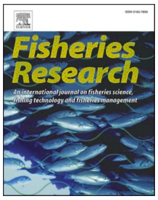
Fisheries Research 268 (2023) 106849

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres



Assessing ecological connectivity of blacktip sharks (*Carcharhinus limbatus*) in the Gulf of Mexico using stable isotope analysis of multiple tissues

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ARTICLE INFO

Handled by Ivone Figueiredo Rosa

Keywords:
Elasmobranch
Population connectivity
Vertebrae
Muscle
Turnover rates
Ecological population

ABSTRACT

Effective management of blacktip sharks relies on a comprehensive understanding of population distribution and stock structure. Previous stock separations and the implementation of regional quotas have proven successful in maintaining the sustainable harvest of blacktip sharks in both the Western North Atlantic and United States Gulf of Mexico (US GoM), in commercial and recreational fisheries. Within the US GoM, finer scale biological separation between blacktips in western and eastern regions has been supported through tagging, genetic, and diet data. This study provides additional evidence of unique ecological isotopic niche areas and $\delta^{15}N$ and $\delta^{13}C$ values for blacktip sharks from the western, central, and eastern US GoM across muscle and vertebral tissues that reflect different isotope turnover rates. Blacktip sharks from western and central regions exhibited isotope values and niches that were significantly smaller compared to eastern sharks, with enriched $\delta^{13}C$ and depleted $\delta^{15}N$ west of 88ºN. Ontogenetic shifts were noted for most regions and there was no crossover or overlap in average isotope values from early to recent life between regions. These spatiotemporal patterns suggest that in the year following birth and prior to the time of capture, blacktip sharks on the central and western shelves have separated ecologically from blacktips on the eastern US GoM Florida shelf.

1. Introduction

The blacktip shark (*Carcharhinus limbatus*) is a large coastal elasmobranch distributed across the United States Gulf of Mexico (US GoM) (Drymon et al., 2020). Though considered a highly migratory predator that connects disparate food webs and transfers nutrients across ecotones, blacktip sharks are known to form discrete ecological groupings that isolate populations with low geographic proximity (Kohler and Turner, 2019; SEDAR 29, 2018). Isolation by distance has been demonstrated within the US GoM blacktip shark population and the extent to which this contributes to regional differences in life history characteristics has been increasingly explored (Keeney et al., 2003a, 2005; Carlson et al., 2006; Lewis et al., 2016; Matich et al., 2021b; Swift et al. in revision). As a species of ecological and economic importance, effective management relies on a comprehensive understanding of population distribution within the US GoM to ensure regional quotas are appropriate and effective (Drymon et al., 2010, 2020). As such, understanding the potential presence and connectivity of subpopulations is critical to avoid localized overharvesting when dispersal and mixing is limited (Hanski, 1997; Harrison and Taylor, 1997; Fronhofer et al., 2012; Hawkins et al., 2016).

Currently, the US GoM supports more than two-thirds of the recreational and commercial blacktip landings throughout the Western North Atlantic Ocean, and regional quota allocations over the last 10 years have promoted sustainable harvest (Cortes and Baremore, 2012, SEDAR 2018; Pacoureau et al., 2023). As management strategies that have considered the eastern and western groups of US GoM blacktips separately have proven effective to maintaining sustainable harvest, further research into the ecological stock structure is warranted to better understand the degree of connectivity between these isolated groups (SEDAR 2018). Indeed, data from 52 years of the NOAA Fisheries Cooperative Shark Tagging program suggests little exchange among blacktip sharks residing in different regions of the US GoM (Kohler and Turner, 2019). Differences in diet composition, habitat use, and home

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https://doi.org/10.1016/j.fishres.2023.106849
Received 2 May 2023; Received in revised form 7 August 2023; Accepted 30 August 2023

ranges have been demonstrated for blacktip sharks that occupy different regions within the US GoM (Kohler and Turner, 2019; Matich et al., 2021b, Swift et al. in revision, Hayne et al. unpubl data). Recent work has also shown two genetically distinct subpopulations of blacktips in the eastern and western US GoM that align with managerial stock separation (Swift et al., 2023). These studies represent traditional tagging and genetic stock delineation methods; however, stable isotope analysis (SIA) has not yet been used as an ecological tracer to distinguish blacktip sharks residing in different regions of the US GoM. Though various studies have used SIA to explore trophic ecology of US GoM blacktip sharks (Plumlee and Wells, 2016; Seubert et al., 2019; Matich et al., 2021b), using SIA for ecological delineation allows for complementary metrics that define ecological separation to be explored by examining trophic histories and movement through baseline isotopic gradients.

Stable isotope analysis of nitrogen (^{15}N) and carbon (^{13}C) is an effective method of analyzing fish tissues to provide comprehensive ecological insights into migration and residency (Peterson and Fry, 1987; Hobson, 1999; Trueman et al., 2012; Mohan et al., 2023). Stable isotope ratios (δ^{15}N and δ^{13}C) vary across different regions due to baseline variations in biogeochemical and oceanographic processes (Peterson and Fry, 1987; Hobson, 1999; Glibert et al., 2019). The unique location-based isotopic components of primary producers are assimilated by consumers in predictable ways, where δ^{15}N values approximate trophic level and δ^{13}C values indicate primary carbon sources, allowing inferences on trophic ecology and foraging location to be made (DeNiro and Epstein, 1978; Schoeninger and DeNiro, 1984; Peterson and Fry, 1987). Presenting δ^{15}N and δ^{13}C values as a bi-plot creates a two-dimensional visualization of an organism's δ-space, which represents the overall isotopic niche area occupied (Newsome et al., 2007). Comparison of different groups of consumer's isotopic values and δ-space can then be used to evaluate the degree of separation or ecological connectivity related to regional residency over distinct spatiotemporal scales (Shiffman et al., 2012; Zeichner et al., 2017; Munroe et al., 2018). Additionally, resident behavior or movement patterns on local and regional scales may be reflected in isotopic signatures of metabolically active and accretionary tissues (Logan and Lutcavage, 2010; Mohan et al., 2023).

The most common tissues used for isotopic analysis are metabolically active, such as liver, muscle, or blood (Domi et al., 2005; Kinney et al., 2011a; Kim et al., 2012a; Munroe et al., 2018). Due to biochemical catabolic and anabolic cellular processes, these tissues change over time and thus do not represent the entire life history of an organism. In contrast, elasmobranch vertebral cartilage grows by concentric layers of mineral and protein that are metabolically inert after synthesis, providing an unaltered chronology of isotope dynamics over an organism's lifetime (Campana et al., 2002; Estrada et al., 2006; Kerr et al., 2007; Tillett et al., 2011; Carlisle et al., 2015; McMillan et al., 2017b). The isotopic signatures found in discrete vertebral bands provide a snapshot of local biogeochemistry and dietary sources assimilated at the time the band was formed. In this way, the growing vertebral edge corresponds to recent life at the site of capture, while the band-pair closest to the birth band represents local chemistry and diet in the organism's early life, immediately following birth (Campana, 2001; McMillan et al., 2017a). By comparing isotopic compositions from the time of capture to the year after birth, inferences about trophic ecology and foraging habitats over an organism's lifetime can be made (Estrada et al., 2006; Kim et al., 2012b; Carlisle et al., 2015). Using these accretionary structures for SIA to examine ecological population structure at different temporal resolutions can advance knowledge of fine scale population dynamics and inform stock delineation (Edmonds et al., 1999; Tanner et al., 2016; Artetxe-Arrate et al., 2021).

The US GoM represents an ideal ecosystem to use baseline isotope gradients to infer patterns of residency as research has demonstrated significant regional gradients within the northern US GoM, whereby the eutrophic western and central US GoM is enriched in ^{15}N and depleted in ^{13}C relative to the oligotrophic eastern shelf (Radabaugh et al., 2013; Radabaugh and Peebles, 2014; Le-Alvarado et al., 2021). This study reports the first stable isotope assessment of blacktip shark vertebrae, providing a novel method of exploring ecological connectivity and patterns of residency within US GoM blacktip sharks. We evaluate and compare the δ^{15}N and δ^{13}C values in blacktip shark vertebrae and muscle tissue from different regions of the US GoM with respect to both recent life (vertebral edge) and early life (vertebral natal band). It is expected that shark tissues will reflect the local baselines of δ^{15}N and δ^{13}C values across their entire lifetimes, suggesting regional residency. Evidence of isotopic separation will provide further evidence of discrete ecological groupings of blacktip sharks in the US GoM, supporting stock assessment efforts to improve the overall management of the blacktip shark population.

2. Materials and methods

2.1. Sample collection and preparation

Blacktip sharks were collected through fishery independent surveys and by recreational and commercial fishermen in the US GoM from 2020 to 2022 Sharks were collected from Texas to represent the western US GoM, Louisiana, Mississippi and Alabama to represent the central US GoM, and along the Gulf coast of Florida to represent the eastern US GoM ($n = 86$, Fig. 1). Each individual shark was sexed and measured for fork length.

Vertebrae were removed from the thoracic region of the vertebral column of each shark and centrum were isolated by removing excess muscle tissue with scalpels. A portion of the excess muscle tissue sampled near the vertebrae was freeze dried at −40 °C for 48 h but was otherwise untreated. Dried muscle tissue was then ground into a fine powder and a subsample of ~0.8 to 1.2 mg was packaged in 4 mm x 6 mm tin capsules for stable isotope analysis. Vertebral centra were then sectioned longitudinally using a Buehler Isomet 1000 low-speed saw with a diamond wafering blade to obtain a sample with a thickness of 2.5–3.5 mm. The resulting vertebrae cross sections were mounted on a slide using crystal bond for further processing (Fig. 2).

Tissue for SIA from sectioned vertebrae samples of each individual shark was obtained using a MicroMill2 (MM2) (Elemental Scientific Lasers, Omaha, NE, USA). Samples were affixed to the sample plate using tape and visualized using a digital camera to identify the areas of the vertebrae that represented early life and recent life (Fig. 3). To target the early life portion of the vertebrae, the birth band was visually identified as the band with a marked angle change in the intermedalia and the trajectory of the drill was programmed to sample between the birth band and the first band of winter growth. For older individuals, decreased growth rate with age results in a lack or compression of band pairs (Francis et al., 2007; Passerotti et al., 2014; Natanson et al., 2018). Therefore, a standardized pattern of 900x500x600 μm (LxWxD) was set to mill the same amount of tissue from the vertebral edge of each individual (differing lengths) to represent recent life (months to years). The trajectory of the drill was computer programmed to extract tissue from the targeted vertebrae locations using a 400 μm round-tipped carbide bit (model H71.11.004 by Brasseler USA Dental Instruments, Savannah, GA, USA) (Passerotti et al., 2014; Turner Tomaszewicz et al., 2016). Samples from the natal band and vertebral edge were collected on opposite corpus calcareum, and 1–2 mg of the resulting powdered tissue was packaged into tin capsules for SIA.

Due to the limited quantity of material that can be obtained from the milling process, samples were not treated to remove inorganic carbon from the hydroxyapatite within the cartilage. Treatment of elasmobranch tissues have resulted in non-linear results and comparison between treated and untreated tissues have shown no impact on stable isotope values, confounding the utility of removing lipids and urea from muscle tissue and inorganic carbon from vertebrae tissue (Hussey et al., 2012; Christiansen et al., 2014; Carlisle et al., 2015). Even so, some vertebral tissues did not yield adequate sample mass for stable isotope

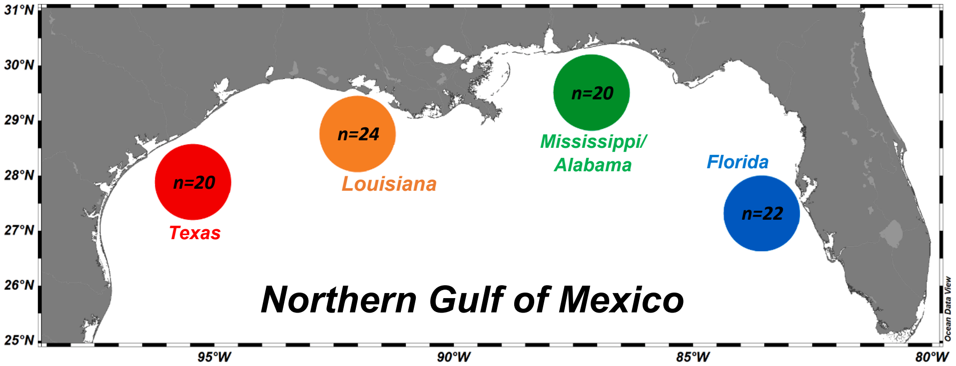

Fig. 1. Map of regions for blacktip sharks captured in the northern US GoM.

Fig. 2. Vertebrae centrum excised from the thoracic region of a blacktip shark harvested from the US US GoM (left). Individual centrum were sagittal sectioned (middle) to produce a 2–3 mm bowtie cross section that was mounted on a glass slide for further processing (right).

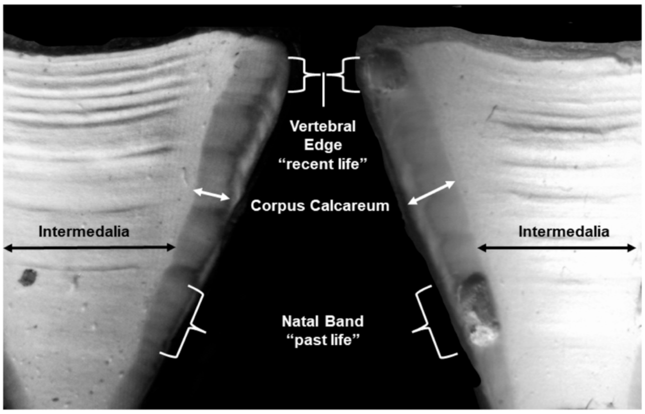

Fig. 3. Microscope image of a blacktip shark vertebral cross-section (left). The microscope camera was used to identify the natal band and vertebral edge from the hypermineralized corpus calcareum of each vertebrae bowtie, and a computer was used to program a specific trajectory of the micromill to extract tissue from the targeted regions (right) for stable isotope analysis.

analysis; these individual tissues were removed from their corresponding groups before assessing average stable isotope values and performing statistical analyses.

2.2. Stable isotope analysis

Powdered vertebrae and muscle tissue were analyzed for δ^{15}N and δ^{13}C values, ‰ carbon, and ‰ nitrogen on an Isoprime isotope ratio mass spectrometer at the University of New Hampshire Stable Isotope Laboratory. Masses of carbon and nitrogen within the powdered tissue samples were measured and converted to moles to calculate the final C:N ratio. Isotope data (precision ~ 0.2‰) was reported relative to Vienna Pee Dee belemnite (VPDB) and atmospheric N_2 for carbon and nitrogen, respectively, in δ notation as follows:

$$\delta_X=[(R_{sample}/R_{standard})-1]\times1000,$$

where X is ^{13}C or ^{15}N and R is the ratio ^{13}C/^{12}C or ^{15}N/^{14}N (Peterson and Fry, 1987; Coplen, 2011).

2.3. Statistical analysis

All statistical analyses were performed in RStudio (version 4.0.3, R Foundation for Statistical Computing) and in PRISM (Graphpad, version 9.1.2, 225, San Diego, CA, USA) with significant values set at $p < 0.05$. The data were found to significantly depart from normality and homogeneity of variance through Shapiro-Wilks and Levene's tests and normalization techniques failed to establish normal distributions for isotope data. Therefore, a non-parametric approach was used for all statistical analyses.

2.4. Stable isotope ratios and niches

Early life isotope ratios in the natal band, and recent life isotope ratios in the muscle and vertebral edge were assessed for significant differences in δ^{13}C and $\delta1^{5}$N values between regions (Kruskal-Wallis, Wilcoxon signed rank tests). To explore variations in isotopic niches between regions, the SIBER (Stable Isotope Bayesian Ellipses) from the SIAR package in R version 4.1.11 as described by Jackson et al. (2011) was utilized for all data visualization and calculations. Isotopic niche ellipses were first plotted using blacktip shark δ^{15}N and δ^{13}C values by region and tissue (vertebral edge, natal band, muscle) to create a biplot encompassing all individuals within each group. Average total isotopic niche breadth was then measured by calculating the convex hull areas, which represent the total occupied niche area (TA) and standard ellipse areas corrected for sample size (SEAc). SEAc values represent the central isotopic niche area of a group with corrections for maximum likelihood, providing a graphical interpretation of standard deviations within the group that can then be compared without the effects of outliers or sample size. The SEAc encompassing 75 % of the data were then used to quantitatively compare overlap between isotopic niches and the proportion of one group that overlaps another (Jackson et al., 2011). Overlap >60 % indicates biologically relevant patterns of shared resource use between groups, as two groups of organisms sharing more than half of their resources would introduce competition (Schoener, 1968; Guzzo et al., 2016; Dance et al., 2018). High isotopic overlap may imply similar isotopic baselines as opposed to competition for the same resources, indicating occupation of the same isotopic niche as opposed to occupation of the same geographical location.

2.5. Ontogenetic shifts

Ontogenetic shifts were examined within each region by comparing δ^{13}C and δ^{15}N values between the vertebral edge and the natal band that included each individual within a region (Paired Wilcoxon signed-rank test). Average natal band and vertebral edge isotope values were then calculated for each region and plotted for visual comparison to explore any crossover or overlap in values between region with ontogeny.

3. Results

Shark vertebrae and muscle tissue were obtained from Texas (TX, $n = 20$, 7 male, 13 female), Louisiana (LA, $n = 24$, 10 male, 14 female), Mississippi and Alabama (MS/AL, $n = 20$, 10 male, 10 female), and Florida (FL, $n = 22$, 11 male, 11 female). The mean size of blacktip sharks sampled was 120 cm FL in TX, 122 cm FL in LA, 113 cm FL in MS/AL, and 130 cm in FL (Table 1). Based on recent estimates of median length at maturity for blacktip sharks in the western (101 cm FL for females and 95 cm for males) and eastern (116 cm FL for females and 106 cm FL for males) US GoM, these individuals were mature (Wells pers. comm., Baremore and Passerotti, 2013).

In vertebral and muscle tissues, FL blacktips had the lowest δ^{15}N values and TX blacktips had the highest δ^{15}N values. In the vertebral tissues, LA blacktip sharks had lower δ^{15}N values than MS/AL blacktip sharks but had higher δ^{15}N values than MS/AL blacktip sharks in the muscle tissue. Across all three tissues, FL blacktips had the highest δ^{13}C values, followed by TX, LA, and MS/AL with the lowest δ^{13}C values (Table 2, Fig. 4).

There were significant intraspecific differences in δ^{15}N values between regions (Kruskal-Wallis test, $p < 0.001$) and multiple comparisons between regions also revealed significant differences ($p < 0.001$), with the exceptions of LA and MS/AL in both the natal and edge vertebrae tissues ($p = 0.38$, 0.28, respectively), and TX and LA in the muscle tissue ($p = 0.71$). Similar significant differences were revealed for δ^{13}C values (Kruskal-Wallis test, $p < 0.001$), and multiple comparisons showed the only regions that were not significantly different were LA and MS/AL ($p = 0.33$, 0.65, respectively) in both the muscle and vertebral edge. In the natal band, only TX and FL were not significantly different for δ^{13}C values ($p = 0.4$, Table 3, Fig. 4).

Table 1
Sample sizes and fork length range and mean for blacktip sharks (*C. limbatus)* taken from four regions of the Gulf of Mexico I in 2020–2022 including Texas (TX), Louisiana (LA), Mississippi/Alabama (MS/AL), and Florida (FL). SD refers to the standard deviation.

State	n	Fork Length Range (cm)	Mean Fork Length (± SD) (cm)
TX	7	M: 89–162	120.4 ± 19.2
	13	F: 97–151	
LA	10	M: 107–124	122.4 ± 9.8
	14	F: 120–140	
MS/AL	10	M:107–122	113 ± 11.2
	10	F: 88–128	
FL	11	M: 90–149	128.9 ± 17.2
	11	F: 121–151	

Table 2
Sample sizes and mean (± SD) δ^{15}N and δ^{13}C values of blacktip shark tissues in each of the four state regions, separated by tissue (natal band, vertebral edge, muscle). Tissues that did not yield adequate sample mass were removed.

		TX	LA	MS/AL	FL
NATAL	n	20	22	20	19
δ^{15}N ‰		14.43 ± 0.4	13.92 ± 0.5	14.05 ± 0.4	11.71 ± 1.6
δ^{13}C ‰		-15.67 ± 1.9	-16.88 ± 2.6	-20.53 ± 3.4	-14.8 ± 2.8
C:N		3.25 ± 0.8	3.05 ± 0.6	3.5 ± 0.9	3.05 ± 0.8
EDGE	n	19	21	20	20
δ^{15}N ‰		14.9 ± 0.5	14.29 ± 0.3	14.36 ± 0.4	11.62 ± 1.2
δ^{13}C ‰		-14.54 ± 2.2	-16.28 ± 1.9	-16.94 ± 2.7	-13.32 ± 1.6
C:N		3.01 ± 0.62	3.03 ± 0.65	3.2 ± 1.05	2.67 ± 0.4
MUSCLE	n	22	24	18	20
δ^{15}N ‰		15.63 ± 0.7	15.57 ± 0.3	14.79 ± 0.6	12.69 ± 1.3
δ^{13}C ‰		-17.45 ± 0.6	-18.19 ± 0.5	-18.31 ± 0.9	-16.16 ± 0.8
C:N		2.82 ± 0.2	2.84 ± 0.2	3.04 ± 0.5	2.91 ± 0.1

In both recent life muscle and vertebral edge tissues, FL blacktip sharks had the largest values for all isotopic niche metrics (SEA, SEAc, TA), followed by blacktip sharks from MS/AL, TX, and LA. In the natal tissue, FL had the largest values for all three isotopic niche metrics, the central states (MS/AL, LA) were in the middle, and TX had the smallest values (Table 4, Fig. 5).

In each of the three tissues, FL blacktip sharks showed low overlap ranging from 0 % to 16 % when compared to TX, LA, and MS/AL. In the natal vertebral tissue, only TX blacktip sharks had significant overlap with LA blacktip sharks (69.3 %), but MS/AL and LA still showed high overlap (58.4 %). In the vertebral edge and muscle tissue, only MS/AL and LA blacktip sharks had substantial overlap (100 %), but TX and MS/AL blacktip sharks still had substantial overlaps of 51.4 % and 57.7 % respectively (Table 5, Fig. 5).

For δ^{15}N, all regions exhibited significant ontogenetic shifts ($p < 0.03$) apart from FL blacktips ($p = 0.38$). For δ^{13}C, all regions exhibited significant ontogenetic shifts ($p < 0.003$) except for blacktips from LA ($p = 0.12$, Fig. 6).

4. Discussion

This study provides further evidence of discrete ecological groupings, regional residency, and low ecological connectivity between blacktip sharks from the eastern US GoM compared to those in the central and western US GoM. Through analysis of δ^{15}N and δ^{13}C values and isotopic niches of vertebral and muscle tissue of blacktip sharks from the US GoM, variations due to ecological separation between four locations and two temporal scales were demonstrated. Previous stock separations and regional quota allocations have been successful in maintaining the sustainable harvest of blacktip sharks in the Southeastern United States (SEDAR 2012, 2018). The recommendation to consider stock separation between eastern and western US GoM blacktips has since been supported by tagging, genetic, and elemental studies that show little mixing between populations across the US GoM (Keeney et al., 2003b, 2005; Kohler and Turner, 2019; Matich et al., 2021b, Hayne et al. unpubl data, Swift et al., 2023). Understanding the presence of subpopulations within the US GoM blacktip shark population assemblage provides a management framework to avoid local overexploitations based on generalized stock structures when ecological connectivity between regions is limited. The existence of genetically and isotopically distinct subpopulations across the northern US Gulf of Mexico (Swift et al., 2023) could indicate the presence of other subpopulations in international GoM water. Therefore, further collaboration with the southern Gulf of Mexico would be warranted to ensure effective international management across the entire region.

4.1. Stable isotope ratios and niches

Isotope ratios in consumer's tissues are influenced by the baseline

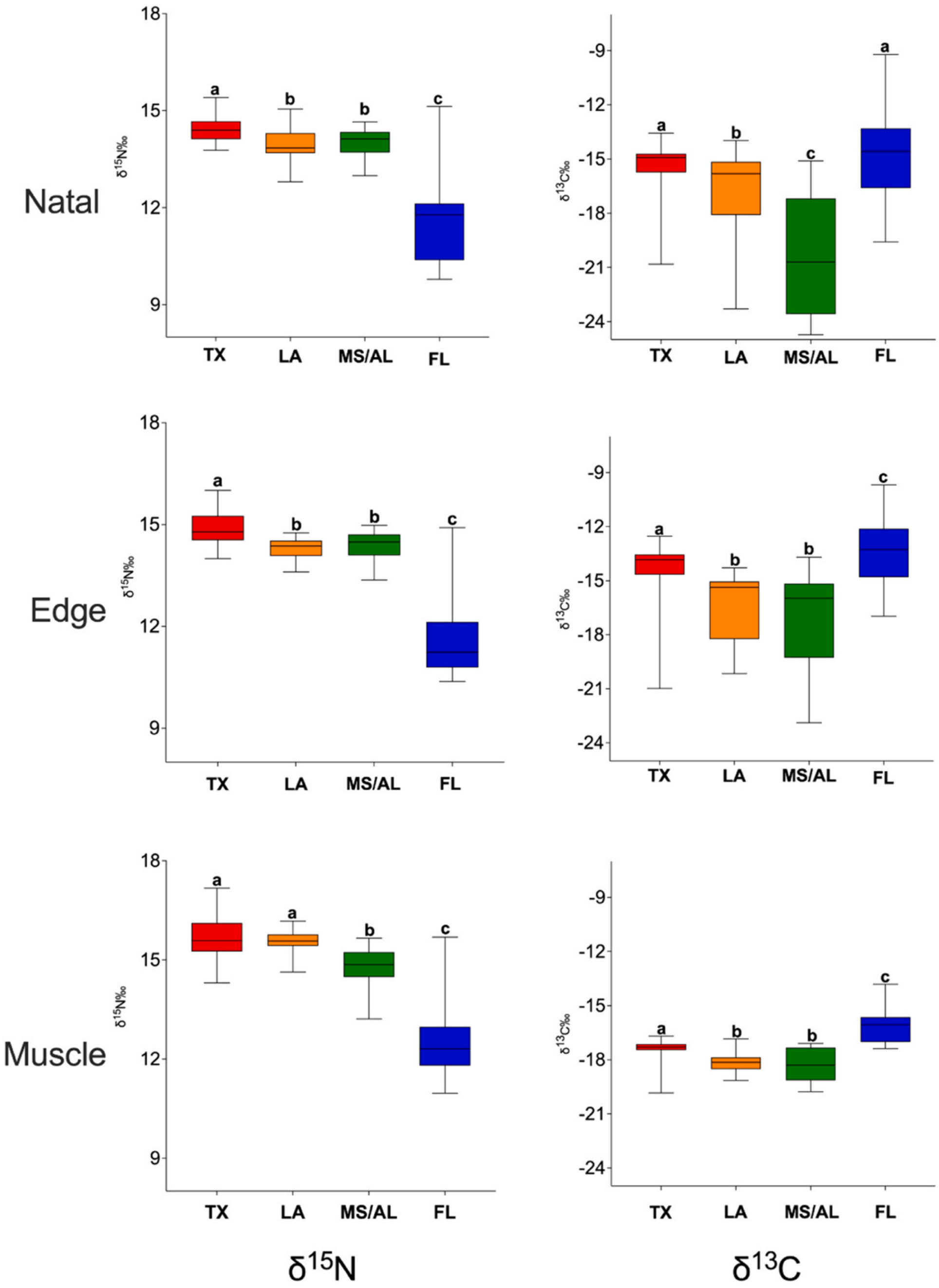

Fig. 4. Boxplots comparing regional δ^{15}N (left) and δ^{13}C (right) values in the natal band (top), vertebral edge (middle), and muscle tissue (bottom) of blacktip sharks collected from the northern US US GoM. Letters indicate significant differences between regions.

δ^{15}N and δ^{13}C values present in foraging locations that vary due to biogeochemical and oceanographic processes (Peterson and Fry, 1987; Hobson, 1999; Glibert et al., 2019). These region-specific baseline isotope values can then be used as natural tracers to explore an organism's habitat use or trophic ecology over different temporal scales (Hobson, 1999). In the US GoM, sampling region has been shown to be a significant driver of isotopic variation, with positive correlations between spatial isotopic gradients and consumers' tissues (Radabaugh et al., 2013; Tucker et al., 2014; Seubert et al., 2019; Le-Alvarado et al., 2021). Comparison of isotopes assimilated in both recent and early life revealed significant differences in blacktips from the eastern US GoM compared to those in the central and western US GoM, suggesting regional separation may be occurring throughout these sharks' lifetimes. In agreement with other studies demonstrating residency in blacktip sharks, we report significant differences in isotopic ratios for different groups of blacktips in the US GoM, implying relatively high site fidelities throughout the northern US GoM (Keeney et al., 2003b, 2005; Hueter et al., 2005; Kohler and Turner, 2019, Swift et al. in revision).

Previous research has demonstrated significant regional δ^{15}N gradients within the northern US GoM, whereby the western and central US GoM is enriched in δ^{15}N relative to the eastern shelf (Radabaugh et al., 2013; Radabaugh and Peebles, 2014; Le-Alvarado et al., 2021). Agriculturally derived nutrient inputs from the Mississippi River and Mobile Bay have been hypothesized mechanisms behind the eutrophication on the western shelf (Rabalais et al., 1996; del Castillo et al., 2001; Dorado et al., 2012; Wells et al., 2017; Le-Alvarado et al., 2021). In contrast, the peninsular Florida shelf is characterized by oligotrophic waters with less nutrient runoff and a large seagrass habitat that gives rise to relatively

Table 3
Results from *post-hoc* Wilcoxon tests comparing δ^{15}N and δ^{13}C values in 3 different blacktip shark tissues collected across the US US GoM. Non-significant differences are shown in bold.

		- δ^{15}N -				- δ^{13}C -	
Natal	TX	LA	MS/AL		TX	LA	MS/AL
LA	<0.001	-	-	LA	0.04	-	-
MS/AL	0.001	**0.28**	-	MS/AL	<0.001	0.002	-
FL	<0.001	<0.001	<0.001	FL	**0.27**	0.03	<0.001
Edge	TX	LA	MS/AL		TX	LA	MS/AL
LA	<0.001	-	-	LA	<0.001	-	-
MS/AL	0.017	**0.38**	-	MS/AL	0.017	**0.33**	-
FL	<0.001	<0.001	<0.001	FL	0.05	<0.001	<0.001
Muscle	TX	LA	MS/AL		TX	LA	MS/AL
LA	**0.71**	-	-	LA	<0.001	-	-
MS/AL	<0.001	<0.001	-	MS/AL	0.017	**0.65**	-
FL	<0.001	<0.001	<0.001	FL	<0.001	<0.001	<0.001

Table 4
Isotopic niche metrics for natal band, vertebral edge, and muscle tissue from blacktip sharks collected across the US US GoM. TA refers to the total convex-hull area, SEA refers to the standard ellipse area, and SEAc refers to the sample-size corrected standard ellipse area encompassing 75 % of the data.

Natal	**TX**	**LA**	**MS/AL**	**FL**
TA	8.31	12.43	11.38	28.44
SEA	2.39	3.69	4.51	11.23
SEAc	2.52	3.87	4.77	11.89
Edge	**TX**	**LA**	**MS/AL**	**FL**
TA	9.01	5.48	9.83	17.64
SEA	3.05	1.87	3.72	6.13
SEAc	3.23	1.97	3.92	6.44
Muscle	**TX**	**LA**	**MS/AL**	**FL**
TA	3.89	2.07	4.77	7.01
SEA	1.15	0.52	1.64	2.54
SEAc	1.21	0.55	1.75	2.68

lower ^{15}N signature compared to the Western US GoM (Mulholland et al., 2006; Dorado et al., 2012; Radabaugh et al., 2013; Radabaugh and Peebles, 2014; Richards et al., 2020). As expected, the δ^{15}N values found in all three blacktip shark tissues reflected this spatial isotopic gradient from the eutrophic waters in the west to the oligotrophic waters in the east.

Florida blacktips had the lowest δ^{15}N values while Texas blacktips had the highest δ^{15}N values in both early and recent life. In addition to the consistency with studies characterizing baseline spatial δ^{15}N gradients across the northern US GoM, this trend has been demonstrated in multiple consumer species in the US GoM and elsewhere, providing a precedent for drawing conclusions about ecological separation based on isotopic separation (Matich et al., 2010, 2021b; Radabaugh et al., 2013; Tucker et al., 2014; Seubert et al., 2019; Whitehead et al., 2020; Dillon et al., 2021). Comparison between isotopes in the skin of loggerhead sea turtles from the West Florida Shelf and Florida Keys to those from the northern US GoM demonstrated significant δ^{15}N depletion for turtles sampled east of Alabama (Tucker et al., 2014). Additionally, both Dillon et al. (2021) and Radabaugh et al. (2013) demonstrated higher δ^{15}N values in the northwest US GoM and lower values near southwest Florida in various teleost species, successfully correlating longitude to δ^{15}N values following the gradient from eutrophic to oligotrophic waters from west to east.

Similarly, δ^{13}C baseline isotopic gradients have been observed at both the primary producer and consumer scale across the US GoM, with enrichment increasing from west to east (Rooker et al., 2006; Dorado et al., 2012; Wells et al., 2017). The Florida shelf has extensive and diverse seagrass habitat within a largely marine environment, which is known to have higher δ^{13}C values compared to other marine primary producers in environments with freshwater inputs, such as the western and central US GoM (Macko and Estep, 1984; Hemminga and Mateo, 1996; Rooker et al., 2006; Bouillon et al., 2008; Peterson et al., 2020). That said, seagrass coverage and salinity increases from north to south along the western US GoM shelf, where southern Texas is a more marine environment relative to northern Texas which has freshwater inputs from the Brazos and Trinity Rivers, as well as the Mississippi River and Mobile Bay.

As expected, based on this isotopic gradient for ^{13}C, blacktip sharks from the central regions generally had the lowest δ^{13}C values, Florida blacktip sharks had the highest, and Texas blacktip sharks were in the middle across both vertebral and muscle tissues. On the western shelf, salinity and seagrass coverage increases from north to south, which may explain the higher δ^{13}C values in Texas blacktip sharks relative those in the central Gulf (Adair et al., 1994; Rooker et al., 2010; Peterson et al., 2020). Indeed, vertebral δ^{13}C values in the natal band of Texas blacktip sharks were not significantly different from Florida blacktip sharks; however, muscle and vertebral edge δ^{13}C values were significantly different between all three regions. Blacktips sharks are known to increase their home range and foraging depths with ontogeny, while juveniles tend to aggregate in shallow, coastal nurseries (Heupel and Heuter, 2001, 2002; Heupel and Simpfendorfer, 2005; Heuter et al., 2005). Therefore, the similarity in the early life for Texas and Florida sharks could be due to the juveniles remaining in the shallow, marine sea-grass habitats while older individuals in Texas make offshore movements and expand their range of foraging grounds, allowing access to the more eutrophic and freshwater habitat of the northwest and central US GoM. As tissues reflecting the most recent isotopes assimilated, demonstrating isotopic separation the vertebral edge and muscle tissue increases our confidence in isotopic separation in the recent life.

The isotopic niche is a concept that integrates specific variables contributing to an organism's ecological niche, which includes the sum of all interactions between an organism and its environment (Newsome et al., 2007). In the same way that ecological niche space can be expressed through a biplot of scenopoetic and bionomic axes' (Hutchinson, 1978), an isotopic niche space can be represented through the multivariate δ-space, whereby δ^{13}C values are a proxy for the scenopoetic axis of bioclimatic region and δ^{15}N values represent the bionomic axis of resource use (Newsome et al., 2007). Isotopic niches encompassing entire groups can then be compared for biologically relevant degrees of overlap that would indicate either separation or shared patterns of resource use (Layman et al., 2007; Jackson et al., 2011). Here, we found FL blacktip sharks had the widest isotopic niche across all tissues sampled and overall niche width generally decreased westward. In a similar study comparing isotopic niches between whale sharks from two locations in Mexico, Whitehead et al. (2020) hypothesized the group with the larger isotopic niche space was related to an increased range of foraging and varied feeding strategies. Similarly, the Florida shelf is a large, 170,000 km^2 habitat with distinct differences in benthic communities, giving rise to an extremely diverse and productive habitat (Walker et al., 2020), whereas the shelf encompassing western and central US GoM is significantly smaller and has far less variation in carbon and nitrogen sources supporting the base of the food web

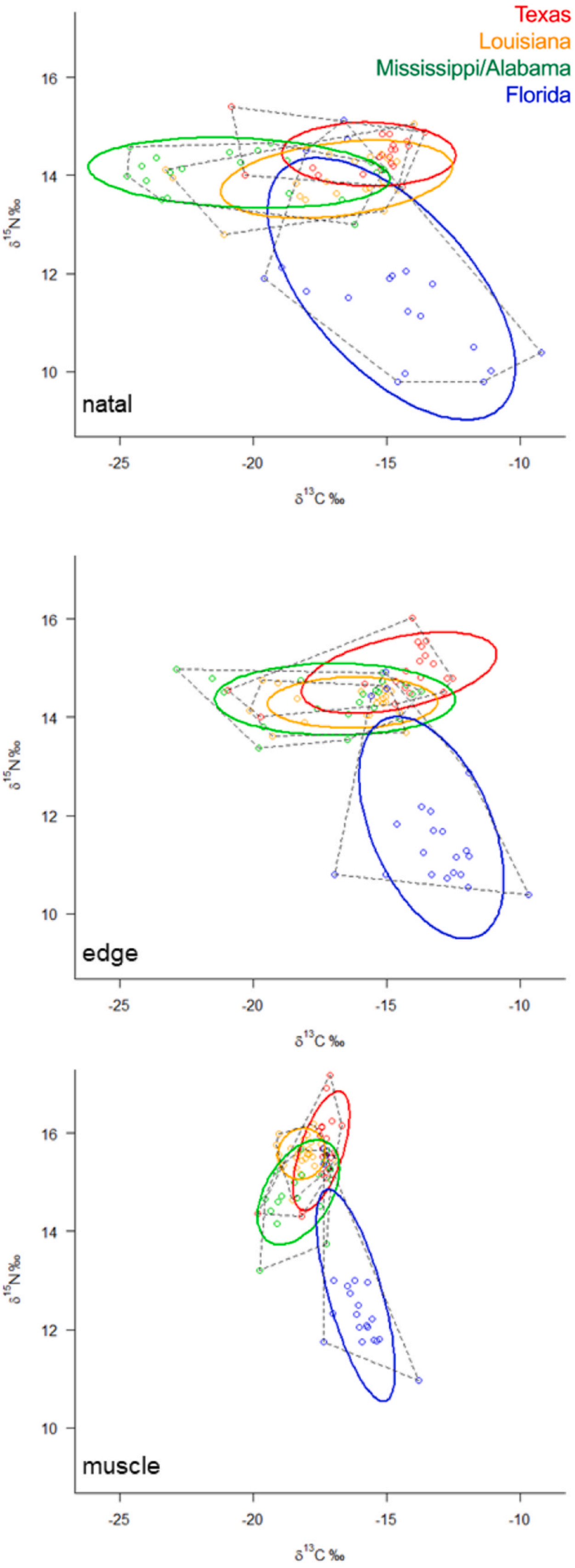

Fig. 5. Total convex-hull area (TA, dotted line) and standard ellipse area (SEA, solid line) of isotopic niches in the natal band (A), vertebral edge (B), and muscle tissue (C) of blacktip shark tissues collected from the northern US US GoM.

Table 5
Total percentage of overlap in sample-size corrected standard ellipse areas (SEAc) encompassing 75 % of the data, among three different blacktip shark tissues collected across the US US GoM. Bold values indicate biologically relevant overlap > 60 %.

Natal	TX	LA	MS/AL
LA	**69.3 %**	-	-
MS/AL	36.4 %	58.4 %	-
FL	5.0 %	16.0 %	11.6 %
Edge	TX	LA	MS/AL
LA	35.0 %	-	-
MS/AL	51.4 %	**100.0 %**	-
FL	0.0 %	1.6 %	4.5 %
Muscle	TX	LA	MS/AL
LA	26.50 %	-	-
MS/AL	57.70 %	**100.00 %**	-
FL	0.9 %	0.00 %	4.5 %

(Rabalais et al., 2007; Justić and Wang, 2014). Therefore, this difference in isotopic niche space could be attributed to differences in overall habitat available and isotopic baseline diversity between the eastern and western US GoM that would be reflected in the isotopic baselines.

In the US GoM, the probability of niche overlap has been shown to decrease with increasing distance between habitats, whereby geographic overlap will give rise to isotopic niche overlap for different groups of fish (Dillon et al., 2021). Here, we utilized the sample size corrected standard ellipse area (SEAc) encompassing 75 % of the data to calculate the proportion of overlap between blacktips from different regions in the US GoM and our results supported the general trend where percent overlap decreased with increasing distance between regions. Florida blacktip isotopic niches consistently separated from the other regions in all three tissues sampled, with all overlap <16 %, suggesting that Florida sharks occupy a distinct isotopic niche relative to the other regions both in both recent and early life.

TX and MS/AL blacktip sharks also exhibited separation across all three tissues (range 36.4–57.7 %); however, overlap was much higher than the substantial separation between FL and the rest of the blacktip sharks. In both the vertebral edge and muscle tissue, TX and LA blacktip sharks also exhibited biologically relevant separation in their isotopic niches (range 26.5 %–35 %), suggesting diversification between these two populations recently, but not in early life as overlap was 69.3 %. The west coast of the US GoM has an isotopic gradient whereby north Texas more closely resembles the central isotopic baseline than the isoscape south of Freeport, Texas that has been demonstrated in both sediments and shrimp (Fry, 1983). Additionally, south Texas has higher salinity due to the geographic separation from the freshwater inputs in north Texas and the central US GoM. As juvenile blacktip sharks use shallow water nurseries in northern Texas, while older individuals expand home ranges and include deeper waters (Matich et al., 2022), isotopic niche overlap in the natal band of LA and TX blacktip sharks could be an artifact of similar isotopic baselines between the north Texas and Louisiana nurseries, as opposed to a homogenization of populations. The high degree of overlap between LA and MS/AL blacktip sharks (range 58.4 %–100 %) in all tissues was expected due to the geographic proximity between these two areas. These results add isotopic evidence of separation to existing genetic, trace element, and tagging evidence of separation between eastern and western US GoM blacktip sharks.

Interestingly, muscular isotopic niche widths were considerably smaller for each region compared to vertebral tissues. Elasmobranch $\delta^{13}C$ values vary based on the lipid content in the tissue analyzed, where a lipid-heavier tissue, such as muscle, will be depleted in ^{13}C relative to more proteinaceous structures such as vertebrae (Kim and Koch, 2012; Carlisle et al., 2017). As the tissues analyzed for this study were not lipid extracted, the smaller range for $\delta^{13}C$ in the muscle tissue could be due to biochemical composition differences between muscle and cartilage. Additionally, the vertebral edge has been hypothesized to be reflective of accumulated $\delta^{15}N$ over a multiyear period, as uncertainties with

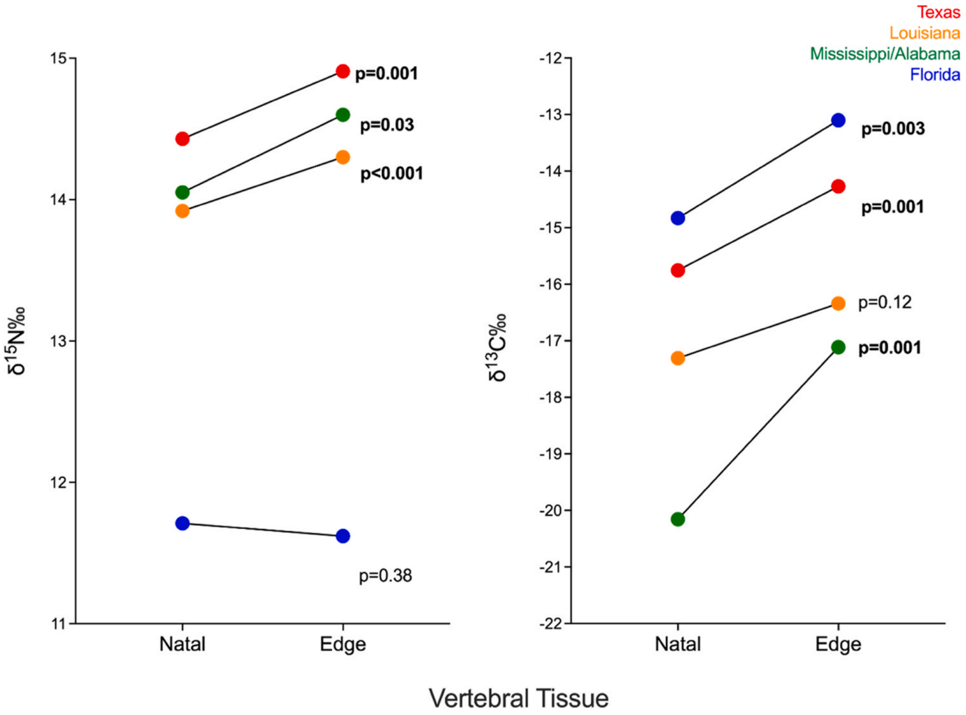

Fig. 6. Average natal band and vertebral edge $\delta^{15}N$ (left) and $\delta^{13}C$ (right) for each region. P-values resulting from Wilcox matched pairs analysis of each region's constituents to explore differences in median values between early and recent life isotopes for each individual within a given region. Bold p-values indicate significant differences between early life isotopes and recent life isotopes.

cartilage accretion rates and slowed deposition with increasing fish size can decrease temporal resolution (Estrada et al., 2006; Francis et al., 2007; Kerr et al., 2007; Natanson et al., 2018). In contrast, controlled laboratory diet studies with small model species have suggested a 95 % diet equilibrium in muscle tissue is reached within 300 days for $\delta^{15}N$ and 500 days for $\delta^{13}C$ (MacNeil et al., 2006; Logan and Lutcavage, 2010; Hussey et al., 2012). For species that have fast metabolisms and use multiple prey sources or foraging locations, isotopic turnover of $\delta^{15}N$ values in the muscle should occur quick enough to capture recent basin-scale movements through a spatial isotopic gradient, which could contribute to smaller isotopic niches, while vertebral cartilage, particularly at the edge, provides more generalized information on the average isoscape experienced (Loor-Andrade et al., 2015).

Conclusions drawn from isotopic niche comparisons based on two dimensions should be considered with caution. The complete suite of components that determine a group's isotopic niche is likely greater than just the specific isotopic ratios of carbon and nitrogen (Bearhop et al., 2004; Newsome et al., 2007). Though the methodology used here employs a Bayesian approach that is more refined than previous statistical methods of isotopic niche characterization, future studies can include further metrics to explore multidimensional isoscapes that include isotope ratios from multiple elements (e.g., sulfur, oxygen) (Swanson et al., 2015; Rossman et al., 2016). Though we suggest the isotopic niche should be considered separately from overall trophic ecology and geographic distribution, low intraspecific overlap between groups of sharks from a range of size/age classes is still indicative of variations contributing to ecological separation (Shipley and Matich, 2020).

4.2. Ontogenetic Shifts

Ontogenetic diet shifts have been demonstrated for many elasmobranch species, including the blacktip shark, where trophic position and foraging area generally increase with increasing size (Livingston, 1982; Wetherbee et al., 2004; Plumlee and Wells, 2016; Matich et al., 2021b). Stable isotope analysis of accretionary structures has been used to demonstrate ontogenetic shifts in various elasmobranch species through the sequential sampling of multiple annuli from one individual (Estrada et al., 2006; Kerr et al., 2007; Kim et al., 2012b; Carlisle et al., 2015; Mohan et al., 2023). Intraspecific comparisons of ontogenetic isotopic shifts between regions provides further insight into lifelong patterns of diet and habitat use that demonstrate geographic variability. In this study, ontogenetic shifts were assessed within each region through paired analyses of each constituents' isotope values found in the natal band compared to the vertebral edge. Significant enrichment between the early life and recent life was demonstrated for both isotopes in all regions, apart from $\delta^{15}N$ values in FL blacktips and $\delta^{13}C$ values for LA blacktips. This finding is supported by other studies that have explored ontogenetic shifts through SIA across the northern US GoM (Peterson et al., 2020; Matich et al., 2021b).

In particular, the lack of a distinct shift for $\delta^{15}N$ values in FL blacktip shark vertebral tissues agrees with a previous study on blacktip sharks in the eastern US GoM, which was attributed to insufficient variation at the base of the food chain for shifts to be detected through SIA (Peterson et al., 2020). In contrast, Matich et al. (2021a) pointed towards increased baseline variability of $\delta^{15}N$ within an ecosystem limiting the ability of $\delta^{15}N$ enrichment to be detected, suggesting corrections need to be applied to account for baseline variability when considering bulk stable isotope ratios. To our knowledge, this is the first study to explore ontogenetic shifts in blacktip sharks using paired analysis of accretionary structures as opposed to exploring $\delta^{15}N$ enrichment with increasing length. Future work should utilize multiple annuli throughout the organism's lifetime to gain a more comprehensive understanding of $\delta^{15}N$ values, particularly for species that have known foraging plasticity that may confound results when just looking at the natal and most recent isotopes experienced.

For $\delta^{13}C$ values, ontogenetic shifts in blacktip sharks have been demonstrated in the northwestern US GoM and are attributed to increases in foraging ground or home range with increasing age (Plumlee and Wells, 2016). In Louisiana, the lack of a detectable ontogenetic shift for δ^{13}C values could be a function of bathymetry, where the shelf drop-off occurs much closer to the shoreline than in the other regions. This could present a geographic barrier preventing the expansion of home range for blacktip sharks that are generally found in shallow waters <10 m in the northern US GoM (Drymon et al., 2010, Ward-Paige, 2014). If any shifts in δ^{13}C values had occurred for LA blacktips, sampling only the vertebral edge may not have been sensitive enough to short term changes in δ^{13}C; therefore, future work may consider using tissues with increased metabolic activity (e.g., blood, liver) to capture short-term isotopic shifts.

Lifetime regional separation for each group was further implied from the lack of crossover in ontogenetic isotopic shifts between regions. Analysis of vertebral tissue from other elasmobranchs has demonstrated intraspecific variation in habitat and resource use, indicative of regional residency. Reconstruction of the trophic history from the sequential sampling of Pacific white shark (*C. carcharias*) vertebrae showed a consistent isotopic pattern correlated to regional baseline isotope ratios, suggesting lifetime residency within one isoscape, while other individuals showed enrichment patterns indicative of migrations between regions (Mohan et al., 2023). Similarly, Kim et al. (2012a,b) found isotopic variation among white sharks was not only due to geographic differences, but also attributable to differences in individual preference accounting for 48 % of isotopic variance. Though white sharks are generalist predators at the population level, individual differences within the population were attributed to local availability of preferrable prey when geographic constraints prohibited movement to more preferred feeding grounds. Similarly, diet descriptions for blacktip sharks in the northern US GoM have shown geographic variability contributing to regional differences in trophic diversity (Matich et al., 2021a). For example, blacktips from the eastern US GoM have a diet comprised of primarily Sparidae species (Heupel and Heuter, 2002), with blacktips in the central US GoM rely heavily on Gulf menhaden (Bethea et al., 2004), while Atlantic croaker makes up over >60 % of the diet for blacktips on the western US GoM (Plumlee and Wells, 2016). When considered with lifelong isotopic variation between regions demonstrated here, these patterns could be indicative of residency with limited overall connectivity between regions.

Ethical statement

All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee (s) have approved them.

CRediT authorship contribution statement

Conceptulization – Addie L. Binstock, J. Marcus Drymon, R.J. David Wells, John A. Mohan. Methodology - Addie L. Binstock, Travis M. Richards, John A. Mohan. Software - Addie L. Binstock. Validation - Addie L. Binstock, Travis M. Richards, John A. Mohan. Formal Analysis - Addie L. Binstock. Investigation - Addie L. Binstock. Resources - Addie L. Binstock, Travis M. Richards, Kesley Gibson-Banks, John A. Mohan, J. Marcus Drymon, R.J. David Wells - Data Curation - Addie L. Binstock, Travis M. Richards, John A. Mohan - Writing- Addie L. Binstock. Visualization - Addie L. Binstock. Administration - Addie L. Binstock, Travis M. Richards, J. Marcus Drymon, John A. Mohan. Funding - Addie L. Binstock, J. Marcus Drymon, R.J. David Wells, John A. Mohan.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Brett Falterman of Fisheries Research LLC and Chris Cremeen for their efforts to collect blacktip shark vertebrae samples. We thank Dr. K. Curiel for her guidance and expertise on developing the micromill methodology. We thank the University of New Hampshire Stable Isotope Facility for performing the stable isotope analysis. This study was performed with the support of Small Grant 614 from the Save Our Seas Foundation. We thank Save Our Seas Foundation for supporting this work and providing a platform to communicate our results. Lastly, we thank the students in the Mohan Shark and Fish Ecology lab for their support with this project.

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