Feeding Ecology of the Sandbar Shark in South Carolina Estuaries Revealed through δ^{13}C and δ^{15}N Stable Isotope Analysis

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ARTICLE

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Abstract
    Stable isotope ratios of carbon and nitrogen (δ^{13}C and δ^{15}N) from muscle samples were used to examine the feeding ecology of a heavily exploited shark species, the Sandbar Shark *Carcharhinus plumbeus*. Two hundred and sixty two Sandbar Sharks were sampled in five South Carolina estuaries. There were no significant differences in average δ^{13}C or δ^{15}N signatures between estuaries, between sampling years, or between male and female Sandbar Sharks, suggesting that these variables do not affect diet. A potential ontogenetic diet shift between young-of-year and juvenile Sandbar Sharks in South Carolina, similar to a shift previously described in Virginia and Hawaii populations, is suggested by significant differences in average δ^{13}C and average δ^{15}N signatures between these age-classes. Results confirm that Sandbar Sharks in South Carolina are generalist predators and that juvenile Sandbar Sharks have a wider diet breadth than young-of-year sharks, a pattern common in elasmobranchs. Sandbar Shark diet in South Carolina is similar to that found in previous stomach content analysis studies. This study also demonstrates that nonlethal sampling methods can be applied to sharks to obtain diet and trophic information, including the detection of ontogenetic shifts in diet.

Many species of sharks (subclass Elasmobranchii) are ecologically important animals because of their role as predators in marine environments (Chapman et al. 2006), though decades of global overfishing have led to reported population declines in many shark species (Dulvy et al. 2008). The U.S. National Marine Fisheries Service plans to eventually institute a new ecosystem-based fishery management plan to improve the management of U.S. shark species (SEDAR 2006). Ecosystem-based fisheries management plans differ from traditional fishery management by focusing not just on a target population but also on diet, trophic interactions, and environment (Pikitch et al. 2004).

One shark species of particular concern to the National Marine Fisheries Service is the heavily exploited Sandbar Shark *Carcharhinus plumbeus* (SEDAR 2006). Sandbar Sharks, which are seasonally abundant in South Carolina (Castro 1993; Abel et al. 2007; Ulrich et al. 2007), have declined in population size in the western North Atlantic by 60–80%; but populations have begun to stabilize since 2007 due to catch restrictions (Romine et al. 2011; SEDAR 2011). Sandbar Sharks are born near the mouths of shallow estuaries in late May or early June and enter the estuaries as primary nurseries, remaining there until October or November (Castro 1993; Ulrich et al. 2007). After overwintering offshore, young juvenile Sandbar Sharks return to estuaries the following spring and utilize them as secondary nurseries. (Conrath and Musick 2008).

The traditional method for characterizing shark diet is stomach content analysis, which has typically involved opening the shark's stomach and identifying the prey items found inside (Cortes 1999). Alternative nonlethal methods, such as gastric lavage and stomach eversion, have also been utilized (Shurdak and Gruber 1989). Stomach content analysis provides high-resolution "snapshot" diet data (Hyslop 1980; Pinnegar and Polunin 1999), though there are many limitations to the method. For example, predatory fishes often have a high percentage of empty stomachs (Arrington et al. 2002), which can result in having to lethally sample a larger number of specimens in order to accumulate enough prey items to characterize a species' diet. Additionally, sharks may regurgitate due to capture stress, which increases the number of animals with empty stomachs (Stevens 1973).

An alternative method to study elasmobranch diet is stable isotope analysis (Hussey et al. 2011; Hussey et al. 2012; Shiffman et al. 2012). This method utilizes the isotopic signatures of carbon and nitrogen isotopes in tissues to examine trophic status and other relevant ecological relationships, such as sources of carbon to the food web (Peterson and Fry 1987). This technique can provide long-term, temporally integrated diet estimates compared with stomach content analysis, which reflects only recently ingested prey (Pinnegar and Polunin 1999). Gathering samples for stable isotope analysis can also be nonlethal and minimally invasive when restricted to the use of certain tissues (Sanderson et al. 2009).

This study examines the ratios of carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) stable isotopes in muscle tissue of Sandbar Sharks in South Carolina's estuaries. Carbon isotopic ratio levels are commonly slightly enriched relative to a food source, approximately 0–1‰ relative to a standard with each trophic level increase, while nitrogen isotopic ratios typically enrich approximately 3.4‰ per trophic level (Minagawa and Wada 1984; Peterson and Fry 1987). Carbon isotopic ratios are therefore useful to differentiate between food web carbon sources (i.e., benthic versus pelagic, coastal versus offshore) and indicate diet, while nitrogen isotopic ratios can indicate different trophic levels (Peterson and Fry 1987; Post 2002).

While the values of 3.4‰ and 0–1‰ are typical diet–tissue discrimination factors, these values can vary significantly by study species and tissue. A review of diet–tissue discrimination factors (Caut et al. 2009) found that the mean discrimination factor for nonelasmobranch fish muscle is approximately 2.5‰ for nitrogen isotopes and 1.8‰ for carbon isotopes. Recent research on elasmobranchs has shown that the diet–tissue discrimination factor values can be slightly different for these fishes, ranging from 2.4‰ for nitrogen isotopes and 0.9‰ for carbon isotopes in the muscle of the Sand Tiger *Carcharias taurus* (Hussey et al. 2010) to 3.7‰ for nitrogen isotopes and 1.7‰ for carbon isotopes in the muscle of the Leopard Shark *Triakis semifasciata* (Kim et al. 2012).

Though Sandbar Shark diet has never been characterized in South Carolina, stomach content analyses have been conducted on Sandbar Sharks from the coastal waters of the Hawaiian Islands (McElroy et al. 2006) and the estuarine and coastal waters of Virginia (Medved et al. 1985; Ellis and Musick 2007). These past studies noted an ontogenetic shift in diet in both regions, with young-of-year (age-0) Sandbar Sharks preying primarily on benthic crustaceans, including blue crab *Callinectes sapidus* and mantis shrimp *Squilla empusa*, and older, larger juveniles relying increasingly on small elasmobranchs and teleost fishes. However, Sandbar Sharks have many allopatric subpopulations (Compagno et al. 2005) and it is unknown if this diet shift occurs throughout their entire range. Other shark species, such as the Shortfin Mako *Isurus oxyrinchus* (Stevens 1984; Cliff et al. 1990; Maia et al. 2006) and the Spiny Dogfish *Squalus acanthias* (Ellis et al. 1996; Smith and Link 2010), are known to consume radically different types of prey in various parts of their range.

Determining whether ontogenetic diet shifts occur is important to consider when attempting to create effective ecosystem-based fisheries management plans (Lucifora et al. 2009; Simpfendorfer et al. 2011). Stable isotope analysis comparing δ^{13}C and δ^{15}N tissue signatures of individuals of different age-classes within the same species has been used to detect ontogenetic diet shifts in animals such as the green sea turtle *Chelonia mydas* (Arthur et al. 2008) and Red Snapper *Lutjanus campechanus* (Wells et al. 2008), though rarely in wild populations of sharks. Though detecting an ontogenetic shift in diet was not the focus of their studies, Matich et al. (2010) noted a difference in inter-tissue isotopic signature variability between smaller and larger Bull Sharks *Carcharhinus leucas* and Vaudo and Heithaus (2011) noted differences in average isotopic signatures between different size-classes of three species of coastal elasmobranchs. Ontogenetic diet shifts in

sharks have been detected using other analyses of isotopic data that involved either sacrificing sharks to obtain liver samples or opportunistically utilizing vertebrae samples from sharks sacrificed for other studies (MacNeil et al. 2005; Estrada et al. 2006; Hussey et al. 2011; Malpica-Cruz et al. 2013).

Since many shark species are live bearing, the maternal contribution of isotopes to age-0 sharks must be considered when analyzing isotopic signatures of age-0 specimens (McMeans et al. 2009; Vaudo et al. 2010; Olin et al. 2011). Maternal investment results in higher $\delta^{15}N$ and either higher or lower $\delta^{13}C$ values in age-0 sharks relative to mothers (McMeans et al. 2009; Vaudo et al. 2010). Maternal contribution can also be detected by analyzing the change in isotopic signature of age-0 sharks over time as they shift to a dietary-influenced isotopic signature (Shaw 2013). Additionally, while isotope turnover rates are generally slow in shark muscle (requiring up to 2 years for complete turnover), significant and ecologically relevant changes in Sandbar Shark muscle isotopic signature ($\sim2‰$ for ^{13}C and $\sim5‰$ for ^{15}N) are detectable within 2 months of a diet switch (Logan and Lutcavage 2010). Isotopic turnover rates must also be considered when analyzing isotopic ratios from species that undergo seasonal migrations, such as Sandbar Sharks that migrate between estuarine and offshore waters (Castro 1993; Abel et al. 2007).

The goals of this study were to use $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures of muscle tissue to characterize the diets and trophic levels of Sandbar Sharks in South Carolina estuaries and coastal waters and to determine if there are any ontogenetic, sex-based, or geographic differences in diet and trophic level. The South Carolina estuarine systems sampled differ geographically and ecologically from the more northern habitats of Virginia (Dame et al. 2000) and the reef-dominated habitats of Hawaii, where previous stomach content analyses of this species have been conducted. Isotopic data from sympatric potential prey species in South Carolina were also analyzed.

METHODS

Sample collection.—Sandbar Shark muscle samples were obtained opportunistically from three coastal shark surveys. Sandbar Sharks were captured using longlines by the South Carolina Department of Natural Resources (SCDNR) Cooperative Atlantic States Shark Pupping and Nursery survey, the SCDNR Adult Red Drum *Sciaenops ocellatus* survey, and the Coastal Carolina University shark survey. Five South Carolina estuaries were sampled from May through November in 2009 and 2010: Winyah Bay, Bulls Bay, Charleston Harbor, St. Helena Sound, and Port Royal Sound (Figure 1). All Sandbar Sharks captured were sexed, measured (both fork length [FL] and stretch total length [TL]), tagged through the dorsal fin with Dalton roto-tags, and released. Dorsal muscle samples of approximately 2 g were taken from the captured Sandbar Sharks prior to release using a 2.0-mm disposable biopsy punch (Premier Medical Products Unipunch). Muscle samples were kept on ice in 2.0-mL cryovials while in the field and upon return to the laboratory were frozen at $-80°C$ until processing.
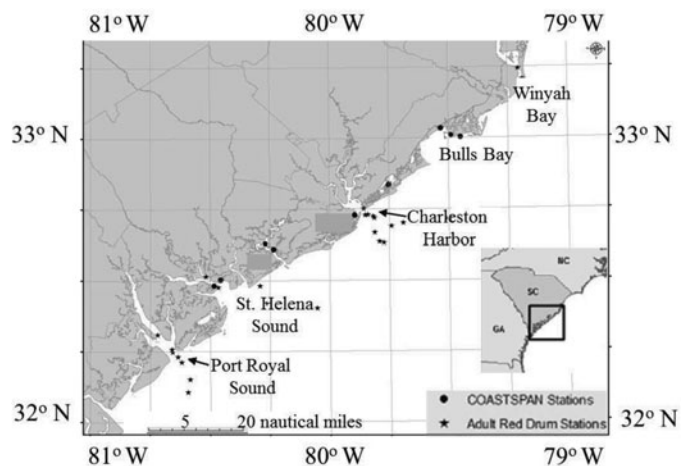

FIGURE 1. Sampling sites in South Carolina estuaries and coastal waters. The dots represent longline and gillnet survey locations from the SCDNR Cooperative Atlantic States Shark Pupping and Nursery survey (COASTSPAN), while the stars represent the longline survey locations from the SCDNR Adult Red Drum project.

Young of year were defined as Sandbar Sharks less than 1 year old (age 0) and were identified by the presence of umbilical scarring and a FL less than 580 mm (Ulrich et al. 2007). Juveniles were older than 1 year (>580 mm FL) and had no umbilical scarring but had not yet reached the reproductively mature size of approximately 1,400 mm FL (Sminkey and Musick 1996). Sandbar Sharks over 1,400 mm FL were considered adults, and since only eight adult sharks were captured during this study, adults were excluded from most analyses. Samples of co-occurring possible prey species in South Carolina estuarine waters, including a variety of invertebrate and fish species, were obtained opportunistically from SCDNR inshore fisheries surveys. Whenever possible, samples of each prey species were obtained from multiple estuaries, but individuals from different estuaries were grouped together for analysis.

Sample processing.—Residual skin, shell, or scales were removed from biopsy samples (Sandbar Sharks and co-occurring possible prey were analyzed to elucidate Sandbar Shark diet) using a scalpel so that only muscle tissue was analyzed (following Davenport and Bax 2002). Preliminary analysis was performed to determine whether urea removal and lipid removal were needed. This consisted of processing multiple samples from the same individual shark in four different ways (no lipid removal and no urea removal, urea removal and no lipid removal, lipid removal and no urea removal, and removal of both lipids and urea) and comparing results. This process was repeated for samples from 10 individual sharks.

To remove urea, all elasmobranch muscle tissue (Sandbar Sharks as well as rays and sharks analyzed as potential prey species) were sonicated three times in 1.0 mL of deionized water for 15 min, decanting the water in between each sonication (Kim and Koch 2011). Preliminary analysis indicated that urea removal lowered $\delta^{15}N$ signatures in elasmobranch muscle by

an average of 0.5‰ and therefore urea removal was performed on all elasmobranch muscle tissue (Sandbar Sharks and co-occurring potential prey species) analyzed in this study.

Lipid extraction is occasionally performed on muscle tissues (MacNeil et al. 2005), but preliminary trials indicated that this method had no effect on the $\delta^{13}C$ signatures of shark muscle ($\delta^{13}C$ signatures of the samples analyzed in the preliminary trials were extremely similar and considered equal between lipid extraction and nonlipid extraction processing methods). Additionally, C:N ratios were low for Sandbar Sharks (approximately 1.2), suggesting low lipid content (Post et al. 2007). Therefore lipid extraction was not utilized on elasmobranch samples in this study. Lipid extraction was, however, utilized on all muscle samples from nonelasmobranch co-occurring potential prey species. One milliliter of dichloromethane was added to each sample tube containing nonelasmobranch muscle tissue, tubes were placed in an ultrasonic water bath for 15 min, and the dichloromethane was then decanted, repeating the process a total of three times (John Kucklick, National Institutes of Science and Technology, personal communication).

All samples were then lyophilized (SP Scientific Virtis Genesis) overnight and homogenized into a fine powder using a Biospec mini bead-beater 8 with 1.0-mm beads. Aliquots of these powdered samples (1 mg) were measured, placed into tin capsules, and analyzed using a Thermo Flash EA coupled to a ThermoFisher Scientific Delta V Plus Isotope-ratio mass spectrometer located at the isotope laboratory at the Skidaway Institute of Oceanography (Savannah, Georgia), which has a precision of ± 0.1 for both carbon and nitrogen isotopes. Sample stable isotope values were calibrated against internally calibrated laboratory chitin powder standards (-0.90‰ ^{15}N,-18.95‰ ^{13}C), which are cross-checked against the U.S. Geological Survey 40 international isotope standard and National Institute of Standards and Technology Standard Reference Material 8542 ANU-Sucrose.

Statistical analysis.—Stable isotope ratios were expressed in parts per thousand (‰), a ratio of the isotopes in a sample relative to a reference standard. Delta notation (δ) is defined using the following equation:

$$\delta X = \left(\frac{R_{sam}}{R_{sta}} - 1 \right) \cdot 1{,}000\text{‰},$$

where X is defined as the heavy isotope, either ^{13}C or ^{15}N, R_{sam} is the ratio of heavy to light isotopes within each sample, and R_{sta} is the heavy to light ratio in a reference standard.

Isotopic data ($\delta^{13}C$ and $\delta^{15}N$) from each muscle sample were analyzed by sampling month, sampling year, sex, location (estuary), and age-class. Differences in $\delta^{13}C$ and $\delta^{15}N$ between sampling months, sampling years, sexes, estuaries, and age-classes were assessed by multiple-factor analysis of variance (ANOVA). First, all Sandbar Sharks were compared. Second, in order to avoid maternal input bias in age-0 sharks (Olin et al. 2011) and recent offshore feeding bias in migrating ju-

venile sharks, only samples collected after July 15th (approximately 2 months after juvenile Sandbar Sharks typically reenter the estuary and most young of year have been born, Ulrich et al. 2007) were compared. This was considered to be enough time for the Sandbar Sharks' slow muscle isotopic turnover rate (Logan and Lutcavage 2010) to reflect evidence of an estuarine diet-influenced isotope signature, though likely not enough time to allow for full isotopic equilibrium to the estuarine environment. Sandbar Sharks captured after July 15 are referred to as "summer–fall" sharks hereon. Finally, to account for the unbalanced sample design, multiple ANOVAs were performed focusing on each variable to avoid interaction effects (i.e., almost all age-0 sharks were captured in just two estuaries, complicating analysis by estuary, and certain estuaries were sampled more in certain months, complicating analysis by month). Tests were run for both the complete set of all Sandbar Sharks and for summer–fall sharks only, and a Holm correction was used on the resulting *P*-values to reduce the chance of type I error. We hypothesized significant differences in both $\delta^{13}C$ and $\delta^{15}N$ between age-classes, which would indicate an ontogenetic diet shift, but did not expect differences between sampling years, sampling months, or sampling locations. Statistical calculations throughout the study were performed using R (R Development Core Team 2010).

Metrics for comparison of isotope ratios between age-classes followed methods by Layman et al. (2007a). Metrics include $\delta^{15}N$ range and $\delta^{13}C$ range (the difference between the largest and smallest $\delta^{15}N$ and $\delta^{13}C$ values within each age-class), and total occupied niche area (the convex hull area of the polygon represented by all of the $\delta^{13}C$ or $\delta^{15}N$ data for each age-class). Unlike raw isotopic data, these values are suitable for comparisons between species from different habitats.

The relative trophic position of Sandbar Sharks was calculated using Post's (2002) formula. The species used to estimate $\delta^{15}N_{base}$ was Summer Flounder *Paralichthys dentatus*, a secondary consumer that was assigned a trophic level of 3.0. A value of 3.7 was used for the initial value of $\Delta^{15}N$, the increase in the ratio of ^{15}N associated with one increasing trophic level, following Kim et al. (2012). Trophic position calculation requires appropriately selected diet–tissue discrimination factors. The primary diet–tissue discrimination factor utilized comes from Kim et al. (2012), to date the only discrimination factors calculated for elasmobranchs using completely controlled feeding conditions. For the purpose of testing sensitivity, trophic position calculations were also run with diet–tissue discrimination factors from Hussey et al. (2010), a "semicontrolled" feeding study, and the mean values for nonelasmobranch fishes from Caut et al. (2009).

Current stable isotopic analytical techniques do not allow for the precise determination of the specific prey species consumed by generalist predators. Though several advanced statistical mixing models exist, many have very precise data requirements that were not met by this study due to the opportunistic sampling regime (samples were provided by the SCDNR inshore

TABLE 1.   Biological and demographic data for all Sandbar Sharks sampled (total) and those from summer–fall (SF) months.

Location, sex, and length	Total age-0	Total juvenile	Total adult	SF age-0	SF juvenile	SF adult
Winyah Bay	1	64	6	1	63	4
Bulls Bay	27	34	0	19	13	0
Charleston Harbor	0	38	0	0	28	0
Port Royal Sound	0	12	1	0	12	1
St. Helena Sound	49	29	1	38	24	1
All estuaries	77	180	8	58	140	6
Males	43	69	2	31	53	2
Females	34	111	6	27	89	4
Minimum TL (mm)	550	715	1,684	561	715	1,684
Mean TL (mm)	645	1,113	1,785	647	1,175	1,738
Maximum TL (mm)	713	1,681	2,000	713	1,681	1,800

fisheries survey whenever possible, and samples obtained did not include primary producers). The sample size of many prey species was insufficient to infer diet with accuracy using many mixing models, and baseline data (i.e., primary producer carbon signature) was unavailable. Multiple samples of each prey species were averaged together with the assumption that specimens from different estuaries had similar isotopic signatures.

RESULTS

A total of 262 Sandbar Sharks were sampled in South Carolina waters for this study, including 177 juveniles, 77 young of year, and 8 adults (Table 1). All but one young of year were captured in Bulls Bay and St. Helena Sound, while juveniles were captured in all sampled estuaries (Table 1). The δ^{15}N signatures of age-0 Sandbar Sharks were significantly lower in summer–fall than in spring (Figure 2; Table 2) and did not decrease any further within the course of this study, validating the choice of sampling approximately 2 months after most young of year are born (a July 15th cutoff) for reducing maternal contribution bias to age-0 Sandbar Sharks.

Initial multifactor ANOVA analysis of summer–fall Sandbar Sharks (Table A.1 in the appendix) indicated significant differences in δ^{15}N between estuaries ($F = 8.6$, $P < 0.001$) and no significant differences between age-classes ($F = 2.01$, $P = 0.15$). Analysis of summer–fall Sandbar Sharks indicated significant differences in δ^{13}C between age-classes ($F = 8.2$, $P = 0.005$), estuaries ($F = 12.9$, $P < 0.005$), month ($F = 8.4$, $P < 0.005$), and year ($F = 19.35$, $P < 0.005$).

To account for the unbalanced sampling design (i.e., uneven numbers of young of year between estuaries, unequal sampling of different estuaries in different months), each variable's effect on δ^{15}N and δ^{13}C was also analyzed with individual ANOVAs (Table A.2 in the appendix). When only young of year ($n = 53$) and juveniles ($n = 140$) captured after July 15 (summer–fall) were analyzed separately to minimize potential maternal input or offshore feeding signals (Olin et al. 2011), ANOVA

results indicated no significant differences for δ^{15}N or δ^{13}C signatures between years (Table A.2 in the appendix). When only summer–fall juveniles or only summer–fall young of year were compared between estuaries, there were no significant differences in δ^{13}C or δ^{15}N between estuaries (Table A.2 in the appendix). The significant differences between estuaries appear to have been driven not by real isotopic differences between different estuaries, but by unequal catch rates of young of year between estuaries (Table 1), providing additional support to our decision to utilize multiple individual ANOVAs to analyze this dataset.

When all summer–fall Sandbar Sharks were pooled together from both years and all estuaries, δ^{15}N and δ^{13}C varied significantly between young of year and juveniles (Figure 3), with higher δ^{15}N values ($F = 6.4$, $P = 0.048$) and more negative
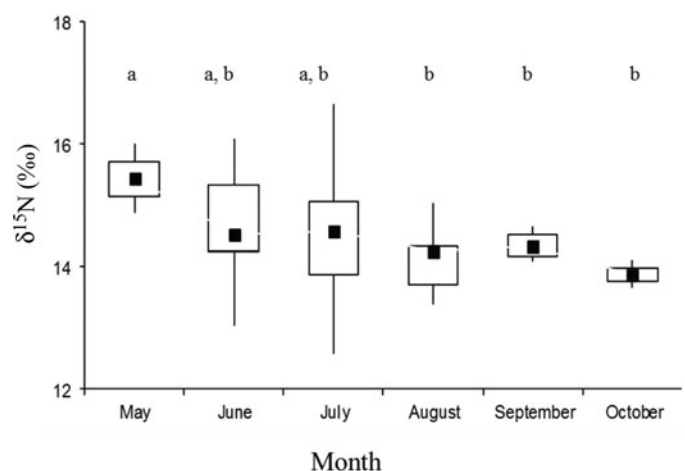

FIGURE 2.   Box plot of mean δ^{15}N signature of age-0 Sandbar Sharks by capture month. The black squares represent the means, the box dimensions represent the 25th–75th percentile ranges, and the whiskers show the 10th–90th percentile ranges. Boxes labeled with the same letter are not significantly different.

TABLE 2.   Carbon and nitrogen stable isotopic signatures for Sandbar Shark muscle tissue from each life history stage. Values from all Sandbar Sharks, those collected before July 15th (spring), and those collected after July 15th (summer–fall) are shown.

Category	N	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
		Mean	Range	SD	Mean	Range	SD
All sharks							
Age-0	77	−17.5	−16.0 to −19.0	0.56	14.8	12.6 to 16.7	0.85
Juvenile	180	−18.5	−15.8 to −20.4	0.85	14.6	12.0 to 16.6	0.79
Adult	8	−18.1	−17.4 to −19.8	0.75	14.8	13.9 to 15.9	0.76
Summer–fall sharks							
Age-0	53	−17.4	−16.0 to −19.0	0.60	14.5	12.6 to 16.5	0.89
Juvenile	140	−18.5	−16.2 to −20.3	0.83	14.8	12.0 to 16.6	0.81
Adult	6	−18.2	−17.4 to −19.8	0.83	14.8	13.9 to 15.9	0.89
Spring sharks							
Age-0	22	−17.4	−16.6 to −18.2	0.60	14.6	13.4 to 16.1	0.87
Juvenile	40	−18.7	−17.2 to −20.4	0.76	14.5	12.4 to 16.0	0.77
Adult	2	−17.7	−17.5 to −17.8	0.13	14.9	14.8 to 15.0	0.08

$\delta^{13}C$ values ($F = 62.9$, $P < 0.001$) in juveniles than in young of year (Table A.2 in the appendix). Adults were excluded from this analysis due to low sample size.

Juveniles had a larger $\delta^{15}N$ range (4.5 versus 4.0), $\delta^{13}C$ range (4.1 versus 3.0), and total occupied niche area (14.1 versus 7.1) than young of year (Figure 4). Layman metrics of $\delta^{15}N$ range, $\delta^{13}C$ range, and total occupied niche area were very similar when comparing these metrics calculated for all Sandbar Sharks with those calculated for only summer–fall Sandbar Sharks (nearly all of the outer points of the convex hull were summer–fall sharks), and the results presented here represent all Sandbar Sharks. Adults were excluded from Layman metric analysis due to small sample size. Regression analysis showed statistically significant effects of total length on both $\delta^{13}C$ ratio

($T = 4.18$, $P < 0.0005$) and $\delta^{15}N$ ratio ($T = 3.6$, $P < 0.0005$) (Figure 5).

When using diet–tissue discrimination factors from Kim et al. (2012), age-0 Sandbar Sharks in South Carolina were assigned a trophic position of 3.8, while juveniles and adults were assigned a trophic position of 3.9 using the formula from Post (2002). The use of discrimination factors from Caut et al. (2009) resulted in trophic positions of 4.1 for young of year and 4.3 for juveniles and adults, and the use of discrimination factors from Hussey et al. (2010) resulted in trophic position calculations of
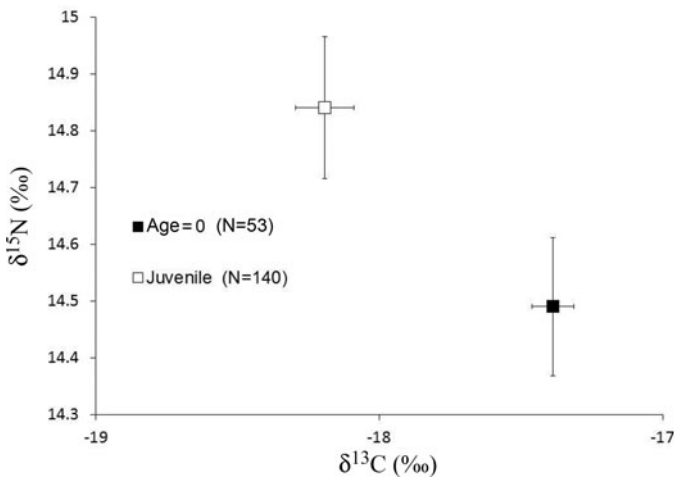

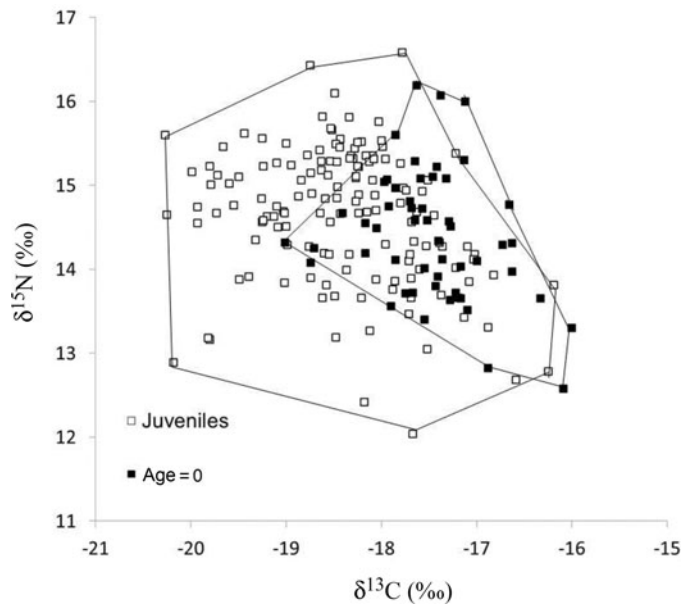FIGURE 3.   Mean $\delta^{15}N$ and $\delta^{13}C$ values (error bars are ± 1 SE) of summer–fall Sandbar Sharks.

FIGURE 4.   Values of $\delta^{15}N$ and $\delta^{13}C$ from individual muscle samples of all Sandbar Sharks. Polygons represent the total occupied niche area (and overlap) of all age-0 and juvenile Sandbar Sharks.
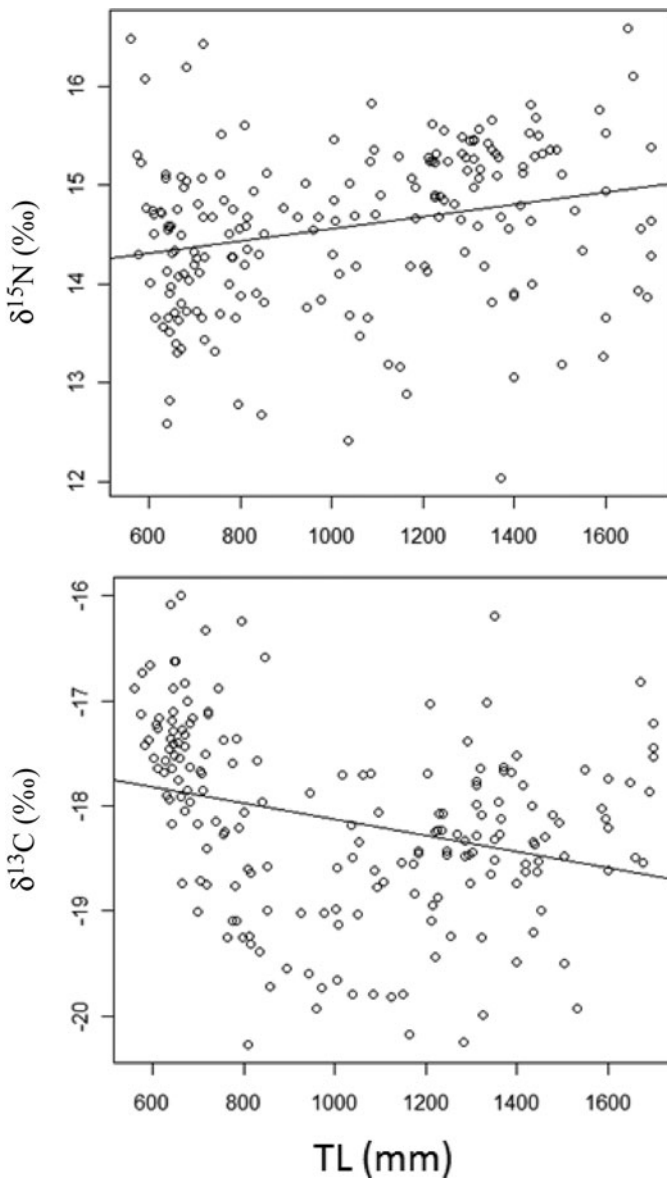
FIGURE 5. Regression of $\delta^{15}N$ (top panel) and $\delta^{13}C$ (bottom panel) by stretch total length for summer–fall Sandbar Sharks.
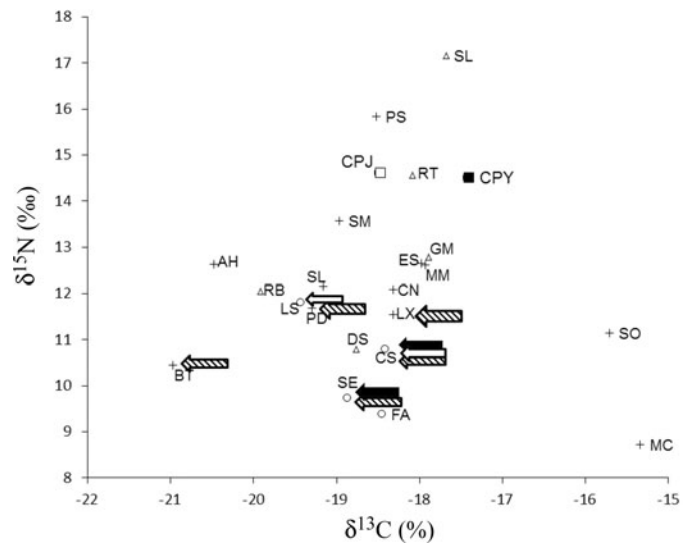

FIGURE 6. Mean isotopic values of age-0 and juvenile Sandbar Sharks and co-occurring potential prey species. The squares represent Sandbar Sharks (CPJ are juveniles, CPY are young of year), circles represent invertebrates, triangles represent elasmobranchs, and pluses represent teleost fishes. See Table 3 for species abbreviations. Filled arrows indicate species identified as being an important part of the diet of age-0 Sandbar Sharks by Ellis and Musick 2007, empty arrows indicate important prey species for juveniles identified by Ellis and Musick 2007, and crosshatched arrows indicate prey species identified as being important by Medved et al. 1985 (which did not distinguish by age-class).

2012) below age-0 Sandbar Sharks, suggesting that diets are similar between the regions (Figure 6).

DISCUSSION

Our results suggest the presence of an ontogenetic diet shift between age-0 and juvenile Sandbar Sharks in South Carolina estuarine waters, indicated by differences in average $\delta^{15}N$ and $\delta^{13}C$ signatures between these two age-classes. This ontogenetic diet shift is consistent with young of year feeding mainly on small benthic animals (crustaceans such as mantis shrimp and blue crab, elasmobranchs such as Atlantic Stingray, and teleosts such as Summer Flounder) during the first year of life and expanding their diets to include additional pelagic animals (teleosts such as Atlantic Menhaden and invertebrates such as squid *Loligo* spp.) during the juvenile years. This diet shift, from mostly benthic invertebrates to mostly pelagic teleosts, has been previously described from stomach content analyses of Sandbar Sharks in Hawaii (McElroy et al. 2006) and Virginia (Ellis and Musick 2007). Caution should be utilized interpreting these data due to concerns about maternal contribution influencing the age-0 values and offshore feeding influencing the juvenile values, since the time for complete tissue isotopic turnover (Kim et al. 2012) exceeded the 2 months allowed by this study. However, the many similarities between our conclusions and previous stomach-content-based Sandbar Shark diet analysis, including evidence of an ontogenetic diet shift from benthic invertebrates to pelagic teleosts, give us confidence in the robustness of our results.

4.2 for young of year and 4.3 for juveniles and adults. No difference in trophic position was found between using all Sandbar Sharks and only summer–fall Sandbar Sharks, so all samples were pooled for trophic analysis.

The potential prey samples collected included 146 specimens of 21 species (Table 3). All specimens of a single species were pooled for prey analysis to generate mean isotopic values for that species (Table 3). Benthic invertebrates identified as being important to the diet of age-0 Sandbar Sharks and squid *Loligo* sp. identified as being important to the diet of juveniles in Virginia by Ellis and Musick (2007) are approximately one trophic level (using diet–tissue discrimination factors from Kim et al.

TABLE 3. Carbon and nitrogen stable isotopic signatures of all South Carolina potential prey samples. Blue crab size is carapace width, and ray size is disc width. All other sizes are total length.

Species	Species code	Average size (cm)	N	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
				Mean	Range	SD	Mean	Range	SD
Rays									
Atlantic Stingray *Dasyatis sabina*	DS	~30	1	−18.8			10.8		
Cownose Ray *Rhinoptera bonasus*	RB	~75	5	−19.9	−19.7 to −20.3	0.25	12.1	11.6 to 12.3	0.38
Smooth Butterfly Ray *Gymnura micrura*	GM	~30	3	−17.9	−16.7 to −19.6	1.53	12.8	12.2 to 13.6	0.69
Teleosts									
Striped Anchovy *Anchoa hepsetus*	AH	6.4	6	−20.5	−19.7 to −21.5	0.63	12.8	11.7 to 13.0	0.50
Bluefish *Pomatomus saltatrix*	PS	~20	4	−18.5	−17.7 to −19.8	0.98	15.8	14.8 to 16.5	0.72
Summer Flounder *Paralichthys dentatus*	PD	10.1	10	−19.3	−17.6 to −22.2	1.52	11.7	9.6 to 12.7	0.82
Ladyfish *Elops saurus*	ES	15	1	−18.0			12.6		
Atlantic Menhaden *Brevoortia tyrannus*	BT	~15	11	−21.0	−19.2 to −22.7	1.24	10.5	9.3 to 11.6	0.75
Striped Mullet *Mugil cephalus*	MC	~25	5	−15.3	−12.9 to −16.7	1.57	8.7	6.8 to 9.4	1.15
Red Drum *Sciaenops ocellatus*	SO	24	3	−15.7	−15.6 to −15.9	0.14	11.2	10.9 to 11.4	0.21
Spanish Mackerel *Scomberomorus maculatus*	SM	48.4	2	−19.0	−18.8 to −19.2	0.31	13.6	13.4 to 13.8	0.29
Spot *Leiostomus xanthurus*	LX	16.2	16	−18.6	−15.8 to −21.5	1.28	11.6	10.4 to 13.0	0.81
Spotted Seatrout *Cynoscion nebulosus*	CN	12.9	6	−18.3	−17.6 to −19.8	0.75	11.8	11.1 to 13.3	0.76
Star Drum *Stellifer lanceolatus*	SL	12.4	10	−19.2	−18.7 to −19.9	0.38	12.2	11.7 to 12.4	0.28
Southern Kingfish *Menticirrhus americanus*	MA	35.5	2	−17.9	−17.6 to −18.3	0.46	12.6	12.5 to 12.7	0.16
Invertebrates									
Squid *Loligo* sp.	LS	6.3	16	−19.4	−17.8 to −21.1	1.11	11.8	11.1 to 13.2	0.63
Blue crab *Callinectes sapidus*	CS	~15	6	−18.4	−16.8 to −19.2	0.84	10.8	8.9 to 12.7	1.39
Brown shrimp *Farfantepenaeus aztecus*	FA	10.2	17	−18.5	−16.1 to −22.7	1.74	9.4	7.5 to 10.9	1.21
Mantis shrimp *Squilla empusa*	SE	7.5	8	−18.9	−18.1 to −20.0	0.78	9.7	9.1 to 10.6	0.46
Shark pups									
Atlantic Sharpnose Shark *Rhizoprionodon terraenovae*	RT	33.4	11	−18.1	−16.6 to −19.2	1.06	14.6	13.0 to 16.5	1.22
Scalloped Hammerhead *Sphyrna lewini*	SL	46.5	4	−17.7	−17.4 to −18.4	0.51	17.2	16.5 to 17.9	0.80

The ontogenetic diet shift between summer–fall age-0 and juvenile Sandbar Sharks in this study was represented by a difference in $\delta^{15}N$ of ~0.3‰ and a difference in $\delta^{13}C$ of ~1‰ between the two age-classes. Wells et al. (2008) studied juvenile and adult Red Snapper and, due to a diet shift from zooplankton (primary consumers) to small teleosts and benthic crustaceans (secondary consumers), found a difference of ~1.3‰ in $\delta^{15}N$—as expected, a larger ontogenetic difference in $\delta^{15}N$ than what we observed in Sandbar Sharks in this study because of a larger

transition within the food chain. The change in $\delta^{13}C$ that Wells et al. (2008) found (~1‰) is similar to changes observed in this study, and in both cases the predator changed feeding habitats within an ecosystem (benthic to pelagic for summer–fall estuarine Sandbar Sharks, sandy bottom to reef for continental shelf Red Snapper). Estrada et al. (2006) found a $\delta^{15}N$ shift of ~3‰ in the vertebrae of White Shark *Carcharodon carcharias* that was associated with a diet shift from teleosts to marine mammals that feed on teleosts. MacNeil et al. (2005) found differences

in $\delta^{15}N$ comparable to those in this study ($\sim 0.5\permil$) between liver and cartilage samples within individual Blue Sharks *Prionace glauca* and Common Thresher Sharks *Alopias vulpinus*, but larger $\delta^{15}N$ differences ($\sim 3\permil$) were found between liver and cartilage samples of Shortfin Makos. Blue and Thresher sharks switch diets between preferred teleost prey, a lesser diet change than that of Shortfin Makos, which switch from preying on cephalopods to piscivorous Bluefish, and therefore have a larger difference in $\delta^{15}N$ signature than what was observed in this study. While regression analysis of total length by $\delta^{15}N$ and by $\delta^{13}C$ showed a significant effect of size on isotopic signature, the diet transition is not as abrupt as that found in Bluefin Tuna *Thunnus thynnus* by Graham et al. (2007).

South Carolina juvenile Sandbar Sharks had a larger $\delta^{15}N$ range, $\delta^{13}C$ range, and total occupied niche area than age-0 sharks, indicating a more diverse diet among juvenile individuals (Layman et al. 2007a). This is consistent with the increase in diet diversity observed in adult Sandbar Sharks in Hawaiian waters (McElroy et al. 2006). Additionally, the high degree of overlap in total occupied niche area between young of year and juveniles suggests that while Sandbar Sharks consume additional prey species as they grow, older and larger juvenile sharks still consume preferred young-of-year prey. This feeding strategy has been observed in multiple shark species (Grubbs 2010), such as the Tiger Shark *Galeocerdo cuvier* (Lowe et al. 1996), Broadnose Sevengill Shark *Notorynchus cepedianus* (Ebert 2002), Lemon Shark *Negaprion brevirostris* (Wetherbee et al. 1990), and Bonnethead *Sphyrna tiburo* (Bethea et al. 2007). The sample sizes between young of year and juveniles are significantly different, which could influence these calculations, but Vaudo and Heithaus (2011) performed a bootstrapping analysis and found asymptotes at a sample size of approximately 25–30, less than our smaller sample size, for several different coastal elasmobranch species.

As a higher total occupied niche area indicates a higher diet breadth, the generalist feeding behavior of juvenile Sandbar Sharks observed in western North Atlantic estuaries (Ellis and Musick 2007) is reflected in the relatively high Layman metrics calculated in this study compared with other marine species. Layman metrics have been calculated for few other elasmobranch species to date. The $\delta^{15}N$ range, $\delta^{13}C$ range, and total occupied niche area calculations for the juvenile Sandbar Sharks in this study were larger than those for 9 of the 10 studied coastal elasmobranch species in Australia (Vaudo and Heithaus 2011). The Indo-Pacific Spotted Eagle Ray *Aetobatus ocellatus,* the largest batoid found in coastal Australian waters and the only local species with jaw morphology capable of crushing the shells of bivalve and gastropod prey, displayed higher Layman metric values than the Sandbar Sharks in our study (Vaudo and Heithaus 2011). Additionally, a marine piscivorous teleost in the coastal Bahamas, the Gray Snapper *Lutjanus griseus*, has a total occupied niche area of 8.9 (Layman et al. 2007b), intermediate to that of age-0 (7.1) and juvenile (14.1) Sandbar Sharks in South Carolina. It is important to note that the present study

grouped together Sandbar Sharks from different estuaries while Vaudo and Heithaus (2011) sampled in a single system, which may artificially increase the isotopic niche width of our samples if there are significant differences in baseline isotopic signatures between estuaries sampled in this study. Future calculations of Layman metrics for other marine predatory fishes will allow for interesting comparisons between species and habitats.

This study assigned age-0 Sandbar Sharks a mean trophic level of 3.8 and juvenile Sandbar Sharks a mean trophic level of 3.9 using the formula from Post (2002) and diet–tissue discrimination factors from Kim et al. (2012). Adult Sandbar Sharks, which annually migrate between coastal and offshore waters, had a trophic level of 3.9 (despite a small sample size [$n = 8$] that limits our confidence in these results), indicating a similar diet to the juveniles. Based on seven Sandbar Shark diet studies included in a meta-analysis by Cortes (1999), four of which included adults (Wass 1973; Cliff et al. 1988; Stevens and McLaughlin 1991; Stillwell and Kohler 1993), Sandbar Sharks had a mean trophic level of 4.1, not a significantly different value from our calculation of 3.8 ($\chi^2 = 0.9$, $P = 0.75$). Trophic level can increase with increasing total length due to the ability of larger sharks to capture prey that smaller sharks cannot (Cortes 1999; Grubbs 2010), which explains the slightly lower trophic level observed in our study focusing on young of year and juveniles. The use of diet–tissue discrimination factors from Caut et al. (2009) and Hussey et al. (2010) resulted in very similar (but slightly higher) trophic position values, showing that, in this case, the trophic level estimates were relatively insensitive to diet–tissue discrimination factors.

Differences in the isotopic signature of Sandbar Sharks captured during April–June from that of summer–fall sharks (Table 2) potentially indicated the influence of maternal effects on the isotopic composition of newborn age-0 sharks (McMeans et al. 2009; Vaudo et al. 2010) and the influence of recent offshore feeding that affected the isotopic composition of recently arrived juveniles in the months of May and June (Ulrich et al. 2007). Offshore food webs can have a less negative carbon signature than adjacent estuarine food webs (Leakey et al. 2008), with differences of up to $4\permil$, which would influence the isotopic signatures of juvenile Sandbar Sharks that had recently been feeding offshore.

Once unequal capture rates of young of year and juveniles were taken into account (by analyzing average isotopic signatures of young of year only and juveniles only), no significant differences were found between estuaries. Similar prey species were found in each estuary, although local abundance can be variable (Bill Roumillat, SCDNR, personal communication). Between-estuary movements of age-0 and juvenile Sandbar Sharks in Virginia have been observed, but it is more common for Sandbar Sharks to remain within one estuary during a summer (Grubbs et al. 2007). Within South Carolina, tagging recaptures indicate seasonal fidelity to estuaries (Bryan Frazier, SCDNR, personal communication). No significant differences in $\delta^{15}N$ or $\delta^{13}C$ were found between sexes, which is consistent

with the species' known life history, as age-0 and juvenile Sandbar Sharks are not known to spatially segregate based on sex within South Carolina estuaries (Ulrich et al. 2007).

The methods utilized in this study have important limitations that must be considered when interpreting these results. Different carbon signatures between juvenile and age-0 Sandbar Sharks may reflect a shift from benthic to pelagic feeding within an estuary, or they may reflect evidence of offshore feeding in juveniles despite our efforts to correct for this with a July 15th cutoff date. The use of multiple single-factor ANOVAs, which were performed to correct for the unbalanced and opportunistic sampling regime, increases the chance of a type II error. Additionally, our decision to combine Sandbar Sharks and potential prey from different estuaries assumes that the baseline isotopic signature of these estuaries is very similar, which may or may not be the case. Additional sampling, which would have ideally included primary producers and multiple individuals of each potential prey species from each estuary, would have resolved this but was not possible due to the logistical limitations of the study. Single-tissue stable isotope analysis provides less information than analyses of multiple tissues, since different tissues have different turnover rates (MacNeil et al. 2005), though obtaining samples from commonly used tissues such as liver and vertebrae usually requires the sacrifice of animals. Finally, whenever possible, studies should be designed to obtain the data needed for precise statistical mixing models.

While lethal shark research is sometimes necessary to obtain the data needed by fisheries managers, we agree with Heupel and Simpfendorfer (2010) and Hammerschlag and Sulikowski (2011) that nonlethal methods should be used whenever possible. No Sandbar Sharks were sacrificed for this project, and despite utilizing only one tissue type (muscle), our results showed trends consistent with earlier lethal-sampling dietary research. Sharks have longer isotopic turnover rates than teleosts (Hesslein et al. 1993; Logan and Lutcavage 2010), and slow turnover rates have been observed in shark muscle tissue (MacNeil et al. 2005; Logan and Lutcavage 2010). Comparisons of stable isotope data with detailed stomach content analysis data, ideally obtained though gastric lavages, can provide complimentary dietary information but are very labor intensive (Vaudo and Heithaus 2011). Our study is among the first to detect an ontogenetic diet shift in a wild population of sharks using a nonlethal, single-tissue stable isotope analysis sample design.

Fisheries managers interested in creating an ecosystem-based fisheries management plan for the western North Atlantic Ocean Sandbar Shark population can incorporate data from this study. Sandbar Shark diet appears consistent between estuaries, sexes, and years. A benthic-to-pelagic, crustacean-to-teleost ontogenetic diet shift similar to the shift documented in Virginia's and Hawaii's Sandbar Shark populations appears to also occur in South Carolina's population. Juvenile Sandbar Sharks have a wider diet breadth than age-0 sharks within South Carolina and have some of the highest values of diet breadth metrics ever calculated in an elasmobranch, supporting the idea that they are generalist predators. We encourage future muscle isotope studies of this type to reduce unnecessary lethal sampling of elasmobranchs and to provide basic dietary information to fisheries managers.

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Appendix: ANOVA Results

TABLE A.1.　Results of multifactor ANOVAs comparing the $\delta^{15}N$ and $\delta^{13}C$ signatures of Sandbar Shark muscle samples (summer–fall only). Significant P-values are indicated in bold italics. As no interaction terms (i.e., $\delta^{13}C \times$ year : sex) were significant, interaction terms were omitted from this table.

Comparison	df	Mean square	F	P
$\delta^{13}C \times$ year	1	9.85	19.34	***2.9 × 10⁻⁵***
$\delta^{13}C \times$ sex	2	0.0374	0.074	0.929
$\delta^{13}C \times$ estuary	4	6.58	12.9	***6.08 × 10⁻⁹***
$\delta^{13}C \times$ month	4	4.279	8.4	***4.4 × 10⁻⁶***
$\delta^{13}C \times$ age-class	1	4.18	8.21	***0.0048***
$\delta^{15}N \times$ year	1	0.198	0.358	0.55
$\delta^{15}N \times$ sex	2	1.329	2.4	0.09
$\delta^{15}N \times$ estuary	4	4.773	8.63	***3.4 × 10⁻⁶***
$\delta^{15}N \times$ month	4	0.803	1.447	0.22
$\delta^{15}N \times$ age-class	1	1.115	2.01	0.157

TABLE A.2.　Results of single-factor ANOVAs comparing the $\delta^{15}N$ and $\delta^{13}C$ signatures of Sandbar Shark muscle samples. Significant P-values are in bold italics. The column "Corrected P" uses a Holm correction to adjust P-values to account for the increased type I error rate associated with running multiple single-factor ANOVAs ($N = 5$ for each of the following: all Sandbar Sharks $\delta^{15}N$, all Sandbar Sharks $\delta^{13}C$, summer–fall Sandbar Sharks $\delta^{13}C$, and summer–fall Sandbar Sharks $\delta^{15}N$). Sample sizes (N) of each group are included and are also found in Table 1. Estuary abbreviations are as follows: WB = Winyah Bay, BB = Bulls Bay, CH = Charleston Harbor, PRS = Port Royal Sound, and SHS = St. Helena Sound).

Comparison	df	Mean square	F	P	Corrected P
All Sandbar Sharks					
$\delta^{15}N \times$ estuary (age-0: WB $N = 1$, BB $N = 27$, CH and PRS $N = 0$, SHS $N = 47$; juvenile: WB $N = 64$, BB $N = 34$, CH $N = 38$, PRS $N = 12$, SHS $N = 29$)	4	2.9	4.6	***0.001***	***0.005***
$\delta^{15}N \times$ month	6	0.6	1.0	0.42	0.648
$\delta^{15}N \times$ year	1	2.7	4.3	***0.039***	0.156
$\delta^{15}N \times$ sex	1	1.2	1.8	0.17	0.51
$\delta^{15}N \times$ age-class (age-0: $N = 77$ versus juvenile: $N = 180$)	1	0.6	0.9	0.324	0.648
$\delta^{13}C \times$ estuary (age-0: WB $N = 1$, BB $N = 27$, CH and PRS $N = 0$, SHS $N = 47$; juvenile: WB $N = 64$, BB $N = 34$, CH $N = 38$, PRS $N = 12$, SHS $N = 29$)	4	2.3	4.4	***0.002***	***0.008***
$\delta^{13}C \times$ month	5	1.56	2.9	***0.008***	***0.024***
$\delta^{13}C \times$ year	1	1.1	2.1	0.147	0.294
$\delta^{13}C \times$ sex	1	0.2	0.3	0.734	0.734
$\delta^{13}C \times$ age-class (age-0: $N = 77$ versus juvenile: $N = 180$)	1	61.1	114.6	***<0.0001***	***0.0005***
Summer–fall Sandbar Sharks					
$\delta^{15}N \times$ estuary	4	4.4	7.6	***0.0001***	***0.0005***
$\delta^{15}N \times$ month	4	2.3	4.4	***0.02***	0.06
$\delta^{15}N \times$ year	1	0.2	0.3	0.58	0.58
$\delta^{15}N \times$ sex	1	1.3	1.9	0.149	0.298
$\delta^{15}N \times$ age-class (age-0: $N = 58$ versus juvenile: $N = 140$)	1	4.1	6.4	***0.012***	***0.048***

TABLE A.2. Continued.

Comparison	df	Mean square	F	P	Corrected P
$\delta^{13}C \times$ estuary	4	8.4	13.6	*<0.0001*	*<0.0001*
$\delta^{13}C \times$ month	4	10.6	18.7	*<0.0001*	*<0.0001*
$\delta^{13}C \times$ year	1	0.3	0.4	0.51	1
$\delta^{13}C \times$ sex	1	0.0	0.26	0.974	1
$\delta^{13}C \times$ age-class (age-0: $N = 58$ versus juvenile: $N = 140$)	1	27.0	62.9	*<0.0001*	*<0.0001*
Summer–fall age-class-restricted estuary comparisons					
$\delta^{15}N \times$ estuary (age-0 only: WB $N = 1$, BB $N = 19$, CH and PRS $N = 0$, SHS $N = 38$)	1	0.1	0.2	0.70	
$\delta^{15}N \times$ estuary (juveniles only: WB $N = 63$, BB $N = 13$, CH $N = 28$, PRS $N = 12$, SHS $N = 24$)	3	0.5	1.2	0.31	
$\delta^{13}C \times$ estuary (age-0 only: WB $N = 1$, BB $N = 19$, CH and PRS $N = 0$, SHS $N = 38$)	1	1.1	3.8	0.058	
$\delta^{13}C \times$ estuary (juveniles only: WB $N = 63$, BB $N = 13$, CH $N = 28$, PRS $N = 12$, SHS $N = 24$)	3	1.6	2.5	0.067	