

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

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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 behaviours 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

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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 (i) resource scarcity, (ii) interhabitat differences in resource availability, (iii) fitness trade-offs that result in individual-specific behaviour, (iv) cultural transmission of foraging traditions and/or (v) 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, Svanback & Eklov 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).

Materials and 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 to 2009 on *c.* 500 m longlines fitted with 40–55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to *c.* 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 *c.* 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 1 min at 3000 r.p.m. 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

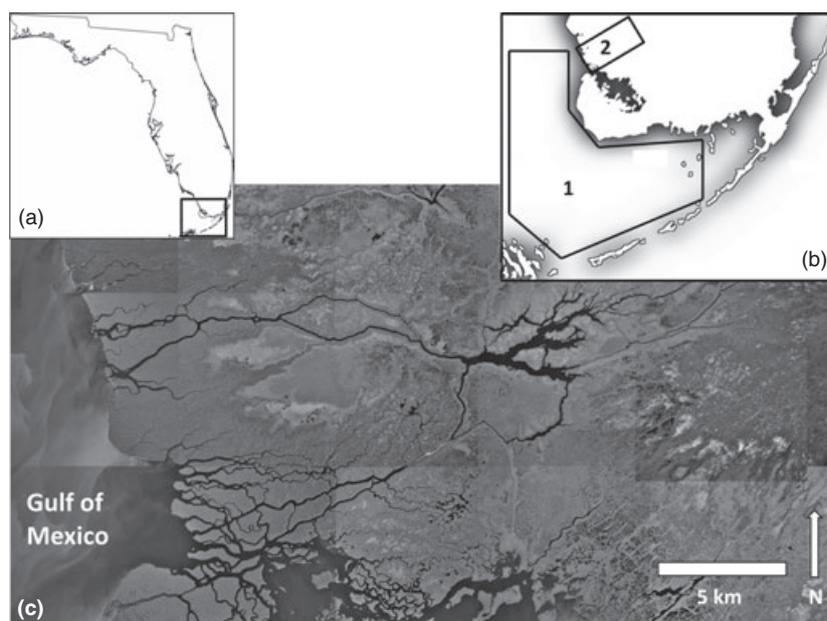


Fig. 1. Coastal habitats of south Florida (a) can be divided into marine (1) and freshwater/estuarine (2) food webs (b). Juvenile bull sharks were sampled in the Shark River Estuary (c), which is within the freshwater/estuarine food web.

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 99 cm in total length (c. 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 $\delta^{13}\text{C}$ that were representative of two 'endpoint' habitats: (i) 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 (ii) 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 (< 4 m 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 > 2 m are tiger sharks (Heithaus 2001; Wirsing, Heithaus & Dill 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 to 2009 on drumlines equipped with a single Mustad shark hook (12/0–14/0) (see Wirsing, Heithaus & Dill 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 cm² 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 $\delta^{13}\text{C}$ that were representative of two discrete food webs to establish the ecosystem context of trophic interactions in Shark Bay: (i) 'benthic food web' (likely based on seagrass and associated microphytobenthos) and (ii) 'pelagic food web', which would be expected to be based on autochthonous seston production.

STABLE ISOTOPE ANALYSIS

All shark samples were analysed 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 ± 0.3SD, mean blood = 2.7 ± 0.2SD, mean plasma = 2.0 ± 0.2SD; tiger shark mean fin = 2.7 ± 0.1SD, mean

blood = 2.4 ± 0.0SD, mean plasma = 2.1 ± 0.1SD) 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 $\delta^{13}\text{C}$ values of shark muscle (Hussey *et al.* 2010). Samples from producers and invertebrates with carbonate shells were acidified for $\delta^{13}\text{C}$ values ($\delta^{15}\text{N}$ run separately). Producer and community consumer samples were analysed at Yale Earth System Center for Stable Isotopic Studies.

To verify analytical consistency, we randomly selected samples to be analysed at both Florida International University and Yale University, for which the variation between resulting $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ values were 0.13‰ ± 0.20SE. The standard deviations of standards run for Yale were 0.14‰ for $\delta^{13}\text{C}$ and 0.22‰ for $\delta^{15}\text{N}$, and 0.29‰ for $\delta^{13}\text{C}$ and 0.24‰ for $\delta^{15}\text{N}$ 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 niche width (Bolnick *et al.* 2003). The 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 (i) stable $\delta^{13}\text{C}$ 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 (ii) 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 and 576 days) and whole blood has an intermediate turnover rate (complete isotope turnover in 240–300 days; MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Matich, Heithaus & Layman 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 = 0.1 kg; *Carcharhinus plumbeus*: mean mass = 6.4 kg), and isotope turnover rates can vary with body size (e.g. Carleton & Martinex del Rio 2005, Martinez del

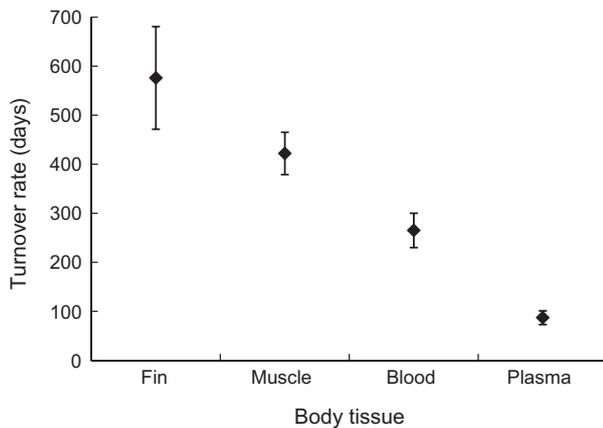


Fig. 2. Estimated turnover rates (\pm SE) of body tissues from freshwater stingray (*Potamotrygon motoro*: MacNeil, Drouillard and Fisk 2006 – fin, muscle, and blood) and leopard shark (*Triakis semifasciata*: Sora Kim personal communication – plasma). These turnover rates are from controlled studies 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).

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, Heithaus & Layman 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 $\delta^{13}\text{C}$ values). Therefore, to normalize isotope data for bull sharks and tiger sharks, we converted $\delta^{13}\text{C}$ values for tissues to proportional values (P -values; Newsome *et al.* 2007). Each system has two discrete basal resource pools with distinct $\delta^{13}\text{C}$ values: the Shark River has a 'freshwater/estuarine food web' (mean $\delta^{13}\text{C} = -29.7\text{‰} \pm 0.7\text{SE}$) and a 'marine food web' ($-14.5\text{‰} \pm 0.3\text{SE}$), and Shark Bay has a 'benthic food web' ($-8.5\text{‰} \pm 0.3\text{SE}$) and a 'pelagic food web' ($-16.1\text{‰} \pm 0.8\text{SE}$). Therefore, ' P -values' were calculated based on mean $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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): (i) 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, (ii) 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, (iii) 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 (iv) 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

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

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

$$E(\text{SS}_W) = \frac{\left[\sum_{i=1}^m \sum_{j=1}^n (Y_{ij} - Y_i)^2 \right]}{m(n-1)} \quad \text{eqn 2}$$

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

$$\text{Var}(X) = E[(X - \mu)^2] \quad \text{eqn 3}$$

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

$$\text{TNW} = \text{Var}(X_{ij}) \quad \text{eqn 4}$$

$$\text{WIC} = E[\text{Var}(x_j|i)] \quad \text{eqn 5}$$

$$\text{BIC} = \text{Var}[E(x_j|i)] \quad \text{eqn 6}$$

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

Results

CHARACTERIZATION OF FOOD WEBS

The $\delta^{13}\text{C}$ values of primary producers and consumers of the Shark River Estuary (freshwater/estuarine food web) dif-

fered substantially from those found in the marine food web (Fig. 3a). Resident consumers' mean $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values between -11‰ and -19‰ . Although consumers with intermediate $\delta^{13}\text{C}$ values (-19‰ to -25‰) are found in the Shark River Estuary (e.g. snook, *Centropomus undecimalis*, $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values relatively close to freshwater/estuarine consumers (e.g. snook mean $\delta^{13}\text{C}$ = $-25.0\text{‰} \pm 0.6\text{SE}$; M. Heithaus, unpublished data). Several species are found in both the freshwater/estuarine and marine habitats, and often have a $\delta^{13}\text{C}$ value of the habitat where they were captured (e.g. blue crabs, *Callinectes sapidus*, Florida Bay $\delta^{13}\text{C}$ = -14.3‰ ; Shark River mid-estuary $\delta^{13}\text{C}$ = $-27.8\text{‰} \pm 0.3\text{SE}$; gray snapper, *Lutjanus griseus*, Florida Bay $\delta^{13}\text{C}$ = $-13.4\text{‰} \pm 1.2\text{SE}$; Shark River $\delta^{13}\text{C}$ = $-28.4\text{‰} \pm 0.4\text{SE}$; Chasar *et al.* 2005; C. McIvor *et al.*, personal communication).

Consumers of Shark Bay, Australia were not as separated in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values lower than -16‰ , while those from

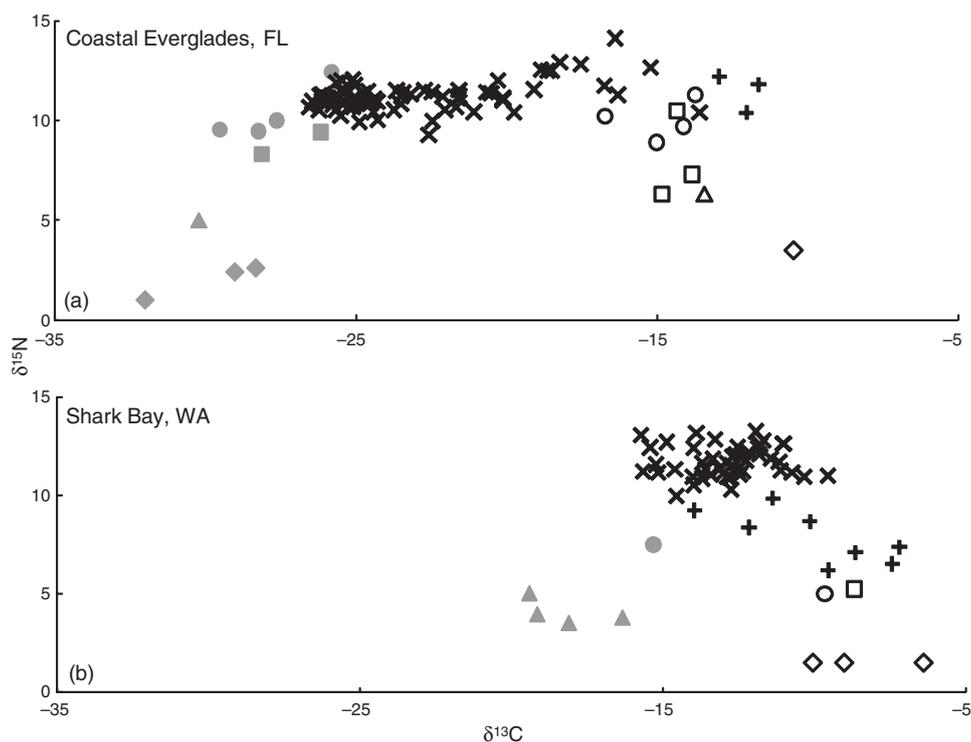


Fig. 3. (a) Mean isotope values for producers and consumers in the Shark River Estuary (freshwater/estuarine food web) and surrounding marine waters. Producers and consumers from the freshwater/estuarine food web are gray and those from the marine food web are white. Producers are diamonds (\blacklozenge), molluscs are triangles (\blacktriangle), crustaceans are squares (\blacksquare), teleosts are circles (\bullet), 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 (\blacklozenge), molluscs are triangles (\blacktriangle), other invertebrates are squares (\blacksquare), megagrassers (i.e. dugongs and turtles) are circles (\bullet), other elasmobranchs are crosses ($+$), and tiger sharks (whole blood) are X's. Standard deviations around mean values are omitted for simplicity.

the benthic food web had $\delta^{13}\text{C}$ values higher than -10‰ . Unlike the Shark River ecosystem, consumers with intermediate values were common in Shark Bay. As the trophic position (inferred by $\delta^{15}\text{N}$ value) of taxa increased, taxa mean $\delta^{13}\text{C}$ values converged toward intermediate values.

GENERAL ISOTOPE TRENDS IN SHARKS

Overall, we sampled 71 bull sharks (100–187 cm TL) in the Shark River Estuary and 46 tiger sharks (160–396 cm TL) in Shark Bay, Australia. The mean $\delta^{13}\text{C}$ of bull sharks were: $-22.8\text{‰} \pm 0.4\text{SE}$ (muscle), $-22.9\text{‰} \pm 0.4\text{SE}$ (whole blood) and $-21.5\text{‰} \pm 0.7\text{SE}$ (plasma). Mean $\delta^{13}\text{C}$ values, however, masked considerable variability, i.e. a $\delta^{13}\text{C}$ range of 12.7‰ , which was 60% of the $\delta^{13}\text{C}$ range of producers and consumers in the Shark River Estuary and surrounding coastal waters of Florida Bay (*c.* 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 $\delta^{13}\text{C}$ values that fell between these two food webs (Fig. 3a). Tiger sharks had a narrower $\delta^{13}\text{C}$ range (5.9‰) that was 42% of the entire $\delta^{13}\text{C}$ range of producers and consumers in Shark Bay (14‰), and all individuals, except for one, had $\delta^{13}\text{C}$ 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

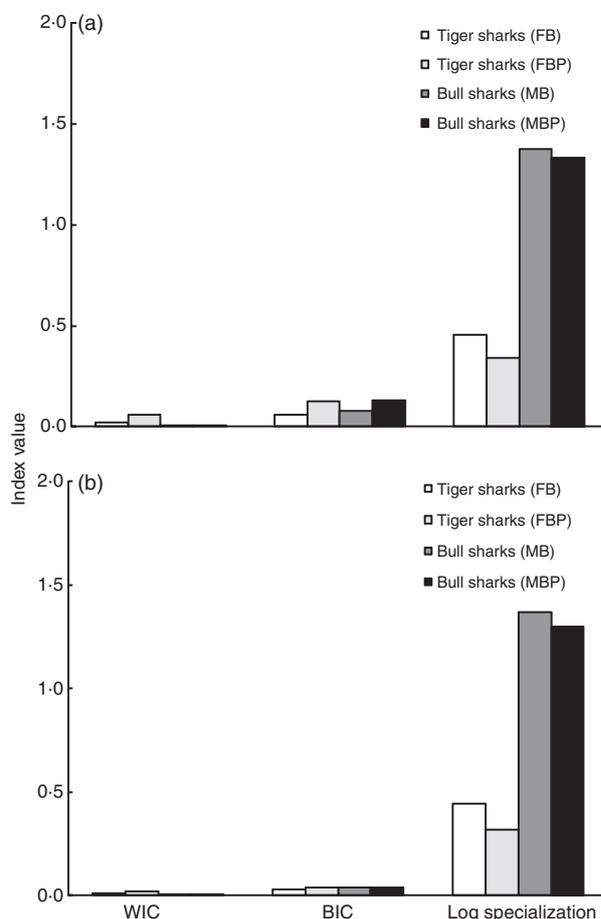


Fig. 4. (a) Specialization indices of bull sharks and tiger sharks based on isotope P -values derived from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks); (b) specialization comparison between bull sharks and tiger sharks using isotope P -values from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks), derived from IndSpec.

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 87% of 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 behaviour in marine mammals (e.g. *Orcinus orca*: Williams *et al.* 2004; *Arctocephalus gazella* and *Arctocephalus tropicalis*: Chérel *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: Chérel *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.

Table 1. ANOVA results for effects of size, sex, season, year, and condition on dietary specialization in bull sharks and tiger sharks

	Two-tissue ANOVA			Three-tissue ANOVA		
	<i>n</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>F</i>	<i>P</i>
Bull Sharks						
Total length	71	0.36	0.55	15	0.16	0.69
Sex	71	0.68	0.41	15	<0.01	0.99
Capture season	71	2.84	0.10	15	NA	NA
Capture year	71	0.21	0.89	15	NA	NA
Body condition	13	0.82	0.39	13	0.07	0.79
Tiger Sharks						
Total length	46	1.12	0.30	21	0.83	0.38
Sex	46	0.34	0.57	15	NA	NA
Capture season	46	1.28	0.28	21	0.83	0.57
Capture year	46	1.55	0.22	21	NA	NA

NA, sample sizes not adequate for tests.

The two species of sharks studied here are widely considered to be generalist top predators in their respective ecosystems (e.g. Simpfendorfer, Goodreid & McAuley 2001; Weatherbee & Cortes 2004), but both the GLMs and Ind-Spec revealed considerable differences in the patterns of trophic specialization between them. Tiger sharks apparently were relatively generalized in their diets. Values of $\delta^{13}\text{C}$ 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 behaviour of particular individuals over time. But for sharks and many other upper trophic level marine predators, this is not possible. Analysing stable isotopic signatures of multiple tissues with differing rates of turnover is a powerful tool for assessing individual specialization when

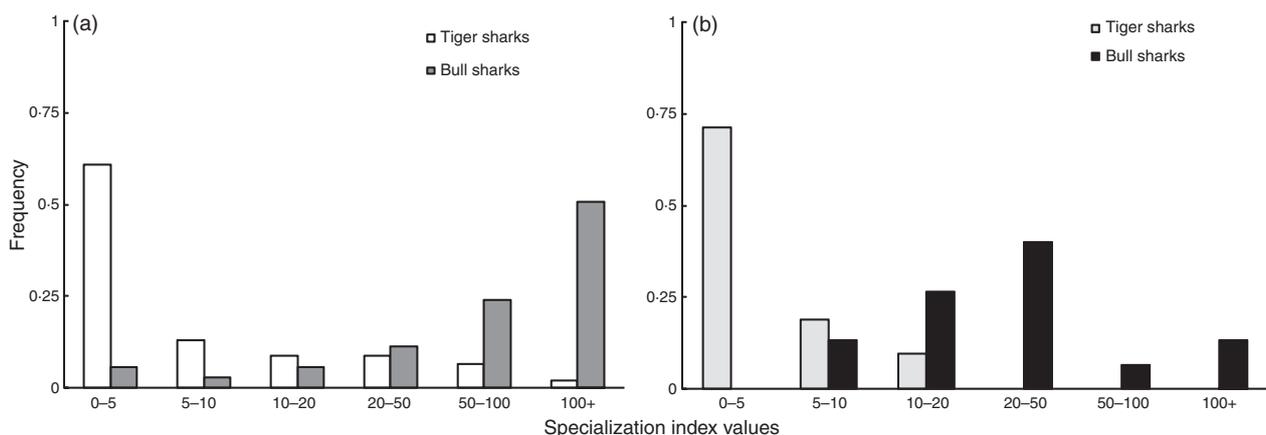


Fig. 5. (a) Frequency of tiger sharks and bull sharks within each range of specialization values calculated from the (a) two-tissue and (b) three-tissue individual variance analyses. Higher specialization index values indicates greater dietary specialization.

an individual can only be sampled once (e.g. Bearhop *et al.* 2004; Quevedo, Svanback & Eklov 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 $\delta^{15}\text{N}$ more than $\delta^{13}\text{C}$ (e.g. Hobson, Alisauskas & Clark 1993; Kurle & Worthy 2001; Polischuk, Hobson & Ramsay 2001), and there was no effect of body condition on $\delta^{13}\text{C}$ 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 ($3.1 \pm 0.3\text{SD}$), suggesting lipid content resulted in minimal variation in muscle $\delta^{13}\text{C}$ between individuals (i.e. little effect on BIC). Mean C : N ratios of fin ($2.7 \pm 0.1\text{SD}$; tiger sharks), whole blood ($2.7 \pm 0.2\text{SD}$, $2.4 \pm 0.0\text{SD}$) and plasma ($2.0 \pm 0.2\text{SD}$, $2.1 \pm 0.1\text{SD}$) 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 $\delta^{13}\text{C}$ 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, Heithaus & Layman 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 and 390–540 days, respectively; blood 265 and 240–300 days respectively) despite an order of magnitude difference in body mass (0.1 and 6.4 kg; MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010). Previous studies of bull sharks (Matich, Heithaus & Layman 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 *c.* 230 days for blood plasma, *c.* 720 days for whole blood, and *c.* 1500 days for fin (back-calculations based on turnover times of sandbar sharks; Logan & Lutcavage 2010; and regression model from Carleton & Martinez 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, Bentall & Estes 2008) compared to more generalized sea otters along the Washington coastline where diverse food sources were

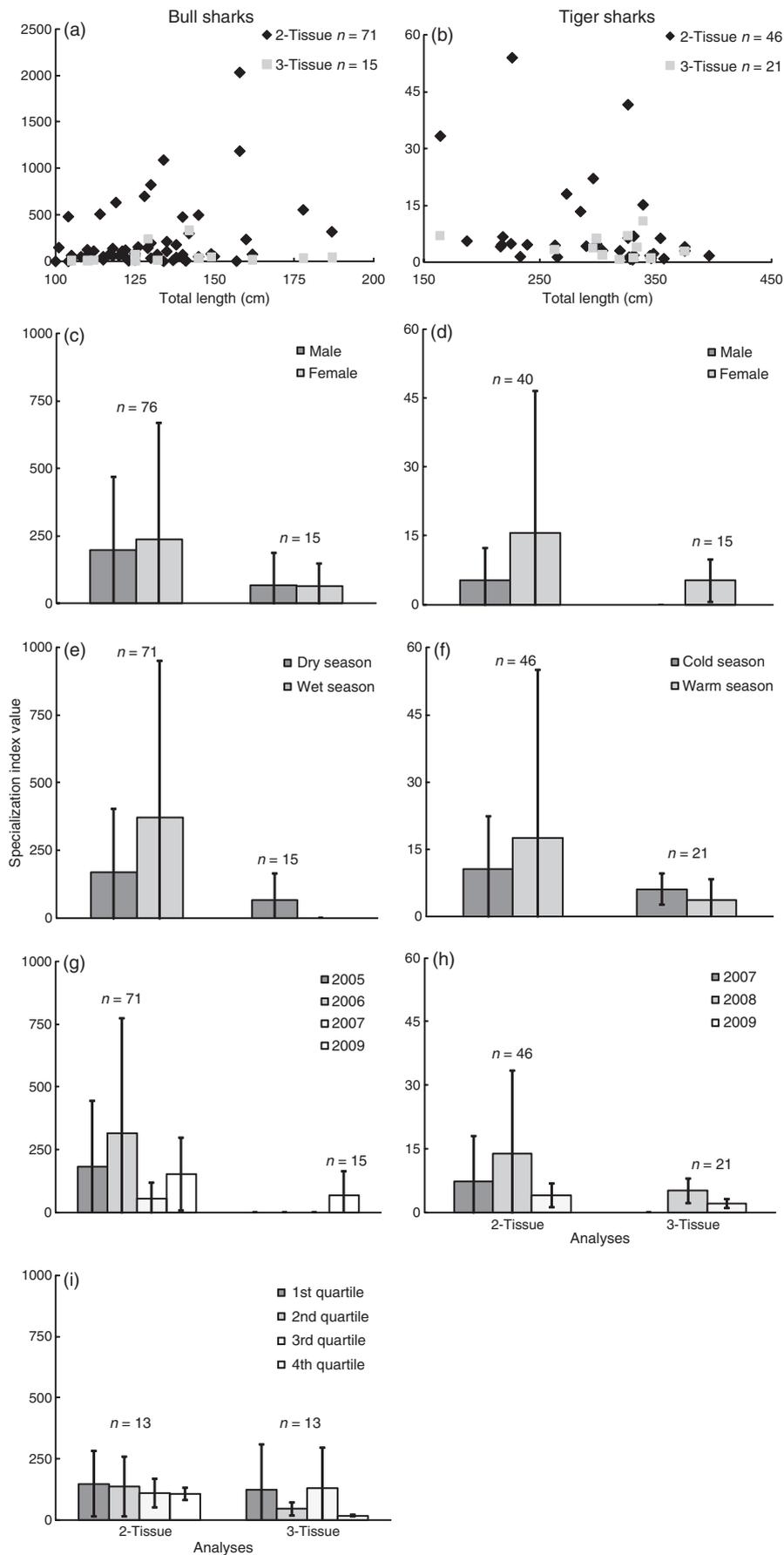


Fig. 6. Individual specialization index values based on two- (◆) and three-tissue (■) GLMs of (a) bull sharks and (b) tiger sharks in relation to shark total length (cm). Mean individual specialization index values (\pm SD) based in two- and three-tissue GLMs of bull sharks separated by (c) sex, (e) 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.

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, Paquet & Reimchen 2009; Quevedo, Svanback & Eklov 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 $\delta^{13}\text{C}$ values suggest bull shark individuals derive carbon from multiple food webs, but the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$, 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 behaviour 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 favours 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 behavioural 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, Svanback & Eklov 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, $\delta^{13}\text{C}$ 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.

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