ARTICLE

Vertebral chemistry distinguishes regional populations of Blacktip Sharks in the northern Gulf of Mexico

Abigail H. P. Hayne[1] ⓘ | Travis M. Richards[2] | J. Marcus Drymon[3,4] ⓘ |
Brett Falterman[5] | Nathan R. Miller[6] | R. J. David Wells[2,7] | John A. Mohan[1] ⓘ

[1]School of Marine and Environmental Programs, University of New England, Biddeford, Maine, USA

[2]Department of Marine Biology, Texas A&M University Galveston, Galveston, Texas, USA

[3]Coastal Research and Extension Center, Mississippi State University, Biloxi, Mississippi, USA

[4]Mississippi–Alabama Sea Grant Consortium, Ocean Springs, Mississippi, USA

[5]Fisheries Research Support LLC, Mandeville, Louisiana, USA

[6]Jackson School of Geosciences, University of Texas at Austin, Austin, Texas, USA

[7]Department of Ecology and Conservation Biology, Texas A&M University, Texas, USA

Correspondence
Abigail H. P. Hayne
Email: ahayne@une.edu

Abstract

Objective: Understanding the spatial connectivity of elasmobranch populations is critical for regional fisheries management. The Blacktip Shark *Carcharhinus limbatus* is abundant in coastal waters of the Gulf of Mexico (GoM) and important in recreational and commercial fisheries. Based on genetic and tagging studies, GoM Blacktip Sharks are currently managed under separate quotas between the eastern and western GoM (divided at 88°W), but no studies have used vertebral chemistry to assess the population structure of adult Blacktip Sharks.

Methods: We compared vertebral elemental signatures (barium [Ba], magnesium [Mg], manganese [Mn], strontium [Sr], and zinc [Zn]) for the first year of life with last occupied habitats (vertebral edges) in Blacktip Sharks collected from the western (Texas and Louisiana) and eastern (Alabama and Florida) coastal waters of the northern GoM.

Result: We found significant regional differences in Ba, Mg, Mn, and Sr vertebral edge signatures, suggesting ecological separation of Blacktip Sharks. Significant correlation between first-year and edge signatures suggested a high degree of residency between life stages. Cross-validated discriminant function analyses yielded highest regional classification accuracies when Florida sharks were grouped separately west of 88°W (90%), demonstrating the unique elemental signatures of eastern versus western GoM Blacktip Sharks under current management delineations.

Conclusion: Combined, these findings demonstrate that trace element markers can distinguish regional populations of Blacktip Sharks and provide a complimentary approach in addition to genetics and physical tagging to support current stock management efforts.

KEYWORDS
Blacktip Sharks, Gulf of Mexico, population connectivity, vertebral chemistry

INTRODUCTION

Knowledge of spatial ecology—particularly population connectivity—is central to elasmobranch (shark, skate, and ray) management efforts (Bonfil 1997; Fogarty and Botsford 2007; Simpfendorfer et al. 2011). An understanding of region-specific demographics is particularly crucial for managing species with complex genetic and population structures (Policansky and Magnuson 1998). Shark tagging studies provide valuable high-resolution data on individual movements that have traditionally been used to understand population distributions. However, data interpretation is often limited by variable tag deployment times. Similarly, genetic studies of population connectivity are helpful in summarizing spatial patterns of gene flow (Hedgecock et al. 2007). However, methods capable of tracking habitats that are occupied throughout ontogeny, which tagging and genetic studies cannot provide, are needed (e.g., Speed et al. 2010).

The Blacktip Shark *Carcharhinus limbatus* is a recreationally and commercially important shark species found throughout coastal waters of the southeastern United States and Gulf of Mexico (GoM; Cortés and Baremore 2012; Southeast Data, Assessment, and Review [SEDAR] 2018). They are highly mobile and make large-scale coastal migrations (Kohler and Turner 2019), but they form separate stocks between the western North Atlantic Ocean and the GoM and are consequently managed in two groups under Southeast Data, Assessment, and Review. However, historical mark–recapture data from the National Marine Fisheries Service (Kohler and Turner 2019) and mitochondrial DNA analyses (Keeney et al. 2005) show little exchange of sharks between the eastern and western GoM. As such, the most recent GoM assessment (SEDAR 2012) recommended that future assessments should consider and evaluate the eastern and western GoM sharks as different stocks divided at 88°W, a meridian that is frequently used by managers to delineate stocks in the GoM. Currently, Blacktip Sharks are managed under two different quotas between the eastern and western GoM (National Marine Fisheries Service 2022). The most recent estimated commercial landings for Blacktip Sharks west of 88°W was 226 metric tons compared to estimated landings of 10.2 metric tons for Blacktip Sharks east of 88°W (National Marine Fisheries Service 2022). The high quantity of Blacktip Shark landings in the GoM necessitates sustainable management efforts to ensure stability and prevent overexploitation. However, few studies to date have assessed the extent of spatial connectivity across multiple regions of the GoM for Blacktip Sharks.

Trace element analysis of elasmobranch vertebrae is increasingly used in studies of spatial connectivity.

Impact statement

Stocks of Blacktip Sharks are managed under separate quotas in the eastern and western Gulf of Mexico. In this study, elemental signatures in mineralized vertebral cartilage of Blacktip Sharks confirm ecological population separation. Natural chemical tags in shark vertebrae offer an additional tool to characterize population connectivity and stock delineation for highly migratory shark species.

Elasmobranch vertebrae are composed of calcified cartilage encased in a protein membrane that is mineralized by calcium phosphate hydroxyapatite crystals (Urist 1976; Dean and Summers 2006). Trace elements from the diet and the surrounding water enter the blood plasma via the gills, skin, intestines, and kidneys (Pentreath 1973; Dacke 1979) and are incorporated within the vertebral organic matrix as the centra grow radial concentric bands (Dean and Summers 2006). Unlike other biogenic apatites, elasmobranch hydroxyapatite does not undergo remodeling or resorption over time; thus, the chemical history of biomineralization can be assessed for environmental and/ or ecological proxies of habitats occupied over the shark's lifetime (Ashhurst 2004; Dean et al. 2015). Consequently, elemental signatures can act as natural environmental proxies that may be used to study ecological population connectivity (Tillett et al. 2011; Lewis et al. 2016; Smith et al. 2016).

Vertebral elemental analysis has also been employed to study elasmobranch stock structure (Schroeder et al. 2010; McMillan et al. 2017a, 2017b), natal origin (Lewis et al. 2016; TinHan et al. 2020; LaFreniere et al. 2023), age determination (Scharer et al. 2012; Mohan et al. 2018; Coiraton et al. 2019), age-related movements (Smith et al. 2016; Coiraton et al. 2020), ontogenetic habitat shifts (Livernois et al. 2021), and habitat use (Izzo et al. 2016; Feitosa et al. 2021; Brodbeck et al. 2023; Mohan et al. 2023). Both essential (i.e., magnesium [Mg], manganese [Mn], and zinc [Zn]) and non-essential (i.e., barium [Ba] and strontium [Sr]) trace elements have proven useful in these studies. The uptake of essential trace elements into the blood and vertebrae is believed to be controlled by diet and physiological pathways, whereas non-essential trace elements are considered as proxies for environmental conditions, such as salinity and temperature (Smith et al. 2013; Pistevos et al. 2019). When combined, multi-element signatures can provide powerful classification tools for studies of population connectivity and stock separation (McMillan et al. 2017a, 2017b; Coiraton et al. 2020;

Sanchez et al. 2020). As individuals in populations are exposed to the same water chemistries, eat similar prey, and experience similar physiological controls, their vertebrae should incorporate comparable, region-specific elemental signatures (McMillan et al. 2017a, 2017b). Chemical signatures at the vertebral edge correspond to the known spatial and temporal point of capture, reflecting the shark's most recent habitat use (Smith et al. 2016; Coiraton et al. 2020), whereas signatures isolated from the first year of life can be used to investigate residency over ontogeny (Izzo et al. 2016). Together, these stage-specific life records can be used to assess and delineate boundaries between groups of spatially distinct and/or connected populations.

In this study, we assessed multi-elemental vertebral signatures as spatial markers of juvenile (first year of life) and capture locations to characterize Blacktip Shark population connectivity in the western and eastern regions of the northern GoM (nGoM). We evaluated (1) the efficacy of vertebral edge signatures as discriminators of capture region and the potential to successfully assign Blacktip Sharks to their region of capture and (2) the consistency of edge and first-year signatures for elucidating residency.

METHODS

Sample collection and preparation

Blacktip Sharks from the nGoM were collected through recreational and commercial fishing and via fishery-independent surveys along the Texas, Louisiana, Alabama, and Florida coasts between May and September (except Louisiana sharks, which were landed in early February) from 2020 to 2021 ($n = 117$; Figure 1). Each shark was sexed and measured for precaudal length, fork length (FL), and total length (TL) to the nearest centimeter, with FL ranging from 51.6 to 162 cm (average ± standard deviation $= 117 \pm 19$ cm FL; Table 1; Figure 2). Of the 32 individuals collected from Texas, 19 lacked FLs; thus, TL-to-FL conversions for these sharks were estimated from the linear relationship between TL and FL for 455 fully measured individuals (males: $r^2 = 0.97$; females: $r^2 = 0.99$). As part of a long-term tagging and sampling effort, one male shark that was captured on October 30, 2006, and injected with oxytetracycline (OTC) to mark the vertebrae was recaptured 14.5 years later (on June 28, 2021; Table 2). Based on visible band pair identification, that shark was caught
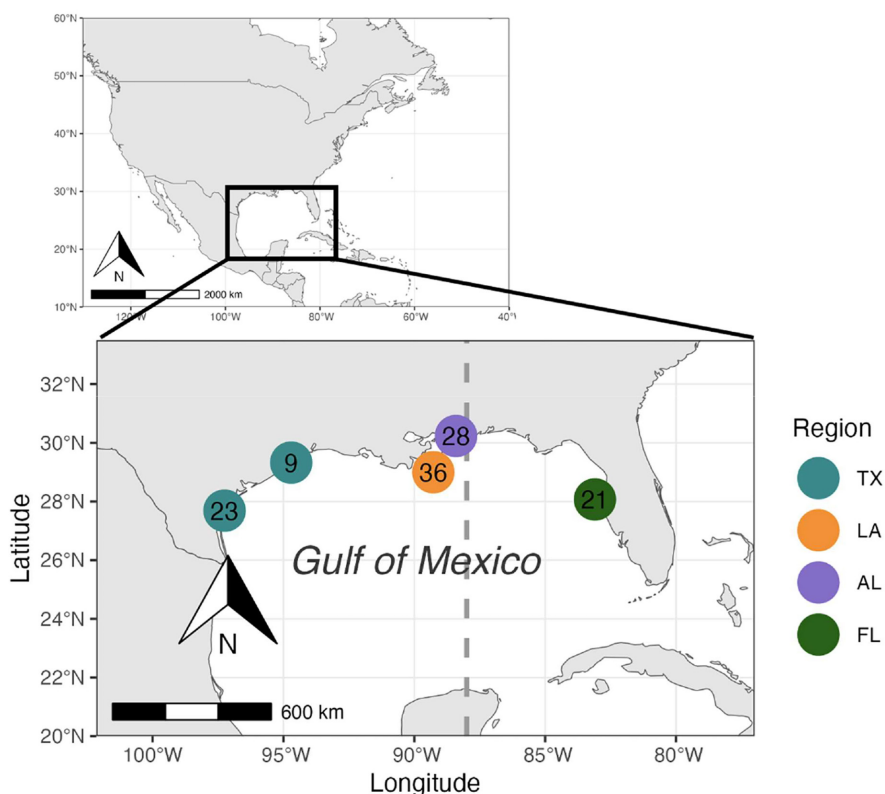

FIGURE 1 Map of sample collection regions for Blacktip Sharks in the northern Gulf of Mexico (GoM). Circles represent the area of collection, and numbers indicate the sample size of vertebrae collected from each region, with colors corresponding to each state. The gray dashed line represents the 88°W line of longitude, which is the meridian currently used by managers to delineate Blacktip Shark stock structure in the GoM. AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas.

TABLE 1 Collection region, total sample size, sample size by sex (F = female; M = male), and average fork length (±standard deviation [SD]) for Blacktip Sharks in the present study. nGOM, northern Gulf of Mexico.

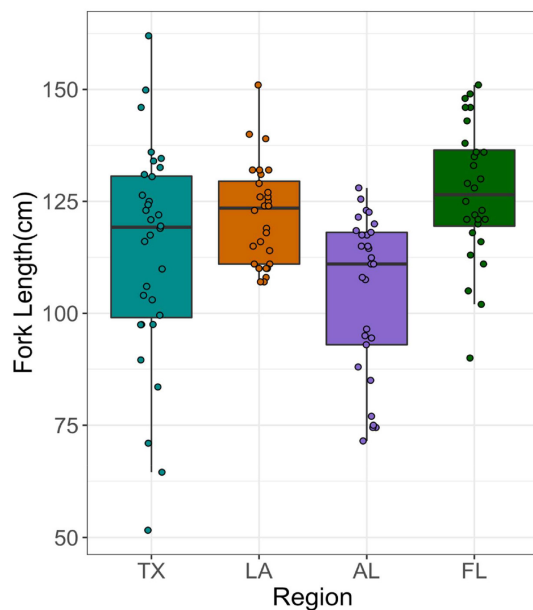| Region	Total	F	M	Average fork length (cm; ±SD)
Western nGoM	68	42	26	118 ± 19
Texas	32	22	10	113 ± 15
Louisiana	36	20	16	121 ± 10
Eastern nGoM	49	25	24	115 ± 21
Alabama	28	14	14	105 ± 18
Florida	21	11	10	128 ± 17
Total	117	66	51	117 ± 19

FIGURE 2 Fork lengths (cm) for 117 Blacktip Sharks collected in the northern Gulf of Mexico. AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas. The line within each box represents the respective median value; top and bottom box ends represent the third and first quartile, respectively; whisker ends represent the largest and smallest values within 1.5 times the interquartile range above and below the third and first quartiles; open circles represent individual data points.

during the first year of life. Since the time at liberty was known, this vertebral sample allowed for validation of time and habitat occupation during the shark's first year of life.

Vertebral centra were isolated from the vertebral column of sample specimens with a scalpel. Hemal and neural arches as well as excess tissue were also removed. Centra were air-dried and then sectioned longitudinally using a Buehler IsoMet 1000 low-speed circular saw with a diamond wafering blade to obtain 1-mm-thick "bow tie"-shaped cross sections. Bow tie halves were affixed to petrographic slides using thermoplastic cement (Crystalbond).

Elemental data collection and analysis

Vertebral elemental concentrations in the direction of radial (outward) growth were determined by laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS). Elemental concentrations in the direction of radial growth were quantified using an ESI NWRI93 excimer LA system (193 nm; 4-ns pulse width) coupled to an Agilent 7500ce ICP-MS at the Jackson School of Geosciences, University of Texas at Austin. After preablation (125-μm spot; 100-μm/s scan rate; 4.2-J/cm^2 fluence) to remove shallow surface contaminants, corpus calcareum transects beginning at the vertebral focus and ending at the marginal edge were performed on each specimen by using a 25- × 100-μm rectangular aperture, with the long axis maintained parallel to concentric Liesegang growth lines (Dean et al. 2015); a 25-μm/s scan rate; 4.45 ± 0.05-J/cm^2 fluence; a 15-Hz repetition rate; and carrier gas flows of 0.8 L/min for argon and 0.8 L/min for helium. The quadrupole time-resolved methods measured 10 masses with integration times of 10 ms (^{24}Mg, ^{25}Mg, ^{43}Ca, ^{44}Ca, ^{55}Mn, and ^{88}Sr) and 20 ms (^{7}Li, ^{63}Cu, ^{66}Zn, and ^{138}Ba). Measured intensities were converted to elemental concentrations (μg/g) using Iolite software (Paton et al. 2011), with ^{43}Ca as the internal standard and a Ca index value of 35wt% (Mohan et al. 2018; Livernois et al. 2021). U.S. Geological Survey (USGS) MAPS-4 (synthetic bone) was used as the primary calibration standard. National Institute of Standards and Technology (NIST) 612 and USGS MACS-3 were used as external reference standards. The derived elemental time series were smoothed by consecutive moving median and average filters using a 7-point box-car width (28-μm equivalent distance). Time series were converted to distance (μm) from the core based on scan rate and duty cycle.

Following LA-ICP-MS analysis, digital images of individual vertebral centra were obtained using transmitted light on a Leica dissecting microscope fitted with a digital camera (Leica Microsystems). Vertebral laser transect distances (μm) were measured in triplicate from digital images by using ImageJ version 1.53a (Schneider et al. 2012), and the mean distance was used in subsequent analyses. Prenatal data were excluded from analysis, and the birth band location was used as the starting point of each adjusted transect. The birth band was defined as a pronounced change in angle in the intermedialia and was formed on an arbitrary birth date of May 1

TABLE 2 Tag and recapture details for an oxytetracycline-injected male Blacktip Shark that was at liberty in the northern Gulf of Mexico for 14.5 years. (This shark was tagged and recaptured by J.M.D).

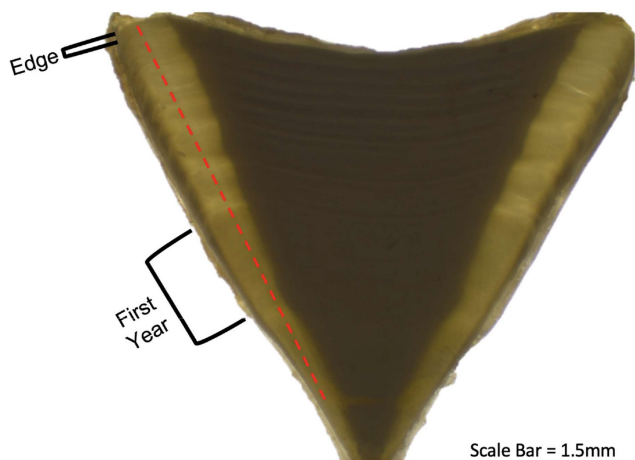| Event	Date	Fork length (cm)	Weight (kg)	Stage	Latitude	Longitude
Tagging	Oct 30, 2006	67	3.5	Immature	30.17883	−88.4702
Recapture	Jun 28, 2021	115	18	Mature	30.25288	−87.9925

FIGURE 3 Sagittal cross section of a Blacktip Shark vertebra, depicting the laser path (red dashed line) and the locations of the first-year and edge signatures isolated from the laser path.

(Carlson et al. 2006). Adjusted transects thus ran from the birth band to the exterior edge of the vertebra.

To isolate elemental signatures from the first year of life, transect data from the birth mark to the end of the first growth band were used. To characterize elemental signatures from the regional collection site at the end of adult life, transect data from the start of the last visible growth band to the marginal edge were used. Elemental concentrations from these transect segments were averaged to characterize first-year and edge (i.e., capture location) life stages (Figure 3). The use of average values here avoided the common complexities of time series analysis, such as autocorrelation and a lack of independence.

Statistical analysis

Univariate normality of elemental concentrations was assessed using the Shapiro–Wilk test of normality. Average elemental concentrations for Mg, Mn, and Zn edge signatures and Sr first-year signatures did not meet normality assumptions. For consistency and ease of analysis, these data were not transformed and nonparametric tests were instead used for all statistical analyses.

Sharks were assigned to three groups to test three predictions. First, sharks were grouped according to the state of collection (hereafter, "state"). Second, sharks were separated into two groups, with a split at 88°W (hereafter, "west/east"). Third, Florida sharks were compared to all remaining sharks (hereafter, "west/Florida"). To explore variability and visualize how the elemental concentrations in vertebrae differed among regions, edge signatures in all individuals were first reduced to two principal components (PC1 and PC2) and multi-element principal components analyses (PCAs) were conducted using the prcomp function within the stats package in RStudio (R Core Team 2024) by using region as the grouping variable. We constructed PCA biplots for each of the three regional grouping types. Differences among regions in single-element vertebral first-year and edge signatures were assessed with a Kruskal–Wallis test and a pairwise Wilcoxon's multiple comparison post hoc test. Quadratic discriminant function analysis (QDFA) was used to evaluate the ability of vertebral edge signatures (Ba, Mg, Mn, Sr, and Zn) to distinguish between regional collection sites using the caret package in RStudio (R Core Team 2024). Group classification accuracy was assessed using a leave-one-out jackknife procedure. We conducted QDFA for each regional grouping type to gauge shifts in classification accuracy across multiple spatial scales. Spearman's correlation analysis was performed to test whether significant relationships existed between first-year and edge signatures for each element pooled across all states. A separate Spearman's correlation analysis was conducted between state-specific first-year and edge signatures.

RESULTS

Average elemental signatures among Blacktip Shark vertebrae are reported in Tables S1 and S2 (available in the Supplement separately online). For the overall study data set, average elemental concentrations between first-year and edge values were comparable (within 1 standard deviation) and increased from tens of micrograms per gram (Ba < Zn < Mn) to thousands of micrograms per gram (Sr < Mg). Elemental signatures, particularly those of Sr and Ba, from the OTC-validated tag–recapture individual showed little change between the first year of life and the edge (Table S3). Differences among multi-element composition in edge signatures were found among the four states (Figure 4). In the state-specific grouping PCA, PC1
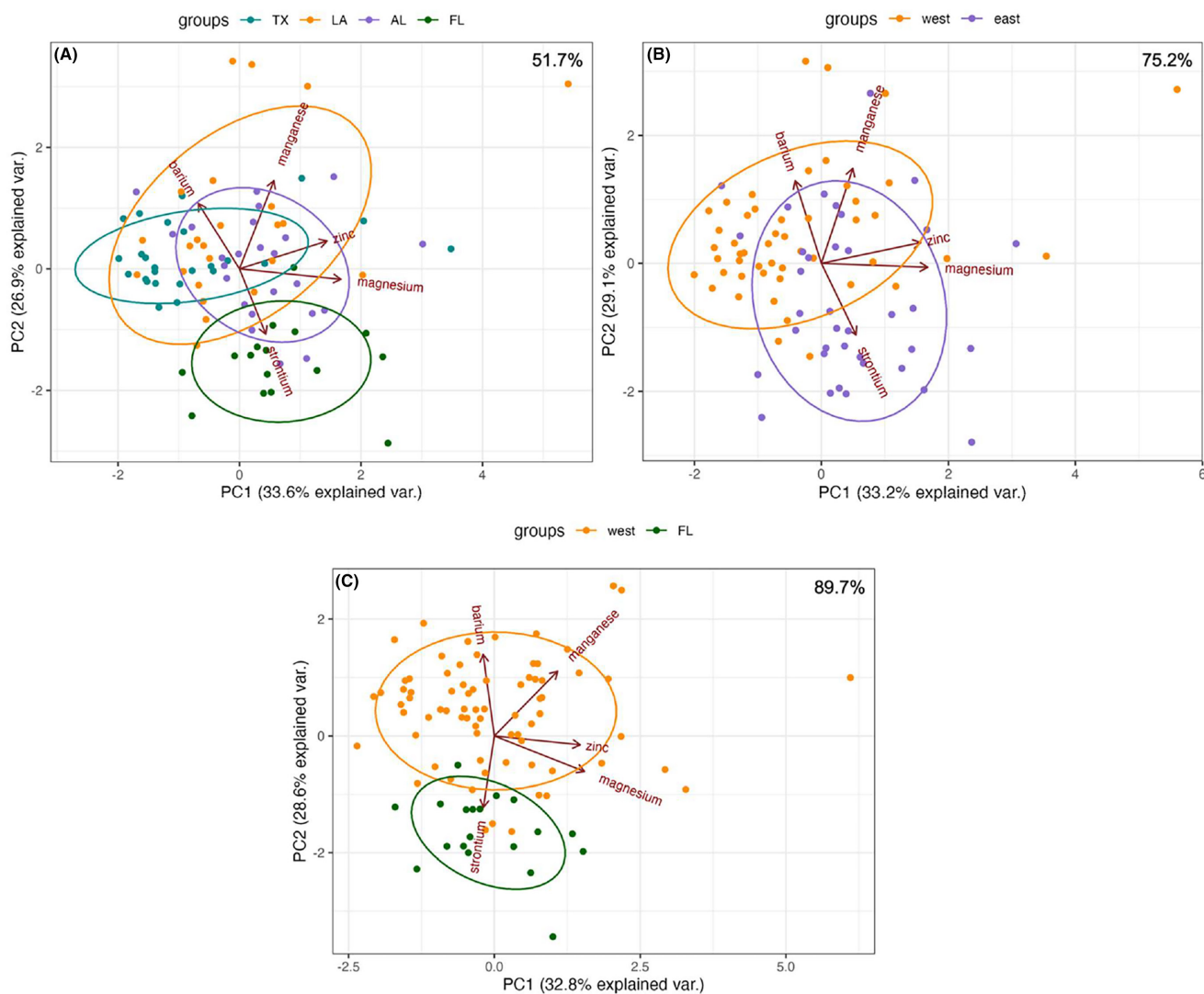
FIGURE 4 Visualized principal components analysis for edge signatures of Blacktip Sharks by region: (A) state-specific grouping, (B) west/east grouping, and (C) west/Florida grouping. Principal components 1 and 2 (PC1 and PC2) are represented as the *x*- and *y*-axes, respectively. The percentages of total variation explained by PC1 and PC2 are given in parentheses. Arrows represent the loading for each labeled element. The percentage in the top right-hand corner of each plot indicates the jackknifed classification accuracy. AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas.

explained 33.6% of the variation whereas PC2 explained less variation (26.9%). Elements with the most influence in PC1 with positive loading were Mg, Mn, and Zn. Barium showed negative loadings for PC2. Separation between states was not clearly defined, with a large amount of overlap for the Louisiana ellipse. The Florida ellipse showed the most prominent separation of the four states. Under the west/east grouping, PC1 and PC2 explained 33.2% and 29.1% of the variability, respectively. Strontium showed the most influence in PC2, with negative loading. Under the west/Florida grouping, PC1 explained 32.8% of the variability whereas PC2 explained 28.6% of the variability. The corresponding biplot showed the least amount of overlap between ellipses, with Sr showing strong negative loading in PC2.

Lengths of first-year signature intervals among individuals averaged $1887 \pm 376\,\mu m$. Average Ba, Mg, Mn, and Sr concentrations showed distinct separation between states, particularly for Florida sharks (Figure 5). Vertebral edge length measurements averaged $291 \pm 221\,\mu m$ among individuals and showed significant regional differences in average concentrations of Ba, Mg, Mn, and Sr (Figure 6). Notably, Louisiana and Alabama showed elevated edge Mn concentrations ($\sim$25–50 μg/g) in comparison with Texas ($\sim$25–30 μg/g) and Florida ($\sim$5–25 μg/g). Average Sr edge signatures were constant throughout Texas, Alabama, and Louisiana ($\sim$1500–1800 μg/g) and were relatively elevated in Florida ($\sim$1700–1800 μg/g). Average edge Ba showed a decline in concentration, with the lowest concentrations being observed in Florida ($\sim$4–8 μg/g).
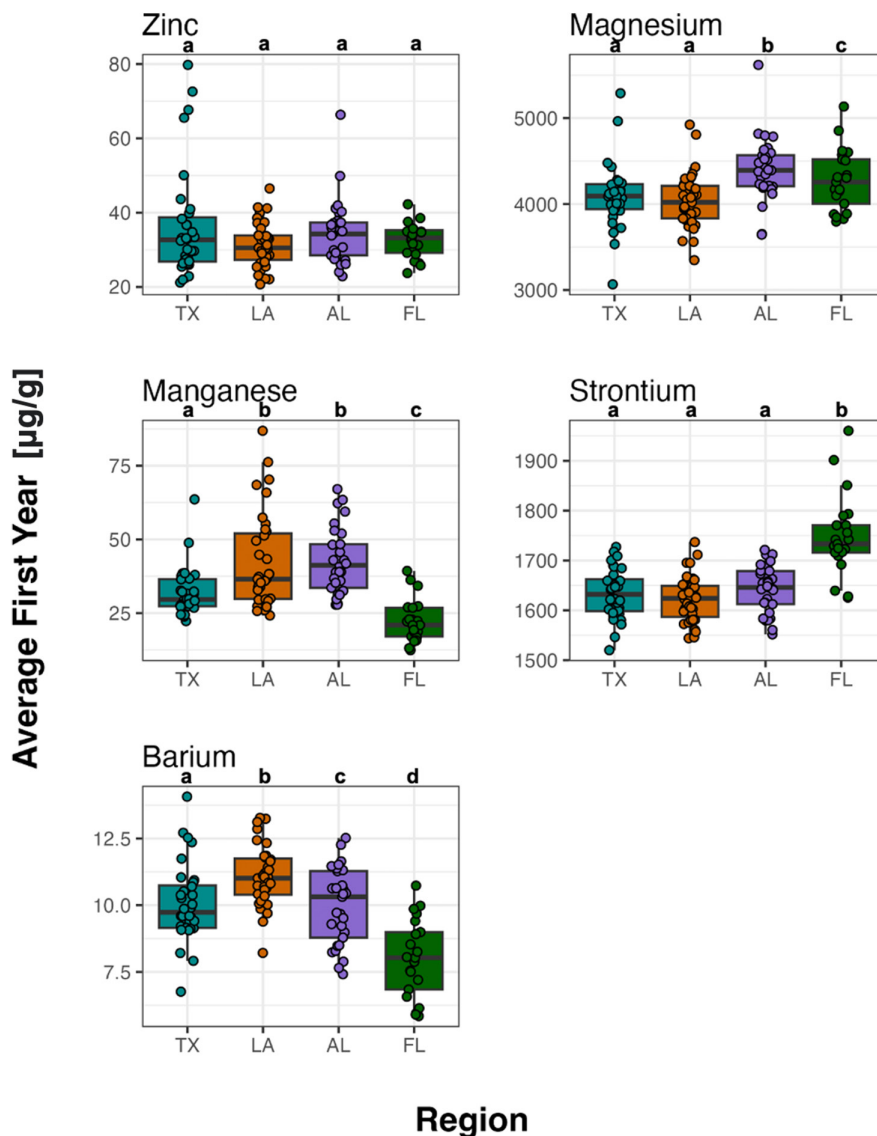
FIGURE 5 Average zinc, magnesium, manganese, strontium, and barium concentrations (µg/g) for first-year signatures of 117 Blacktip Sharks, grouped by collection region. Letters a–c denote Wilcoxon test significance ($p < 0.001$). AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas.

The average jackknifed cross-validated classification accuracy from the QDFA was 51.7% for the four-region grouping but increased to 76.7% under the west/east grouping. Under the west/Florida grouping, the QDFA yielded a further increased cross-validated accuracy of 89.7% (Table 3).

Spearman's correlation coefficients between pooled mean first-year and edge signatures were significant for all elements except Mg (Table 4). Strontium and Zn had the highest correlation coefficients, and Mn showed a significant strong correlation. Strontium and Zn were also strongly correlated in all four states, whereas Mn was not significantly correlated in any state (Figure 7; Table S4). Notably, Texas was the only state where Ba, Mg, Sr, and Zn were found to be significantly correlated between first-year and edge signatures.

DISCUSSION

Regional patterns in elemental signatures

When considered in aggregate by state, west/east, and west/Florida groupings, analyses of trace element vertebral chemistry revealed regionally distinct edge signatures, a high correlation over ontogeny, and high classification accuracies, indicating that Blacktip Shark population structure in the nGoM is complex and may comprise more than one subpopulation. Vertebral edge Ba, Mg, Mn, and Sr values showed significant, spatially distinct differences, suggesting that individuals within each state share water chemistries, environments, and/ or diets that may ultimately represent unique western
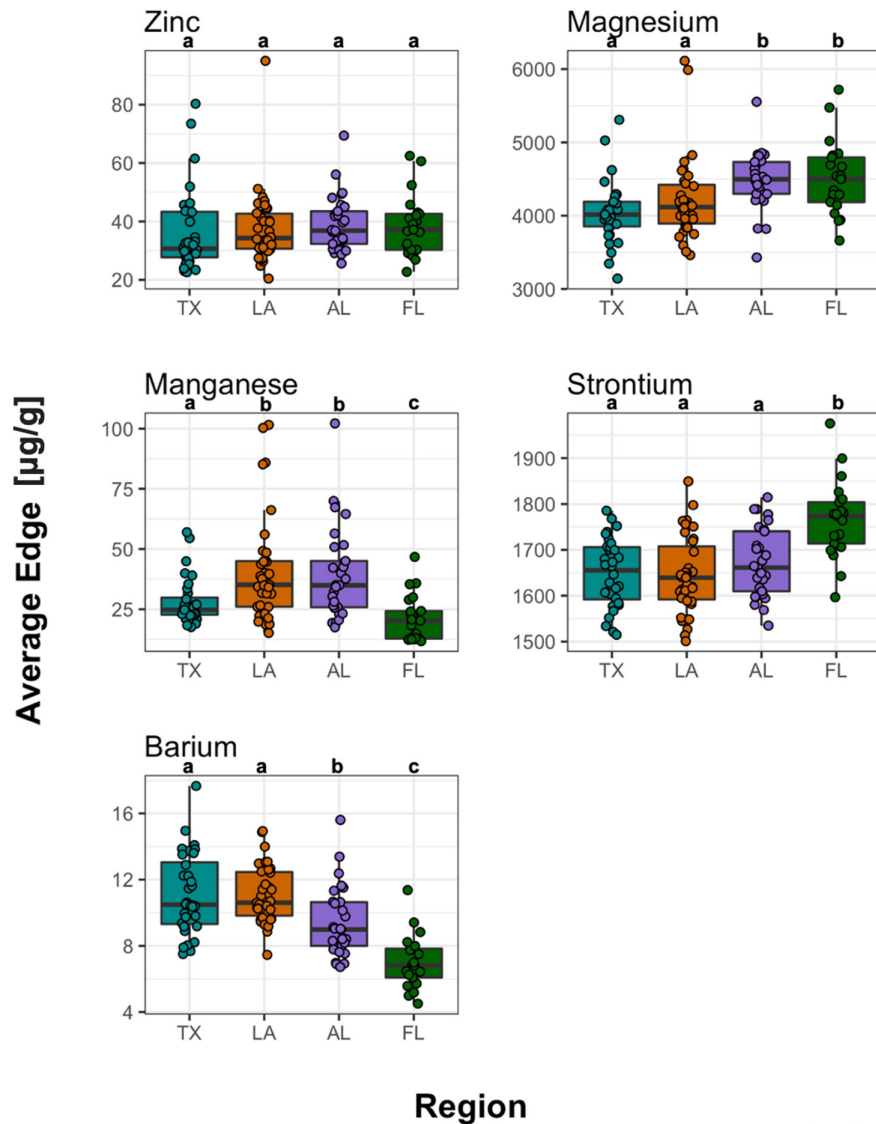
FIGURE 6 Average zinc, magnesium, manganese, strontium, and barium concentrations (μg/g) for edge signatures of 117 Blacktip Sharks, grouped by collection region. Letters a–c denote Wilcoxon test significance ($p < 0.001$). AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas.

TABLE 3 Jackknifed classification accuracy (%) from quadratic discriminant function analysis for groupings of Blacktip Shark vertebral edge signatures (barium, magnesium, manganese, strontium, and zinc) in the northern Gulf of Mexico (nGoM).

Grouping	Accuracy (%)
Four regions: Texas (TX), Louisiana (LA), Alabama (AL), Florida (FL)	51.7
Two regions: western nGoM (TX, LA); eastern nGoM (AL, FL)	75.2
Two regions: western nGoM (TX, LA, AL); eastern nGoM (FL)	89.7

TABLE 4 Results of Spearman's correlation analysis between first-year and edge signatures for 117 Blacktip Sharks in the northern Gulf of Mexico, pooled across individual states.

Element	Correlation coefficient	p-value
Zinc	0.81	<0.0001
Magnesium	0.12	0.205
Manganese	0.57	<0.0001
Strontium	0.81	<0.0001
Barium	0.77	<0.0001

and eastern delineations, with little spatial connectivity across western and eastern regions. These four elements are often identified as environmental proxies because they can directly substitute for Ca during hydroxyapatite

formation (Schoenberg 1963; Aoba et al. 1992; Wells et al. 2000; Dean and Summers 2006; Pon-On et al. 2008).

Edge Mg showed significantly different, elevated signatures in the eastern nGoM compared to the western
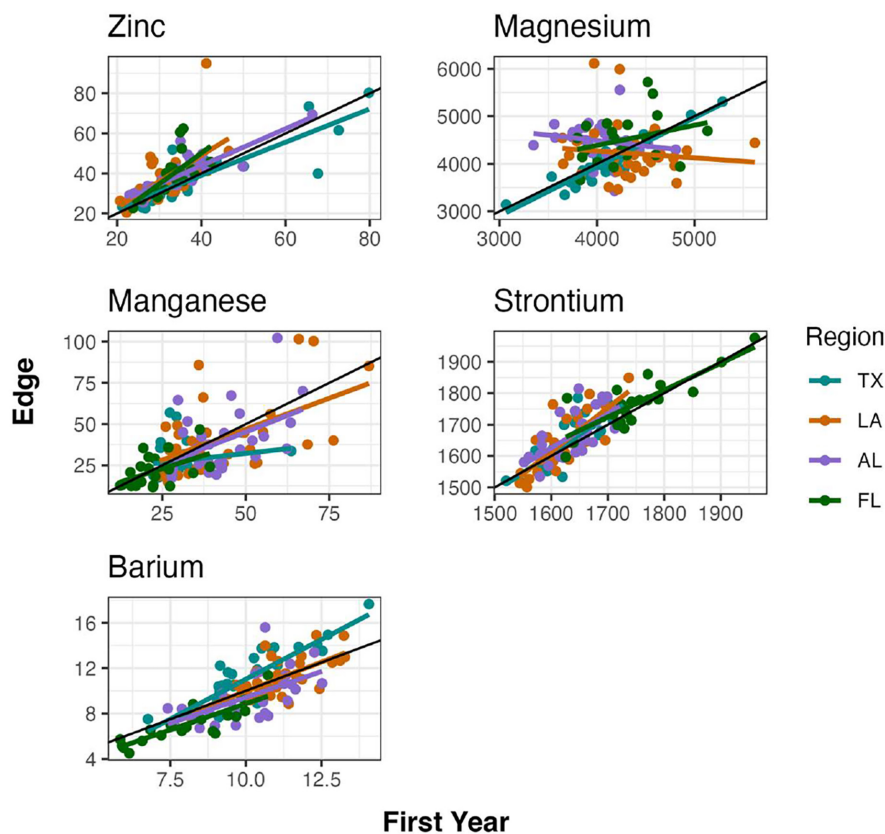
FIGURE 7 Scatterplots for all zinc, magnesium, manganese, strontium, and barium (µg/g) first-year and edge signatures from 117 Blacktip Sharks, grouped by collection region. AL, Alabama; FL, Florida; LA, Louisiana; TX, Texas.

nGoM. Magnesium is the second most abundant cation in seawater and is one of the most abundant minor elements found within the tissues of marine organisms (Cox 1989). Unsurprisingly, edge Mg had the highest concentrations among the five studied elements, with a mean value (4287 µg/g) that was three orders of magnitude higher than edge Ba (10 µg/g), Mn (34 µg/g), and Zn (28 µg/g). Previous studies of GoM Blacktip Sharks have also shown regional vertebral Mg variation in the nGoM. Lewis et al. (2016), for example, documented low Mg concentrations in juvenile Blacktip Sharks collected off Mississippi and Alabama compared to vertebrae from Blacktip Sharks collected off Florida.

Controls on Mg uptake in Blacktip Shark vertebrae are not well understood. Magnesium is an essential nutrient for the catalysis of over 300 enzymes, including those that use or synthesize ATP and those that synthesize nucleic acids (Vernon 1988). Regional differences in vertebral Mg concentration of Longnose Stingrays *Hypanus guttatus* have been attributed to dietary preferences rather than habitat use (Feitosa et al. 2021). Diet and growth may also serve as controls on Mg incorporation in teleost fish otoliths (Hüssy et al. 2020). Although the state-specific diet composition of Blacktip Sharks is well studied (Hoffmayer and Parsons 2003; Bethea

et al. 2004; Barry et al. 2008; Plumlee and Wells 2016), direct comparisons of diet across states and the western/eastern GoM have not been conducted. However, preliminary findings suggest age and growth differences between the western and eastern GoM (T.M.R., personal observation), which may contribute in part to regional variation in Mg. Additionally, element incorporation studies on the vertebrae of Round Stingrays *Urobatis halleri* indicated an inverse relationship between water temperature and vertebral Mg uptake (Smith et al. 2013). Temperature in Florida waters is controlled partially by the input of warm water from the Loop Current relative to cooler coastal shelf waters (15–20°C; Oey et al. 2005), while the Mississippi River outflow is also known to affect the temperature of Texas–Louisiana coastal shelf waters (Nowlin et al. 2005), leading to relatively cool surface waters. Given this, we would expect to see lower Mg in Florida sharks based on the results of Smith et al. (2013), but species-specific relationships between temperature and elemental uptake may exist (McMillan et al. 2017a, 2017b).

Blacktip Sharks that were collected in Alabama and Louisiana showed significantly higher average Mn edge signatures than sharks collected in Texas and Florida. Manganese uptake in shark vertebrae is believed to be

controlled by both physiological and environmental mechanisms. As a transition metal and active redox participant with multiple oxidation states, Mn has been commonly analyzed in studies of teleost otolith chemistry (Limburg et al. 2011, 2015; Mohan and Walther 2016). Manganese cycles between dissolved (Mn^{2+} and Mn^{3+}) and particulate (Mn^{4+}) phases in sediment and water as a function of pH and dissolved oxygen levels. Since low oxygen levels favor dissolved forms, dissolved Mn is more abundant in anoxic seawater. Changing dissolved oxygen levels may also liberate insoluble oxidized Mn (as oxides/hydroxides) from sediments and sedimentary pore waters to bottom waters, where it may substitute for Ca^{2+} during active vertebral hydroxyapatite calcification (Pakhomova et al. 2007). Teleost fish that occupy and feed in low-oxygen zones incorporate Mn at higher levels (Limburg et al. 2015). The effects of hypoxic conditions on Mn uptake in elasmobranch vertebrae remain unstudied, but Mn uptake could follow pathways similar to that of Mn incorporation in otolith aragonite (McMillan et al. 2017a, 2017b). The Mississippi and Atchafalaya River systems are known to affect nGoM water chemistries (Rabalais et al. 2002) and notably contain abundant Mn^{2+} (Shim et al. 2012). Blacktip Sharks that occupy and feed on teleost fish in hypoxic zones within the Mississippi–Atchafalaya River plumes could explain the enriched edge Mn levels documented in Louisiana and Alabama sharks, particularly at known hypoxic conditions along the Louisiana shelf (Rabalais and Turner 2001), where most Louisiana sharks were collected. Lewis et al. (2016) documented similarly high vertebral Mn levels in juvenile Blacktip Sharks from these regions. Given the significantly lower edge Mn signatures of Texas and Florida sharks, these findings indicate that regional population differences are present and may be driven in part by hypoxic water chemistries.

Average Ba and Sr edge signatures for Florida sharks showed striking patterns. Blacktip Sharks in Florida displayed significantly elevated Sr compared to sharks in the remaining three states, which had relatively constant Sr signatures. Conversely, Ba edge signatures showed a decrease eastward across nGoM collection sites, with Florida sharks showing significantly lower concentrations. Strontium has relatively high, uniform concentrations in marine waters, whereas Ba is relatively enriched in areas close to riverine discharge (Thorrold et al. 1997; McCulloch et al. 2005; Crook and Gillanders 2006; McMillan et al. 2017a, 2017b). The Mississippi–Atchafalaya River system is the primary freshwater input source for the nGoM (Justíc et al. 2002; Rabalais et al. 2002; Dagg and Breed 2003) and is known to affect water chemistries in the western nGoM (Walker et al. 2005; Mohan and Walther 2016). Freshwater runoff from the Mississippi–Atchafalaya rivers to Texas,

Louisiana, and Alabama likely plays a role in the relative incorporation of Ba and Sr into the vertebrae of Blacktip Sharks in these coastal waters. Previous vertebral chemistry studies have shown findings similar to the present results (Tillett et al. 2011; Werry et al. 2011; Coiraton et al. 2020); notably, Lewis et al. (2016) found higher Ba and lower Sr in the vertebrae of Blacktip Sharks from the western and central nGoM compared to those from the eastern nGoM. Limited but informative experimental evidence supports the notion that Ba and Sr vertebral incorporation in hydroxyapatite is driven by ambient dissolved concentrations in water, as mediated by temperature and salinity (Smith et al. 2013; Brodbeck et al. 2023). To summarize, vertebral edge Sr and Ba are spatially distinct in the nGoM, particularly in Florida sharks.

Average Zn edge signatures showed no significant regional differences among states. Incorporation of Zn into the vertebrae is thought to occur by entrapment in the interstitial spaces of expanding matrix during accretion (Tang et al. 2009), distinct from the putative incorporation pathways of Ba, Mg, Mn, and Sr. As an essential trace metal, Zn is also a common cofactor for metabolic reactions (Fletcher and Fletcher 1980) and is associated with maternal offloading (Raoult et al. 2018; Livernois et al. 2021) and dietary intake (Mathews and Fisher 2009). As there are no known regional differences in these factors, it is unsurprising that Zn showed no regional differences, and our findings suggest that this element is not a useful indicator of regional separation for Blacktip Sharks.

Assessing ontogeny and residency

We found high correlations between state-specific first-year and edge signatures for Ba, Mn, Sr, and Zn, suggesting that individuals occupy similar and consistent water conditions over ontogeny. These findings, particularly from environmentally mediated elements (e.g., Ba, Mn, and Sr), further suggest a high degree of residency and a lack of movement between the eastern and western nGoM. Notably, the OTC-validated male was recaptured only 47 km from its original capture location after 14.5 years at liberty. It is unknown whether or where this individual migrated during those years, but it is nonetheless a validated example of potential localized residency. Combined with over 50 years of tag–recapture efforts, which show nearly no physical movement between the eastern and western nGoM (Hueter et al. 2004; Kohler and Turner 2019), and significant state-specific differences in mitochondrial DNA (Keeney et al. 2005; Swift et al. 2023), a pattern of localized residency in Blacktip Sharks within the nGoM seems to be emerging.

In all vertebrae studied, Zn showed an unexpectedly high correlation between early life and edge signatures, remaining relatively constant. This contrasts with the work of Livernois et al. (2021), who found higher Blacktip Shark vertebral Zn in early life stages, followed by a consequent decline and stabilization. Zinc is associated with maternal loading (Raoult et al. 2018), which explains the elevated concentrations in early life and diet. Blacktip Sharks in the GoM are specialized, piscivorous feeders but show ontogenetic shifts in dietary preference as they move offshore during later life stages (Hoffmayer and Parsons 2003; Bethea et al. 2004; Barry et al. 2008; Plumlee and Wells 2016). However, since Zn uptake is likely controlled by physiological parameters and was found in relatively low concentrations within the vertebrae, it is possible that long-term concentrations over ontogeny do not shift enough to result in notable changes between life stages (LaFreniere et al. 2023). At this point, given these contrasting results and the controls on the uptake of vertebral Zn, it remains unclear whether this element is an indicator of residency in Blacktip Sharks.

In contrast to Zn, Mg in the first year of life and edge had the lowest overall correlation, increasing slightly over ontogeny. Livernois et al. (2021) found a decrease in Mg over ontogeny for Blacktip Sharks in the western nGoM, potentially related to temperature fluctuations (Smith et al. 2013), but vertebral Mg was also found to be constant throughout life in Longnose Stingrays (Feitosa et al. 2021). The high concentrations of vertebral Mg and the difference in magnitude between measurements of edge and first-year signatures may have driven the low correlation found herein. Given the low correlation between first-year and edge signatures, we are unable to draw conclusions surrounding Blacktip Shark residency patterns from Mg alone.

Manganese displayed significant correlations between life stages, particularly in Alabama and Louisiana. This was anticipated, given the prominent regional differences found in edge Mn. The validated individual (tagged and OTC marked in Alabama and later recaptured in Alabama) showed only a 14-μg/g decrease in Mn over ontogeny. The high correlation seen in these regions suggests that Blacktip Sharks occupy similar water chemistries over ontogeny and that Mn may be used as an environmental indicator of residency for Blacktip Sharks in Louisiana and Alabama. Other Blacktip Shark vertebral studies documented a spike in Mn during early life, followed by a decrease and stabilization, which has been attributed to potential maternal offloading (Lewis et al. 2016; Livernois et al. 2021). Since the precise drivers of vertebral Mn remain largely unknown, some combination of maternal offloading or residence in hypoxic waters could play a role.

Our findings of Mn correlation in Louisiana and Alabama sharks support a consistent habitat preference between the first year of life and adulthood.

Barium and Sr displayed the strongest correlations among regions over ontogeny and showed little change in overall concentrations. Prior studies have also found stable concentrations of these elements over ontogeny for Blacktip Sharks in the western nGoM (Livernois et al. 2021). Both adult and juvenile Blacktip Sharks occupy coastal areas, but juveniles prefer to inhabit estuaries whereas adults migrate longitudinally on a seasonal basis (Heupel and Simpfendorfer 2002; Hueter et al. 2004), occupying warm and saline coastal waters during the periods between migrations (Ward-Paige et al. 2014; Plumlee et al. 2018). Since prior studies demonstrate that Ba and Sr are strong environmental tracers of salinity, our findings may indicate (1) that salinity conditions did not substantially change throughout ontogeny, (2) that this species remains localized between migrations, or (3) both.

Conclusion

Analysis of regional classifications exhibited the lowest accuracy (51.7%) when samples were grouped into four regions (states), whereas the highest accuracy (89.7%) was observed when sharks collected in Florida were grouped separately from sharks collected in the remaining three states. Under this two-region grouping, PCA ellipses showed a distinct separation of Florida sharks, particularly in the loading direction of edge Sr signatures. Regional nursery areas for juvenile Blacktip Sharks in the nGoM were distinguished with similar success (80% and 90%; Lewis et al. 2016). High classification accuracies have also been reported in similar studies of regional population connectivity in elasmobranchs (Smith et al. 2016). Combined with the significantly different Ba, Mn, Mg, and Sr edge signatures and the highly correlated Ba, Mn, Sr, and Zn first-year and edge signatures, these findings support current management of separate quotas in the eastern and western GoM.

It is important to note that a complete understanding of the drivers of regional variability in these signatures is not necessary for their use as indicators of spatial and population connectivity so long as multi-elemental regional comparisons are made (Campana et al. 2000; Elsdon and Gillanders 2003; Izzo et al. 2016; Lewis et al. 2016). Despite the limited understanding of element uptake into cartilaginous structures (Smith et al. 2013; Pistevos et al. 2019), the application of chemical signatures has proven successful in numerous teleost and elasmobranch species and regions, including the nGoM. Our findings add to the evidence that vertebral chemistry is a useful

tool for investigating shared environmental histories. Still, advancement in the field of elasmobranch vertebral chemistry relies on laboratory validation studies. Future studies should specifically investigate the element- and species-specific controls on vertebral chemistry to disentangle the effects of intrinsic (e.g., sex, physiology, growth, and diet) and extrinsic (e.g., temperature, salinity, and hypoxia) factors.

Our results support that vertebral trace element analysis is a promising tool for studying Blacktip Shark population connectivity in the nGoM and likely elsewhere. Along with previous tagging and genetic studies, our results support current multi-stock management efforts. Average vertebral edge signatures of Ba, Mg, Mn, and Sr differed significantly among regions, demonstrating the ability of elemental markers to distinguish sharks based on the site of capture. Region-specific differences in vertebral edge signatures also suggest that connectivity between eastern and western regions is low. In particular, Sr and Ba appear to be powerful discriminators for sharks collected in Florida waters, suggesting that sharks remain within specific regions from early life to adulthood, in agreement with previous literature demonstrating philopatric behavior of this species. Furthermore, Ba, Mg, Mn, Sr, and Zn signatures were shown to discriminate between the west/Florida regional groups with a high degree of accuracy. Overall, the present study demonstrates the success of vertebral chemical signatures in distinguishing between Blacktip Sharks across the GoM and supports current multi-stock management efforts.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, A. H. P. Hayne, upon request.

ETHICS STATEMENT

The collection and use of shark vertebrae were conducted under scientific permit SPR-0912-981 issued from the Texas Parks and Wildlife Department to R. J. D. W. as well as Institutional Animal Care and Use Committee protocols 974304 (University of South Alabama) and 17-620 (Mississippi State University) issued to J. M. D.

ORCID

Abigail H. P. Hayne https://orcid.org/0000-0002-2968-5977
J. Marcus Drymon https://orcid.org/0000-0002-2104-004X
John A. Mohan https://orcid.org/0000-0002-2758-163X

REFERENCES

Aoba, T., Moreno, E., & Shimoda, S. (1992). Competitive adsorption of magnesium and calcium ions onto synthetic and biological apatites. *Calcified Tissue International*, *51*, 143–150. https://doi.org/10.1007/BF00298503

Ashhurst, D. E. (2004). The cartilaginous skeleton of an elasmobranch fish does not heal. *Matrix Biology*, *23*, 15–22. https://doi.org/10.1016/j.matbio.2004.02.001

Barry, K. P., Condrey, R. E., Driggers, W. B., III, & Jones, C. M. (2008). Feeding ecology and growth of neonate and juvenile Blacktip Sharks *Carcharhinus limbatus* in the Timbalier–Terrebone Bay complex, LA, U.S.A. *Journal of Fish Biology*, *73*, 650–662. https://doi.org/10.1111/j.1095-8649.2008.01963.x

Bethea, D. M., Buckel, J. A., & Carlson, J. K. (2004). Foraging ecology of the early life stages of sympatric shark species. *Marine Ecology Progress Series*, *268*, 245–264. https://doi.org/10.3354/meps268245

Bonfil, R. (1997). Status of shark resources in the southern Gulf of Mexico and Caribbean: implications for management. *Fisheries Research*, *29*, 101–117. https://doi.org/10.1016/S0165-7836(96)00536-X

Brodbeck, B. E., Lyons, K., Miller, N. R., & Mohan, J. A. (2023). Sex influences elemental variation in the mineralized vertebrae cartilage of Round Stingray (*Urobatis halleri*). *Marine Biology*, *170*, Article 117. https://doi.org/10.1007/s00227-023-04265-6

Campana, S. E., Chouinard, G. A., Hanson, J. M., Frechet, A., & Brattey, J. (2000). Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research*, *46*, 343–357. https://doi.org/10.1016/S0165-7836(00)00158-2

Carlson, J. K., Sulikowski, J. A., & Baremore, I. E. (2006). Do differences in life history exist for Blacktip Sharks, *Carcharhinus limbatus*, from the United States South Atlantic Bight and eastern Gulf of Mexico? *Environmental Biology of Fishes*, *77*, 279–292. https://doi.org/10.1007/978-1-4020-5570-6_8

Coiraton, C., Amezcua, F., & Ketchum, J. T. (2020). New insights into the migration patterns of the Scalloped Hammerhead Shark *Sphyrna lewini* based on vertebral microchemistry. *Marine Biology*, *167*, Article 58. https://doi.org/10.1007/s00227-020-3668-0

Coiraton, C., Tovar–Ávila, J., Garcés-García, K., Rodríguez-Madrigal, J., Gallegos-Camacho, R., Chávez-Arrenquín, D., & Amezcua, F. (2019). Periodicity of the growth–band formation in vertebrae of juvenile Scalloped Hammerhead Shark *Sphyrna lewini* from the Mexican Pacific Ocean. *Journal of Fish Biology*, *95*(4), 1072–1085. https://doi.org/10.1111/jfb.14100

Cortés, E., & Baremore, I. (2012). *Updated catches of Gulf of Mexico Blacktip Sharks* (SEDAR 29–WP–08). Southeast Data, Assessment, and Review.

Cox, P. (1989). *The elements: their origin, abundance, and distribution*. Oxford University Press.

Crook, D. A., & Gillanders, B. M. (2006). Use of otolith chemical signatures to estimate carp recruitment sources in the mid-Murray River, Australia. *River Research and Applications*, *22*, 871–879. https://doi.org/10.1002/rra.941

Dacke, C. G. (1979). *Calcium regulation in sub–mammalian vertebrates*. Academic Press.

Dagg, M. J., & Breed, G. (2003). Biological effects of Mississippi River nitrogen on the northern Gulf of Mexico: A review and synthesis. *Journal of Marine Systems*, *43*, 133–152. https://doi.org/10.1016/j.jmarsys.2003.09.002

Dean, M. N., Ekstrom, L., Monsonego-Orngan, E., Ballantyne, J., Witten, P. E., Riley, C., Habraken, W., & Omelon, S. (2015). Mineral homeostasis and regulation of mineralization processes in the skeletons of sharks, rays, and relatives (Elasmobranchii). *Seminars in Cell & Developmental Biology*, *46*, 51–67. https://doi.org/10.1016/j.semcdb.2015.10.022

Dean, M. N., & Summers, A. P. (2006). Mineralized cartilage in the skeleton of chondrichthyan fishes. *Zoology*, *109*, 164–168. https://doi.org/10.1016/j.zool.2006.03.002

Elsdon, T. S., & Gillanders, B. M. (2003). Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. *Reviews in Fish Biology and Fisheries*, *13*, 217–235. https://doi.org/10.1023/B:RFBF.0000033071.73952.40

Feitosa, L., Queiroz, A. P. N., Labonne, M., Dressler, V. L., & Lessa, R. P. (2021). Habitat use and nursery evaluation for the Longnose Stingray *Hypanus guttatus* (Bloch & Schneider, 1801) using vertebral microchemistry. *Journal of Fish Biology*, *99*, 1538–1549. https://doi.org/10.1111/jfb.14858

Fletcher, P. E., & Fletcher, G. L. (1980). Zinc- and copper-binding proteins in the plasma of Winter Flounder (*Pseudopleuronectes americanus*). *Canadian Journal of Zoology*, *58*(4), 609–613. https://doi.org/10.1139/z80-086

Fogarty, M. J., & Botsford, L. W. (2007). Population connectivity and spatial management of marine fisheries. *Oceanography*, *20*, 112–123. https://doi.org/10.5670/oceanog.2007.34

Hedgecock, D., Barber, P. H., & Edmands, S. (2007). Genetic approaches to measuring connectivity. *Oceanography*, *20*, 70–79. https://doi.org/10.5670/oceanog.2007.30

Heupel, M. R., & Simpfendorfer, C. A. (2002). Estimation of mortality of juvenile Blacktip Sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 624–632. https://doi.org/10.1139/f02-036

Hoffmayer, E. R., & Parsons, G. R. (2003). Food habits of three shark species from the Mississippi Sound in the northern Gulf of Mexico. *Southeastern Naturalist*, *2*(2), 271–280. https://doi.org/10.1656/1528-7092(2003)002[0271:FHOTSS]2.0.CO;2

Hueter, R. E., Heupel, M. R., Heist, E. J., & Keeney, D. B. (2004). Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, *35*, 239–247. https://doi.org/10.2960/J.v35.m493

Hüssy, K., Limburg, K. E., de Pontual, H., Thomas, O. R. B., Cook, P. K., Heimbrand, Y., Blass, M., & Sturrock, A. M. (2020). Trace element patterns in otoliths: The role of biomineralization. *Reviews in Fisheries Science & Aquaculture*, *29*(4), 445–477. https://doi.org/10.1080/23308249.2020.1760204

Izzo, C., Huveneers, C., Drew, M., Bradshaw, C. J. A., Donnellan, S. C., & Gillanders, B. M. (2016). Vertebral chemistry demonstrates

movement and population structure of bronze whaler. *Marine Ecology Progress Series*, *556*, 195–207. https://doi.org/10.3354/meps11840

Justíc, D., Rabalais, N. N., & Turner, R. E. (2002). Modeling the impacts of decadal changes in riverine nutrient fluxes on coastal eutrophication near the Mississippi River delta. *Ecology Modelling*, *152*, 33–46. https://doi.org/10.1016/S0304-3800(01)00472-0

Keeney, D. B., Heupel, M. R., Hueter, R. E., & Heist, E. J. (2005). Microsatellite and mitochondrial DNA analyses of the genetic structure of the Blacktip Shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology*, *14*, 1911–1923. https://doi.org/10.1111/j.1365-294X.2005.02549.x

Kohler, N. E., & Turner, P. A. (2019). Distributions and movements of Atlantic shark species: A 52-year retrospective atlas of mark and recapture data. *Marine Fisheries Review*, *81*(2), 1–93. https://doi.org/10.7755/MFR.81.2.1

LaFreniere, B. R., Sosa-Nishizaki, O., Herzka, S. Z., Snodgrass, O., Dewar, H., Miller, N., Wells, R. J. D., & Mohan, J. A. (2023). Vertebral chemistry distinguishes nursery habitats of juvenile Shortfin Mako (*Isurus oxyrinchus*) in the eastern North Pacific Ocean. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *15*, Article e10234. https://doi.org/10.1002/mcf2.10234

Lewis, J. P., Patterson, W. F., Carlson, J. K., & McLachlin, K. (2016). Do vertebral chemical signatures distinguish juvenile Blacktip Shark (*Carcharhinus limbatus*) nursery regions in the northern Gulf of Mexico? *Marine and Freshwater Research*, *67*, 1014–1022. https://doi.org/10.1071/MF15088

Limburg, K. E., Olson, C., Walther, Y., Dale, D., Slomp, C. P., & Hoie, H. (2011). Tracking Baltic hypoxia and cod migration over millennia with natural tags. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(22), E177–E182. https://doi.org/10.1073/pnas.1100684108

Limburg, K. E., Walther, B. D., Lu, Z., Jackman, G., Mohan, J. A., Walther, Y., Nissling, A., Weber, P. K., & Schmit, A. K. (2015). In search of the dead zone: Use of otoliths for tracking fish exposure to hypoxia. *Journal of Marine Systems*, *141*, 167–178. https://doi.org/10.1016/j.jmarsys.2014.02.014

Livernois, M. C., Mohan, J. A., TinHan, T. C., Richards, T. M., Falterman, B. J., Miller, N. R., & Wells, R. J. D. (2021). Ontogenetic patterns of elemental tracers in the vertebrae cartilage of coastal and oceanic sharks. *Frontiers in Marine Science*, *8*, Article 704134. https://doi.org/10.3389/fmars.2021.704134

Mathews, T., & Fisher, N. S. (2009). Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Science of the Total Environment*, *407*, 5156–5161. https://doi.org/10.1016/j.scitotenv.2009.06.003

McCulloch, M., Cappo, M., Aumend, J., & Muller, W. (2005). Tracing the life history of individual barramundi using laser ablation MC-ICP-MS Sr-isotopic and Sr/Ba ratios in otoliths. *Marine and Freshwater Research*, *56*, 637–644. https://doi.org/10.1071/MF04184

McMillan, M. N., Izzo, C., Junge, C., Albert, O. T., Jung, A., & Gillanders, B. M. (2017). Analysis of vertebral chemistry to assess stock structure in a deep-sea shark, *Etmopterus spinax*. *ICES Journal of Marine Science*, *74*, 793–803. https://doi.org/10.1093/icesjms/fsw176

McMillan, M. N., Izzo, C., Wade, B., & Gillanders, B. M. (2017). Elements and elasmobranchs: Hypotheses, assumptions and

limitations of elemental analysis. *Journal of Fish Biology*, *90*, 559–594. https://doi.org/10.1111/jfb.13189

Mohan, J. A., Miller, N. R., Herzka, S. Z., Sosa-Nishizaki, O., Dewar, H., Kinney, M., Snodgrass, O., & Wells, R. J. D. (2018). Elements of time and place: Manganese and barium in shark vertebrae reflect age and upwelling histories. *Proceedings of the Royal Society B*, *285*, Article 20181760. https://doi.org/10.1098/rspb.2018.1760

Mohan, J. A., Romo-Cureil, A. E., Herzka, S. Z., Wells, R. J. D., Miller, N. R., Sosa-Nishizaki, O., & Garcia-Rodriguez, E. (2023). Inferring habitat use of the Pacific White Shark using vertebral chemistry. *Frontiers in Marine Science*, *9*, Article 10882219. https://doi.org/10.3389/fmars.2022.1082219

Mohan, J. A., & Walther, B. (2016). Out of breath and hungry: Natural tags reveal trophic resilience of Atlantic Croaker to hypoxia exposure. *Marine Ecology Progress Series*, *56*, 207–221. https://doi.org/10.3354/meps11934

National Marine Fisheries Service. (2022). *Atlantic Shark commercial fishery landings and retention limit updates*. National Marine Fisheries Service. https://www.fisheries.noaa.gov/atlantic-highly-migratory-species/2022-atlantic-shark-commercial-fishery-landings-and-retention

Nowlin, W. D., Jr., Jochens, A. E., DiMarco, S. F., Reid, R. O., & Schroeder, W. W. (2005). Low-frequency circulation over the Texas–Louisiana continental shelf. In W. Sturges & A. Lugo-Fernandez (Eds.), *Circulation in the Gulf of Mexico: Observations and models* (Geophysical Monograph Series 161, pp. 219–240). American Geophysical Union. https://doi.org/10.1029/161GM17

Oey, L. Y., Ezer, T., & Lee, H. C. (2005). Loop current, rings, and related circulation in the Gulf of Mexico: a review of numerical models and future challenges. In W. Sturges & A. Lugo-Fernandez (Eds.), *Circulation in the Gulf of Mexico: Observations and models* (Geophysical Monograph Series 161, pp. 31–56). American Geophysical Union. https://doi.org/10.1029/161GM04

Pakhomova, S. V., Hall, P. O. J., Kononets, M. Y., Rozanov, A. G., Tengberg, A., & Vershinin, A. V. (2007). Fluxes of iron and manganese across the sediment–water interface under various redox conditions. *Marine Chemistry*, *107*, 319–331. https://doi.org/10.1016/j.marchem.2007.06.001

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., & Hergt, J. (2011). Iolite: Freeware for the visualization and processing of mass spectrometric data. *Journal of Analytical Atomic Spectrometry*, *26*, 2508–2518. https://doi.org/10.1039/c1ja10172b

Pentreath, R. J. (1973). The accumulation from seawater of ^{65}Zn, ^{54}Mn, ^{58}Co, and ^{59}Fe by the Thornback Ray, *Raja clavata L. Journal of Experimental Marine Biology and Ecology*, *12*, 327–334. https://doi.org/10.1016/0022-0981(73)90062-2

Pistevos, J. C. A., Reis-Santos, P., Izzo, C., & Gillanders, B. M. (2019). Element composition of shark vertebrae shows promise as a natural tag. *Marine and Freshwater Research*, *70*, 1722–1733. https://doi.org/10.1071/MF18423

Plumlee, J. D., & Wells, R. J. D. (2016). Feeding ecology of three coastal shark species in the northwest Gulf of Mexico. *Marine Ecology Progress Series*, *550*, 163–174. https://doi.org/10.3354/meps11723

Policansky, D., & Magnuson, J. J. (1998). Genetics, metapopulations, and ecosystem management of fisheries. *Ecological Applications*, *8*(Supplement), S119–S123. https://doi.org/10.2307/2641369

Pon-On, W., Meejoo, S., & Tang, I. M. (2008). Substitution of manganese and iron into hydroxyapatite: Core/shell nanoparticles.

Materials Research Bulletin, *43*, 2137–2144. https://doi.org/10.1016/j.materresbull.2007.09.004

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rabalais, N. N., Turner, R. E., & Wiseman, W. J. (2002). Gulf of Mexico hypoxia, a.k.a. "The Dead Zone." *Annual Review of Ecology and Systematics*, *33*, 235–263. https://www.jstor.org/stable/3069262

Raoult, V., Howell, N., Zahra, D., Peddemors, V. M., Howard, D. L., de Jonge, M. D., Buchan, B. L., & Williamson, J. E. (2018). Localized zinc distribution in shark vertebrae suggests differential deposition during ontogeny and across vertebral structures. *PLoS One*, *13*(1), Article e0190927. https://doi.org/10.1371/journal.pone.0190927

Sanchez, P. J., Rooker, J. R., Zapp Sluis, M., Pinsky, J., Dance, M. A., Falterman, B., & Allman, R. J. (2020). Application of otolith chemistry at multiple life history stages to assess population structure of Warsaw Grouper in the Gulf of Mexico. *Marine Ecology Progress Series*, *651*, 111–123. https://doi.org/10.3354/meps13457

Scharer, R. M., Patterson, W. F., III, Carlson, J. K., & Poulakis, G. R. (2012). Age and growth of endangered Smalltooth Sawfish (*Pristis pectinata*) verified with LA-ICP-MS analysis of vertebrae. *PLoS One*, *7*(10), Article e47850. https://doi.org/10.1371/journal.pone.0047850

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Natural Methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Schoenberg, H. P. (1963). Extent of strontium substitution for calcium in hydroxyapatite. *Biochimica et Biophysica Acta*, *75*, 96–103. https://doi.org/10.1016/0006-3002(63)90583-3

Schroeder, R., Simpfendorfer, C. A., & Welch, D. J. (2010). Population structure of two inshore shark species (*Sphyrna lewini* and *Rhizoprionodon acutus*) using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) along the east coast of Queensland, Australia. In D. J. Welch, J. Ovenden, C. Simpfendorfer, A. Tobin, J. A. T. Morgan, R. Street, J. White, A. Harry, R. Schroeder, & W. G. Macbeth (Eds.), *Stock structure of exploited shark species in north-eastern Australia* (Fishing & Fisheries Research Centre Technical Report No. 12, pp. 39–48). James Cook University Press.

Southeast Data, Assessment, and Review. (2012). *SEDAR 29: HMS Gulf of Mexico Blacktip Shark*. Southeast Data, Assessment, and Review.

Southeast Data, Assessment, and Review. (2018). *Update assessment to SEDAR 29: Gulf of Mexico Blacktip Shark*. Southeast Data, Assessment, and Review.

Shim, M. J., Swarzenski, P. W., & Shiller, A. M. (2012). Dissolved and colloidal trace elements in the Mississippi River delta outflow after Hurricanes Katrina and Rita. *Continental Shelf Research*, *42*, 1–9. https://doi.org/10.1016/j.csr.2012.03.007

Simpfendorfer, C. A., Heupel, M., White, W., & Dulvy, N. (2011). The importance of research and public opinion to conservation management of sharks and rays: A synthesis. *Marine and Freshwater Research*, *62*, 518–527. https://doi.org/10.1071/MF11086

Smith, W. D., Heppell, S. S., & Miller, J. A. (2013). Elemental markers in elasmobranchs: Effects of environmental history and growth on vertebral chemistry. *PLoS One*, *8*, Article e62423. https://doi.org/10.1371/journal.pone.0062423

Smith, W. D., Miller, J. A., Fernando Marquez–Farias, J., & Heppell, S. S. (2016). Elemental signatures reveal the geographic origins of a highly migratory shark: Prospects for measuring population connectivity. *Marine Ecology Progress Series*, *556*, 173–193. https://doi.org/10.1371/journal.pone.0062423

Speed, C. W., Field, I. C., Meekan, M. G., & Bradshaw, C. J. A. (2010). Complexities of coastal shark movements and their implications for management. *Marine Ecology Progress Series*, *408*, 275–305. https://doi.org/10.3354/meps08581

Swift, D. G., O'Leary, S. J., Grubbs, R. D., Frazier, B. S., Fields, A. T., Gardiner, J. M., Drymon, J. M., Bethea, D. M., Wiley, T. R., & Portnoy, D. S. (2023). Philopatry influences the genetic population structure of the Blacktip Shark (*Carcharhinus limbatus*) at multiple spatial scales. *Molecular Ecology*, *32*(18), 4953–4970. https://doi.org/10.1111/mec.17096

Tang, Y., Chappell, H. F., Dove, M. T., Reeder, R. J., & Lee, Y. J. (2009). Zinc incorporation into hydroxylapatite. *Biomaterials*, *30*, 2864–2872. https://doi.org/10.1016/j.biomaterials.2009.01.043

Thorrold, S. R., Jones, C. M., & Campana, S. E. (1997). Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic Croaker (*Micropogonias undulatus*). *Limnology and Oceanography*, *42*(1), 102–111. https://doi.org/10.4319/lo.1997.42.1.0102

Tillett, B. J., Meekan, M. G., Parry, D., Munksgaard, N., Field, I. C., Thorburn, D., & Bradshaw, C. J. A. (2011). Decoding fingerprints: Elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. *Marine Ecology Progress Series*, *434*, 133–142. https://doi.org/10.3354/meps09222

TinHan, T. C., O'Leary, S. J., Portnoy, D. S., Rooker, J. R., Gelpi, C. G., & Wells, R. J. D. (2020). Natural tags identify nursery origin of a coastal elasmobranch *Carcharhinus leucas*. *Journal of Applied Ecology*, *57*, 1222–1232. https://doi.org/10.1111/1365-2664.13627

Urist, M. R. (1976). Biogenesis of bone: Calcium and phosphorous in the skeleton and blood in vertebrae evolution. In R. O. Greep & E. B. Astwood (Eds.), *Handbook of physiology* (pp. 183–213). American Physiological Society.

Vernon, W. B. (1988). The role of magnesium in nucleic-acid and protein metabolism. *Magnesium*, *7*(5–6), 234–248.

Walker, N. D., Wiseman, W. J., Rouse, L. J., & Babin, A. (2005). Effects of river discharge, wind stress, and slope eddies on circulation and the satellite-observed structure of the Mississippi River plume. *Journal of Coastal Research*, *216*, 1228–1244. https://doi.org/10.2112/04-0347.1

Ward-Paige, C. A., Britten, G. L., Bethea, D. M., & Carlson, J. K. (2014). Characterizing and predicting essential habitat features for juvenile sharks. *Marine Ecology Progress Series*, *36*, 419–431. https://doi.org/10.1111/maec.12151

Wells, B. K., Bath, G. E., Thorrold, S. R., & Jones, C. M. (2000). Incorporation of strontium, cadmium and barium in juvenile Spot (*Leiostomus xanthurus*) scales reflects water chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*, 2122–2129. https://doi.org/10.1139/f00-178

Werry, J. M. A., Lee, S. Y. A., Otway, N. M. C., Hu, Y. D., & Sumpton, W. E. (2011). A multi-faceted approach for quantifying the estuarine-nearshore transition in the life cycle of the Bull Shark, *Carcharhinus leucas*. *Marine and Freshwater Research*, *62*, 1421–1431. https://doi.org/10.1071/MF11136

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.