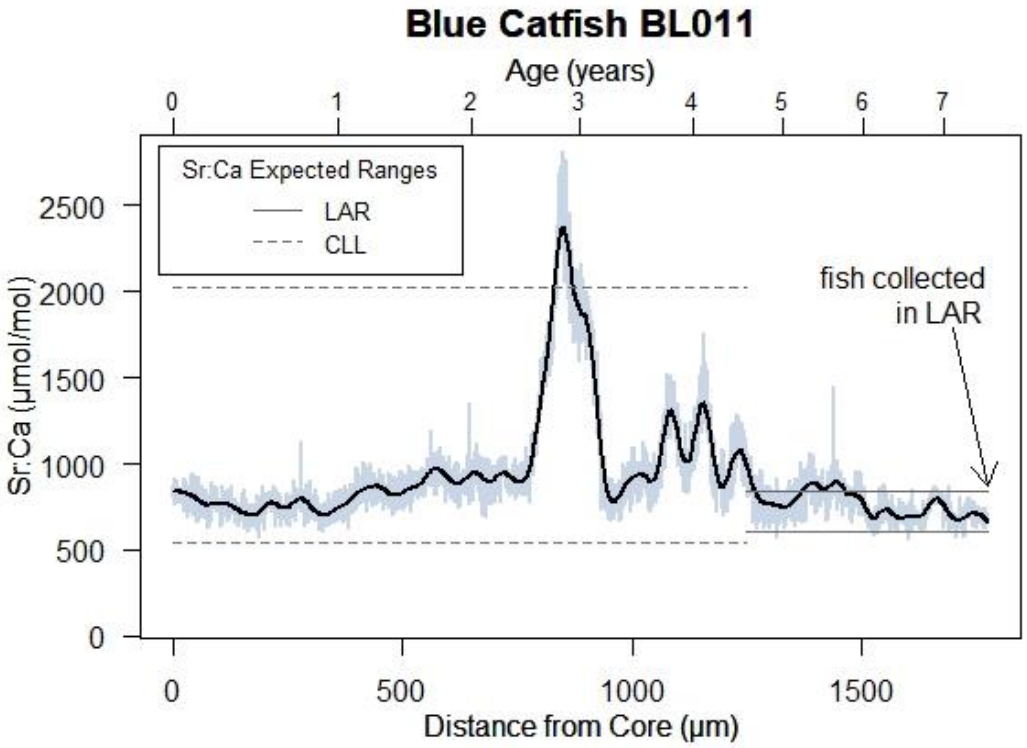

Figure 44. Lifetime Sr:Ca profile of a 7 yr.-old Blue Catfish (BL011) collected from the lower Alabama River (LAR) at rkm 73. The Sr:Ca profile shows a potential downstream passage from Claiborne Lake (CLL) across Claiborne Dam into the lower Alabama River (LAR), possibly occurring around age 4. Alternatively, since this fish was collected in LAR, the large fluctuations in the Sr:Ca profile from ages 2-5 may have resulted from periodic movements between LAR, the Mobile-Tensaw River Delta, or the Tombigbee River.