Intraspecific differences in relative isotopic niche area and overlap of co-occurring sharks

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Abstract Few studies have assessed whether patterns of resource partitioning among sympatric marine predators vary between different habitats. This type of data is important for understanding food web functioning as well as for supporting an ecosystem-based fisheries management plan. In this study, we used $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis to assess the relative isotopic niche area (i.e., which species has the largest and smallest isotopic niche area within a study site) and core isotopic niche overlap between 299 sharks of 11 co-occurring shark species that vary in size, diet and behavior in three coastal study areas in South Florida. Overall, results show that the relative isotopic niche area and patterns of core isotopic niche overlap between some sympatric shark species varied between sites, suggesting plasticity in resource use and competitive interactions between shark species (e.g., 85% of blacktip shark *Carcharhinus limbatus* standard ellipse area overlapped with the blacknose shark *C. acronotus* ellipse in Biscayne Bay, but there was no overlap between these species' ellipses in nearby Florida Bay). Therefore, patterns of resource use and trophic interactions documented among species from one site may not be applicable to a neighboring location. These findings may have implications for food web models that incorporate trophic data from

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different areas for a species when local data are unavailable.

Introduction

The study of predator–prey interactions is complicated by interspecific and intraspecific variation in the resource use of predators and in their competitive interactions with other species (Polis and Holt 1992). In some cases, co-occurring predator species may compete with one another for the same prey resources, but in other cases they may minimize competition by partitioning available resources (Schoener 1974). Determination of the diet and associated trophic relationships of sharks has been identified as research priorities for the conservation and management of shark species (Simpfendorfer et al. 2011), many of which are threatened (Dulvy et al. 2014).

The assessment of shark diets and trophic interactions is complex, because sharks are an ecologically diverse group with considerable variation in size, diet and trophic level both within and between species (Cortés 1999; Hussey et al. 2015; Frisch et al. 2016). In general, relatively few studies to date have compared the spatiotemporal variation in diet breadth and overlap of multiple co-occurring predators (Sanford et al. 2003; Wieters et al. 2008). Both resource partitioning (e.g., Papastamatiou et al. 2006) and competition for resources (e.g., Bethea et al. 2004) have been documented in communities of co-occurring elasmobranch fishes. Spatiotemporal variation in diets is common, but it is important to understand the degree of variation in diet and dietary overlap within and among shark species, and across habitats in order to create ecosystem-based fisheries management plans (Pikitch et al. 2004) and/or for generating ecological risk assessments of threatened species (Gallagher et al. 2012).

Stable isotope analysis provides a non-lethal means of studying aspects of elasmobranch resource use and trophic dynamics (see Shiffman et al. 2012 for a review). While there have been numerous diet and stable isotope studies involving marine predators, many focus on a single species or a single location, and there has been a relative lack of research which has considered how diet and trophic relationships among an assemblage of sympatric marine predators could vary intraspecifically across the range of those species (Sanford et al. 2003; Wieters et al. 2008). Stable isotopic signatures of consumers may vary spatially, temporally or by consumer body size, but few studies have examined all of these variables simultaneously (but see Davenport and Bax 2002; Heithaus et al. 2013; Matich and Heithaus 2014).

It is important to consider the possible effects of temporal variation in baseline isotopic signature (i.e., not distinct patterns of resource consumption among consumers) on the interpretation of consumer isotopic signatures (e.g., Goering et al. 1990; Harvey et al. 2002). Baseline isotopic signature may vary seasonally because of changing environmental conditions that influence the growth of primary producers (e.g., Goering et al. 1990), or there may be interannual variation (e.g., Anderson and Fourqurean 2003). Additionally, the size of an individual consumer may also influence its isotopic signature (more so than just variation in diet between different-sized consumers), and it is important to test for the effects of size (reviewed in Martínez del Rio et al. 2009).

The coastal waters of South Florida provide an opportunity to evaluate potential intraspecific (defined here as between populations of a species located in different habitats) differences in the trophic ecology of sympatric sharks across distinct nearby habitats. South Florida is home to a diverse community of sharks in several distinct habitats which differ in depth, bottom type, biodiversity and fishing pressure (Trent et al. 1997; Torres et al. 2006; Heithaus et al. 2007; Graham et al. 2016). While these habitats are geographically proximate and isotopically similar (described in the study sites section of methods) and some shark species regularly migrate much greater distances than the distance between study sites used here, these study sites are ecologically (in terms of relative biodiversity and biomass of prey and possible competitive interactions) and environmentally (in terms of bottom type and depth) distinct enough to be considered separate habitats. The present study used $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis to investigate patterns of relative isotopic niche area and core isotopic niche overlap among 11 species of co-occurring sharks within three distinct coastal areas of South Florida.

Specifically, this study investigated the following research question: Do patterns of relative isotopic niche area, core isotopic niche overlap and other standard isotopic metrics between sympatric shark species vary across different habitats? We hypothesized that since prey availability is generally similar throughout South Florida and study species' ranges overlap, co-occurring species patterns of relative isotopic niche area, core isotopic niche overlap and other trophic interactions would remain constant across different habitats.
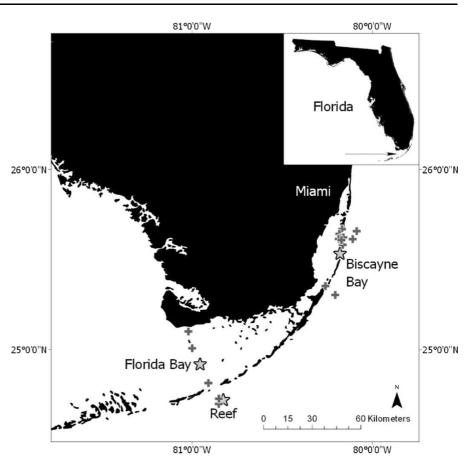
Methods

Study sites

Sampling occurred year round (see supplementary materials for *N* by month and year) in three geographically proximate, isotopically similar, but ecologically and environmentally distinct coastal habitat in South Florida: Biscayne Bay, Florida Bay and the Florida Keys Reef, locations that vary in depth, bottom type, prey biodiversity and biomass, and fishing pressure (Fig. 1). These sites also vary slightly (but are generally similar) in isotopic signature of primary producers and prey species (see Kieckbusch et al. 2004 for Biscayne Bay; Chasar et al. 2005 for Florida Bay; and both Swart et al. 2005; Behringer and Butler 2006 for the Reef, with differences described briefly below).

In the Biscayne Bay study area, sampling occurred year round from 2011 to 2013 on the sand, sponge flat and sea grass habitats within Biscayne National Park. Depth ranged from 5 to 20 m. The mean temperature in this area is 26 °C, with a range from 19 to 33 °C, while salinity is typically 30–40‰ but can occasionally decrease rapidly with freshwater pulses (Lirman and Cropper 2003). Twenty-three families of fishes reside within Biscayne Bay, and the most common are planktivores like *Clupeidae,* low-level predators like *Gerridae* and *Cyprinodontidae,* and small mesopredators like *Lutjanidae* and *Haemulidae* (Serafy et al. 2003). Although it is administered by the Federal National Parks Service, fishing within Biscayne National Park is governed by Florida's state fishing regulations, with commercial and recreational fishing generally permitted except in seasonally closed areas (Graham et al. 2016). The average $\delta^{13}C$ values of primary producers in this habitat have been found to

range from -27 (mangroves) to -6.8 (epiphytic algae), though Kieckbusch et al. (2004) noted that 79% of all values were in the narrower range of -21.9 to -11.9 and that mangroves were not a major carbon source for the fish community. Low-level predatory teleosts such as pinfish (*Lagodon rhomboides)* have an average signature of ($-12.5, 9.5$), and mesopredatory jacks (*Caranx ruber*) have an average signature of ($-10.5, 10.2$), with a total isotopic range of the sample food web of approximately -32 to -7 $\delta^{13}C$ and approximately 1–12 $\delta^{15}N$.

In the Florida Bay study area, sampling primarily occured in the shallow (3–5 m) and turbid waters of Everglades National Park, year round from 2009 to 2012. Due to pulses of freshwater input from the nearby Everglades, salinity in this location can vary from ~ 9 to $\sim 35‰$ (Fourqurean and Schrlau 2003), though measurements in this location taken by our laboratory were almost all over 28‰ (Hammerschlag, unpublished data). Mean temperature is 24.5 °C (range 20–28 °C) (Fourqurean and Robblee 1999). The benthos is primarily carbonate mud with some sea grass beds (Chasar et al. 2005). This region has high biomass of prey fishes (including planktivorous and low-level predatory fishes like *Engraulidae, Blenniodei, Gobiidae* and *Clupeidae,* and mesopredatory fishes from the family *Sciaenidae*, Thayer and Powell 1999), but these prey fishes are mostly relatively small, and there is relatively low fish biodiversity compared to coral reefs. Commercial fishing is prohibited within the waters of Everglades National Park (Graham et al. 2016), but recreational fishing is permitted with some restrictions on gear and bag limit. This habitat has been found to have a distinct isotopic range from Biscayne Bay, approximately -18 to -10 $\delta^{13}C$ and approximately 1–14 $\delta^{15}N$, with pinfish having an average signature of ($-14.07, 9.77$) and mesopredatory crevalle jacks (*Caranx hippos*) measured at ($-13.65, 11.45$) (Chasar et al. 2005).

Lastly, for the Reef study area, sampling occurred on and around a 30-m-deep coral reef near Islamorada, Florida, year round from 2010 to 2013. Salinity in this region is a nearly constant 35‰ (Vaslet et al. 2012), and water temperature ranges from 21 to 29 °C (NOAA National Centers for Environmental Information). The Florida Keys coral reef system has hundreds of species of fish of all trophic levels (Ault and Johnson 1998), including non-elasmobranch mesopredators like grouper and barracuda in higher

Fig. 1 GPS locations of South Florida sampling sites from this study. Larger stars indicate the general location of each study area, while smaller pluses indicate specific sampling sites within each study area

abundance than found in the other two study areas. Our sampling area is not part of one of the Florida Keys National Marine Sanctuary's no-take zones (ecological reserves, sanctuary preservation areas or research natural areas), and therefore, general National Marine Fisheries Service fisheries regulations for US Federal waters apply (Graham et al. 2016). The average $\delta^{13}C$ of coral zooxanthellae in the region has been found to be approximately $(-12, 5)$ (Swart et al. 2005, measured in 1996), while sea grass primary producers here have an average signature of $(-6.9, 2.9)$ while encrusting algae have an average signature of $(-14.9, 4.3)$ (Behringer and Butler 2006). The total isotopic range of the sampled food web was approximately -18 to -5 $\delta^{13}C$ and approximately 0–8 $\delta^{15}N$ (Behringer and Butler 2006), but this study did not include any top predators (though secondary consumer spiny lobster *Panulirus argus* had an average signature of $(-11.8, 7.3)$.

Sample collection and processing

Sharks of 11 species (Table 1) were captured by drum line as part of the University of Miami Shark Research and Conservation Program's ongoing coastal shark survey (following Gallagher et al. 2014). For every shark captured, 5 mL of blood was drawn from the caudal vein. Sharks were measured (stretch total length, in centimeters), sexed and released. Whole blood (including plasma and red blood cells) was used for this analysis, and this tissue has a turnover time of approximately 8 months in chondrichthyans (Macneil et al. 2006).

Samples were stored in vacutainers (not containing anticoagulant) on ice in the field before being stored in a -30 °C degree freezer in the laboratory. Stable isotope analysis was performed in the stable isotope laboratory at Boston University using glycine $(-34, 10.73)$ and peptone $(-14.73, 7.4)$ standards obtained from the National Bureau of Standards. Between 2 and 3 mg of wet weight whole blood was added to tins,

Table 1 Sampled sharks by location, species and sex with average, minimum and maximum length (stretch total length, cm). The standard deviation of length values is also reported. Locations are Biscayne Bay (B), Florida Bay (F) and Reef (R). G. Hammer is great hammerhead shark and S. hammer is scalloped hammerhead shark

Species	N	N (males)	N (females)	Average length (cm)	Min length	Max length	SD length
B							
Sharpnose	6	0	6	88	70	106	13.6
Blacknose	12	6	6	118	100	132	10.9
Blacktip	8	7	1	166	151	191	11.7
Bull	21	6	15	230	183	269	30.2
C. Reef	2	2	0	195	182	208	18.38
G. Hammer	2	1	1	193	157	230	51.6
Lemon	18	6	12	207	152	259	25.1
Nurse	28	12	16	218	80	255	43.6
Sandbar	13	2	11	202	193	216	6.89
Tiger	9	3	6	214	180	268	31.4
F							
Blacknose	7	5	2	113	101	122	8.9
Blacktip	23	2	21	138	99	165	21.8
Bull	16	5	11	190	159	252	26.4
G. Hammer	1	0	1	146	N/A	N/A	N/A
Lemon	33	10	23	208	164	251	23.6
Nurse	23	12	11	217	175	255	25.4
Tiger	3	0	3	190	159	231	37.0
R							
Sharpnose	1	0	1	82	N/A	N/A	N/A
Blacknose	4	0	4	112	96	121	11.4
Blacktip	3	0	3	177	154	193	20.6
Bull	5	3	2	244	189	288	39.1
C. Reef	4	0	4	170	120	246	53.8
Lemon	1	0	1	272	N/A	N/A	N/A
Nurse	19	5	14	229	132	258	28.1
Sandbar	25	6	19	196	176	213	8.74
S. Hammer	2	1	1	261	224	297	N/A
Tiger	9	5	4	214	106	289	56.3

which were dried overnight at 60 °C. Samples were combusted in a EuroVector Euro EA elemental analyzer and then passed through a GV Instruments diluter before being introduced to a GV Instruments IsoPrime isotope ratio mass spectrometer. One replicate per ten samples was run, any outliers were rerun, and laboratory standards of glycine and peptone were run once per 15 samples. Urea was not removed from blood samples. (See Kim and Koch 2012 for a description of this technique and how it impacts isotopic values, and see the limitations section of this manuscript for a discussion of how it could bias our results.) Ratios of stable isotopes were expressed as parts per thousand using standard δ notation.

Stable isotope analysis metrics

Isotopic ratios of carbon (C13/C12) and nitrogen (N15/N14) are enriched relative to a food source in a stepwise and relatively predictable manner (Peterson and Fry 1987), and are reported ‰. Carbon ratios primarily indicate the carbon source (e.g., pelagic vs.

benthic, coastal vs. offshore, Minagawa and Wada 1984) and nitrogen ratios suggesting trophic level (i.e., those with higher N15/N14 ratios are feeding relatively higher in the food web, Post 2002). Analysis of these isotopic ratios can provide measurements of a species' isotopic niche, as well as whether the isotopic niches of co-occurring species overlap. Although an isotopic niche provides insights into the combination of basal resources supporting a consumer's diet and relative trophic level, isotopic niches are distinct from ecological or dietary niches because they do not provide direct insights into particular food sources (reviewed in Layman et al. 2012). While caution is needed in interpreting isotopic data relative to spatiotemporal variation in isotopic values at the base of food webs and dynamics of isotopic discrimination among species, isotopic niche overlap can provide insights into whether dietary overlap might occur.

Five metrics of relative isotopic niche breadth (i.e., comparisons between co-occurring species within the same study site) and area were calculated for each species in each study site with a sample size of greater than five individuals. This included calculations of (1) $\delta^{13}C$ range, (2) $\delta^{15}N$ range, (3) total occupied niche area, (4) standard ellipse area (SEAc) and (5) the mean distance to the centroid (CD), which are defined below. Both $\delta^{13}C$ range and $\delta^{15}N$ range are the differences between the smallest and largest $\delta^{13}C$ and $\delta^{15}N$ values within a sample, respectively (Layman et al. 2007). The total occupied niche area is the area of the convex hull surrounding the outermost points in a sample when those points are plotted on a $\delta^{13}C$ vs. $\delta^{15}N$ biplot, and this measures the total isotopic niche area of sampled individuals (Jackson et al. 2011). The small sample size-corrected standard ellipse area (SEAc) represents the core isotopic niche area of sampled individuals after factoring in maximum likelihood and is similar to a two-dimensional measurement of standard deviation (Jackson et al. 2011). Mean distance to the centroid (CD) measures trophic diversity within a sample (Layman et al. 2007). SEAc is used for overlap calculations and discussed in more detail because it is more suitable for the smaller sample sizes found for some species in this study than total occupied niche area (Jackson et al. 2011; Syväranta et al. 2013).

For $\delta^{13}C$ range, $\delta^{15}N$ range, total occupied niche area and standard ellipse area, larger values indicate relatively broader isotopic niches, likely suggesting a relatively generalist diet. A larger mean distance to the centroid indicates more isotopic dispersion (two-dimensional spread from the mean) within a group of individuals, likely suggesting more individual variation in diet among a population. Non-overlapping ellipses between sympatric species suggest the presence of resource partitioning between those species either in basal resources supporting diets, foraging locations or specific prey (e.g., Heithaus et al. 2013). However, the presence of overlapping ellipses does not necessarily suggest shared patterns of resource use between species. In the case of complex habitats with multiple carbon sources and many available prey resources, multiple distinct patterns of resource consumption can result in similar stable isotope signatures (reviewed in Layman et al. 2012).

Data analysis

All statistical analysis was performed using R version 3.1.2 (R Core Team 2013), including the SIAR and SIBER packages. For each habitat, the mean $\delta^{13}C$ and $\delta^{15}N$ values ± one standard deviation for each species were plotted (following Davenport and Bax 2002). SEAc and convex hull area was only calculated for species with an N greater than or equal to five within a study site.

Model selection

Following Tamburello et al. (2015), we used a model selection approach assessing the support of multiple GLMs to explore the influence of capture month, capture year and individual size (total length) on mean isotopic signature for species which had sufficient sample size across these variables. A species was considered to have sufficient sample size for the capture month variable if at least three sharks were sampled in at least three non-consecutive months, for the year variable if at least five sharks were captured in at least two different years (which sometimes meant comparing species caught in different months between years) and for the length variable if the range of sampled shark length was greater than 1 m. The relative support for each model was assessed using the Akaike information criterion (AIC), with lower AIC scores indicating a better-supported model. The log likelihood of each model was also calculated and reported, with higher values indicating a better-fitting

model coefficient. The number of estimated model parameters, K, was also reported.

Results

Blood samples from 299 sharks of 11 species were analyzed, ranging in size from a 70 cm Atlantic sharpnose shark (*Rhizoprionodon terranovae*) to a 297 cm scalloped hammerhead shark (*Sphyrna lewini*) (Table 1). Species sampled also included blacknose (*Carcharhinus acronotus*), blacktip (*C. limbatus*), bull (*C. leucas),* Caribbean reef (*C. perezi*), great hammerhead (*S. mokarran),* lemon (*Negaprion brevirostris*), nurse (*Ginglymostoma cirratum*), sandbar *(C. plumbeus*) and tiger (*Galeocerdo cuvier*) sharks. Ten species were captured at both Biscayne Bay and the Reef, and seven species were captured at Florida Bay (Table 1). See supplementary materials for N by month and year for each species.

Biscayne Bay

Nurse sharks had the highest values for standard ellipse area (3.36), convex hull area (9.82) and $\delta^{15}N$ range (4.63, Table 2) of any shark species captured at Biscayne Bay. Lemon, bull and sandbar sharks also had relatively high isotopic niche areas. Atlantic sharpnose sharks had the smallest isotopic niche, indicated by their standard ellipse area (1.05) and convex hull area (1.06). Lemon, great hammerhead, bull and sandbar sharks had the highest mean $\delta^{15}N$ values of any species captured at Biscayne Bay, followed by blacktip and blacknose sharks (Fig. 2a, Table 2). The highest overlap between ellipses of sharks captured in Biscayne Bay was observed between blacknose and blacktip sharks, between sharpnose and tiger sharks, and between tiger sharks and nurse sharks (Fig. 3a, Table 3) The ellipses of the three species with the highest $\delta^{15}N$ values (bull, lemon and tiger sharks) did not overlap at all (Fig. 3a). Nurse sharks had the largest CD (1.42, Table 2), and Atlantic sharpnose sharks had the smallest CD (0.69). Mean $\delta^{13}C$ isotopic values ranged from -14.9 ‰ (sandbar shark) to -12.6 (lemon shark), and mean $\delta^{15}N$ values ranged from 11.23 ‰ (Atlantic sharpnose shark) to 13.24 ‰ (sandbar shark).

Table 2 Summary of isotopic metrics for sharks captured at Biscayne Bay (B), Florida Bay (F) and the Reef (R). Sharks are sorted by decreasing SIBER ellipse area

Species	SEAc	$\delta^{13}C$ range	$\delta^{15}N$ range	TA	CD
B					
Nurse	3.36	4.19	4.63	9.82	1.42
Sandbar	2.53	3.8	2.74	5.36	1.06
Lemon	2.28	2.57	2.99	5.4	1.06
Bull	1.95	3.36	3.79	6.41	0.89
Blacknose	1.84	4.61	1.71	4.13	0.90
Tiger	1.72	2.56	2.4	2.67	0.84
Blacktip	1.69	2.83	1.49	2.11	0.93
Sharpnose	1.05	1.47	1.34	1.06	0.69
F					
Bull	5.51	7.03	2.94	12.89	1.99
Lemon	4.96	6.13	3.76	13.98	1.84
Nurse	2.82	5.7	3.5	6.36	1.77
Blacktip	2.04	4.61	3.01	5.62	1.24
Blacknose	0.55	1.07	1.87	0.67	0.66
R					
Bull	6.1	6.1	6.7	5	2.98
Nurse	2.62	4.25	4.71	6.68	1.43
Tiger	2.16	3.16	2.01	3.51	0.96
Sandbar	1.02	1.29	3.86	2.76	0.96

Florida Bay

Bull and lemon sharks had the highest convex hull area (12.89 and 13.98, respectively) and standard ellipse area (5.51 and 4.96, respectively), demonstrating that these species have the largest isotopic niche area of any species captured in Florida Bay. Bull sharks had the highest $\delta^{15}N$ values (Fig. 2b, Table 2). The most overlap between standard ellipses of sharks captured at the Florida Bay study area occurred between ellipses of lemon and blacktip sharks, lemon and bull sharks, blacktip and nurse sharks, and lemon and nurse sharks (Fig. 3b, Table 3). In Florida Bay, the ellipses of shark species with the highest $\delta^{15}N$ values (bull shark, blacktip shark and lemon sharks) overlapped extensively with each other (Fig. 3b). Bull sharks had the largest CD (1.99, Table 2), and blacknose sharks had the smallest (0.66). Mean $\delta^{13}C$ isotopic values ranged from -17.38 ‰ (great hammerhead shark) to
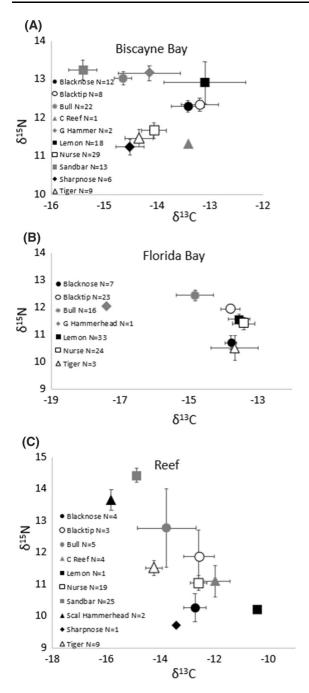
Fig. 2 **a** Average stable isotope ratios of shark species sampled in the Biscayne Bay study area ± 1 standard deviation. **b** Average stable isotope ratios of sharks species sampled in the Florida Bay study area ± 1 standard deviation. **c** Average stable isotope ratios of sharks species sampled in the Reef study area ± 1 standard deviation

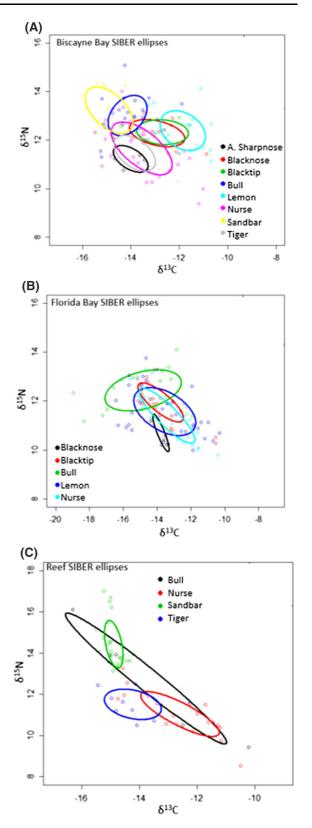− 13.41 ‰ (nurse shark), and mean δ^{15}N values ranged from 10.51 ‰ (tiger shark) to 12.44 ‰ (bull shark).

◄ **Fig. 3** **a** SIBER ellipses of shark species sampled in the Biscayne Bay study area. **b** SIBER ellipses of shark species sampled in the Florida Bay study area. **c** SIBER ellipses of shark species sampled in the Reef study area

Reef

Bull and nurse sharks had the largest isotopic niche areas of any species captured in the Reef study area. Bull sharks had the highest standard ellipse area (6.1), $\delta^{13}C$ range (6.1) and $\delta^{15}N$ range (Table 2). Nurse sharks had the highest convex hull area (6.68), and the second-highest standard ellipse area (2.62), $\delta^{13}C$ range (4.25) and $\delta^{15}N$ range (4.71). Sandbar sharks had the smallest isotopic niche area of any species captured in the Reef study area, demonstrated by the comparatively low standard ellipse area (1.02) and convex hull area (2.76) of this species. Sandbar, scalloped hammerhead and bull sharks had the highest $\delta^{15}N$ values of any species captured in the Reef study

area (Fig. 2c). The highest overlap between standard ellipses of species captured in the Reef study area occurred between nurse and bull sharks, and between two of the species with the highest $\delta^{15}N$ values (sandbar and bull sharks, Fig. 3c, Table 3). There was no overlap between the ellipses of sandbar sharks and tiger sharks, or between the ellipses of sandbar and nurse sharks (Fig. 3c, Table 3). Bull sharks had the largest CD (2.98, Table 2), and tiger and sandbar sharks had the smallest CD of 0.96. Mean $\delta^{13}C$ isotopic values ranged from -15.82 ‰ (scalloped hammerhead sharks) to -10.41 ‰ (lemon shark), and mean $\delta^{15}N$ values ranged from 9.7 ‰ (Atlantic sharpnose shark) to 14.43 ‰ (sandbar shark).

Comparisons of isotopic niche patterns between study areas

Bull, sandbar and hammerhead sharks had among the highest $\delta^{15}N$ levels in each of the three study areas

Table 3 Area of overlap between small sample size-corrected SIBER ellipses of different shark species captured at Biscayne Bay (B), Florida Bay (F) and the Reef (R). Proportion of overlap is indicated in parentheses and should be read as "the proportion of ROW SPECIES ellipse that overlaps with COLUMN SPECIES ellipse" (e.g., 85% the blacktip SIBER ellipse in Biscayne Bay overlaps with the blacknose SIBER ellipse, but that same area of overlap is only 78% of the blacknose SIBER ellipse)

B	Sharpnose	Blacknose	Blacktip	Bull	Lemon	Nurse	Sandbar	Tiger
Sharpnose	X	0	0	0	0	0.62 (0.59)	0	0.84 (0.8)
Blacknose	0	X	1.44 (0.78)	0.15 (0.08)	0.61 (0.33)	0.55 (0.3)	0.03 (0.02)	0
Blacktip	0	1.44 (0.85)	X	0	0.81 (0.48)	0.33 (0.19)	0	0
Bull	0	0.15 (0.08)	0	X	0	0.45 (0.23)	1.03 (0.53)	0
Lemon	0	0.61 (0.27)	0.81 (0.36)	0	X	0	0	0
Nurse	0.62 (0.18)	0.55 (0.3)	0.33 (0.1)	0.45 (0.13)	0	X	0.31 (0.09)	1.48 (0.44)
Sandbar	0	0.03 (0.01)	0	1.03 (0.41)	0	0.31 (0.12)	X	0
Tiger	0.84 (0.49)	0	0	0	0	1.48 (0.86)	0	X

F	Blacknose	Blacktip	Bull	Lemon	Nurse
Blacknose	X	0	0	0.23 (0.42)	0.03 (0.06)
Blacktip	0	X	1.06 (0.52)	1.87 (0.91)	1.38 (0.68)
Bull	0	1.06 (0.19)	X	1.46 (0.27)	0.72 (0.32)
Lemon	0.23 (0.05)	1.87 (0.2)	1.46 (0.29)	X	2.52 (0.51)
Nurse	0.03 (0.01)	1.38 (0.48)	0.72 (0.26)	2.52 (0.89)	X

R	Bull	Nurse	Sandbar	Tiger
Bull	X	1.7 (0.28)	0.64 (0.1)	0.01 (< 1%)
Nurse	1.7 (0.65)	X	0	0.37 (0.14)
Sandbar	0.64 (0.63)	0	X	0
Tiger	0.01 (< 1%)	0.37 (0.36)	0	X

where they were caught (Fig. 2). Atlantic sharpnose sharks, the smallest species caught during this study, consistently had among the lowest $\delta^{15}N$ levels in both habitats where they were found (Fig. 2a, c). Nurse sharks and blacknose sharks had consistently intermediate $\delta^{15}N$ signatures relative to co-occurring species, and the $\delta^{15}N$ of blacktip sharks varied from relatively high to intermediate (Fig. 2). The $\delta^{15}N$ levels of lemon and tiger sharks were the fourth highest of co-occurring species in some study areas, but the eighth highest in others, demonstrating the most variability in relative $\delta^{15}N$ of any species. (Although raw tiger shark $\delta^{15}N$ value was similar between sample sites, how it compared with the $\delta^{15}N$ values of co-occurring species varied).

Some pairs of species had consistent patterns of core isotopic niche overlap across different study areas. The ellipses of sandbar sharks and nurse sharks captured in the Reef study area did not overlap at all, and there was only a small area of overlap between the ellipses of these species captured in the Biscayne Bay study area. There was no standard ellipse overlap between blacknose and bull sharks captured in the Florida Bay study area, and there was only a very small area of overlap between the ellipses of these species captured in the Biscayne Bay study area. Tiger shark and bull shark ellipses did not overlap at all in either the Biscayne Bay or Reef study areas. In Biscayne Bay, only species with high $\delta^{15}N$ values such as sandbar, bull and lemon sharks had notable unique isotopic niche space, the niches for the other species overlapped almost entirely with those of another species. In the Florida Bay area and at the Reef, only bull sharks had a notable amount of unique isotopic niche space.

Other pairs of species had widely variable patterns of core isotopic niche overlap between different study areas. Eighty-six percent of the standard ellipse of tiger sharks captured in Biscayne Bay overlapped with that of nurse sharks captured in Biscayne Bay. In contrast, among sharks captured in the Reef study area, only 17% of the tiger shark ellipse overlapped with the nurse shark ellipse. Eighty-five percent of the ellipse of blacktip sharks captured in Biscayne Bay overlapped with that of blacknose sharks, but in the Florida Bay study area there was no overlap between the ellipses of these species (Fig. 4). Ninety-two percent of the blacktip shark ellipse overlapped with that of lemon sharks, compared with 44% overlap between the

ellipses of these species captured in the Biscayne Bay study area. Eighty-nine percent of the ellipse of nurse sharks captured in Florida Bay overlapped with that of lemon sharks, compared with 0% overlap between the standard ellipses of these species in Biscayne Bay (Fig. 4). Patterns of overlap were similar when using relatively larger convex hulls instead of small sample size-corrected SEAc values (Supplementary material); blacktip and blacknose sharks still showed notably higher isotopic niche overlap in Biscayne Bay than in Florida Bay, and nurse and lemon sharks still showed notably higher isotopic niche overlap in Florida bay than in Biscayne Bay.

Some shark species had consistent patterns of relative isotopic niche area compared with co-occurring species. Lemon and nurse sharks consistently had high ellipse areas relative to other species captured in the same study area. Bull sharks had the highest ellipse areas of any species captured in the Florida Bay (Fig. 3b) and in the Reef (Fig. 3c) study areas, but an intermediate ellipse area relative to species captured at Biscayne Bay (which was driven by a much smaller bull shark ellipse area in Biscayne Bay, rather than notable increases in the ellipse area of the other species, Fig. 3a). Blacknose, blacktip and tiger sharks had intermediate to relatively low ellipse areas in every study area where they were captured.

Model selection

In Biscayne Bay, there was sufficient sample size to test for the effects of all three variables on the isotopic signatures of lemon and nurse sharks and sufficient sample size to assess the effects of capture year and length on bull shark isotopic signature. In Florida Bay, the sample size was adequate to test for the effects of all three variables on lemon and nurse shark isotopic signatures. There was sufficient sample size to assess the influence of length and capture year on bull shark isotopic signature and to assess the influence of length and capture month on the isotopic signature of blacktip sharks. At the Reef, sample size was adequate to investigate the effects of all three variables on nurse sharks. These effects could not be evaluated for other shark species because of little variation in shark total length (most were large juvenile or adult sharks), capture month or capture year.

The best-supported model never included a month × year interaction effect, suggesting that

(A)

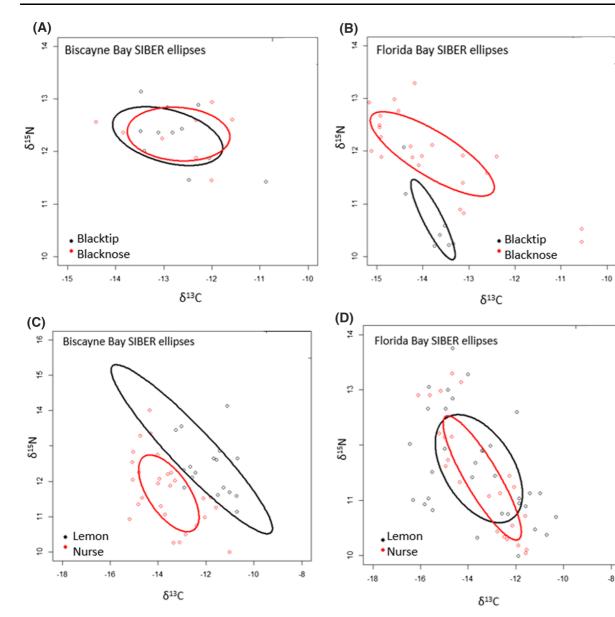**(B)**

(C)

(D)

Fig. 4 Variable patterns in SIBER ellipse overlap for two selected species pairs between two habitats, highlighting the most significant shifts in patterns of isotopic niche overlap discovered in this study. **a** Blacknose (black) and blacktip shark (red) SIBER ellipse overlap in Biscayne Bay compared with

b Blacknose and blacktip shark SIBER ellipse overlap in Florida Bay. **c** Lemon (black) and nurse (red) shark SIBER ellipse overlap in Biscayne Bay compared with **d** Lemon and nurse shark SIBER ellipse overlap in Florida Bay

month and year can be safely analyzed as separate factors. When all three sample areas were analyzed together, the best-supported model did not incorporate study area, but all three study areas were analyzed separately for easier comparison with the rest of this study's results.

The best-supported model for nitrogen signature of lemon sharks captured in Biscayne Bay incorporated length (with larger sharks generally having a lower $\delta^{13}C$ and a higher $\delta^{15}N$ value) and capture year (with later years showing a generally higher $\delta^{13}C$ value and lower $\delta^{15}N$ value, Supplementary Material). For every other species captured in Biscayne Bay, the best-

supported models incorporated only length (with larger sharks generally having a lower $\delta^{13}C$ and a higher $\delta^{15}N$ value). The best-supported models for the carbon and nitrogen signatures of species sampled in Florida Bay all incorporated a temporal factor, either month, year or both, with inconsistent patterns between species (Supplementary material). The best-supported models for the nitrogen signatures of bull, lemon and nurse sharks captured in Florida Bay also incorporated length, with larger individuals having a generally higher $\delta^{15}N$ value. The best-supported model for the isotopic signatures of nurse sharks captured at the Reef incorporated capture month only (Supplementary material).

Discussion

Though studies of the diet of a single predator species or studies of dietary overlap between predators in a single location are relatively common, comparatively few prior studies have compared how patterns of resource use between multiple co-occurring marine top predators may vary among different locations within their range (Sanford et al. 2003; Wieters et al. 2008). The results of this study demonstrate that for some shark species in coastal South Florida, there appears to exist spatial variation in the relative size of core isotopic niches between sympatric species, as well as variation in patterns of core isotopic niche overlap between sympatric species. This leads us to reject our null hypothesis that these isotopic relationships would remain consistent across neighboring study areas where these species co-occur, and given that the isotopic signatures of food webs are similar between these study sites, it suggests that some patterns of resource use and competition among co-occurring shark species may differ between nearby habitats. Accordingly, ecological interactions between sympatric shark species may vary across their ranges, which should be considered when applying ecological data from one location to another location.

Similar patterns of spatial variation in isotopic and trophic interactions between sympatric predators have occasionally been noted in both freshwater and marine systems. A study of trophic interactions between communities of co-occurring freshwater teleost predators noted that the species with the relatively largest isotopic niche varied between nearby lakes (Hayden

et al. 2013). For example, in Lake Roppi, SEAc values were 2.4 for whitefish (*Coregonus lavaretus*) and 4.7 for ruffe *(Gymnocephalus cernuus)*, but in lake Oiko, whitefish had a larger standard ellipse area than ruffe (2.1 vs. 1.7, Hayden et al. 2013), which was attributed to different resource use patterns of the same species between these lakes despite similar prey availability and total system isotopic range in both lakes. A similar pattern was discovered in a marine system, where stomach content analysis showed that gray reef sharks (*Carcharhinus amblyrhynchos*) and sandbar sharks (*Carcharhinus plumbeus*) both primarily consume teleosts (found in 85.2% of gray reef shark stomachs and 70.5% of sandbar shark stomachs) and cephalopods (found in 29.5% of gray reef shark stomachs and 26.5% of sandbar shark stomachs) when either is the only shark species present in a location (Papastamatiou et al. 2006). However, at study sites with large numbers of both gray reef sharks and sandbar sharks, dietary overlap between these species was very low, suggesting resource partitioning (Papastamatiou et al. 2006).

Studies focusing on the diet of a single elasmobranch species across their range have shown that several species have geographic plasticity in their diet. It was found that the diet of the bonnethead sharks (*Sphyrna tiburo)* is approximately 50% crabs and 5% other crustaceans in Tampa Bay, Florida, but approximately 10% crabs and 30% other crustaceans in Florida Bay, despite similar prey availability in both locations (Bethea et al. 2007). Isotopic mixing model results suggest that the diet of Australian sharpnose sharks was split evenly between benthic and pelagic sources in one study site, but was almost entirely composed of benthic sources in another site (Munroe et al. 2015), which was attributed primarily to these dietary generalists taking advantage of distinct prey availability in different study sites. Our results are consistent with these past studies and build upon them by incorporating multiple co-occurring predators.

By demonstrating that some of these shark species can vary their trophic interactions relative to co-occurring species, our results suggest the potential for trophic plasticity in these animals. Trophic plasticity is believed to benefit predators by making them more resilient to changing environmental conditions, such as declines in the availability of certain prey species (Munroe et al. 2014), while also further complicating any analysis of that species' ecological role (since that

role is variable). A change in dietary preference can also occur as a result of increased competition for resources due to the presence of another predator. For example, African wild dogs (*Lycaon pictus*) have similar diets as hyenas (*Crocuta crocuta*), but significantly alter their diet when hyenas are present (Creel and Creel 1996, 1998), resulting in resource partitioning and associated reduced competition for available prey. This shift from possible competition for resources to resource partitioning is similar to what was observed for some species pairs between habitats, possibly as a result of different prey availability between habitats. When voles declined in population, a predatory bird species (the pallid harrier *Circus macrourus*) capable of trophic plasticity was able to adapt to consume 40% more alternative prey biomass than a co-occurring species that specialized in feeding on voles (the Montagu's harrier *C. pygargus*, Terraube et al. 2011). Following a coral mortality event, a species of corallivorous butterflyfish that specialized in consuming one species of coral (*Chaetodon trifascialis*) suffered 98% population declines, while a co-occurring butterflyfish species capable of changing its diet (*C. trifasciatus*) suffered still problematic but comparatively lesser 73% population declines (Graham 2007). Co-occurring elasmobranch, teleost and mammal top predators within a single study site have been shown to vary their degree of resource partitioning seasonally (Matich et al. 2017). The shifts in patterns of isotopic niche overlap and relative isotopic signature between sympatric shark species in different habitats may demonstrate trophic plasticity not only in diet, but in competitive interactions for prey.

Patterns of relative isotopic niche area and core isotopic niche overlap

Bull, sandbar and hammerhead sharks had among the highest mean $\delta^{15}N$ values in each study area where they were sampled. This suggests that these species may regularly occupy higher trophic positions than the other sampled species in these systems, consistent with a multi-species analysis of shark trophic positions focusing on stomach content analysis results (Cortés 1999). Atlantic sharpnose sharks had among the lowest mean $\delta^{15}N$ of sampled species, suggesting that they may feed lower in the food web than the other sampled species from this study. This is consistent with stomach content studies of Atlantic sharpnose

diet, which show that this species primarily consumes small teleost fishes (ranging from 93.2% IRI to 100% IRI, Drymon et al. 2012). On the other hand, tiger sharks, which are large animals often considered to be apex predators, had relatively low mean $\delta^{15}N$ values in each study area where they were sampled. The generalist diet of tiger sharks includes large but low trophic-level prey like herbivorous turtles (Lowe et al. 1996). This may be responsible for the relatively low mean $\delta^{15}N$ values of this species, a pattern also found in Dicken et al. (2017). This finding furthers the assertion (Frisch et al. 2016) that the trophic level of a shark should not be inferred just from a relatively large size.

In this study, bull, sandbar and nurse sharks consistently had large isotopic niche area compared with co-occurring sampled species. Bull sharks had the highest standard ellipse area of any sampled shark species in two of the three habitats included in this study. Similarly, it was found that bull sharks had a much larger isotopic niche area co-occurring predatory than teleost fishes (including wahoo *Acanthocybium solandri,* dorado *Coryphaena hippurus*, sailfish *Istiophorus platypterus* and kawakawa *Euthynnus affinis*) (Daly et al. 2013). Shiffman et al. (2014) also noted a comparatively large isotopic niche area for sandbar sharks in South Carolina (true here for sandbar sharks in Biscayne Bay but not in the Reef study area), and another study noted a relatively large standard ellipse area for nurse sharks in Belize (Tilley et al. 2013).

Several combinations of shark species studied here that had high core isotopic niche overlap in one location had low or no core isotopic niche overlap in other locations, suggesting that whether two sympatric species partition or compete for available prey can vary dramatically even across a short geographic distance if the habitats are different enough. This variability in resource partitioning between co-occurring predators between locations has been noted in some freshwater systems. For example, it was found that the overlap between white (*Morone americana*) and yellow perch (*Perca flavescens*) ellipses was 0% in one study site and 88% in another (Guzzo et al. 2013).

These observed changes in patterns of resource partitioning versus competition for prey may be explained by available prey resources, which varied between the geographically proximate study sites.

When a variety of prey resources suitable for both species is available, the predators may partition resources, but when fewer prey types are available, competition may occur. In this study, blacknose and blacktip sharks had 85% core isotopic niche overlap in Biscayne Bay, but 0% overlap in Florida Bay, suggesting a possible shift from consuming similar resources to resource partitioning. Stomach content analysis of these species has revealed that blacktip sharks in the Northwestern Atlantic primarily eat filter-feeding menhaden (*Brevoortia tyrannus*, Castro 2000), while blacknose sharks eat primarily small piscivorous sciaenids (Ford 2012). However, some teleost, mollusk and crustacean species are consumed by both shark species, and if these prey species are the primary resources available in a location, it may lead to these shark species competing for prey when they would otherwise partition available resources. Similarly, lemon and nurse sharks in this study exhibited high core isotopic niche overlap in one study area, but no overlap in another. Stomach content analysis has shown that both lemon sharks (Cortés and Gruber 1990; Newman et al. 2010) and nurse sharks (Castro 2000) are primarily piscivorous, but also eat benthic invertebrates. When both small invertebrates and fishes are abundant, lemon and nurse sharks may partition available resources between them, but competition may result if a more limited selection of prey is available.

The total isotopic range of the shark community from the Reef study area was highest, likely because of high diversity of prey species in that habitat resulting in complex trophodynamics. With the exception of the lone highly migratory great hammerhead shark in Florida Bay, the total isotopic range of the shark community in Florida Bay was the narrowest, possibly reflecting the comparatively low prey biodiversity in this habitat. These results largely fit into past trophic analyses of these habitats that used stable isotope analysis. The isotopic range of sharks sampled in this study in Biscayne Bay is notably narrower than that reported for the entire food web by Kieckbusch et al. (2004), but comparable to what that study found for the teleost predator community in Biscayne Bay. The relatively enriched $\delta^{13}C$ values of sharks in this study further the findings of Kieckbusch et al. (2004) that mangroves are unlikely to be a major carbon source of predators in this habitat. The average isotopic signature of nurse sharks in Florida Bay was very similar to those reported for mesopredatory teleosts in Chasar et al. (2005), with the average N15 signature of the apex predatory bull shark in this study being higher than anything found in Chasar et al. (2005). The lemon sharks analyzed in this study, however, had a slightly lower $\delta^{15}N$ signature than those analyzed in Chasar et al. (2005), but those were from a museum collection originally sampled in the 1950s. At the Reef study area sharks appear to be feeding in a food web supported more by algae and coral than the more enriched sea grass-supported food web, and this study's nurse sharks, a known predator of spiny lobster, have an average isotopic signature approximately one trophic level higher than what Behringer and Butler (2006) found for local spiny lobster.

Model selection

While it is still important to test for the effects of seasonal variation on the isotopic signatures of elasmobranch predators, those effects were expected to be comparatively small. This is because the effects of temporal variation on baseline isotopic signatures on upper-level consumers are comparatively low (Harvey et al. 2002), and few past studies have documented significant seasonal or interannual variation in elasmobranch isotopic signatures (but see Matich and Heithaus 2014 for an analysis of seasonal prey pulses in a similar system). Additionally, Florida waters have less dramatic seasonal changes in environmental conditions than those of Alaska (Goering et al. 1990) or the Mediterranean (Vizzini and Mazzola 2003), and therefore, a less dramatic seasonal change in baseline isotopic signature would be expected for this study than in those previous studies. Additionally, while it is important to test for the effects of size-based variation in the isotopic signature of consumers, this study was not expected to detect ontogenetic shifts in diet or length-based variation in isotopic routing. This is because most of the individuals captured in this study are at or near adult sizes.

Assumptions and limitations

Interpretation of stable isotope analysis results requires making several assumptions that could affect our conclusions if not met (Hussey et al. 2012; Layman et al. 2012). It is possible that two species can have distinct diets but very similar isotopic signatures

(for example, if one consumer species' isotopic signature represents the average of two isotopically extreme resource bases), which would influence our interpretation of overlapping ellipses between species (reviewed in Layman et al. 2012).

Similarly, we are assuming that sharks are consuming resources primarily in the food web associated with the study area where they were captured, but the distances between study sites are below known migration distances of many species included in this study. These annual migrations, when combined with the long isotopic turnover time of elasmobranch blood (see MacNeil et al. 2006; Matich and Heithaus 2014), may mean that few of these sharks ever came into total isotopic equilibrium with the food web where they were captured. This likely means that the isotopic signatures of many or all sharks in this study represent a mix of feeding in their current capture location and elsewhere, an important consideration when interpreting results. However, the fact that we noted such distinct differences in isotopic niche area and overlap between the same species in different study areas suggests that this is not a major concern; if all sampled sharks were all feeding on a shared pool of resources regardless of capture location, these different patterns would not have been observed.

The relatively low sample size of some species included in this study may have underestimated the true isotopic niche variability of these species (e.g., an N of 25–40 captures a population's isotopic variability well, Vaudo and Heithaus 2011), and all sample sizes were below 25 except for Biscayne Bay nurse sharks, Florida Bay lemon sharks and Reef sandbar sharks (though several other species were close to an N of 25). Similarly, the low sample size of some species in some habitats may have contributed to observed patterns in SEAc overlap—while SEAc is better suited than total occupied niche area for comparing isotopic niche width between small populations (Syväranta et al. 2013), it is certainly possible that a higher sample size would have resulted in more consistent patterns of isotopic niche overlap between study sites as a result of capturing more of the total range in resource use among populations (i.e., our small sample size may have captured outliers in both populations).

While several species of sharks have been known to exhibit ontogenetic shifts in diet (reviewed in Grubbs 2010), this study focused mostly on large juvenile or adult individuals who had likely already undergone their dietary shift. [In many but not all species this shift occurs between small and large juveniles during a transition out of a nursery area, not between large juveniles and adults (Grubbs 2010)]. However, some species do shift habitats and associated diets later in their life history which could cause issues in our interpretation of the data, though our statistical analysis shows that it was likely not a major factor.

In this study, we used only a single tissue type for stable isotope analysis, and this tissue has a relatively long turnover time. Comparing isotopic signatures between tissue types with different turnover rates can reveal whether or not observed patterns in isotopic niche overlap are temporally consistent (e.g., Gallagher et al. 2017, which found that for one of the study sites included in this study, patterns of overlap between species are consistent temporally by comparing isotopic signatures from two distinct tissue types). Also, most of the study species used here have never had their isotopic discrimination factors explicitly measured in laboratory conditions (few elasmobranchs have to date), and different discrimination between species may cause bias in our results (i.e., showing different isotopic signatures even for species with very similar diets because these species may have distinct patterns of isotopic incorporation from their diets).

Not removing urea from samples may result in artificially depleted $\delta^{15}N$ values due to nitrogen (Hussey et al. 2012; Carlisle et al. 2016), but this is expected to be more of an issue when comparing elasmobranch isotopic signatures to co-occurring teleosts and invertebrates, and less of an issue when only comparing results between elasmobranchs (Kim and Koch 2012). While this study showed that there were intraspecific differences between animals in different habitats, those habitats were too varied (in terms of bottom type, depth and prey availability) to reliably assess a single cause for observed differences in isotopic niche area between study areas.

Conclusions

This study provides evidence of intraspecific variability in the relative isotopic niche area and patterns of core isotopic niche overlap in several species of co-occurring marine predators, showing that within different habitats the resource usage and trophic

interactions of sympatric species may vary. Overall, this suggests that the ecological roles of shark species are quite complex and varied. Therefore, results from a dietary overlap study in one habitat cannot necessarily be extrapolated to a separate habitat, even if that separate habitat is nearby. Our results suggest the need to generate empirical assessments of resource use and trophic interactions among sympatric predators specific to a particular area when trying to understand and model trophodynamics for that area.

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