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Philip Matich
Department of Biological Sciences and Marine Sciences Program, Florida International University, pmati001@fiu.edu

Michael R. Heithaus
Marine Sciences Program, Florida International University, heithaus@fiu.edu

Craig A. Layman
Marine Sciences Program, Florida International University

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Contrasting patterns of individual specialization and trophic coupling in two marine apex predators

Philip Matich*, Michael R. Heithaus, and Craig A. Layman

Marine Sciences Program
Florida International University
3000 NE 151st
North Miami, FL 33181, USA

Running head: specialization in top marine predators

*To whom correspondence should be addressed, pmati001@fiu.edu, (305) 919-5602 voice, (305) 919-4030 fax
Summary

1. Apex predators are often assumed to be dietary generalists and, by feeding on prey from multiple basal nutrient sources, serve to couple discrete food webs. But there is increasing evidence that individual level dietary specialization may be common in many species, and this has not been investigated for many marine apex predators.

2. Because of their position at or near the top of many marine food webs, and the possibility that they can affect populations of their prey and induce trophic cascades, it is important to understand patterns of dietary specialization in shark populations.

3. Stable isotope values from body tissues with different turnover rates were used to quantify patterns of individual specialization in two species of “generalist” sharks (bull sharks, *Carcharhinus leucas*, and tiger sharks, *Galeocerdo cuvier*).

4. Despite wide population-level isotopic niche breadths in both species, isotopic values of individual tiger sharks varied across tissues with different turnover rates. The population niche breadth was explained mostly by variation within individuals suggesting tiger sharks are true generalists. In contrast, isotope values of individual bull sharks were stable through time and their wide population level niche breadth was explained by variation among specialist individuals.

5. Relative resource abundance and spatial variation in food-predation risk tradeoffs may explain the differences in patterns of specialization between shark species.

6. The differences in individual dietary specialization between tiger sharks and bull sharks results in different functional roles in coupling or compartmentalizing distinct food webs.

7. Individual specialization may be an important feature of trophic dynamics of highly mobile marine top predators and should be explicitly considered in studies of marine food webs and the ecological role of top predators.
Key Words: estuary, food webs, foraging ecology, marine community dynamics, predator-prey interactions, seagrass ecosystems, sharks, stable isotopes, trade-offs, trophic coupling
Introduction

Populations of large marine predators are declining rapidly worldwide (e.g. Myers & Worm 2003; Estes et al. 2007; Ferretti et al. 2010), which may lead to marked changes in community structure and ecosystem function (Heithaus et al. 2008). While numerous studies have shown that removal of top predators can have significant consequences for marine communities, the scope, magnitude, and context-dependence of these effects are only starting to be realized (Heithaus et al. 2008). In many cases, our understanding of the ecological role of large marine predators, and potential consequences of their declines, is hindered by a lack of data on their trophic ecology.

In addition to top-down impacts on prey species, an important ecological function of predators is the coupling of energy pathways from distinct food webs (Rooney et al. 2006). This occurs when lower trophic level consumers derive their energy from a single source (i.e. primary producer base), but at increasing trophic levels consumers tend to incorporate energy from a wider range of prey serving to couple multiple energetic pathways (Rooney et al. 2006; Rooney, McCann & Moore 2008). Such coupling is often evaluated at a population level, ignoring the behaviors and habits of individuals. Populations of “generalist” predators may in fact be a collection of individual-level trophic specialists that vary considerably in their resource use (e.g. Urton & Hobson 2005; Woo et al. 2008). At a population level, predator species may incorporate prey taxa from multiple food webs into their diets, but individual-level dietary specialization may serve to keep energy pathways from discrete food webs separate. For example, Eurasian perch (Perca fluviatilis), which have a wide niche width at the population level, segregate into littoral and pelagic specialists, and consequently individuals do not couple these two components of freshwater food webs (Quevedo, Svanback & Eklov 2009).
Individual specialization within populations may be more likely under conditions of 1) resource scarcity, 2) interhabitat differences in resource availability, 3) fitness trade-offs that result in individual-specific behavior, 4) cultural transmission of foraging traditions, and/or 5) cognitive constraints that limit the use of diverse sets of resources (e.g. Rendell & Whitehead 2001; Estes et al. 2003; Svanback & Persson 2004; Araujo & Gonzaga 2007; Darimont, Paquet & Reimchen 2009). Recent studies have investigated individual dietary specialization in birds (e.g. Inger et al. 2006; Martinez del Rio et al. 2009a), mammals (e.g. Urton & Hobson 2005; Newsome et al. 2009), and bony fishes (e.g. Beaudoin et al. 1999; Quevedo et al. 2009), but few studies have considered individual specialization in large, non-mammalian, marine predators that use multiple ecosystems.

Here we investigate whether two species of sharks, in two distinct ecosystems, exhibit individual trophic specialization. Specifically, we used stable isotope analysis of multiple tissues with different turnover rates, to reveal patterns of variation in diets within and among individual bull sharks (Carcharhinus leucas Müller & Henle, 1839) inhabiting an oligotrophic coastal estuary, and among individual tiger sharks (Galeocerdo cuvier Peron & LeSueur, 1822) in a relatively pristine seagrass community. Our study investigates if predator populations can be treated as homogeneous units, or if an individual level approach is essential to understand the full range of trophic roles that these populations fill (Estes et al. 2003; Svanback & Persson 2004; Ravigne, Dieckmann & Olivieri 2009).
Methods

Coastal Everglades, Florida

The Shark River Estuary of Everglades National Park, Florida, USA (Fig. 1), is the main drainage basin for the Everglades (Childers 2006). The ecosystem is primarily a braided stream lined by mangroves that extend more than 30 km upstream from the Gulf of Mexico, before giving way to freshwater vegetated marshes. It is considered a relatively oligotrophic, phosphorus-limited system (Childers et al. 2006). The Shark River Estuary is a nursery for juvenile bull sharks, which may be found from the mouth of the river to more than 27 km upstream (Wiley & Simpfendorfer 2007; Heithaus et al. 2009). Bull sharks are one of the largest-bodied predators in the ecosystem. Bull sharks in coastal ecosystems have a relatively wide dietary niche at the population level, preying on teleosts, mollusks, crustaceans, cephalopods, and other elasmobranchs (Snelson & Williams 1981; Snelson, Mulligan & Williams 1984; O’Connell et al. 2007).

Bull sharks were captured from 2005-2009 on ~500 m longlines fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (Mugil sp.) and attached to ~2 m of 400 kg monofilament line (see Heithaus et al. 2009 for details). Captured sharks were processed alongside the sampling vessel, or within a water-filled, aerated cooler on board. We used a biopsy punch to collect a 0.5 cm³ muscle tissue biopsy ca. 5 cm lateral to the first dorsal fin, and an 18 gauge needle to collect 4 ml of blood from the caudal vein. From the blood, 3 ml was placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and separated into its components, including plasma, using a centrifuge spun for one minute at 3000 rpm. The remaining 1 ml of blood was retained in its original composition (whole blood, “blood” hereafter). Tissues were placed on ice and frozen upon return to the laboratory. Skin
was removed from muscle samples before laboratory preparations. Because muscle tissue of
sharks may incorporate isotopes from their diet over a temporal scale of many months (e.g.
MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Matich, Heithaus & Layman 2010;
S. Kim personal communication), only bull sharks over 99cm in total length (approximately 1-2
years old and older) were included in analyses to eliminate any potential maternal isotopic
influence.

To determine the community context of trophic interactions in the Shark River Estuary,
we defined ranges of $\delta^{13}$C that were representative of two “endpoint” habitats: (1) mangrove
creeks and estuarine marshes within the Shark River Slough (i.e. from the mouth of the estuary
and upstream, termed the “freshwater/estuarine food web”) (Fry & Smith 2002; Williams &
Trexler 2006), and (2) fully marine habitats (e.g., seagrass beds) in Florida Bay (“marine food
web”) (Chasar et al. 2005) (Fig. 1). From the existing literature and our own analyses, we
compiled both primary producers and “resident” consumers, i.e., taxa that are largely restricted
in their distribution to one of the two habitat “endpoints” and would be unlikely to move between
them. Sampling of bull sharks in this study was entirely within the boundaries of the
“freshwater/estuarine food web”.

Shark Bay, Australia

Shark Bay is a large, seagrass-dominated, subtropical bay located along the central
Western Australian coast. The study took place in the Eastern Gulf, offshore of Monkey Mia
Dolphin Resort. The study site is made up of a series of shallow (<4m depth) seagrass-covered
banks and deep channels (see Wirsing, Heithaus & Dill 2006 for detailed description). Tiger
sharks are the top predator in the ecosystem, and more than 95% of catches of sharks >2m are
tiger sharks (Heithaus 2001; Wirsing et al. 2006). Tiger sharks are widely considered to be one of the most generalized of sharks in terms of diet, which may include mollusks, cephalopods, elasmobranchs, teleosts, reptiles (sea snakes, sea turtles), and marine mammals (Compagno 1984; Lowe et al. 1996; Simpfendorfer, Goodreid & McAuley 2001).

Tiger sharks were captured from 2007-2009 on drumlines equipped with a single Mustad shark hook (12/0-14/0) (see Wirsing et al. 2006 for details). Captured sharks were processed alongside the sampling vessel. Blood and plasma were collected in the same manner as with bull sharks, and scissors were used to collect a 0.5 cm$^2$ tissue clip from the dorsal fin (fin tissue was collected and used for analyses rather than muscle tissue because of the difficulty in collecting muscle from large tiger sharks). Samples were processed in the same manner as those for bull sharks.

Similar to the Shark River Estuary, we defined ranges of $\delta^{13}$C that were representative of two discrete food webs to establish the ecosystem context of trophic interactions in Shark Bay: (1) “benthic food web” (likely based on seagrass and associated microphytobenthos) and (2) “pelagic food web”, which would be expected to be based on autochthonous seston production.

**Stable isotope analysis**

All shark samples were analyzed at the Florida International University Stable Isotope Facility (43 *C. leucas* blood samples and 50 *C. leucas* muscle samples) or the Yale Earth System Center for Stable Isotopic Studies (15 *C. leucas* plasma samples, 28 *C. leucas* blood samples, 21 *C. leucas* muscle samples, 21 *G. cuvier* plasma samples 46 *G. cuvier* blood samples, and 46 *G. cuvier* fin samples). Lipid extraction was not performed because C:N ratios (bull shark mean muscle = 3.1 ± 0.3 SD, mean blood = 2.7 ± 0.2 SD, mean plasma = 2.0 ± 0.2 SD; tiger shark
mean fin = 2.7 ± 0.1 SD, mean blood = 2.4 ± 0.0 SD, mean plasma = 2.1 ± 0.1 SD) were generally below those suggested for extraction or mathematical correction (3.5; Post et al. 2007), and lipid extraction appears have minimal effects (<0.6‰) on δ\(^{13}\)C values of shark muscle (Hussey et al. 2010). Samples from producers and invertebrates with carbonate shells were acidified for δ\(^{13}\)C values (δ\(^{15}\)N run separately). Producer and community consumer samples were analyzed at Yale Earth System Center for Stable Isotopic Studies.

To verify analytical consistency, we randomly selected samples to be analyzed at both Florida International University and Yale University, for which the variation between resulting δ\(^{13}\)C values and δ\(^{15}\)N values were 0.13‰ ± 0.20SE. The standard deviations of standards run for Yale were 0.14‰ for δ\(^{13}\)C and 0.22‰ for δ\(^{15}\)N, and 0.29‰ for δ\(^{13}\)C and 0.24‰ for δ\(^{15}\)N for Florida International.

Quantitative Analysis

Trophic specialization can be assessed by measuring the variation in the diets of individuals, and is accomplished by calculating the dietary variation within individuals (WIC: within individual component of variation) and between individuals (BIC: between individual component of variation) of a population (Roughgarden 1972, Bolnick et al. 2002). The WIC of a population measures how variable an individual’s diet is over a given time frame. This is typically expressed as a mean value for an entire population, but also can be calculated for individuals (see ‘individual variance’ calculations below). The BIC of a population measures how different each individual’s diet is from the other members of the population (Bolnick et al. 2002). For individuals and populations that are more specialized, WIC should be relatively small because individual diets show little variation and should be consistent over time. Generalist
individuals should have a relatively higher WIC because these individuals have a broader dietary
iche width (Bolnick et al. 2003). The variation between individuals (BIC) varies based on total
niche width (TNW), but in general, the degree of individual specialization should increase as the
BIC:WIC specialization ratio increases for a given TNW (Newsome et al. 2009).

Stable isotope analysis has become a popular method for addressing questions about
trophic ecology and dietary specialization because 1) stable $\delta^{13}$C isotopes can be used to assess
the flow of basal nutrients through food webs and gain insights into trophic coupling (e.g. France
1997; Vander Zanden & Vadeboncoeur 2002), and 2) body tissues of individuals incorporate
isotope values of their diets at various rates (e.g. Hobson & Clark 1992, Bearhop et al. 2004).
Comparing isotopic values of multiple tissues that vary in turnover rate within an individual,
therefore, provides insight into the relative temporal stability of an individual’s diet, and can be
used to investigate questions about individual trophic specialization (Bearhop et al. 2004).

Isotopic turnover rates of elasmobranchs studied to date suggest that muscle and fin have
relatively long turnover rates (complete isotope turnover in 390-540 days and 576 days) and
whole blood has an intermediate turnover rate (complete isotope turnover in 240-300 days;
MacNeil et al. 2006; Logan & Lutcavage 2010, Matich et al. 2010) (Fig. 2). Blood plasma turns
over at an even faster rate than whole blood (S. Kim, personal communication; complete isotopic
turnover in plasma occurs in 72-102 days; Fig. 2), so we used plasma to provide insight into diets
at shorter temporal scales. Although most of these isotope turnover rates were calculated for
relatively small elasmobranchs in captive trials (Potamotrygon motoro: mean mass = 0.1kg;
Carcharhinus plumbeus: mean mass = 6.4kg), and isotope turnover rates can vary with body size
(e.g. Carleton & Martinez del Rio 2005, Martinez del Rio et al. 2009b), field studies of size-
based variation among fin, muscle, and blood of bull sharks suggest that these lab-based
estimates are likely similar to those found in natural settings (Matich et al. 2010; see discussion for further consideration of turnover rates). Furthermore, even if there is variation in absolute turnover rates based on body size, the relative turnover times of tissues (muscle/fin > whole blood > plasma) is expected to be the same.

Delta values (δ) are often used to express stable isotope data, but in order to make comparisons in specialization between tiger sharks and bull sharks, it was necessary to account for difference in their potential isotope niche width (i.e. differences in the range of δ^{13}C values). Therefore, to normalize isotope data for bull sharks and tiger sharks, we converted δ^{13}C values for tissues to proportional values (p-values; Newsome et al. 2007). Each system has two discrete basal resource pools with distinct δ^{13}C values: the Shark River has a “freshwater/estuarine food web” (mean δ^{13}C = -29.7‰ ± 0.7SE) and a “marine food web” (-14.5‰ ± 0.3SE), and Shark Bay has a “benthic food web” (-8.5‰ ± 0.3SE) and a “pelagic food web” (-16.1‰ ± 0.8SE). Therefore, “p-values” were calculated based on mean δ^{13}C values of available food sources for each system using a two-source mixing model (Phillips & Gregg 2001). These p-values provide a measure of the relative position of δ^{13}C values between endpoints of potential energy pathways.

To quantify dietary specialization in bull sharks and tiger sharks, we employed four different models (see below for details of each): 1) General Linear Model (GLM) using isotope p-values of two body tissues (bull sharks: muscle and whole blood; tiger sharks: fin and whole blood) with individual included as a random effect, 2) GLM using isotope p-values of three body tissues (bull sharks: muscle, whole blood, and plasma; tiger sharks: fin, whole blood, and plasma) with individual included as a random effect, 3) calculation of variance for each individual using isotope p-values of two body tissues (bull sharks: muscle and blood; tiger
sharks: fin and blood), and 4) calculation of variance for each individual using isotope p-values of three body tissues (bull sharks: muscle, blood, and plasma; tiger sharks: fin, blood, and plasma). In addition, we calculated individual specialization metrics using IndSpec (Bolnick et al. 2002) to supplement our novel analytical framework.

Two-tissue GLM

The mean sum of squares of the two-tissue model ($E(\text{SS}_B)$), which is defined as

$$E(\text{SS}_B) = \frac{n \sum_{i=1}^{m} (\bar{Y}_i - \bar{Y})^2}{n(n-1)} \quad (1)$$

measures the variability between individuals (a proxy for the between individual component of variation – BIC), where $m$ is the total number of individuals, $i$ is any individual, and $n$ is the total number of tissues. The mean sum of squares of the error ($E(\text{SS}_W)$), where

$$E(\text{SS}_W) = \frac{\sum_{i=1}^{m} \sum_{j=1}^{n} (Y_{ij} - \bar{Y}_i)^2}{n(m-1)} \quad (2)$$

measures the variability within individuals (a proxy for the within individual component of variation – WIC), where $j$ is any tissue. The resulting F-ratio ($E(\text{SS}_B):E(\text{SS}_W)$) is a proxy for individual specialization within the population (a proxy of BIC:WIC). As the variation between individuals increases (i.e. BIC increases), and/or the variation within individuals decreases (i.e. WIC decreases), the ratio, and therefore relative degree of individual specialization, increases (Bolnick et al. 2003).

Three-tissue GLM
Employing plasma with muscle and blood (bull sharks), and fin and blood (tiger sharks), is a more rigorous test of specialization because of the rapidity with which plasma turns over. If the relatively short term isotope values of plasma are consistent with those of the slower turnover tissues, this provides additional evidence for specialization within a population. GLMs were conducted as described above, but with three tissue types employed.

Two-tissue individual variance

A GLM produces values that can be used to assess the relative specialization of a population, and these can be compared between populations. Yet, the two- and three-tissue GLMs do not provide a way to make multiple pair-wise comparisons among individuals within a population, and assess the frequency of individuals that are more or less specialized. To this end, variance of p-values for bull sharks (muscle and blood) and for tiger sharks (fin and blood) was calculated for each individual separately, using

$$Var(X) = E[(X - \mu)^2]$$ (3)

resulting in a WIC value for each individual in the two populations. The population BIC (estimated from the two-tissue random effects models) was then divided by each individual WIC, yielding a relative specialization value for each individual shark. Higher values of this index suggest a greater degree of dietary specialization, i.e., the different tissue types had more similar isotope p-values. Analysis of variance (ANOVA) was run to determine if specialization index values were related to body size (total length in cm), body condition (based on residual of length-mass relationship; only bull sharks), sex, capture season, or capture year.

Three-tissue individual variance
Similar to the three-tissue GLM, plasma was included in the individual variance analyses as a more rigorous test of individual specialization. Calculations were carried out in the same fashion as the two-tissue individual variance analysis. ANOVA was run to test the significance of the same factors (body size, condition, sex, season, and year) on specialization.

IndSpec

IndSpec is a program developed to calculate the specialization parameters described by Bolnick et al. (2002) from diet data. The program calculates the variability between each isotope value and relates this to individuals within the population using

\[ TNW = \text{Var}(x_{ij}) \]  
\[ WIC = E[\text{Var}(x_j|i)] \]  
\[ BIC = \text{Var}[E(x_j|L)] \]

where \( x \) is the diet parameter (\( \delta^{13}C \) value for our study), \( j \) is tissue type, and \( i \) is individual.

Results

Characterization of food webs

The \( \delta^{13}C \) values of primary producers and consumers of the Shark River Estuary (freshwater/estuarine food web) differed substantially from those found in the marine food web (Fig. 3a). Resident consumers’ mean \( \delta^{13}C \) values from the freshwater/estuarine food web were always lower than -25‰, and usually lower than -28‰. In contrast, consumers feeding in marine habitats had \( \delta^{13}C \) values between -11‰ and -19‰. Although consumers with intermediate \( \delta^{13}C \) values (-19‰ to -25‰) are found in the Shark River Estuary (e.g. snook, Centropomus undecimalis, \( \delta^{13}C \) range = -18.9‰ to -27.3‰, M. Heithaus unpublished data), a
review of the literature and our own sampling suggests that these consumers are relatively uncommon, and they tend to have δ¹³C values relatively close to freshwater/estuarine consumers (e.g. snook mean δ¹³C = -25.0‰ ± 0.6SE, M. Heithaus unpublished data). Several species are found in both the freshwater/estuarine and marine habitats, and often have a δ¹³C value of the habitat where they were captured (e.g. blue crabs, Callinectes sapidus, Florida Bay δ¹³C = -14.3‰; Shark River mid-estuary δ¹³C = -27.8‰ ± 0.3SE; gray snapper, Lutjanus griseus, Florida Bay δ¹³C = -13.4‰ ± 1.2SE; Shark River δ¹³C = -28.4‰ ± 0.4SE; Chaser et al. 2005; C. McIvor et al. personal communication).

Consumers of Shark Bay, Australia were not as separated in δ¹³C values as consumers in the Shark River, but there were still distinctions between taxa of the benthic and pelagic food webs (Fig. 3b). Primary consumers from the pelagic food web had δ¹³C values lower than -16‰, while those from the benthic food web had δ¹³C values higher than -10‰. Unlike the Shark River ecosystem, consumers with intermediate values were common in Shark Bay. As the trophic position (inferred by δ¹⁵N value) of taxa increased, taxa mean δ¹³C values converged toward intermediate values.

General isotope trends in sharks

Overall, we sampled 71 bull sharks (100-187cm TL) in the Shark River Estuary and 46 tiger sharks (160-396 cm TL) in Shark Bay, Australia. The mean δ¹³C of bull sharks were: -22.8‰ ± 0.4SE (muscle), -22.9‰ ± 0.4SE (whole blood), and -21.5‰ ± 0.7SE (plasma). Mean δ¹³C values, however, masked considerable variability, i.e. a δ¹³C range of 12.7‰, which was 60% of the δ¹³C range of producers and consumers in the Shark River Estuary and surrounding coastal waters of Florida Bay (~22‰). Nineteen bull sharks fell within the range of isotope
values for those taxa identified in the freshwater/estuarine food web and eight fell within values of the marine food web; the rest (N= 44) had δ^{13}C values that fell between these two food webs (Fig. 3a). Tiger sharks had a narrower δ^{13}C range (5.9‰) that was 42% of the entire δ^{13}C range of producers and consumers in Shark Bay (14‰), and all individuals, except for one, had δ^{13}C values that were intermediate between mean values of species in the benthic and pelagic food webs (Fig. 3b).

Patterns of individual specialization

Two-tissue GLMs based on muscle and blood (bull sharks), and fin and blood (tiger sharks) revealed that bull sharks tended to be more specialized and tiger sharks tended to be more generalized in their respective diets (Fig. 4a). Within-individual variation (WIC) of bull sharks (0.003) was considerably lower than that of tiger sharks (0.021), while between individual variation (BIC) was greater in bull sharks (0.08) than tiger sharks (0.06). The specialization index for tiger sharks was relatively low (2.84), suggesting that individuals were more generalized in their diet. In contrast, the specialization index was much greater for bull sharks (23.7; Fig. 4a), indicating individuals were more specialized in their diet.

Three-tissue GLM based on muscle, blood, and plasma (bull sharks), and fin, blood, and plasma (tiger sharks) strengthened trends found in the two-tissue random effects models (Fig. 4a). For individuals with more specialized diets, the value of the three-tissue specialization index should be comparable, or increase, relative to that based on two tissues, because short-term and long-term dietary variation should be similar when diets are temporally stable. In contrast, for generalists, variation within individuals (WIC) should be greater on average when including fast turnover tissues, and therefore should result in lower specialization values for analyses based
on three tissues than those based on two tissues. Consistent with these predictions, when plasma was included with fin, muscle, and blood, the specialization index was considerably greater than that of the two-tissue analysis for bull sharks (42.5). And, while the specialization index increased for tiger sharks (4.37), suggestive of a more specialized diet, it was still considerably smaller than that of the bull sharks quantified using the two and three tissue models, and tiger shark WIC was greater for the three-tissue analysis (0.06), indicating tiger sharks are more generalized.

IndSpec revealed very similar patterns in the dietary specialization of bull sharks and tiger sharks (Fig. 4b) when compared to the GLMs (Fig. 4a). WIC was considerably lower for bull sharks (0.002 for both the two- and three-tissue analyses) than for tiger sharks (0.01 and 0.02 for the two- and three-tissue analyses, respectively), and BIC was comparable for the two species (0.04 for both bull shark analyses, and 0.03 and 0.04 for the tiger shark two- and three-tissue data sets, respectively). The specialization index values for bull sharks (23.4 and 19.8 for the two- and three-tissue data sets, respectively) were considerably higher than those for tiger sharks (2.8 and 2.1 for the two- and three-tissue data sets, respectively).

The two-tissue individual variance analysis revealed similar trends. A large proportion of bull shark individuals had relatively high specialization indexes (92% had specialization index values greater than ten), while most tiger shark individuals had relatively low specialization index values (74% had a specialization index less than ten; Fig. 5a). The distribution of sharks falling into each range of specialization values was significantly different for tiger sharks and bull sharks (Kolmogorov-Smirnov test: $T_{\text{samples}} = 0.96; p < 0.01$). Similarly, the three-tissue individual variance analysis showed that tiger sharks were less specialized than bull sharks (Kolmogorov-Smirnov test: $T_{\text{samples}} = 1; p < 0.01$; Fig. 5b). In this analysis, more than 71% of
tiger sharks had specialization values below ten, while all bull sharks had specialization values above ten. Specialization index values were not directly related to shark body size, body condition, sex, season, or year (Table 1; Fig. 6).

Discussion

Variation in trophic specialization

Individual-level specialization is relatively widespread, and can be an important factor driving population-level trophic dynamics (Bolnick et al. 2003). Yet, with the exception of foraging behavior in marine mammals (e.g. Orcinus orca: Williams et al. 2004; Arctocephalus gazella and Arctocephalus tropicalis: Cherel et al. 2007; Tursiops aduncus: Mann et al. 2008; Enhydra lutris nereis: Newsome et al. 2009) and marine birds (e.g. Phalacrocorax albiventer: Kato et al. 2000; five penguin species: Cherel et al. 2007; Uria lomvia: Woo et al. 2008), individual specialization has been largely overlooked in marine systems, and the implications of specialization on food web dynamics has not been adequately investigated. Because of the important role sharks can play in ecosystems (see Heithaus et al. 2008; 2010), it is especially important to elucidate patterns of individual specialization in this group of elasmobranch fishes, and the implications this may have for food web structure and ecosystem function.

The two species of sharks studied here are widely considered to be generalist top predators in their respective ecosystems (e.g. Simpfendorfer et al. 2001; Weatherbee & Cortes 2004), but both the GLMs and IndSpec revealed considerable differences in the patterns of trophic specialization between them. Tiger sharks apparently were relatively generalized in their diets. Values of δ¹³C over multiple time scales revealed that there was considerable variation in the diet of tiger sharks over time, indicating that individuals have relatively unspecialized diets.
In contrast, bull sharks showed temporal stability in their diets, and most individuals were relatively specialized despite having a broad isotopic niche width at the population level. Therefore, although both species are “generalists” at the population level, they differ considerably at the individual level.

Often, individual specialization can be documented by observing the behavior of particular individuals over time. But for sharks and many other upper trophic level marine predators, this is not possible. Analyzing stable isotopic signatures of multiple tissues with differing rates of turnover is a powerful tool for assessing individual specialization when an individual can only be sampled once (e.g. Bearhop et al. 2004; Quevedo et al. 2009; Jaeger et al. 2010). Because analytical techniques for determining specialization patterns using isotope data from tissues with different turnover rates are still being developed, we used two methods to assess specialization: GLMs and the computer program IndSpec. Despite differences in output, both analytical frameworks produced the same trends in individual dietary specialization, or lack thereof, for bull sharks and tiger sharks – bull sharks are relatively more specialized than tiger sharks.

Body condition (reviewed by Vanderklift & Ponsard 2003) and the presence of lipids (Post et al. 2007) can be important factors to consider when interpreting isotopic values. Neither of these factors though, appeared to likely confound the results in our study. First, body condition tends to affect δ¹⁵N more than δ¹³C (e.g. Hobson, Alisauskas & Clark 1993; Kurle and Worthy 2001; Polischuk, Hobson & Ramsay 2001), and there was no affect of body condition on δ¹³C of bull sharks (body condition data were not available for tiger sharks). Lipid content of tissues also is likely to have little effect on our results. Although sharks store lipids in muscle and liver tissues (Bone 1999; Remme et al. 2006), the mean C:N ratio of bull shark muscle tissue
was low with little variation (3.1 ± 0.3 SD), suggesting lipid content resulted in minimal variation in muscle δ¹³C between individuals (i.e. little effect on BIC). Mean C:N ratios of fin (2.7 ± 0.1 SD; tiger sharks), whole blood (2.7 ± 0.2 SD, 2.4 ± 0.0 SD), and plasma (2.0 ± 0.2 SD, 2.1 ± 0.1 SD) were also low for bull sharks and tiger sharks, respectively.

Shifts in metabolic activity in response to variation in abiotic conditions (e.g. temperature) can modify isotope discrimination and routing, and lead to variability in δ¹³C values (reviewed by Kelly 2000). However, this likely did not affect the interpretation of the results from this study. Seasonal variation in water temperature occurs in the Shark River Estuary and Shark Bay, Australia, but they occur over a similar range (Shark River: 15-33°C; Heithaus et al. 2009; Shark Bay: 13-28°C; Wirsing, Heithaus & Dill 2007). Therefore, it seems unlikely that the differing patterns of specialization we observed can be attributed to differential effects of temperature on isotopic routing and discrimination.

Interpretation of isotope values can also be affected by whether tissues are in dietary equilibrium (reviewed by Martinez del Rio et al. 2009b), which may be influenced by seasonal changes in diets or prey switching within the timescale of a tissue’s turnover (e.g. Matich et al. 2010). It is quite possible that tissues – especially those with longer turnover times – are not in equilibrium (at least for larger tiger sharks). The possibility of non-equilibrium of tissues in tiger sharks and some bull sharks, however, does not confound our basic findings of interspecific differences in individual specialization, because we are explicitly making use of differential rates of change in signatures of various tissues and do not need to assume that they are in equilibrium.

Finally, knowing the timeframe over which isotopic values are incorporated into tissues is important for determining the timescale over which specialization is measured using our methods. Isotopic turnover rates generally decrease with increasing body size (Martinez del Rio
et al. 2009b), at a rate of $x^{-0.25}$ (Carleton & Martinez del Rio 2005). For slow-growing species like elasmobranchs, this relationship however, may overestimate differences in turnover rates. For example, freshwater stingrays (*Potamotrygon motoro*) and sandbar sharks (*Carcharhinus plumbeus*) had comparable turnover rates (muscle 422 days and 390-540 days, respectively; blood 265 days and 240-300 days, respectively) despite an order of magnitude difference in body mass (0.1kg and 6.4kg; MacNeil et al. 2006; Logan & Lutcavage 2010). Previous studies of bull sharks (Matich et al. 2010) suggest that turnover rates of muscle and whole blood of bull sharks in the Shark River are similar to laboratory estimates for freshwater stingrays, leopard sharks, and sandbar sharks, and body size differences may not result in major changes in isotopic turnover rates in this group of fishes. Nonetheless, if the standard scaling relationships apply, then tiger sharks should exhibit complete turnover times on the order of ~230 days for blood plasma, ~720 days for whole blood, and ~1500 days for fin (back-calculations based on turnover times of sandbar sharks; Logan & Lutcavage 2010; and regression model from Carleton & Martinex del Rio 2005). It is important to note, that if tiger sharks exhibit these longer turnover times, it would be expected to result in patterns of specialization that are opposite to those we found. Because the faster turnover tissues (i.e. plasma, whole blood) would incorporate diets over longer time frames, short-term variation in diets would not be reflected to the same degree and WIC should be smaller than in species with faster turnover rates.

**Drivers of specialization and implications**

Our results suggest that individual dietary specialization in elasmobranchs, and resulting community trophodynamics, is context dependent. Differences in resource availability and distribution as well as intraspecific competition, between the Shark River Estuary and Shark Bay
ecosystems suggest that density-dependence may be an important factor affecting individual
trophic specialization in sharks (see Estes et al. 2003; Svanback & Persson 2004; Tinker, Bentall
& Estes 2008 for non-shark examples). Density-dependence generally occurs in early life-stages
of sharks, including in nursery habitats like the Shark River Estuary, where population sizes are
relatively large with respect to resource availability (see Heithaus 2007; Heupel, Carlson &
Simpfendorfer 2007 for reviews). Conditions of resource scarcity can lead to specialization in
trophic niches, because individuals exploiting a narrow range of resources can be more efficient
than those exploiting more diverse resources (Bolnick et al. 2003). For example, sea otters
(Enhydra lutris nereis) foraging in resource-limited habitats of the central Californian coastline
were relatively specialized in their diets (Tinker et al. 2008) compared to more generalized sea
otters along the Washington coastline where diverse food sources were readily available (Laidre
& Jameson 2006). Resources for tiger sharks are relatively abundant in Shark Bay (Heithaus et
al. 2002), likely leading to lower levels of competition, which may result in individual tiger
sharks being relatively unspecialized in their diets. In comparison, the oligotrophic nature of the
Shark River Estuary leads to low aquatic productivity and limited resource availability in much
of the system (Childers 2006). Such resource limitation is likely a driver of the individual
trophic specialization found in the bull shark population.

In ecosystems with multiple potential energetic pathways, the spatial arrangement of
discrete food webs may also be an important factor determining levels of individual
specialization. Ecosystems with discrete food webs that have a high degree of geographical
overlap are more likely to support generalist individuals, because individuals can readily exploit
resources from both food webs without significant movements between resource patches (e.g.
Miller, Karnovsky & Trivelpiece 2009; Montevecchi et al. 2009). When food webs are spatially
distinct with little or no geographic overlap, however, individual dietary specialization may be relatively widespread across a population (e.g. Darimont et al. 2009; Quevedo et al. 2009). In Shark Bay, both pelagic and benthic food webs overlap spatially, providing tiger sharks with access to each food web within the same habitat. In contrast, within the Shark River Estuary the marine and freshwater/estuarine food webs are spatially distinct. Specialization would be expected if sharks segregated into individuals that strictly resided in marine waters and those that stayed within the estuary. However, the bull sharks used for this study were all captured within the estuary, suggesting they move between habitats that encompass each food web.

Mean population $\delta^{13}$C values suggest bull shark individuals derive carbon from multiple food webs, but the $\delta^{13}$C values and specialization index values indicated that many individuals specialized in feeding from the marine food web despite being captured within the estuary. Indeed, taxa representing the marine food web are found more than 30 km from the capture locations of some sharks that had enriched $\delta^{13}$C, suggesting that they had moved long distances to feed. Movement data from bull sharks in the Shark River suggest that some individuals do move from the estuary into marine waters and back (P. Matich unpublished data) and further work will be needed to link individual behavior with patterns of specialization. Why wouldn’t all sharks move into, or remain in, the potentially more resource-rich marine food web?

Although the juvenile bull sharks in our study are among the largest-bodied predators in the Shark River Estuary, larger sharks that can prey upon these juvenile sharks inhabit the furthest areas downstream in the marine food web where risk is greatest for juvenile sharks (P. Matich unpublished data). Therefore, in addition to the trade-off between opportunistic feeding and foraging efficiency that favors specialization in resource-poor environments (Bolnick 2004), specialization in the bull sharks of the Shark River Estuary may also occur because of a trade-off
between foraging opportunities and the risk of predation. These data support the view that behavioral and dietary specialization may be closely linked (Bolnick *et al.* 2007).

Individual specialization affects trophic dynamics, and previous studies suggest it may prevent resources of spatially distinct food webs from being coupled by individual predators (e.g. Quevedo *et al.* 2009). However, highly mobile predators, like sharks, have the potential to forage at a great distance from sites where they spend considerable amounts of time, and may serve to couple ecosystems through this trophic role. Isotope values suggest that some bull sharks move into and out of the system, which may enable them to feed on taxa from both the marine and freshwater/estuarine food webs, and would likely result in low specialization index values based on a generalized diet. Yet, $\delta^{13}$C values indicated that these individuals were dietary specialists and fed primarily in the marine food web located outside of the nursery in which they were captured. These specialized individuals may serve as important links in the connectivity of multiple food webs through a bottom-up mechanism of nutrient transport to the oligotrophic upper reaches of the Everglades (Polis, Anderson & Holt 1997), while tiger sharks of Shark Bay may serve a more traditional role of a generalist top predator that couples discrete food webs (Rooney *et al.* 2006). Food web structure and dynamics may be more complex in ecosystems with specialist top predators, and a “species-level” approach to conservation and management may be over simplistic in such situations. Therefore, studies of foraging ecology of highly mobile marine predators should explicitly consider the possibility of individual specialization. The use of stable isotopes sampled from multiple tissues would allow such studies to be conducted non-lethally and/or alongside traditional diet studies employing stomach contents analysis.
In summary, our study suggests that individual specialization can occur in non-mammalian marine top predators, but is not ubiquitous. Factors including resource availability, competition, food-predation risk trade-offs, and spatial overlap of food webs may contribute to the observed levels of specialization. Future studies should explore the level of individual dietary specialization that occurs within other shark populations, as well as other highly mobile apex predators, and the potential effects this may have on ecosystem processes. Studies that investigate the mechanisms by which among individual specialization is manifest in highly mobile predators, the heritability or drivers of this variation in trophic niches, and the effects specialization has on the trophic dynamics within and across ecosystems will be particularly important for future conservation efforts, especially in light of widespread top predator declines in marine ecosystems.

Acknowledgements

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International University IACUC approval. This is publication 44 of the Shark Bay Ecosystem Research Project.

References


spatula), in Lake Pontchartrain, an oligohaline estuary in southeastern Louisiana.


Table 1. ANOVA results for effects of size, sex, season, year, and condition on dietary specialization in bull sharks and tiger sharks. NA: sample sizes not adequate for tests.

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Figure Legends

Figure 1. Coastal habitats of south Florida (a) can be divided into marine (1) and freshwater/estuarine (2) food webs (b). Juvenile bull sharks were sampled in the Shark River Estuary (c), which is within the freshwater/estuarine food web.

Figure 2: Estimated turnover rates (± SE) of body tissues from freshwater stingray (*Potamotrygon motoro*: MacNeil *et al.* 2006 – fin, muscle, and blood) and leopard shark (*Triakis semifasciata*: Sora Kim personal communication – plasma). These turnover rates are from controlled studies using relatively small individuals, which are comparable in size to the bull sharks in this study. Tiger sharks in Shark Bay are considerably larger, and therefore turnover rates may be slower (see Discussion).

Figure 3: (A) Mean isotope values for producers and consumers in the Shark River Estuary (freshwater/estuarine food web) and surrounding marine waters. Producers and consumers from the freshwater/estuarine food web are gray and those from the marine food web are white. Producers are diamonds (♦), molluscs are triangles (▲), crustaceans are squares (■), teleosts are circles (●), other elasmobranchs are crosses (+), and bull sharks (whole blood) are X’s. (B) Mean isotope values for producers and consumers in Shark Bay. Producers and consumers from the pelagic food web are gray and those from the benthic food web are white. Producers are diamonds (♦), molluscs are triangles (▲), other invertebrates are squares (■), megagrazers (i.e. dugongs and turtles) are circles (●), other elasmobranchs are crosses (+), and tiger sharks (whole blood) are X’s. Standard deviations around mean values are omitted for simplicity.
Figure 4: a) Specialization indices of bull sharks and tiger sharks based on isotope p-values derived from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks); b) specialization comparison between bull sharks and tiger sharks using isotope p-values from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks), derived from IndSpec.

Figure 5: a) Frequency of tiger sharks and bull sharks within each range of specialization values calculated from the a) two-tissue and b) three-tissue individual variance analyses. Higher specialization index values indicates greater dietary specialization.

Figure 6: Individual specialization index values based on two- (♦) and three-tissue (■) GLMs of a) bull sharks and b) tiger sharks in relation to shark total length (cm). Mean individual specialization index values (± S.D.) based in two- and three-tissue GLMs of bull sharks separated by c) sex, e) capture season, g) capture year, and i) body condition, and mean individual specialization index values of tiger sharks separated by d) sex, f) capture season, and h) capture year.
Figure 1
Figure 2
Figure 3

(a) Coastal Everglades, FL

(b) Shark Bay, WA