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A MICROCHEMICAL ANALYSIS TO ASSESS CONTRIBUTIONS OF STOCKED AND WILD CHANNEL CATFISH (ICTALURUS PUNCTATUS) TO STATE-OWNED LAKES IN ARKANSAS

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A MICROCHEMICAL ANALYSIS TO ASSESS CONTRIBUTIONS OF STOCKED AND
WILD CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) TO STATE-OWNED LAKES IN
ARKANSAS

by

Morgan Reeves Winstead

B.S., North Carolina State University, 2019

A Thesis

Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

School of Biological Sciences
in the Graduate School
Southern Illinois University Carbondale
May 2023

THESIS APPROVAL

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A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Master of Science
in the field of Zoology

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December 16, 2022

AN ABSTRACT OF THE THESIS OF

Morgan Winstead, for the Master of Science degree in Zoology, presented on December 16, 2022, at Southern Illinois University Carbondale.

TITLE: A MICROCHEMICAL ANALYSIS TO ASSESS CONTRIBUTIONS OF STOCKED AND WILD CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) TO STATE-OWNED LAKES IN ARKANSAS

MAJOR PROFESSOR: Dr. Gregory W. Whitledge

Channel Catfish are broadly distributed in the U.S. and are important commercially and recreationally in many rivers, lakes, reservoirs, and streams. Since they are a popular sportfish, many state-owned lakes are stocked with a variety of sizes to enhance population sizes and provide angling opportunities. The goals of this study were to determine the contributions of stocked fish, determine the fish size at stocking, and to assess the contribution of yearling and catchable sizes to the stocked percentage. Fish samples were obtained from three hatcheries and six lakes within different ecoregions across Arkansas to assess whether chemical signatures were different among locations. Sectioned pectoral spines were analyzed for Sr:Ca and Ba:Ca using laser ablation-ICPMS to determine whether location-specific Sr:Ca and Ba:Ca signatures were reflected in pectoral spine samples, and to assess the accuracy with which fish could be assigned to their collection location using spine Sr:Ca and Ba:Ca. Fin spine core Sr:Ca and Ba:Ca data were also used to identify stocked fish and determine size at stocking for hatchery-origin fish sampled from each of the six lakes. Spine microchemistry represents a non-lethal approach to identify stocked catfish and infer size at stocking, which will better inform allocation of hatchery-produced fish. Differences in pectoral spine Sr:Ca edge signatures among locations were detected, which were primarily driven by differences in geology among ecoregions. Assignment accuracy of fish to collection location using Random Forest Modeling was 88% or greater for all but one of the study lakes. This allowed for application of the random forest model

on pectoral spine core Sr:Ca and Ba:Ca to assign individuals sampled from the lakes as hatchery or wild origin. Among all the Channel Catfish sampled from the six lakes, 45% were identified as hatchery origin and 46% of those were stocked as catchable size fish. Contributions of stocked fish varied among study lakes from 0% to 100%. This was the first study to demonstrate that pectoral spine microchemistry can be used for assessing both stocking contribution and inferring fish size at stocking. Overall, this study will aid in the allocation of hatchery-reared catfish by management biologists, and could lead to more projects focused on exploring stocking contribution by microchemistry, such as assessment of how habitat enhancement may influence the contribution of natural reproduction to catfish populations.

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CHAPTER 1

A MICROCHEMICAL ANALYSIS TO ASSESS CONTRIBUTIONS OF STOCKED AND WILD CHANNEL CATFISH TO STATE-OWNED LAKES IN ARKANSAS

INTRODUCTION

Within North America there are 45 species within the catfish (Ictaluridae) family, with Channel Catfish (*Ictalurus punctatus*) being among the popular game species of catfishes. They occurred originally in the Gulf of Mexico and the Mississippi Valley states, but were not found in the Atlantic coastal plain or west of the Rocky Mountains (Wellborn 1998). They have since been introduced throughout most of the United States for recreational and commercial purposes and they are the most widely spread catfish nationwide (Wilson 1991, Quinn 2011). Among recreationally harvested fish species, catfish are the fourth most pursued freshwater fish in the United States based on a U.S. Fish and Wildlife Survey (USFWS 2016). Within the Arkansas, a variety of sport fish are targeted, with catfish angling accounting for 18 percent of the effort statewide (“Catfish”, 2020). With Channel Catfish being such a sought-after species recreationally, this can lead to high harvest and mortality within the fishery. When natural recruitment is low or zero, lakes are stocked with put-take (catchable) and put-grow-take (fingerling or yearling) Channel Catfish; nearly all states within the distribution of Channel Catfish have implemented stocking programs to create and maintain either fishery (Vanderford 1984; Smith and Reeves 1986; Michaletz and Dillard 1999). Reasons for low natural recruitment include predation on smaller Channel Catfish, limited habitat for reproduction, or competition for food. Multiple studies report that natural recruitment is insignificant in small impoundments because of predation, mainly by Largemouth Bass (*Micropterus salmoides*) (Marzolf 1957; Krummrich and Heidinger 1973; Spinelli et al. 1985; Storck and Newman 1988). When it comes

to consistent recruitment and strong year classes, stable water levels and low retention time in reservoirs has a strong influence (Stevens 2013). Channel Catfish growth is often density dependent, which means with high biomass there could be both intraspecific and interspecific competition for food resources (Michaletz 2009). To supplement Channel Catfish populations that have low recruitment and high harvest rates, a variety of size classes are stocked in many lakes throughout their range (Michaletz 2009).

Channel Catfish are often stocked as large fingerlings or at ‘catchable’ size to reduce Largemouth Bass predation on stocked fish. However, rearing fish to this size is relatively expensive and hatchery space is limited. Costs to rear Channel Catfish fingerlings ranged from \$30.00/100 for 6-in fish to \$60.00/100 for 12-in fish (Masser and Hyde 1994). Therefore, choosing stocking locations (i.e., prioritizing stocking where demand and harvest is high, survival of stocked fish is high, and natural recruitment is low to nonexistent), sizes, and number of fish stocked at each location to optimize the stocking program and use of hatchery resources is key. Overstocking where there is a naturally reproducing population can lead to slow growth and poor condition of Channel Catfish (Hill 1984; Mitzner 1999; Mosher 1999). Stocking few fingerlings in a population with low natural recruitment can result in fast-growing Channel Catfish but may not provide a viable fishery, especially if harvest is high (Michaletz 2009). Generally, as the stocking rate increases, the relative abundance and mortality of Channel Catfish increases, and condition, growth, and size structure decrease (Michaletz et al. 2008; Michaletz, 2009; Refaey et al 2018). Depending on existing abundance, the contribution of stocked Channel Catfish can vary among locations; Stewart and Long (2015) saw this when evaluating pre-stocking and post-stocking of two similar lakes in Oklahoma. One lake demonstrated a low contribution of stocked fish (3-30%) and smaller sized fish, whereas the other demonstrated high

contribution of stocked fish (89-94%) and larger sized fish, even though both lakes were stocked at similar pre-determined rate. In another study of the Buffalo River in Arkansas, stocked Channel Catfish made up about 93% of the population one year after stocking (Siegwarth & Johnson 1998). Based on the diverse contributions and exploitation rates of Channel Catfish among lakes, it can be difficult for agencies to know how many fish to stock in a lake. This has resulted in managers trying a variety of stocking rates, stocking sizes, and stocking different species of catfish, with inconstant success (Storck and Newman 1988; Shaner et al. 1996; Michaletz and Dillard 1999; Michaletz et al. 2008). Michaletz (2009) suggested that stocking rates need to be determined for each lake because of variability in responses. It has also been suggested for managers to focus on stocking or harvest restrictions of heavily exploited populations and reduce stocking on lightly exploited lakes (Michaletz et al. 2008). Overall, it is important for managers to know how stocked Channel Catfish are contributing to the natural population to effectively manage Channel Catfish fisheries.

Within Arkansas, catfish are present within most rivers, lakes, reservoirs, and streams, and are important in recreational and commercial aspects. Since the 1940s, the Arkansas Game and Fish Commission has stocked more than 1.5 million fish of different species, with Channel Catfish being the most stocked species (Siegwarth & Johnson, 1998). Management objectives for lakes typically focus on maintaining or achieving a desired catch rate. One way the commission supports the populations of Channel Catfish is through stocking fingerling, yearling, and catchable size fish. In Arkansas, the stocking sizes are defined as; fingerlings (1-3 inches), yearling (8-10 inches), and catchable (12-14 inches and about 1 pound).

One way to figure out how stocking is contributing to the population is through use of tags or marks that enable identification of stocked individuals. There are eight primary tagging

types (fin clips, strap tags, anchor tags, transbody tags, injectable tags, skin alterations, telemetry, and origin markers). Each of these tagging methods have advantages and disadvantages to them when used. Fin clippings are a cost effective, easy to apply to large groups, and can be identified for 10 months and sometimes longer depending on the fin that is clipped (Welker 1967; Bunch et al. 2018; Neely et al. 2021). The main disadvantage is that individuals cannot be differentiated. Strap tags are rarely used in catfish research and literature, because of negative effects such as altered behavior, stunted bone growth, and tissue damage (Neely et al. 2021). One exception to strap tags is the butt-end tag when placed on Flathead Catfish pectoral spines, which allows for individual identification and has high retention with minimal tissue and structure damage (Summerfelt and Turner 1972). Anchor tags allow rapid tagging of high volumes of fish, individual fish identification and highly visible external identification, but there is variable retention among catfish species (Neely et al. 2021). Transbody tags especially dangler tags are used by many researchers when needing individual identification for periods longer than a year, but some disadvantages to this method is the time it takes to attach the tag, trauma to dorsal muscle, open wounds that may be prone to infection, and predator attraction to smaller fish (Strand et al. 2002; Neely et al. 2021). Injectable tags include coded wire tags (CWT), visible implant elastomer (VIE), and passive integrated transponders (PIT). CWT allows for individual (lethal) and batch marking (non-lethal) of fish and have 90% retention of at least 4 months (Heidinger and Cook 1988; Becher et al. 2018). VIE allows for groups to be identified rapidly without sacrifice based on color and can be seen up to nine months (Reeves and Buckmeier 2009; Zeller and Cairns 2010). PIT tags have not been used extensively in stocked catfish, but they allow for individual identification. One concern is that PIT tags could cause damage to anglers that consume tissue from tagged fish since they are encased in a glass capsule

(Daugherty and Buckmeier 2009). Skin alterations such as cold brands have the best promise for long-term retention (38 months for channel catfish) with batch marking of catfish when few groups need to be identified (Pritchard et al. 1974; Neely et al. 2017). Telemetry is an effective tool for monitoring individual fish for up to several years, but is restricted by financial and temporal limitations (Neely et al. 2017). One of the newest methods for identifying stocked fish is hard-part microchemistry, which uses naturally-occurring elemental and isotopic concentrations in calcified structures to infer natal origin of individual fish (Pracheil et al. 2014). Some limitations are the sacrifice of fish for otoliths, lumen formation in center of fin spines, and is a relatively new method that has not been used in many catfish studies (Neely et al. 2017).

This study evaluated the effectiveness of origin markers on hard-part microchemistry, specifically pectoral spines, to determine natal origin of Channel Catfish within Arkansas. Two advantages of using calcified structures over traditional mark-recapture studies involving artificial tags are avoiding the need to physically tag fish and tags have a shorter retention time than calcified structures (Thorrold et al. 2002). Another benefit of using natural ‘tags’ versus conventional tagging, is that the structure’s elemental composition will mirror the environmental characteristics of the water the fish inhabits. Water chemistry can vary spatially, and if a fish moves among areas as it grows, that variability will be recorded in the growth layers of the structure. The structure’s material deposited at different ages can be removed and chemically analyzed to determine where a fish lived at different points in its life (Pine et al. 2012). In cases of determining if a fish is of hatchery or wild origin, the core of the calcified structure can be analyzed to infer the natal origin. Normally trace elements strontium and barium in relation to calcium are used for analysis in structures, because Sr:Ca and Ba:Ca ratios are correlated with element:Ca ratios in environmental water (Campana, 1999). Otoliths are normally used for this

process, but an alternative to this structure is pectoral fin spines. The pectoral spines in Channel Catfish are hardened and thickened fin rays, which are comprised of biological apatite (Whitledge 2017, Willmes 2016). Like otoliths, pectoral spines increase in size as the individual grows and lays down growth rings but can form lumens which can result in loss of the early life history record. A benefit of using pectoral spines in research is that they provide a non-lethal way for aging and using microchemistry to track natal origin of individuals.

Microchemistry with trace element analysis of sectioned fin rays has been demonstrated to be an effective, non-lethal alternative to otolith chemistry for reconstructing individual fish environmental history in a few freshwater and anadromous fish species (Veinott et al. 1999; Arai et al. 2002; Clarke et al. 2007; Allen et al. 2009). Multiple studies have found spines to be strongly correlated with water chemistry. Wolf et al. (2013) found a nearly 1:1 relationship between pelvic fin and otolith $^{87}\text{Sr}/^{86}\text{Sr}$. Smith (2010) found that water, otoliths, and spines were highly correlated in elemental signatures for ictalurid catfishes. This study also found both structures in catfish were able to accurately assign origin of individual fish. Avigliano et al. (2019) also found that spine chemistry is an acceptable non-lethal advantage over otoliths to study different biological aspects of catfish. To be able to measure the success of stocking efforts, hatchery reared individuals need to be distinguishable from naturally spawned fish, typically for many years after release (Taylor et al. 2005). The use of microchemistry has been successful in identifying the natal origin of stocked individuals in a wild population back to the hatchery. Bickford and Hannigan (2005) were able to use walleye (*Sander vitreus*) otoliths and distinguish the hatchery of origin with a high degree of accuracy. Rude and Whitledge (2014) were also able to correctly identify 84% of muskellunge from hatchery origin from pelvic fin ray microchemistry. Currently there are no studies that have used Channel Catfish pectoral spines to

identify natal location (hatchery) of stocked fish; thus this study meets a critical need with a high probability of successfully increasing our understanding of stocked fish population dynamics.

Arkansas has several state-owned lakes across the state that are heavily fished for Channel Catfish, and to support these fisheries many of those lakes are stocked regularly with one or more size classes of fish. However, the contributions of stocked fish to Channel Catfish populations in these lakes are unknown. Therefore, this study was designed to use natural chemical markers to evaluate contributions of stocked and wild Channel Catfish in select state-owned lakes in Arkansas. Three objectives were set for this study, with the first being to evaluate the application of pectoral spine microchemistry for distinguishing stocked and wild Channel Catfish from lakes in different ecoregions, second to identify the contribution of stocking and size those individuals were stocked at, and lastly to use the results to better inform stocking and management strategies of Channel Catfish within Arkansas. There are currently four warmwater state hatchery facilities and one net pen facility that raise Channel Catfish and are located within different ecoregions of the state. Joe Hogan State Hatchery is the main hatchery in Arkansas that produces all Channel Catfish for stocking. Along with producing fish to stock in lakes, they provide fry and fingerlings for other hatchery facilities (C.B. Charlie Craig and Wm. H. Donham hatcheries) and 8-inch catfish to net pens (Jim Collins net pens) for later stocking (“Joe Hogan Fish Hatchery”, 2021).

METHODS

Study Area

State-owned Lakes- Catfish pectoral spine samples were obtained from six state-owned lakes for this project. Lakes included in the study were all 414 hectares or less and distributed across ecoregions in the state (Figure 1). Lake Jack Nolen is the smallest at 84 hectares and is

located east of Greenwood in southeastern Sebastian County in the Arkansas River Valley region. It was constructed in 1991 and is a popular sportfishing area for crappies (*Pomoxis* sp), Bluegill (*Lepomis macrochirus*), Redear Sunfish (*Lepomis microlophus*), Largemouth Bass and Channel Catfish (Leone and Feltz, 2019). Lake Jack Nolen contains standing dead timber and stumps and emergent vegetation along the shoreline. Most of the emergent vegetation is American Water Willow (*Justicia americana*), and a Swamp Smartweed (*Polygonum hydropiperoides*). The lake is shallow with a maximum depth of 7 meters near the dam. The banks and bottom substrate are mainly shale and mud, with many creek channels and root systems of standing timber. Several predatory species of sport fish have been stocked into Lake Jack Nolen, Flathead Catfish (*Pylodictis olivaris*), Channel Catfish, and Largemouth Bass. Lake Jack Nolen has been stocked from Jim Collins net-pen, with Channel Catfish being stocked annually. From 2009 through 2019 there were 7 to 11 Channel Catfish stocked annually per hectare, all of which were catchable size.

Lake Barnett is 101 hectares and located west of Floyd in White County between the Arkansas River Valley and Delta regions. It was constructed in 1979, but not filled until 1984, and is a popular fishing area for crappies, sunfishes, Largemouth Bass, Spotted Bass (*Micropterus punctulatus*) and Channel Catfish (Bly et al, 2021). Lake Barnett is long and narrow with steep sides, with an average lake depth of 6 meters and maximum depth of 27 meters. The lakebed is comprised of mainly rubble, gravel, mud, and sand. Cover in the lake is composed primarily of dead standing timber that is distributed throughout the lake, and in shallow areas the shoreline is covered with Water Willow and Spatterdock (*Nuphar luteum macrophyllum*). Channel Catfish were stocked into Lake Barnett, at a rate of approximately 11 catchable catfish per hectare annually, prior to 2008. After 2008, the stocking rate was reduced to

7 catchable Channel Catfish per hectare annually. Lake Barnett has been stocked by Joe Hogan and Jim Collins net-pens. From 2009 through 2019, there were 4 to 8 catchable Channel Catfish stocked annually per hectare, and 41 yearling catfish stocked per hectare in 2016.

Lake Charles is 233 hectares and is located southwest of Powhatan in Lawrence County, in the foothills of the Ozark Mountain region (Asher and Timmons, 2020). It was constructed in 1964 and is a popular sportfishing area for crappies, sunfish, Largemouth Bass, and catfish. Lake Charles has many coves that feature flooded timber and a shoreline that varies from a gradual to steep slope, with an average lake depth of 2 meters and maximum depth of 6 meters. The bottom substrate is comprised largely of mud flats and bedrock outcroppings. Lake Charles has been stocked from Joe Hogan, Donham, and Jim Collins net-pens. From 2010 through 2020 there were 1 to 22 catchable Channel Catfish stocked per hectare annually, and 2 to 15 yearling Channel Catfish stocked per hectare annually. Table 1 shows the stocking years associated with each lake and what sizes of Channel Catfish were stocked during those years.

Along with the primary study lakes, there were an additional three lakes from which 12-15 Channel Catfish pectoral spines per lake were obtained to assess differences in microchemistry between each of the lakes and hatcheries that served as sources of stocked Channel Catfish. Although few pectoral spine samples were obtained from the additional three lakes, stocked individuals sampled from these locations were identified using pectoral spine core microchemistry when possible. Table 2 shows the stocking years associated with each lake and what sizes of Channel Catfish were stocked during those years. The first additional lake sampled was Bob Kidd Lake, which is 81 hectares and is located 2 miles west of Prairie Grove within Washington County in the Ozark Mountain region (“Bob Kidd”, 2021). It was constructed in 1976, and is a popular sportfishing area for Crappie Bluegill , Redear Sunfish , Largemouth Bass

and Channel Catfish (Stein and Hopkins, 2019). The shoreline of Bob Kidd is characterized by standing dead timber and stumps, buckbrush, and riprap, with an average lake depth of 3 meters and maximum depth of 9 meters. In shallow areas, the shoreline is comprised of waterlilies (Nymphaeaceae) and other emergent plants. The banks and bottom substrate are mainly mud, clay, and shale with slight gravel. Bob Kidd Lake has been stocked by Craig and Jim Collins Net-Pens. From 2009 through 2019, there were 7 to 9 catchable Channel Catfish stocked per hectare annually, 49 to 74 yearling Channel Catfish stocked per hectare annually.

The second additional lake included in the study was Lake Overcup, which is 414 hectares and is located 1 mile north of Morrilton within Conway County in the Arkansas River Valley region. It was constructed in 1964, and is a popular sportfishing area for Crappie, Bluegill, Redear Sunfish, Largemouth Bass, Flathead Catfish, and Channel Catfish (Bly et al., 2019). Lake Overcup is characterized by 50% of the shoreline being cleared and developed land and other areas covered with water willow, standing dead timber, buttonbush (*Cephalanthus occidentalis*), and riprap, with an average lake depth of 2 meters and maximum depth of 6 meters. The bottom substrate is comprised largely of shale, clay and fine sediments. Lake Overcup has been stocked by Joe Hogan and Jim Collins Net-Pens. From 2009 through 2019, there were 1 to 7 catchable Channel Catfish stocked per hectare annually, and 29 fingerling Channel Catfish stocked per hectare in 2016.

The third additional lake included in this study was Upper White Oak Lake, which is 242 hectares and located 15 miles northwest of Camden within Ouachita County in the Coastal Plain region. It was constructed in 1961, and is a popular area for crappie, sunfishes, Largemouth Bass, and catfishes (Yung and Kern, 2019). Upper White Oak is characterized by 25% of the shoreline developed land, and other areas covered with pine and mixed hardwoods, with an average lake

depth of 2 meters and maximum depth of 5 meters. The lakebed is comprised of mainly sand, and dense cover of stumps just below the surface of the water. Upper White Oak Lake has been stocked by Joe Hogan and Jim Collins Net-Pens. From 2008 through 2018 there were 3 to 17 catchable Channel Catfish stocked per hectare annually, and 82 yearling Channel Catfish stocked per hectare in 2016.

Catfish Collection

Fish were sampled from the six study lakes by crews from Arkansas Game and Fish Commission (AGFC). The crews used three, 3-meter-long tandem baited hoop-nets (HNS) to catch Channel Catfish. For lakes 50-202 hectares 8 HNS were used, and lakes 202-809 hectares 16 HNS were used with each net series counting as one unit of effort. Fish from Bob Kidd were collected in June 2017, Upper White Oak Lake in May 2018, and Lake Barnett and Overcup in May 2019, Jack Nolen in May 2019, and Lake Charles in May and June 2020 with total length recorded. Pectoral spines from ten fish per 25 mm (1 in) group were retained for aging by AGFC. Channel Catfish from the hatcheries were collected when fish were moved for stocking or when hatchery personnel checked ponds in 2020 and 2022. Catfish were collected from Joe Hogan Hatchery in January and February 2020 with length, weight, and year class recorded for each fish. Fish from Donham Hatchery were collected in April 2022 with length and weight recorded for each fish. Fish from Jim Collins net pens were collected in October 2020 with length recorded. For this project, 334 individual fish were analyzed, each primary lake had 76 individual spines, each extra lake had 12-15 individual spines, and each hatchery had 14-20 individuals. A subsample of 76 spines from each lake was selected to show a variety of ages and lengths from the whole group.

Pectoral Spine Preparation and Microchemical Analysis

Pectoral spines were removed from each fish in the field by disjuncting the spine and then pulling straight out from the body. Spines were then placed in scale envelopes and stored in a warm, dry location for 10-30 days for drying. After drying, any hardened tissue was removed from the spine using forceps, and spines are sectioned using an ISOMET low-speed saw starting at the articulating process. All removing and sectioning of spines was performed by Arkansas Game and Fish Commission, after which the sections were transported to Southern Illinois University for further processing in preparation for microchemical analysis. Sectioned spines were sanded using wetted 600 grit sandpaper and polished using lapping film to reveal the core and annuli. Polished spine sections were mounted on an acid-washed slide with double-sided tape and stored in an acid washed polypropylene petri dish. Sections were used for aging each individual, the ages were estimated by readers from Arkansas Game and Fish Commission and myself.

One section from the articulating process was used for trace element analysis (Sr:Ca and Ba:Ca). Spines were analyzed using a Thermo X-Series 2 inductively coupled plasma mass spectrometer (ICPMS) combined with a CETAC Technologies LSX-266 laser ablation system (LA-ICPMS). The laser ablated a transect starting approximately 100 μm from the core, traveling through the core and extending to the edge of the spine on the opposite side of the core from the start of the ablation transect. Two standard reference materials (MACS-3 (CaCO_3 matrix) and NIST1486 (bone meal)) were analyzed every 10-15 samples in triplicate, to correct for potential instrumental drift and enable calculation of elemental concentrations from raw isotopic count data. Each sample was preceded by a 30 second gas blank measurement and followed by a 30 second washout period. Results were converted to elemental concentrations ($\mu\text{g/g}$), after

correction of the gas blank, matrix, and drift effects using ElementR (Sirot and Guilhaumon 2020). Isotopes analyzed were ^{43}Ca , ^{86}Sr , and ^{138}Ba , and calcium was used as the internal standard. The mean relative standard deviation (RSD) for replicate measurements of ^{86}Sr and ^{138}Ba in the reference standards were 4.73% and 6.68%, respectively. Limits of detection (LOD) were 419 cps/sec for strontium and 101 cps/sec for barium and elemental concentrations in spines always exceeded detection limits. Strontium and Barium concentrations were converted to molar Sr:Ca and Ba:Ca ratios (mmol/mol).

Statistical Analysis

To characterize lake and hatchery Sr:Ca and Ba:Ca signatures, the mean Element:Ca from the outermost 25 μm of the laser ablation transect across the sectioned fin ray from each fish was assumed to represent the elemental signature indicative of the lake or hatchery that fish was collected from. These means were taken from each individual from each location to create a known signature edge dataset. To detect differences in the elemental signatures among fish from different study lakes and hatcheries, a multivariate analysis of variance (MANOVA) was used on all the spine edge Sr:Ca and Ba:Ca data together. An ANOVA was also done for each element followed by Tukey's Honestly Significant Differences tests to assess differences among individual locations. To determine whether strontium or barium accounted for more variation in spine chemical signature differences among locations, a standardized conical coefficient was used (Scheiner 2001). The conical coefficient plotted the class means on two canonical variables, confidence circles for those means, and variable vectors showing the correlations of variables with the canonical variates.

Random Forest models (Breiman 2001) were used to assess classification accuracy of assigning individual fish to known sources (hatcheries or lakes where fish were collected) using

spine edge Sr:Ca and Ba:Ca. First, multiple edge datasets were created for each study lake by utilizing the pectoral spine edge data from all fish collected from the respective lake and hatcheries that contributed fish. Five hundred trees were generated for each study lake using aggregated bootstrap sampling of the edge data. Within each tree, the model used the bootstrapped sample to predict (i.e., classify origin) the data not included in the bootstrap and generated an out-of-bag (OOB) error, which indicated the ability of the tree to appropriately classify a fish back to its correct location designation based on its edge Sr:Ca and Ba:Ca signature. The OOB errors from each of the 500 trees were aggregated into a single OOB estimate of error rate, which was used to assess the overall classification accuracy of the model (Liaw and Weiner 2002).

To identify natal origin for all Channel Catfish, multiple prediction datasets were created for each lake by using the prediction function, which utilized the random forest edge dataset for the respective lake and the individual core signature. The core signatures were determined from the average of 300 μm after the 100 μm before the core was discarded, this distance was used since that was the average size of spine cores. After the prediction function was run against the edge dataset and core signatures, the predictions determined if an individual was a stocked or natural fish.

After individuals were identified as stocked or natural, breakpoint analysis (Priyadarshana and Sofronov 2015) was run on each individual spine signature that was acquired from the series of data collected from the laser transect to see where changes from hatchery to lake signatures occurred. This change was determined by using the minimum to maximum and averages of edge values from each site. Location of signature change aided in identifying size of stocking, the breakpoint was plotted on the ablated line to determine if it was within or outside

the first annulus. If the signature of the hatchery ended past the first annulus, it would be inferred that it was stocked at a catchable size or if the signature ended prior or at the first annulus, it would be inferred it was stocked as a yearling or smaller. Generally, yearlings are 6-12 months old, but likely stocked just before the first annulus appears, while catchables are typically 18-24 months old, but most are 2 years old. Occasionally, there are slow and fast-growing catfish, the slow growers could be a year or older and be considered yearling size, while fast growers could be a year old and be considered catchable size. The stocking records for each lake and ages of individuals were used in combination with the breakpoint to determine the size at stocking.

All statistical analyses were performed using R Studio (R version 4.1.2, R Core Team 2022). All assumptions of parametric statistics were assessed and met or addressed, and all statistical analyses were evaluated at $\alpha = 0.05$.

RESULTS

Differences in Spine Microchemistry among Locations

The MANOVA indicated that there were differences in Channel Catfish pectoral spine edge microchemistry among locations ($P < 2.2e-16$). The standardized conical coefficient showed that the first conical variable 1 accounted for 96% separation, and strontium was strongly correlated with that dimension (Figure 2). Mean pectoral spine edge Sr:Ca did not differ between Craig-Bob Kidd, Overcup-Jim Collins, or White Oak-Joe Hogan ($P \geq 0.93$). Mean pectoral spine Sr:Ca differed for all other combinations of lakes and the hatcheries that represented sources of stocked Channel Catfish to each lake ($P \leq 0.03$). For pectoral spine edge Ba:Ca, Tukey's HSD tests showed that Joe Hogan-Barnett, Craig-Bob Kidd, Jim Collins-Bob Kidd, Jim Collins-Charles, Joe Hogan- Jack Nolen, Overcup-Jim Collins, and White Oak-Joe Hogan means did not differ ($P \geq 0.1$). The difference in mean pectoral spine edge Ba:Ca between

Joe Hogan Hatchery and Bob Kidd Lake was marginally significant ($P = 0.06$). All other combinations of lakes and hatcheries that served as sources of stocked Channel Catfish to each lake had differing mean pectoral spine edge Ba:Ca values ($P \leq 0.01$). Table and Figure 3 show the means and minimum and maximum pectoral spine edge Sr:Ca and Ba:Ca for each study location.

Random Forest

When broken up individually by lakes and their respective hatcheries, Lake Charles had the highest overall classification accuracy, with 98% being correctly classified back. Upper White Oak Lake had the lowest overall classification accuracy of 71%. Lake Overcup had the second lowest overall classification accuracy of 80%. Lakes Barnett, Jack Nolen, and Bob Kidd had intermediate classification accuracies of 88-91% (Table 4). This is comparing only the edge data for all of the individual fish from each study location, and not using any of the core signatures. For the classification, in the example of Lake Jack Nolen it would be a fair assumption that 9% of the fish could possibly be misclassified between Jack Nolen and Joe Hogan, but fish going through Jim Collins Net Pen should not be misclassified.

Stocking Contribution

With all the study lakes grouped together, 122 of the 267 individuals were of hatchery origin. Based off natal origin signatures, the random forest model classified contributions of hatchery origin fish as follows: Lake Overcup 100%, Bob Kidd 86%, Lake Charles 83%, Lake Barnett 36%, Upper White Oak 33%, and Lake Jack Nolen 0%. Analysis of breakpoints showed that for yearlings $671\mu\text{m}$ and $1242\mu\text{m}$ for catchable was the average distance of the hatchery signatures across the spine (Figure 4).

From the breakpoints in signatures and ages of the individuals identified to have hatchery origins, 83% were stocked as catchable size fish (Figure 4). Broken up by individual lake, 100% of Lake Overcup, 81% of Charles, 68% of Lake Barnett, 69% of Bob Kidd Lake, and 100% of Upper White Oak Lake stocked Channel Catfish were catchable size. Lake Barnett had a unimodal pattern of Channel Catfish length-frequency, with the highest peak at 500 mm. Both Lake Charles and Jack Nolen had a bimodal pattern with the highest peaks for Lake Charles at 250 and 400 mm, and Jack Nolen at 350 and 500 mm (Figure 5). These graphs were created to compare the length-frequency of the fish sampled from each lake in relation to fish origin (stocked or wild). When comparing the size at stocking percentages with the length-frequency, it was discovered that there were more yearling sizes in the length-frequency than in the size at stocking percentages in Lake Charles. Resulting in 56% of Lake Charles stocked Channel Catfish being stocked at catchable size. This could be from the hatcheries holding the slow growing Channel Catfish that are older than a year old, which would be misclassified from aging and breakpoint analysis.

DISCUSSION

Microchemistry as a tool

There was significant variation of chemistry among most of the study lakes and hatcheries throughout Arkansas, which allowed for distinguishing of naturally and hatchery produced fish. The variation observed among the lakes and hatcheries Sr:Ca and Ba:Ca was likely due to the different ecoregions and heterogeneity in the underlying geology. Lakes and hatcheries that did have overlap and did not differ in spine edge Sr:Ca were within similar or the same ecoregions. Study sites that are generally in the Lowlands (e.g., Mississippi Delta and Gulf Coastal Plains) had the highest spine Sr:Ca ratios, while sites in the Ozark Mountains had the

lowest Sr:Ca ratios. Sites located in the Arkansas River Valley and Ouachita Mountains had intermediate Sr:Ca ratios. The Ba:Ca signatures showed the same pattern, although the differences in Ba:Ca among the ecoregions are less pronounced. Water bodies demonstrate specific chemical signatures due to different geological characteristics, weathering processes, and groundwater recharge among respective drainages (Pangle et al. 2010). Studies done on water chemistry have seen similar patterns, with lower water Sr:Ca in the Ozark Highlands and lower Sr:Ca in rivers and lakes in the Mississippi River and Gulf Coastal Plain lowlands (Zeigler and Whitley 2011, Whitley 2022). Within this study, we used both elements for analysis and found that Sr:Ca was the best indicator when distinguishing chemical signatures of locations. This could be due to the greater value range of Sr:Ca of locations, but there have been a few studies that show structure Ba:Ca has been less strongly correlated with water Ba:Ca in comparison to correlations between Sr:Ca in water and Sr:Ca in hard structures of freshwater fishes (Clarke et al. 2007; Zeigler and Whitley 2010; Smith and Whitley 2010).

The random forest model was able to classify fish in the edge dataset to their correct location with greater than 70% classification accuracy, with most lakes having greater than 88% accuracy in assigning fish to their collection location despite overlap in some signatures. For comparison, classification accuracies in a similar study of otoliths on Yellow Perch (*Perca flavescens*) have ranged from 67% to 100% in Lake Erie tributaries (Pangle et al. 2010). Rude and Whitley (year) were also able to identify 84% of muskellunge back to hatchery origin with pelvic fin ray microchemistry and confirm assignments with PIT tags. Lastly, a study on June Suckers (*Chasmistes liorus*) showed that pelvic fins and otolith microchemistry could be used to determine between wild and hatchery origin fish (Wolff et al. 2013). The classification accuracy of these studies depended on geologic differences among sites and the number of hatcheries and

lakes involved, but overall showed that microchemistry is a useful tool in determining natal origins of wild and stocked fish. Since there was high classification accuracy between lakes and hatcheries, the random forest was able to use predictions to give natal origins to unknown fish. This allowed for individuals to be classified as hatchery or wild origin and to further look at contributions within each lake.

Lakes within the lowlands and Arkansas River Valley (e.g., White Oak, Barnett, and Jack Nolen), had overlapping Sr:Ca signatures with Joe Hogan hatchery. Rearing fish through Joe Hogan hatchery and then Jim Collins net pens (Ouachita ecoregion) made stocked fish easier to identify, since the fish had a section of lower Sr:Ca in the spine that reflected the profound shift to lower ratio of Jim Collins net pens signature. For future applications of microchemistry in Arkansas lakes, it would be practical to evaluate stocked fish contributions using fish that have been in at least one hatchery or rearing facility that is in a different ecoregion than the lake they're being stocked into.

This study is one of the first to date that explored microchemistry as a tool for classifying natal origins for stocked and wild fish with the use of pectoral spines, as well as the first to use changes in signatures across sectioned spines to determine size at stocking. Being able to determine how far the hatchery signature extended from the core of the sectioned spine allowed for identification of catchable or yearling fish, which helps enhance the value of microchemistry of stocking evaluations. Past studies have used otoliths for microchemistry in distinguishing stocked and wild fish, but the use of pectoral spines provides the added benefit of a non-lethal method. Otoliths have been found to provide more precise estimates of age than spines for Channel Catfish, but studies have found that Channel Catfish populations across a broad geographic distribution showed spines have a high (>80%) probability of providing accurate ages

for Channel Catfish up to age 4 and providing an age estimate within 1 year of the assumed correct age from otoliths for up to age 16 (Buckmeier et al. 2002, Olive et al. 2011). Colombo et al. (2010) found high agreement and detected no bias between otoliths and spines in Channel Catfish. Pectoral spines were recommended to estimate ages of Channel Catfish in short-lived populations or where older fish are rare or nonexistent (Hall et al. 2022). The oldest fish in this study was 10 years old, but the majority of the fish samples from the study lakes were 3-5 years old. Being able to assess the stocking contribution, size at stocking, and not having to sacrifice fish for the study makes microchemistry an ideal tool for managers.

Stocking Contributions

For natal origins, the predictions from the random forest found that 45% of all the Channel Catfish had hatchery origins, but varied between the lakes. The estimated percent of contribution from stocked fish was lowest in the three study lakes (White Oak, Barnett, and Jack Nolen) that had overlapping Sr:Ca signature with Joe Hogan hatchery. This lower contribution percentage could be partially due to classification error (mis-identifying stocked fish as wild individuals). This would be especially true for Upper White Oak Lake and Lake Barnett, which was stocked with fish transferred directly from Joe Hogan hatchery to the lake. However, fish stocked into Lake Jack Nolen were also reared at Jim Collins net pens, so identification of stocked fish would be enhanced because of the difference in Sr:Ca signature between Jim Collins net pen and Lake Jack Nolen. For all other lakes, the percent of stocking contribution was relatively high, suggesting that stocked fish contribute strongly to populations in those lakes.

Another possible explanation for some of the differences in estimated contribution of stocked fish among lakes could be differences in stocking rates among lakes. When comparing the lakes where 76 individuals were sampled from each, the number of catfish stocked ranged

from 1 to 22 catchable catfish and 5 to 41 yearling catfish per hectare annually. In the case of Lake Jack Nolen, which is the smallest of those lakes with the lowest number of stocked catfish, it had no hatchery individuals in the sample. Conversely, Lake Charles is the largest of those lakes, has been stocked with the most catfish, and had the highest percentage of hatchery individuals identified in our samples. In terms of sizes of fish stocked, Lake Jack Nolen was only stocked catchable size catfish, but Lake Charles was stocked with both catchables and yearlings. This could account for the relatively high percentage of stocked Channel Catfish in the Lake Charles sample compared to Lake Jack Nolen, and could bring attention to the stocking rate that is used at Lake Charles. One thing to note between these two lakes is the length-frequency of Channel Catfish. For both lakes, a similar bimodal pattern in length-frequency occurred, but the peaks at Lake Jack Nolen were at much larger lengths. Then, when comparing Lake Barnett and Lake Charles, since both have hatchery individuals within the study sample, Lake Charles is a little over two times in size as Lake Barnett and has been stocked with about three times as many catfish. When comparing length-frequencies between the two lakes, Lake Barnett has a high number of larger fish within the population than Lake Charles. Lake Barnett had about half the amount of identified stocked fish when compared to Lake Charles, and this could be because fewer fish were stocked into Lake Barnett. Lastly, when comparing Lake Jack Nolen and Lake Barnett both are similar in size, mortality rates, and comparable CPUE based off records from Arkansas Game and Fish Commission. The pair have similar stocking rates of catchable catfish, but Lake Barnett has stocked yearlings where Jack Nolen has not. The length-frequency graphs show they both have a wide range of sizes, with Lake Barnett having a few larger individuals. If the samples are representative of size distributions of Channel Catfish in these lakes, the smaller size of fish collected from Lake Charles may be due to slower growth of fish from a larger

population, since Channel Catfish growth is often density dependent (Michaletz 2009). Overstocking catfish on top of a naturally reproducing population can lead to slow growth and poor condition of Channel Catfish (Hill 1984; Mitzner 1999; Mosher 1999). Other reasons for differences in length-frequency distributions among lakes could be differences in harvest rates, food availability, or size-dependent or age-dependent survival probabilities (Ylikarjula et al. 1999). This could lead to more research projects on these lakes to see if the stocking rates need to be evaluated, or if there are other reasons for the smaller catfish in the Lake Charles population. Lastly, other factors that could potentially explain differences in stocking contribution could be the availability of spawning habitat or predation on small catfish among the different lakes.

Along with a little less than half of the catfish being of a hatchery origin across all of the study lakes, most of them were stocked at a catchable size. In Arkansas, a catchable size stocked catfish is 12 to 14 inches and about 1 pound in size, and a yearling is 8 to 10 inches. Raising fish to these sizes can be costly at \$30.00/100 for 6-in fish to \$60.00/100 for 12-in fish (Masser and Hyde 1994). In most of the lakes that were stocked with yearlings during more than one year, the number of stocked yearlings was equal, to or greater than, the number of catchable catfish stocked. Yet, no more than 44% of the hatchery fish sampled were stocked as yearlings. Some of these sizes could have been misclassification since classification depends: 1. accuracy of age assignments, 2. using a consistent laser path across spine sections, 3. accurate identification of location of Sr:Ca shifts in relation to the location of the first annulus, and 4. the assumption that all fish stocked at age 1 were catchable size when stocked. The breakpoint analysis helped in decreasing this misclassification, by plotting the whole signature and identifying where the signatures changed from hatchery to lake based on the distance from core. The greater number of

catchables than yearlings among stocked fish identified in our samples may have been due to higher survival post-stocking for the larger catchable fish. Stock and Newman (1988) found that for the greatest return on investment, Channel Catfish of a minimum 8-inches should be stocked. Along with the evaluation of Channel Catfish stocked in lakes that contained Largemouth Bass, sizes 6-inches to 8-inches had the highest survival (78-100%) (Dudash et al.1996). Howell and Betsill (1999) suggested that catfish 9-inches were most appropriate in Texas, while Shaner et al. (1996) reported optimum size of 10-inches in Alabama. These sizes all fall within the yearling size class for Arkansas, but as seen it varies between states and studies on the optimal size. For Arkansas, stocking catchable size catfish might be more cost effective when looking at the return of individuals in the population based off the number and size stocked.

Management Implication

Overall, there are many stocked fish throughout most of these study lakes. This information can help shed light on the population dynamics of Channel Catfish on the different study lakes and is a tool for post-stocking assessment. Now managers have an idea of how much stocking is contributing to the population and what sizes are surviving after stocking. From the length-frequency of the primary three study lakes, it shows that there is a range of sizes across the wild and hatchery fish which indicates a variety of age groups. In Lake Charles the lack of smaller fish indicates low natural reproduction but length at age data suggests stunting. These data could lead to investigations into whether there should be changes in stocking practices (e.g. number stocked, frequency, size stocked) so that overstocking does not occur within lakes, along with investigations into available habitat (e.g., spawning), and effects of predation on small Channel Catfish that could be causing low natural reproduction. For Lake Jack Nolen since there were no stocked fish, an exploitation study of hatchery fish to see if they are being fished out

quickly or if hatchery fish aren't doing well post stocking. Since, Lake Barnett recently reduced the stocking rate; conducting this study again 5 years after that change in stocking could provide insight into the relationship between stocking rate, mortality, and size structure. This project is the first to use microchemistry to help identify stocked and wild individuals in a population, and now it has been shown to be a useful tool in the fisheries community, especially if there are distant chemical signatures between lakes and hatcheries.

This project could be expanded on by increasing the samples size from the lakes, especially the ones that only had 15 or less individuals to see how much stocking is contributing to those populations. It has been suggested that stocking rates need to be determined for each lake because of variability in responses to stocking (Michaletz 2009). It has also been suggested for managers to focus on stocking or harvest restrictions of heavily exploited populations and reduce stocking on lightly exploited lakes (Michaletz et al. 2008). Stocking and exploitation rate could help determine if harvest affects population size and if stocking rates affect angler effort and harvest. Adding other lakes from across the state that are of interest to managers can help increase the information available to managers when creating management plans and improving stocking rate. Similar studies could include a pre-study to see how much stocking contributes, followed by a habitat enhancement project, conducted with a post-study to see if stocking contributions changes demographics of natural populations.

EXHIBITS

Table 1: Stocking Records of each primary study lake by number stocked per hectare, fish size, and years stocked.

Lake	Size	Year	Fish per hectare	
<i>Barnett</i>	catch	2009	7	
	catch	2010	7	
	catch	2011	7	
	catch	2012	8	
	catch	2013	7	
	catch	2014	7	
	catch	2015	7	
	catch	2016	7	
	yearling	2016	41	
	catch	2017	8	
	catch	2018	8	
	catch	2019	4	
	<i>Charles</i>	catch	2010	7
		yearling	2010	5
catch		2011	9	
yearling		2011	5	
catch		2012	11	
yearling		2012	5	
catch		2013	9	
yearling		2013	5	
catch		2014	8	
yearling		2014	5	
catch		2015	5	
yearling		2015	2	
catch		2016	22	
yearling		2016	15	
catch		2017	1	
yearling		2017	15	
catch		2018	1	
yearling		2018	15	
catch		2019	1	
yearling		2019	15	
yearling		2020	15	
<i>Jack Nolen</i>	catch	2009	10	
	catch	2010	10	
	catch	2011	7	
	catch	2012	7	
	catch	2013	6	
	catch	2014	7	
	catch	2015	11	
	catch	2016	8	
	catch	2017	7	
	catch	2018	7	
catch	2019	7		

Table 2: Stocking Records of each extra study lake by number stocked per hectare, fish size, and years stocked.

Lake	Size	Year	Fish per hectare	
<i>Bob Kidd</i>	catch	2009	7	
	catch	2010	7	
	catch	2011	7	
	catch	2012	9	
	catch	2012	7	
	catch	2013	7	
	yearling	2013	49	
	catch	2014	7	
	yearling	2014	49	
	yearling	2015	49	
	yearling	2016	49	
	yearling	2017	49	
	yearling	2018	74	
	yearling	2019	49	
	<i>Overcup</i>	catch	2009	5
catch		2010	5	
catch		2011	5	
catch		2012	5	
catch		2013	5	
catch		2014	5	
catch		2015	7	
catch		2016	2	
fingerling		2016	29	
catch		2017	2	
catch		2018	2	
catch		2019	1	
<i>White Oak</i>		catch	2008	10
		catch	2009	9
	catch	2010	9	
	catch	2011	10	
	catch	2012	4	
	catch	2013	14	
	catch	2014	3	
	catch	2015	11	
	catch	2016	17	
	yearling	2016	82	
	catch	2017	12	
	catch	2018	9	

Table 3: Mean, minimum, and maximum pectoral spine edge Sr:Ca ($\mu\text{mol/mol}$; a) and Ba:Ca ($\mu\text{mol/mol}$; b) for each lake and hatchery included in the study.

a

Lake	Mean	Minimum	Maximum	Sample Size
Barnett	984	541	1699	76
Bob Kidd	268	233	310	15
Charles	175	98	260	76
Jack Nolen	1091	702	1627	76
Overcup	452	305	573	14
White Oak	1439	916	2085	12
Craig	212	186	237	15
Donham	580	482	695	20
Jim Collins	549	503	609	17
Joe Hogan	1430	768	2176	14

b

Lake	Mean	Minimum	Maximum	Sample Size
Barnett	37	12	81	76
Bob Kidd	15	10	24	15
Charles	14	4	30	76
Jack Nolen	45	16	145	76
Overcup	12	6	20	14
White Oak	40	22	56	12
Craig	12	8	21	15
Donham	49	20	58	20
Jim Collins	6	4	28	17
Joe Hogan	33	16	50	14

Table 4: Confusion matrix for each lake with respective hatcheries, with estimate error rate.

Confusion Matrix- Lake Charles					
OOB estimate of error rate: 2%					
	Charles	Donham	Jim Collins	Joe Hogan	Class Error
Charles	76	0	0	0	0
Donham	0	19	1	0	0.05
Jim Collins	0	1	16	0	0.05
Joe Hogan	0	0	1	13	0.07

Confusion Matrix- Lake Jack Nolen				
OOB estimate of error rate: 9%				
	Jack Nolen	Jim Collins	Joe Hogan	Class Error
Jack Nolen	73	0	3	0.04
Jim Collins	0	17	0	0
Joe Hogan	7	0	7	0.50

Confusion Matrix- Bob Kidd Lake					
OOB estimate of error rate: 11%					
	Bob Kidd	Craig	Jim Collins	Joe Hogan	Class Error
Bob Kidd	13	2	0	0	0.13
Craig	2	13	0	0	0.13
Jim Collins	0	0	16	1	0.06
Joe Hogan	0	0	2	12	0.14

Confusion Matrix- Lake Barnett			
OOB estimate of error rate: 12%			
	Barnett	Joe Hogan	Class Error
Barnett	73	3	0.04
Joe Hogan	8	6	0.57

Confusion Matrix- Lake Overcup				
OOB estimate of error rate: 20%				
	Overcup	Jim Collins	Joe Hogan	Class Error
Overcup	11	3	0	0.21
Jim Collins	5	12	0	0.29
Joe Hogan	0	1	13	0.07

Confusion Matrix- Upper White Oak Lake				
OOB estimate of error rate: 29%				
	White Oak	Jim Collins	Joe Hogan	Class Error
White Oak	3	0	7	0.70
Jim Collins	0	17	0	0
Joe Hogan	4	1	9	0.36

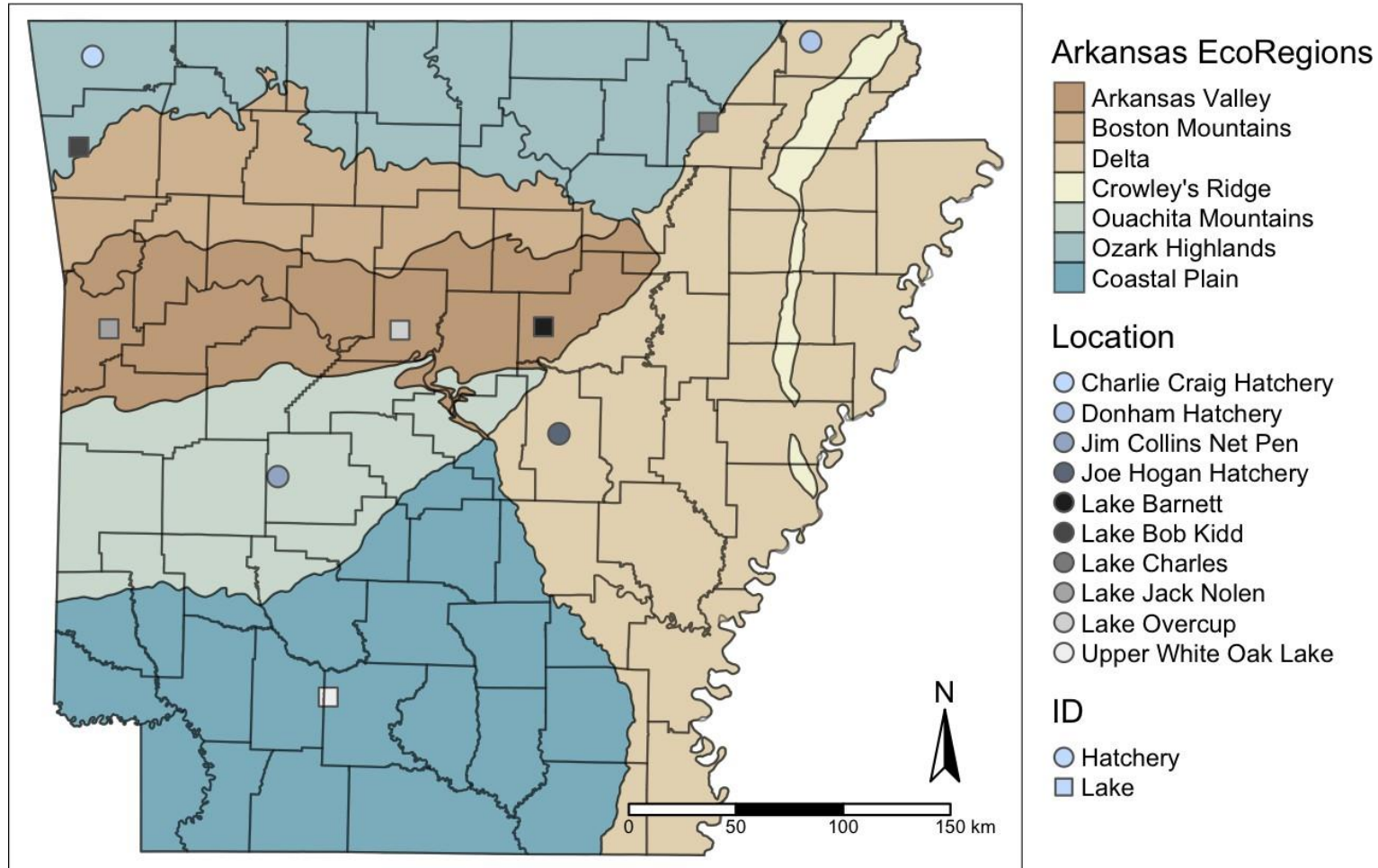


Figure 1: Map of study lakes and hatcheries and their respective ecoregions.

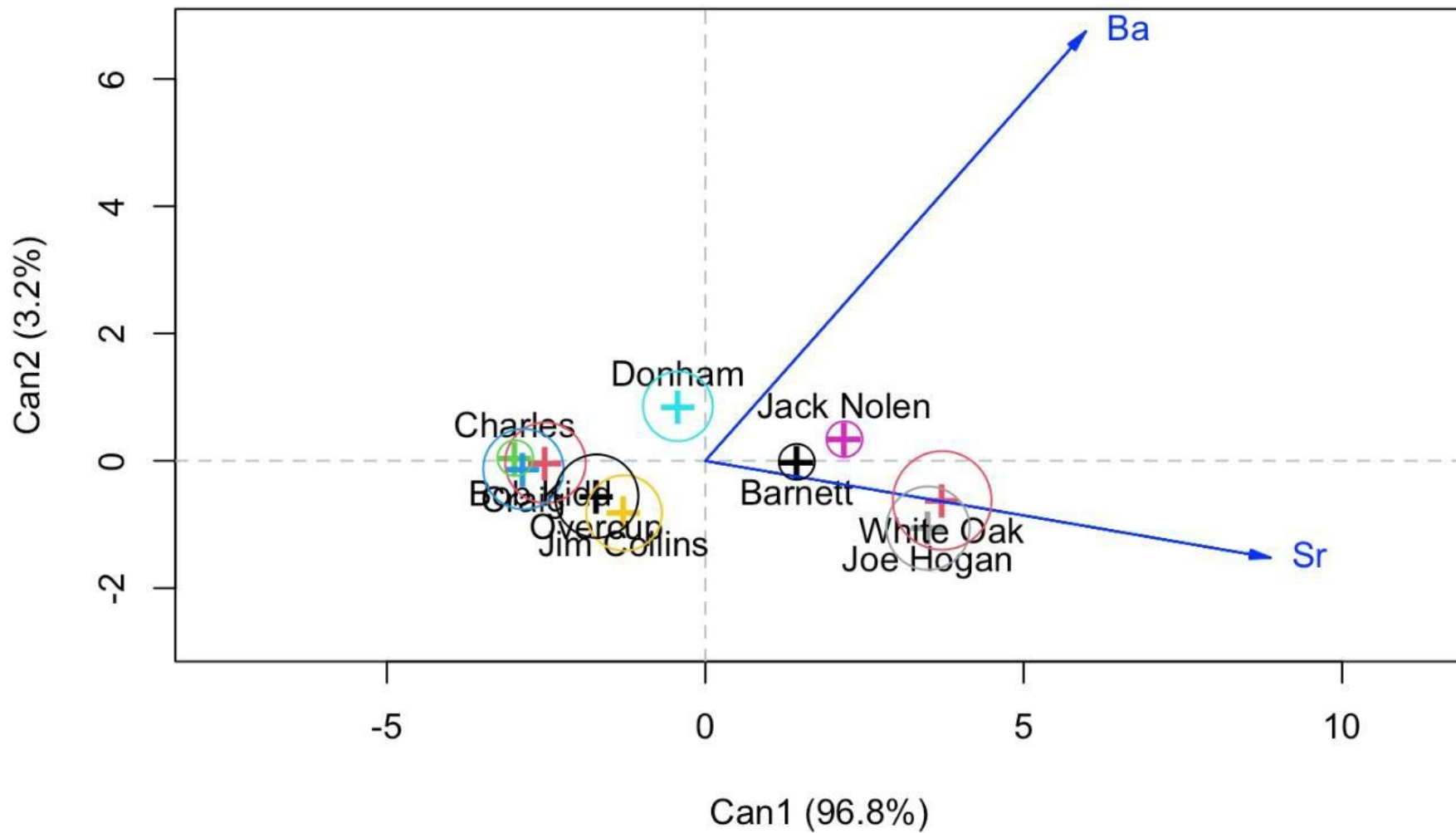


Figure 2: Canonical coefficients for Strontium and Barium signatures for each location.

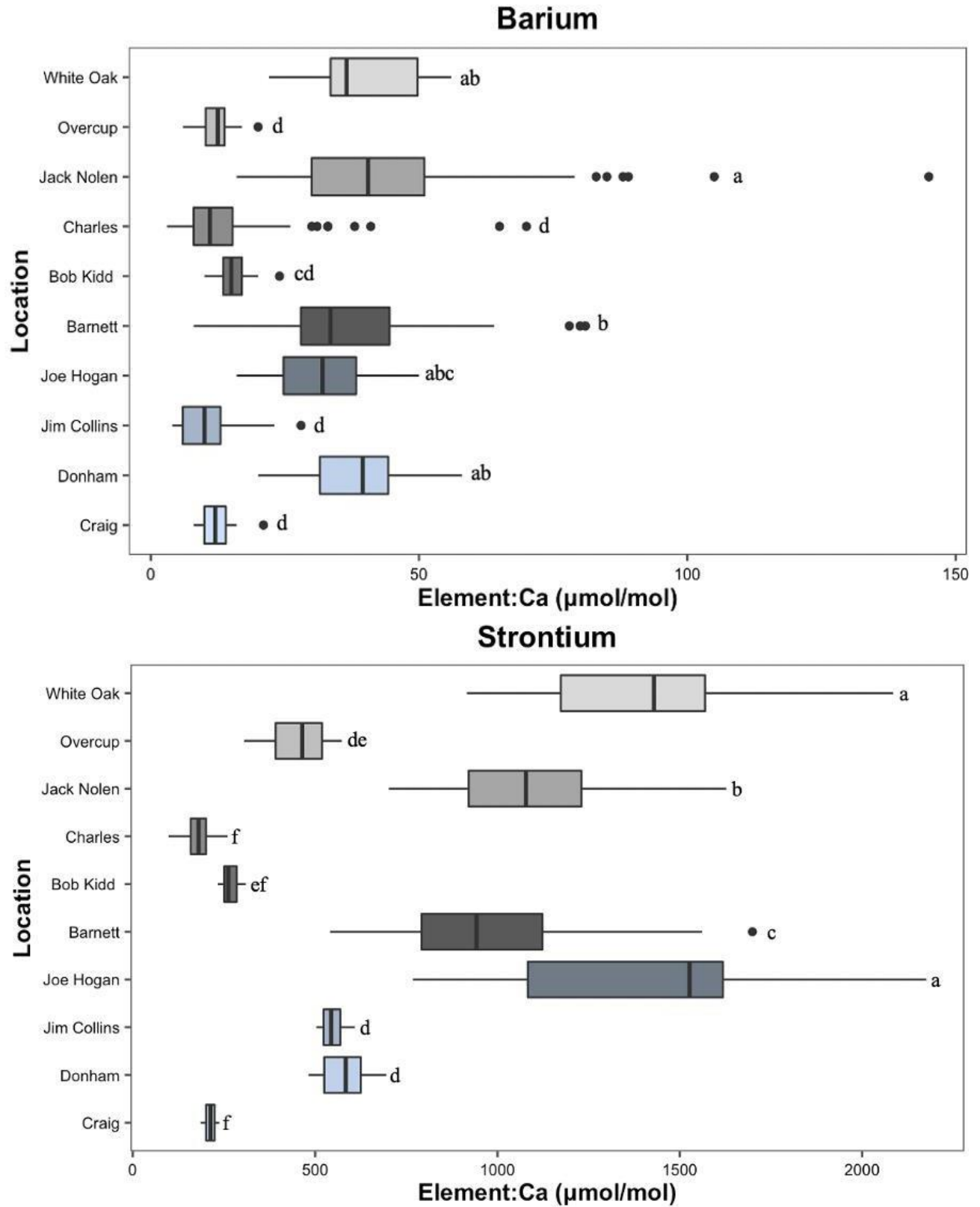


Figure 3: Elemental edge signatures of study lakes and hatcheries. Lakes are represented with grey colors and hatcheries with blue colors.

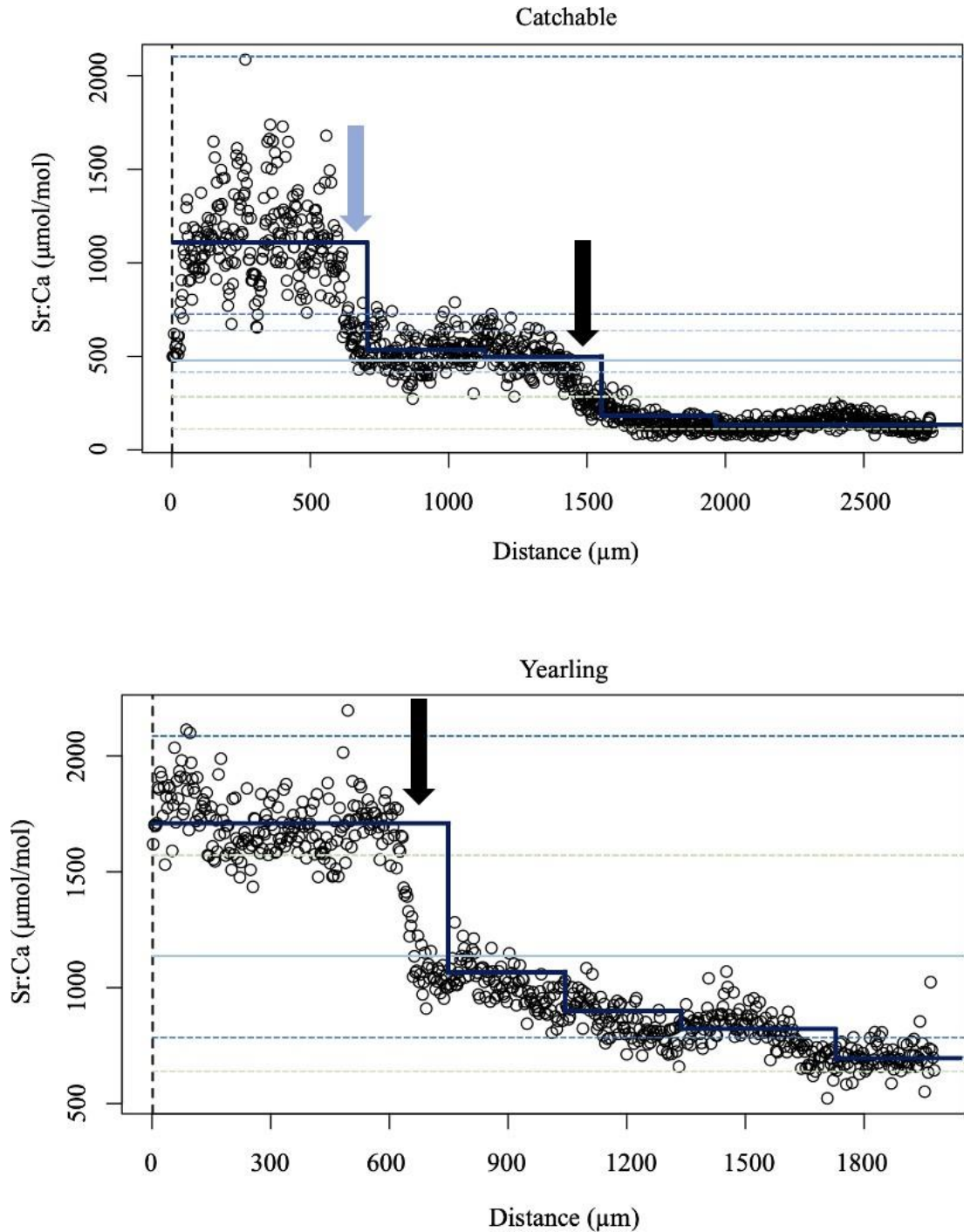


Figure 4: Example of laser ablation transect Sr:Ca for catchable and yearling fish. Solid light blue line shows the mean Sr:Ca for the entire data series, dashed green line shows the minimum and maximum pectoral spine edge Sr:Ca for fish sampled from lakes, the solid dark lines show means of sections of each transect identified by break point analysis, and dashed light and dark blue lines show minimum and maximum pectoral spine edge Sr:Ca for fish obtained from hatcheries. Blue arrow indicates changes from one hatchery to another hatchery. Black arrow indicates where the signature changes from the hatchery to respective lake.

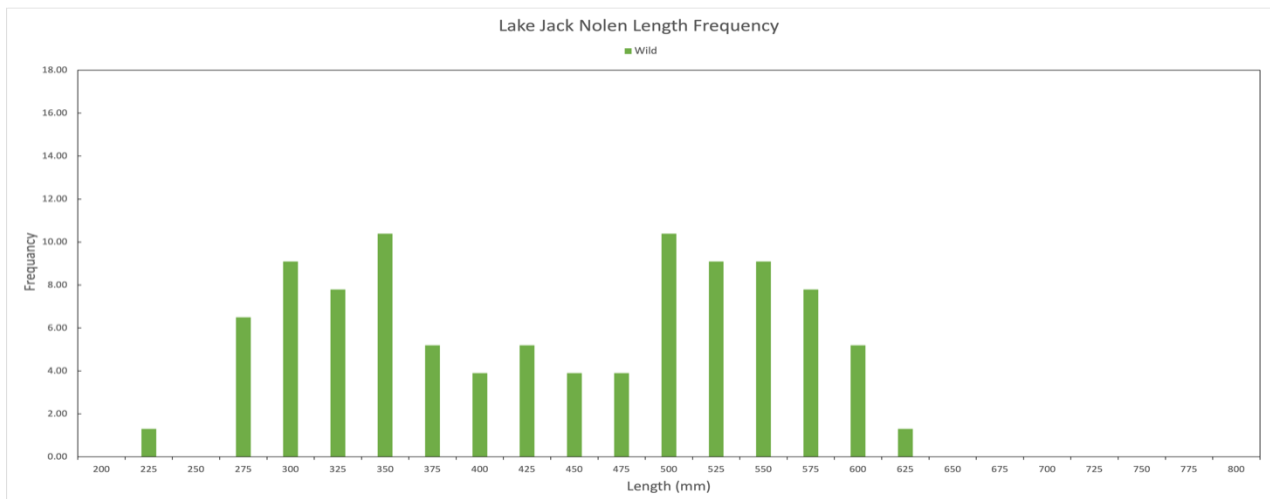
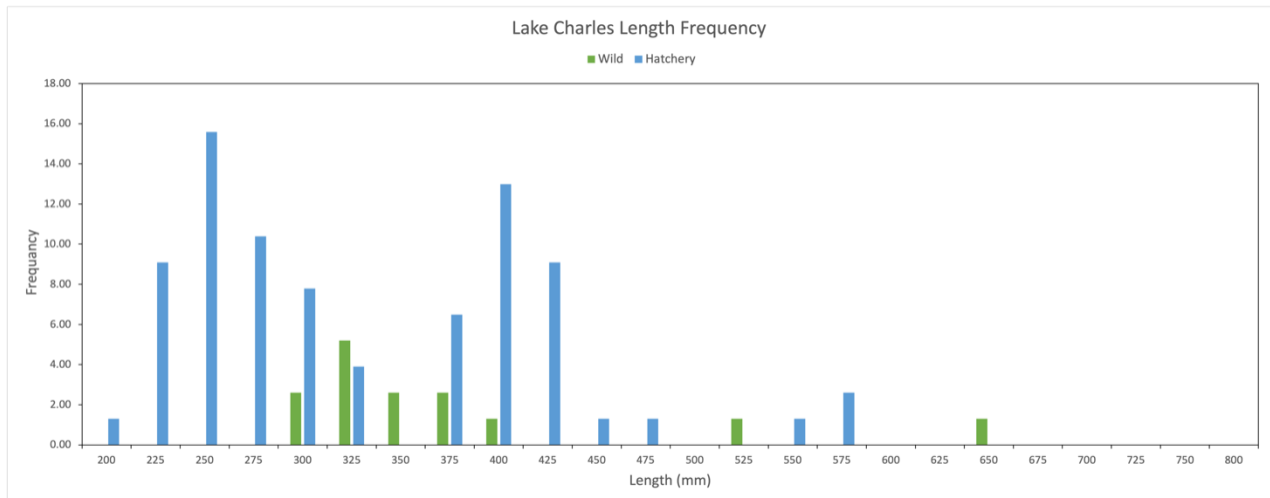
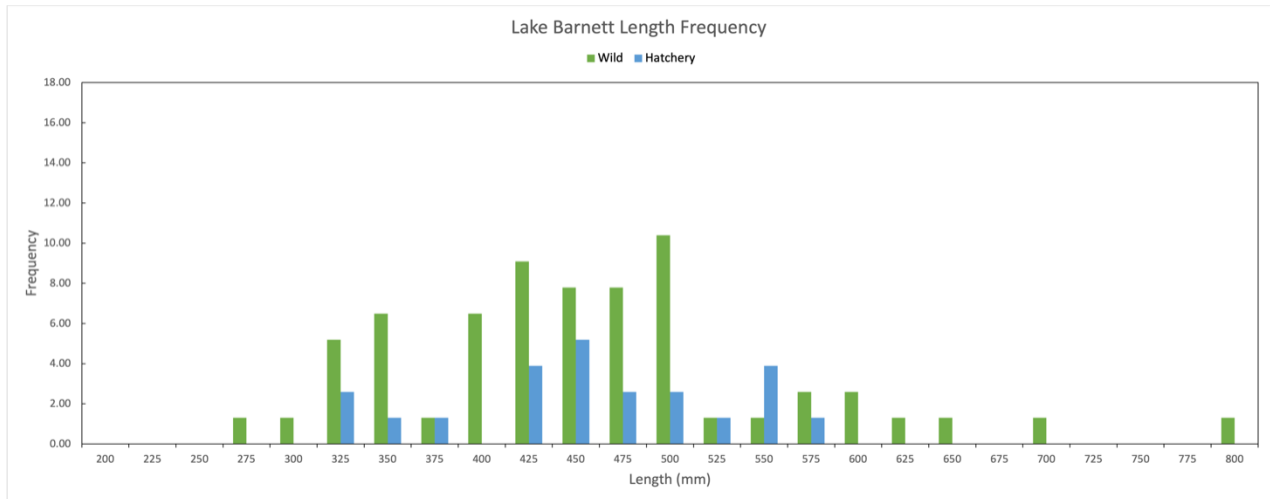


Figure 5: Length-frequency graphs along with natal origin frequency of Lake Barnett, Lake Charles, and Lake Jack Nolen.

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