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

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

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19 Running head: specialization in top marine predators

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23 Summary

- 24 1. Apex predators are often assumed to be dietary generalists and, by feeding on prey from
25 multiple basal nutrient sources, serve to couple discrete food webs. But there is increasing
26 evidence that individual level dietary specialization may be common in many species, and
27 this has not been investigated for many marine apex predators.
- 28 2. Because of their position at or near the top of many marine food webs, and the possibility
29 that they can affect populations of their prey and induce trophic cascades, it is important to
30 understand patterns of dietary specialization in shark populations.
- 31 3. Stable isotope values from body tissues with different turnover rates were used to quantify
32 patterns of individual specialization in two species of “generalist” sharks (bull sharks,
33 *Carcharhinus leucas*, and tiger sharks, *Galeocerdo cuvier*).
- 34 4. Despite wide population-level isotopic niche breadths in both species, isotopic values of
35 individual tiger sharks varied across tissues with different turnover rates. The population
36 niche breadth was explained mostly by variation within individuals suggesting tiger sharks
37 are true generalists. In contrast, isotope values of individual bull sharks were stable through
38 time and their wide population level niche breadth was explained by variation among
39 specialist individuals.
- 40 5. Relative resource abundance and spatial variation in food-predation risk tradeoffs may
41 explain the differences in patterns of specialization between shark species.
- 42 6. The differences in individual dietary specialization between tiger sharks and bull sharks
43 results in different functional roles in coupling or compartmentalizing distinct food webs.
- 44 7. Individual specialization may be an important feature of trophic dynamics of highly mobile
45 marine top predators and should be explicitly considered in studies of marine food webs and
46 the ecological role of top predators.

47

48 **Key Words:** estuary, food webs, foraging ecology, marine community dynamics, predator-prey

49 interactions, seagrass ecosystems, sharks, stable isotopes, trade-offs, trophic coupling

50 **Introduction**

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

59 In addition to top-down impacts on prey species, an important ecological function of
60 predators is the coupling of energy pathways from distinct food webs (Rooney *et al.* 2006). This
61 occurs when lower trophic level consumers derive their energy from a single source (i.e. primary
62 producer base), but at increasing trophic levels consumers tend to incorporate energy from a
63 wider range of prey serving to couple multiple energetic pathways (Rooney *et al.* 2006; Rooney,
64 McCann & Moore 2008). Such coupling is often evaluated at a population level, ignoring the
65 behaviors and habits of individuals. Populations of “generalist” predators may in fact be a
66 collection of individual-level trophic specialists that vary considerably in their resource use (e.g.
67 Urton & Hobson 2005; Woo *et al.* 2008). At a population level, predator species may
68 incorporate prey taxa from multiple food webs into their diets, but individual-level dietary
69 specialization may serve to keep energy pathways from discrete food webs separate. For
70 example, Eurasian perch (*Perca fluviatilis*), which have a wide niche width at the population
71 level, segregate into littoral and pelagic specialists, and consequently individuals do not couple
72 these two components of freshwater food webs (Quevedo, Svanback & Eklov 2009).

73 Individual specialization within populations may be more likely under conditions of 1)
74 resource scarcity, 2) interhabitat differences in resource availability, 3) fitness trade-offs that
75 result in individual-specific behavior, 4) cultural transmission of foraging traditions, and/or 5)
76 cognitive constraints that limit the use of diverse sets of resources (e.g. Rendell & Whitehead
77 2001; Estes *et al.* 2003; Svanback & Persson 2004; Araujo & Gonzaga 2007; Darimont, Paquet
78 & Reimchen 2009). Recent studies have investigated individual dietary specialization in birds
79 (e.g. Inger *et al.* 2006; Martinez del Rio *et al.* 2009a), mammals (e.g. Urton & Hobson 2005;
80 Newsome *et al.* 2009), and bony fishes (e.g. Beaudoin *et al.* 1999; Quevedo *et al.* 2009), but few
81 studies have considered individual specialization in large, non-mammalian, marine predators that
82 use multiple ecosystems.

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

92 **Methods**

93 *Coastal Everglades, Florida*

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

105 Bull sharks were captured from 2005-2009 on ~500m longlines fitted with 40-55 14/0 or
106 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to ~2m of 400kg
107 monofilament line (see Heithaus *et al.* 2009 for details). Captured sharks were processed
108 alongside the sampling vessel, or within a water-filled, aerated cooler on board. We used a
109 biopsy punch to collect a 0.5 cm³ muscle tissue biopsy *ca.* 5 cm lateral to the first dorsal fin, and
110 an 18 gauge needle to collect 4ml of blood from the caudal vein. From the blood, 3ml was
111 placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and
112 separated into its components, including plasma, using a centrifuge spun for one minute at
113 3000rpm. The remaining 1ml of blood was retained in its original composition (whole blood,
114 “blood” hereafter). Tissues were placed on ice and frozen upon return to the laboratory. Skin

115 was removed from muscle samples before laboratory preparations. Because muscle tissue of
116 sharks may incorporate isotopes from their diet over a temporal scale of many months (e.g.
117 MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Matich, Heithaus & Layman 2010;
118 S. Kim personal communication), only bull sharks over 99cm in total length (approximately 1-2
119 years old and older) were included in analyses to eliminate any potential maternal isotopic
120 influence.

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

131

132 *Shark Bay, Australia*

133 Shark Bay is a large, seagrass-dominated, subtropical bay located along the central
134 Western Australian coast. The study took place in the Eastern Gulf, offshore of Monkey Mia
135 Dolphin Resort. The study site is made up of a series of shallow (<4m depth) seagrass-covered
136 banks and deep channels (see Wirsing, Heithaus & Dill 2006 for detailed description). Tiger
137 sharks are the top predator in the ecosystem, and more than 95% of catches of sharks >2m are

138 tiger sharks (Heithaus 2001; Wirsing *et al.* 2006). Tiger sharks are widely considered to be one
139 of the most generalized of sharks in terms of diet, which may include mollusks, cephalopods,
140 elasmobranchs, teleosts, reptiles (sea snakes, sea turtles), and marine mammals (Compagno
141 1984; Lowe *et al.* 1996; Simpfendorfer, Goodreid & McAuley 2001).

142 Tiger sharks were captured from 2007-2009 on drumlines equipped with a single Mustad
143 shark hook (12/0-14/0) (see Wirsing *et al.* 2006 for details). Captured sharks were processed
144 alongside the sampling vessel. Blood and plasma were collected in the same manner as with bull
145 sharks, and scissors were used to collect a 0.5 cm² tissue clip from the dorsal fin (fin tissue was
146 collected and used for analyses rather than muscle tissue because of the difficulty in collecting
147 muscle from large tiger sharks). Samples were processed in the same manner as those for bull
148 sharks.

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

153

154 *Stable isotope analysis*

155 All shark samples were analyzed at the Florida International University Stable Isotope
156 Facility (43 *C. leucas* blood samples and 50 *C. leucas* muscle samples) or the Yale Earth System
157 Center for Stable Isotopic Studies (15 *C. leucas* plasma samples, 28 *C. leucas* blood samples, 21
158 *C. leucas* muscle samples, 21 *G. cuvier* plasma samples 46 *G. cuvier* blood samples, and 46 *G.*
159 *cuvier* fin samples). Lipid extraction was not performed because C:N ratios (bull shark mean
160 muscle = 3.1 ± 0.3 SD , mean blood = 2.7 ± 0.2 SD, mean plasma = 2.0 ± 0.2 SD; tiger shark

161 mean fin = 2.7 ± 0.1 SD, mean blood = 2.4 ± 0.0 SD, mean plasma = 2.1 ± 0.1 SD) were
162 generally below those suggested for extraction or mathematical correction (3.5; Post *et al.* 2007),
163 and lipid extraction appears have minimal effects ($<0.6\%$) on $\delta^{13}\text{C}$ values of shark muscle
164 (Hussey *et al.* 2010). Samples from producers and invertebrates with carbonate shells were
165 acidified for $\delta^{13}\text{C}$ values ($\delta^{15}\text{N}$ run separately). Producer and community consumer samples
166 were analyzed at Yale Earth System Center for Stable Isotopic Studies.

167 To verify analytical consistency, we randomly selected samples to be analyzed at both
168 Florida International University and Yale University, for which the variation between resulting
169 $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ values were $0.13\% \pm 0.20\text{SE}$. The standard deviations of standards run for
170 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
171 Florida International.

172

173 *Quantitative Analysis*

174 Trophic specialization can be assessed by measuring the variation in the diets of
175 individuals, and is accomplished by calculating the dietary variation within individuals (WIC:
176 within individual component of variation) and between individuals (BIC: between individual
177 component of variation) of a population (Roughgarden 1972, Bolnick *et al.* 2002). The WIC of a
178 population measures how variable an individual's diet is over a given time frame. This is
179 typically expressed as a mean value for an entire population, but also can be calculated for
180 individuals (see 'individual variance' calculations below). The BIC of a population measures
181 how different each individual's diet is from the other members of the population (Bolnick *et al.*
182 2002). For individuals and populations that are more specialized, WIC should be relatively small
183 because individual diets show little variation and should be consistent over time. Generalist

184 individuals should have a relatively higher WIC because these individuals have a broader dietary
185 niche width (Bolnick *et al.* 2003). The variation between individuals (BIC) varies based on total
186 niche width (TNW), but in general, the degree of individual specialization should increase as the
187 BIC:WIC specialization ratio increases for a given TNW (Newsome *et al.* 2009).

188 Stable isotope analysis has become a popular method for addressing questions about
189 trophic ecology and dietary specialization because 1) stable $\delta^{13}\text{C}$ isotopes can be used to assess
190 the flow of basal nutrients through food webs and gain insights into trophic coupling (e.g. France
191 1997; Vander Zanden & Vadeboncoeur 2002), and 2) body tissues of individuals incorporate
192 isotope values of their diets at various rates (e.g. Hobson & Clark 1992, Bearhop *et al.* 2004).
193 Comparing isotopic values of multiple tissues that vary in turnover rate within an individual,
194 therefore, provides insight into the relative temporal stability of an individual's diet, and can be
195 used to investigate questions about individual trophic specialization (Bearhop *et al.* 2004).

196 Isotopic turnover rates of elasmobranchs studied to date suggest that muscle and fin have
197 relatively long turnover rates (complete isotope turnover in 390-540 days and 576 days) and
198 whole blood has an intermediate turnover rate (complete isotope turnover in 240-300 days;
199 MacNeil *et al.* 2006; Logan & Lutcavage 2010, Matich *et al.* 2010) (Fig. 2). Blood plasma turns
200 over at an even faster rate than whole blood (S. Kim, personal communication; complete isotopic
201 turnover in plasma occurs in 72-102 days; Fig. 2), so we used plasma to provide insight into diets
202 at shorter temporal scales. Although most of these isotope turnover rates were calculated for
203 relatively small elasmobranchs in captive trials (*Potamotrygon motoro*: mean mass = 0.1kg;
204 *Carcharhinus plumbeus*: mean mass = 6.4kg), and isotope turnover rates can vary with body size
205 (e.g. Carleton & Martinez del Rio 2005, Martinez del Rio *et al.* 2009b), field studies of size-
206 based variation among fin, muscle, and blood of bull sharks suggest that these lab-based

207 estimates are likely similar to those found in natural settings (Matich *et al.* 2010; see discussion
208 for further consideration of turnover rates). Furthermore, even if there is variation in absolute
209 turnover rates based on body size, the relative turnover times of tissues (muscle/fin > whole
210 blood > plasma) is expected to be the same.

211 Delta values (δ) are often used to express stable isotope data, but in order to make
212 comparisons in specialization between tiger sharks and bull sharks, it was necessary to account
213 for difference in their potential isotope niche width (i.e. differences in the range of $\delta^{13}\text{C}$ values).
214 Therefore, to normalize isotope data for bull sharks and tiger sharks, we converted $\delta^{13}\text{C}$ values
215 for tissues to proportional values (p-values; Newsome *et al.* 2007). Each system has two discrete
216 basal resource pools with distinct $\delta^{13}\text{C}$ values: the Shark River has a “freshwater/estuarine food
217 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
218 Bay has a “benthic food web” ($-8.5\text{‰} \pm 0.3\text{SE}$) and a “pelagic food web” ($-16.1\text{‰} \pm 0.8\text{SE}$).
219 Therefore, “p-values” were calculated based on mean $\delta^{13}\text{C}$ values of available food sources for
220 each system using a two-source mixing model (Phillips & Gregg 2001). These p-values provide
221 a measure of the relative position of $\delta^{13}\text{C}$ values between endpoints of potential energy
222 pathways.

223 To quantify dietary specialization in bull sharks and tiger sharks, we employed four
224 different models (see below for details of each): 1) General Linear Model (GLM) using isotope
225 p-values of two body tissues (bull sharks: muscle and whole blood; tiger sharks: fin and whole
226 blood) with individual included as a random effect, 2) GLM using isotope p-values of three body
227 tissues (bull sharks: muscle, whole blood, and plasma; tiger sharks: fin, whole blood, and
228 plasma) with individual included as a random effect, 3) calculation of variance for each
229 individual using isotope p-values of two body tissues (bull sharks: muscle and blood; tiger

230 sharks: fin and blood), and 4) calculation of variance for each individual using isotope p-values
231 of three body tissues (bull sharks: muscle, blood, and plasma; tiger sharks: fin, blood, and
232 plasma). In addition, we calculated individual specialization metrics using IndSpec (Bolnick *et*
233 *al.* 2002) to supplement our novel analytical framework.

234

235 Two-tissue GLM

236 The mean sum of squares of the two-tissue model ($E(SS_B)$), which is defined as

237

$$238 \quad E(SS_B) = \frac{n \sum_{i=1}^m (\bar{Y}_i - \bar{Y})^2}{n(m-1)} \quad (1)$$

239 measures the variability between individuals (a proxy for the between individual component of
240 variation – BIC), where m is the total number of individuals, i is any individual, and n is the total
241 number of tissues. The mean sum of squares of the error ($E(SS_W)$), where

242

$$243 \quad E(SS_W) = \frac{[\sum_{i=1}^m \sum_{j=1}^n (Y_{ij} - \bar{Y}_i)^2]}{m(n-1)} \quad (2)$$

244 measures the variability within individuals (a proxy for the within individual component of
245 variation – WIC), where j is any tissue. The resulting F-ratio ($E(SS_B):E(SS_W)$) is a proxy for
246 individual specialization within the population (a proxy of BIC:WIC). As the variation between
247 individuals increases (i.e. BIC increases), and/or the variation within individuals decreases (i.e.
248 WIC decreases), the ratio, and therefore relative degree of individual specialization, increases
249 (Bolnick *et al.* 2003).

250

251 Three-tissue GLM

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

257

258 Two-tissue individual variance

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

$$265 \quad \text{Var}(X) = E[(X - \mu)^2] \quad (3)$$

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

273

274 Three-tissue individual variance

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

279

280 IndSpec

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

$$284 \quad TNW = Var(x_{ij}) \quad (4)$$

$$285 \quad WIC = E[Var(x_j|i)] \quad (5)$$

$$286 \quad BIC = Var[E(x_j|i)] \quad (6)$$

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

288

289 **Results**

290 *Characterization of food webs*

291 The $\delta^{13}\text{C}$ values of primary producers and consumers of the Shark River Estuary
292 (freshwater/estuarine food web) differed substantially from those found in the marine food web
293 (Fig. 3a). Resident consumers' mean $\delta^{13}\text{C}$ values from the freshwater/estuarine food web were
294 always lower than -25‰ , and usually lower than -28‰ . In contrast, consumers feeding in
295 marine habitats had $\delta^{13}\text{C}$ values between -11‰ and -19‰ . Although consumers with
296 intermediate $\delta^{13}\text{C}$ values (-19‰ to -25‰) are found in the Shark River Estuary (e.g. snook,
297 *Centropomus undecimalis*, $\delta^{13}\text{C}$ range = -18.9‰ to -27.3‰ , M. Heithaus *unpublished data*), a

298 review of the literature and our own sampling suggests that these consumers are relatively
299 uncommon, and they tend to have $\delta^{13}\text{C}$ values relatively close to freshwater/estuarine consumers
300 (e.g. snook mean $\delta^{13}\text{C} = -25.0\text{‰} \pm 0.6\text{SE}$, M. Heithaus *unpublished data*). Several species are
301 found in both the freshwater/estuarine and marine habitats, and often have a $\delta^{13}\text{C}$ value of the
302 habitat where they were captured (e.g. blue crabs, *Callinectes sapidus*, Florida Bay $\delta^{13}\text{C} = -$
303 14.3‰ ; Shark River mid-estuary $\delta^{13}\text{C} = -27.8\text{‰} \pm 0.3\text{SE}$; gray snapper, *Lutjanus griseus*, Florida
304 Bay $\delta^{13}\text{C} = -13.4\text{‰} \pm 1.2\text{SE}$; Shark River $\delta^{13}\text{C} = -28.4\text{‰} \pm 0.4\text{SE}$; Chaser *et al.* 2005; C. McIvor
305 *et al.* personal communication).

306 Consumers of Shark Bay, Australia were not as separated in $\delta^{13}\text{C}$ values as consumers in
307 the Shark River, but there were still distinctions between taxa of the benthic and pelagic food
308 webs (Fig. 3b). Primary consumers from the pelagic food web had $\delta^{13}\text{C}$ values lower than -16‰ ,
309 while those from the benthic food web had $\delta^{13}\text{C}$ values higher than -10‰ . Unlike the Shark
310 River ecosystem, consumers with intermediate values were common in Shark Bay. As the
311 trophic position (inferred by $\delta^{15}\text{N}$ value) of taxa increased, taxa mean $\delta^{13}\text{C}$ values converged
312 toward intermediate values.

313

314 *General isotope trends in sharks*

315 Overall, we sampled 71 bull sharks (100-187cm TL) in the Shark River Estuary and 46
316 tiger sharks (160-396 cm TL) in Shark Bay, Australia. The mean $\delta^{13}\text{C}$ of bull sharks were: -
317 $22.8\text{‰} \pm 0.4\text{SE}$ (muscle), $-22.9\text{‰} \pm 0.4\text{SE}$ (whole blood), and $-21.5\text{‰} \pm 0.7\text{SE}$ (plasma). Mean
318 $\delta^{13}\text{C}$ values, however, masked considerable variability, i.e. a $\delta^{13}\text{C}$ range of 12.7‰ , which was
319 60% of the $\delta^{13}\text{C}$ range of producers and consumers in the Shark River Estuary and surrounding
320 coastal waters of Florida Bay ($\sim 22\text{‰}$). Nineteen bull sharks fell within the range of isotope

321 values for those taxa identified in the freshwater/estuarine food web and eight fell within values
322 of the marine food web; the rest (N= 44) had $\delta^{13}\text{C}$ values that fell between these two food webs
323 (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
324 of producers and consumers in Shark Bay (14‰), and all individuals, except for one, had $\delta^{13}\text{C}$
325 values that were intermediate between mean values of species in the benthic and pelagic food
326 webs (Fig. 3b).

327

328 *Patterns of individual specialization*

329 Two-tissue GLMs based on muscle and blood (bull sharks), and fin and blood (tiger
330 sharks) revealed that bull sharks tended to be more specialized and tiger sharks tended to be
331 more generalized in their respective diets (Fig. 4a). Within-individual variation (WIC) of bull
332 sharks (0.003) was considerably lower than that of tiger sharks (0.021), while between individual
333 variation (BIC) was greater in bull sharks (0.08) than tiger sharks (0.06). The specialization
334 index for tiger sharks was relatively low (2.84), suggesting that individuals were more
335 generalized in their diet. In contrast, the specialization index was much greater for bull sharks
336 (23.7; Fig. 4a), indicating individuals were more specialized in their diet.

337 Three-tissue GLM based on muscle, blood, and plasma (bull sharks), and fin, blood, and
338 plasma (tiger sharks) strengthened trends found in the two-tissue random effects models (Fig.
339 4a). For individuals with more specialized diets, the value of the three-tissue specialization
340 index should be comparable, or increase, relative to that based on two tissues, because short-term
341 and long-term dietary variation should be similar when diets are temporally stable. In contrast,
342 for generalists, variation within individuals (WIC) should be greater on average when including
343 fast turnover tissues, and therefore should result in lower specialization values for analyses based

344 on three tissues than those based on two tissues. Consistent with these predictions, when plasma
345 was included with fin, muscle, and blood, the specialization index was considerably greater than
346 that of the two-tissue analysis for bull sharks (42.5). And, while the specialization index
347 increased for tiger sharks (4.37), suggestive of a more specialized diet, it was still considerably
348 smaller than that of the bull sharks quantified using the two and three tissue models, and tiger
349 shark WIC was greater for the three-tissue analysis (0.06), indicating tiger sharks are more
350 generalized.

351 IndSpec revealed very similar patterns in the dietary specialization of bull sharks and
352 tiger sharks (Fig. 4b) when compared to the GLMs (Fig. 4a). WIC was considerably lower for
353 bull sharks (0.002 for both the two- and three-tissue analyses) than for tiger sharks (0.01 and
354 0.02 for the two- and three-tissue analyses, respectively), and BIC was comparable for the two
355 species (0.04 for both bull shark analyses, and 0.03 and 0.04 for the tiger shark two- and three-
356 tissue data sets, respectively). The specialization index values for bull sharks (23.4 and 19.8 for
357 the two- and three-tissue data sets, respectively) were considerably higher than those for tiger
358 sharks (2.8 and 2.1 for the two- and three-tissue data sets, respectively).

359 The two-tissue individual variance analysis revealed similar trends. A large proportion of
360 bull shark individuals had relatively high specialization indexes (92% had specialization index
361 values greater than ten), while most tiger shark individuals had relatively low specialization index
362 values (74% had a specialization index less than ten; Fig. 5a). The distribution of sharks falling
363 into each range of specialization values was significantly different for tiger sharks and bull
364 sharks (Kolmogorov-Smirnov test: $T_{\text{samples}} = 0.96$; $p < 0.01$). Similarly, the three-tissue
365 individual variance analysis showed that tiger sharks were less specialized than bull sharks
366 (Kolmogorov-Smirnov test: $T_{\text{samples}} = 1$; $p < 0.01$; Fig. 5b). In this analysis, more than 71% of

367 tiger sharks had specialization values below ten, while all bull sharks had specialization values
368 above ten. Specialization index values were not directly related to shark body size, body
369 condition, sex, season, or year (Table 1; Fig. 6).

370

371 **Discussion**

372 *Variation in trophic specialization*

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

384 The two species of sharks studied here are widely considered to be generalist top
385 predators in their respective ecosystems (e.g. Simpfendorfer *et al.* 2001; Weatherbee & Cortes
386 2004), but both the GLMs and IndSpec revealed considerable differences in the patterns of
387 trophic specialization between them. Tiger sharks apparently were relatively generalized in their
388 diets. Values of $\delta^{13}\text{C}$ over multiple time scales revealed that there was considerable variation in
389 the diet of tiger sharks over time, indicating that individuals have relatively unspecialized diets.

390 In contrast, bull sharks showed temporal stability in their diets, and most individuals were
391 relatively specialized despite having a broad isotopic niche width at the population level.
392 Therefore, although both species are “generalists” at the population level, they differ
393 considerably at the individual level.

394 Often, individual specialization can be documented by observing the behavior of
395 particular individuals over time. But for sharks and many other upper trophic level marine
396 predators, this is not possible. Analyzing stable isotopic signatures of multiple tissues with
397 differing rates of turnover is a powerful tool for assessing individual specialization when an
398 individual can only be sampled once (e.g. Bearhop *et al.* 2004; Quevedo *et al.* 2009; Jaeger *et al.*
399 2010). Because analytical techniques for determining specialization patterns using isotope data
400 from tissues with different turnover rates are still being developed, we used two methods to
401 assess specialization: GLMs and the computer program IndSpec. Despite differences in output,
402 both analytical frameworks produced the same trends in individual dietary specialization, or lack
403 thereof, for bull sharks and tiger sharks – bull sharks are relatively more specialized than tiger
404 sharks.

405 Body condition (reviewed by Vanderklift & Ponsard 2003) and the presence of lipids
406 (Post *et al.* 2007) can be important factors to consider when interpreting isotopic values. Neither
407 of these factors though, appeared to likely confound the results in our study. First, body
408 condition tends to affect $\delta^{15}\text{N}$ more than $\delta^{13}\text{C}$ (e.g. Hobson, Alisauskas & Clark 1993; Kurle and
409 Worthy 2001; Polischuk, Hobson & Ramsay 2001), and there was no affect of body condition on
410 $\delta^{13}\text{C}$ of bull sharks (body condition data were not available for tiger sharks). Lipid content of
411 tissues also is likely to have little effect on our results. Although sharks store lipids in muscle
412 and liver tissues (Bone 1999; Remme *et al.* 2006), the mean C:N ratio of bull shark muscle tissue

413 was low with little variation (3.1 ± 0.3 SD), suggesting lipid content resulted in minimal
414 variation in muscle $\delta^{13}\text{C}$ between individuals (i.e. little effect on BIC). Mean C:N ratios of fin
415 (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,
416 2.1 ± 0.1 SD) were also low for bull sharks and tiger sharks, respectively.

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

425 Interpretation of isotope values can also be affected by whether tissues are in dietary
426 equilibrium (reviewed by Martinez del Rio *et al.* 2009b), which may be influenced by seasonal
427 changes in diets or prey switching within the timescale of a tissue's turnover (e.g. Matich *et al.*
428 2010). It is quite possible that tissues – especially those with longer turnover times – are not in
429 equilibrium (at least for larger tiger sharks). The possibility of non-equilibrium of tissues in tiger
430 sharks and some bull sharks, however, does not confound our basic findings of interspecific
431 differences in individual specialization, because we are explicitly making use of differential rates
432 of change in signatures of various tissues and do not need to assume that they are in equilibrium.

433 Finally, knowing the timeframe over which isotopic values are incorporated into tissues
434 is important for determining the timescale over which specialization is measured using our
435 methods. Isotopic turnover rates generally decrease with increasing body size (Martinez del Rio

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

454

455 *Drivers of specialization and implications*

456 Our results suggest that individual dietary specialization in elasmobranchs, and resulting
457 community trophodynamics, is context dependent. Differences in resource availability and
458 distribution as well as intraspecific competition, between the Shark River Estuary and Shark Bay

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

476 In ecosystems with multiple potential energetic pathways, the spatial arrangement of
477 discrete food webs may also be an important factor determining levels of individual
478 specialization. Ecosystems with discrete food webs that have a high degree of geographical
479 overlap are more likely to support generalist individuals, because individuals can readily exploit
480 resources from both food webs without significant movements between resource patches (e.g.
481 Miller, Karnovsky & Trivelpiece 2009; Montevicchi *et al.* 2009). When food webs are spatially

482 distinct with little or no geographic overlap, however, individual dietary specialization may be
483 relatively widespread across a population (e.g. Darimont *et al.* 2009; Quevedo *et al.* 2009). In
484 Shark Bay, both pelagic and benthic food webs overlap spatially, providing tiger sharks with
485 access to each food web within the same habitat. In contrast, within the Shark River Estuary the
486 marine and freshwater/estuarine food webs are spatially distinct. Specialization would be
487 expected if sharks segregated into individuals that strictly resided in marine waters and those that
488 stayed within the estuary. However, the bull sharks used for this study were all captured within
489 the estuary, suggesting they move between habitats that encompass each food web.

490 Mean population $\delta^{13}\text{C}$ values suggest bull shark individuals derive carbon from multiple
491 food webs, but the $\delta^{13}\text{C}$ values and specialization index values indicated that many individuals
492 specialized in feeding from the marine food web despite being captured within the estuary.

493 Indeed, taxa representing the marine food web are found more than 30 km from the capture
494 locations of some sharks that had enriched $\delta^{13}\text{C}$, suggesting that they had moved long distances
495 to feed. Movement data from bull sharks in the Shark River suggest that some individuals do
496 move from the estuary into marine waters and back (P. Matich *unpublished data*) and further
497 work will be needed to link individual behavior with patterns of specialization. Why wouldn't
498 all sharks move into, or remain in, the potentially more resource-rich marine food web?

499 Although the juvenile bull sharks in our study are among the largest-bodied predators in the
500 Shark River Estuary, larger sharks that can prey upon these juvenile sharks inhabit the furthest
501 areas downstream in the marine food web where risk is greatest for juvenile sharks (P. Matich
502 *unpublished data*). Therefore, in addition to the trade-off between opportunistic feeding and
503 foraging efficiency that favors specialization in resource-poor environments (Bolnick 2004),
504 specialization in the bull sharks of the Shark River Estuary may also occur because of a trade-off

505 between foraging opportunities and the risk of predation. These data support the view that
506 behavioral and dietary specialization may be closely linked (Bolnick *et al.* 2007).

507 Individual specialization affects trophic dynamics, and previous studies suggest it may
508 prevent resources of spatially distinct food webs from being coupled by individual predators (e.g.
509 Quevedo *et al.* 2009). However, highly mobile predators, like sharks, have the potential to
510 forage at a great distance from sites where they spend considerable amounts of time, and may
511 serve to couple ecosystems through this trophic role. Isotope values suggest that some bull
512 sharks move into and out of the system, which may enable them to feed on taxa from both the
513 marine and freshwater/estuarine food webs, and would likely result in low specialization index
514 values based on a generalized diet. Yet, $\delta^{13}\text{C}$ values indicated that these individuals were dietary
515 specialists and fed primarily in the marine food web located outside of the nursery in which they
516 were captured. These specialized individuals may serve as important links in the connectivity of
517 multiple food webs through a bottom-up mechanism of nutrient transport to the oligotrophic
518 upper reaches of the Everglades (Polis, Anderson & Holt 1997), while tiger sharks of Shark Bay
519 may serve a more traditional role of a generalist top predator that couples discrete food webs
520 (Rooney *et al.* 2006). Food web structure and dynamics may be more complex in ecosystems
521 with specialist top predators, and a “species-level” approach to conservation and management
522 may be over simplistic in such situations. Therefore, studies of foraging ecology of highly
523 mobile marine predators should explicitly consider the possibility of individual specialization.
524 The use of stable isotopes sampled from multiple tissues would allow such studies to be
525 conducted non-lethally and/or alongside traditional diet studies employing stomach contents
526 analysis.

527 In summary, our study suggests that individual specialization can occur in non-
528 mammalian marine top predators, but is not ubiquitous. Factors including resource availability,
529 competition, food-predation risk trade-offs, and spatial overlap of food webs may contribute to
530 the observed levels of specialization. Future studies should explore the level of individual
531 dietary specialization that occurs within other shark populations, as well as other highly mobile
532 apex predators, and the potential effects this may have on ecosystem processes. Studies that
533 investigate the mechanisms by which among individual specialization is manifest in highly
534 mobile predators, the heritability or drivers of this variation in trophic niches, and the effects
535 specialization has on the trophic dynamics within and across ecosystems will be particularly
536 important for future conservation efforts, especially in light of widespread top predator declines
537 in marine ecosystems.

538

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551

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750 specialization in diet by a generalist marine predator reflects specialization in foraging
751 behavior. *Journal of Animal Ecology*, **77**, 1082-1091.

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

		Two-tissue ANOVA			Three-tissue ANOVA		
		N	F	p	N	F	p
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

754 **Figure Legends**

755

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

759

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

766

767 Figure 3: (A) Mean isotope values for producers and consumers in the Shark River Estuary
768 (freshwater/estuarine food web) and surrounding marine waters. Producers and consumers from
769 the freshwater/estuarine food web are gray and those from the marine food web are white.
770 Producers are diamonds (◆), molluscs are triangles (▲), crustaceans are squares (■), teleosts are
771 circles (●), other elasmobranchs are crosses (+), and bull sharks (whole blood) are X's. (B) Mean
772 isotope values for producers and consumers in Shark Bay. Producers and consumers from the
773 pelagic food web are gray and those from the benthic food web are white. Producers are
774 diamonds (◆), molluscs are triangles (▲), other invertebrates are squares (■), megagrazers (i.e.
775 dugongs and turtles) are circles (●), other elasmobranchs are crosses (+), and tiger sharks (whole
776 blood) are X's. Standard deviations around mean values are omitted for simplicity.

777

778 Figure 4: a) Specialization indices of bull sharks and tiger sharks based on isotope p-values
779 derived from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and
780 plasma (P) (tiger sharks); b) specialization comparison between bull sharks and tiger sharks
781 using isotope p-values from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F),
782 blood (B), and plasma (P) (tiger sharks), derived from IndSpec.

783

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

787

788 Figure 6: Individual specialization index values based on two- (◆) and three-tissue (■) GLMs of
789 a) bull sharks and b) tiger sharks in relation to shark total length (cm). Mean individual
790 specialization index values (\pm S.D.) based in two- and three-tissue GLMs of bull sharks
791 separated by c) sex, e) capture season, g) capture year, and i) body condition, and mean
792 individual specialization index values of tiger sharks separated by d) sex, f) capture season, and
793 h) capture year.

Figure 1

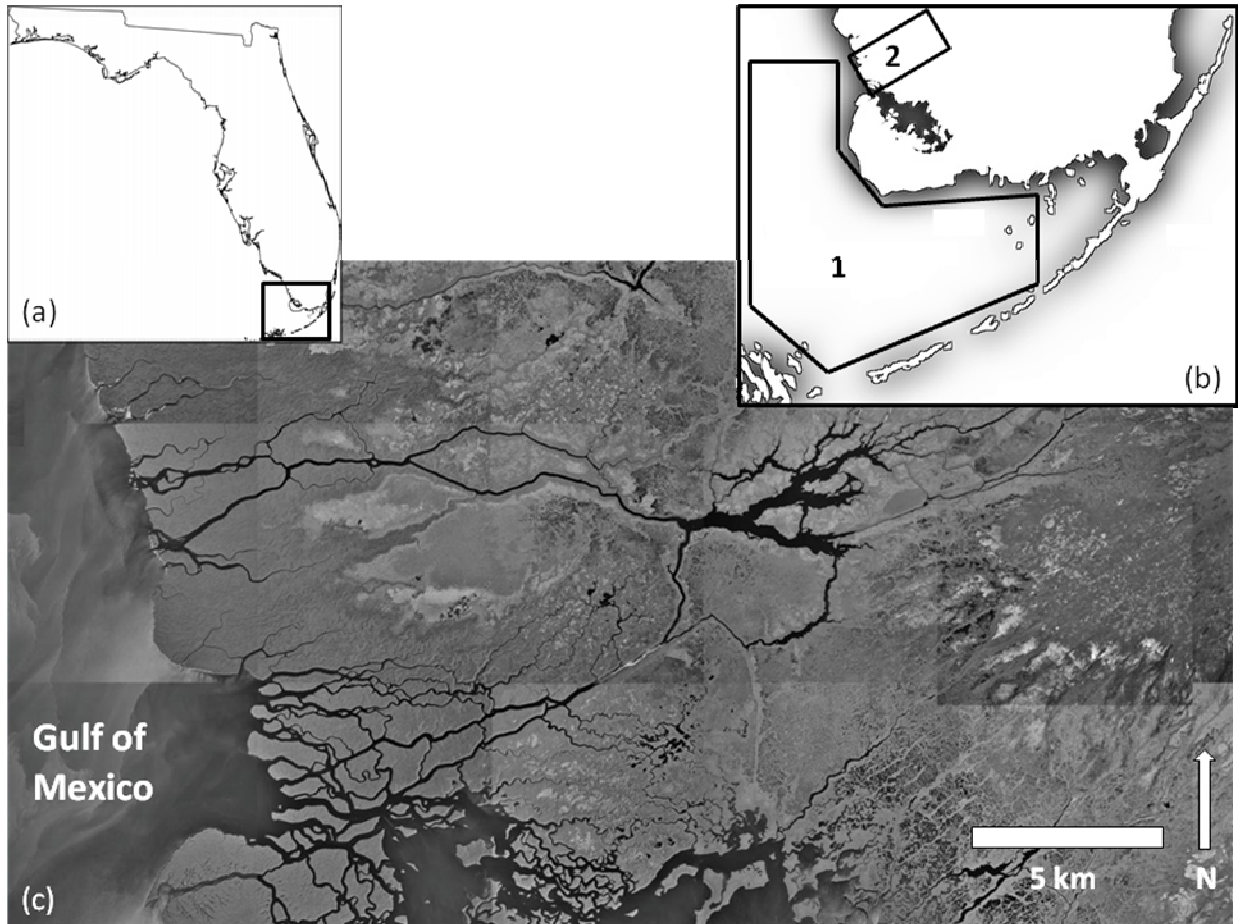


Figure 2

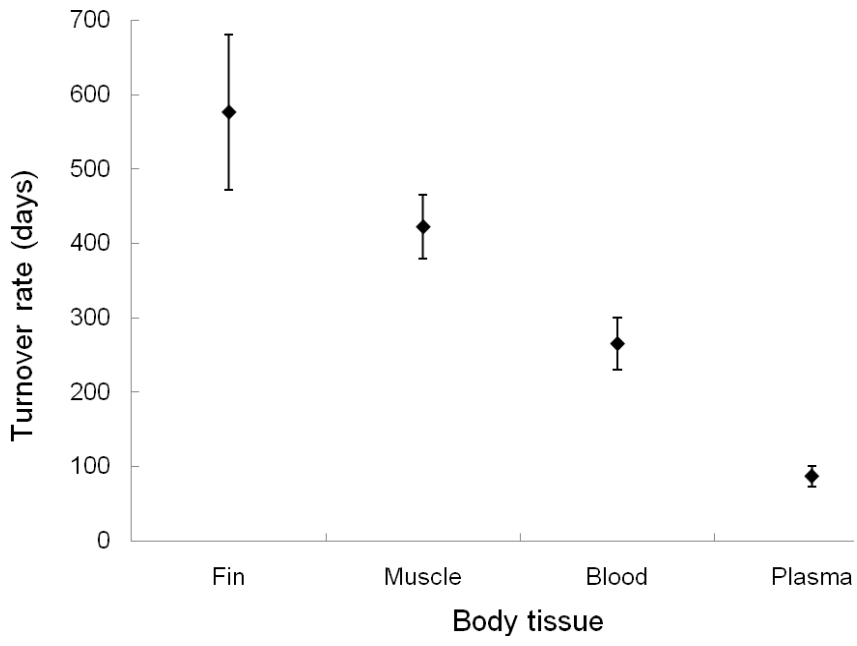


Figure 3

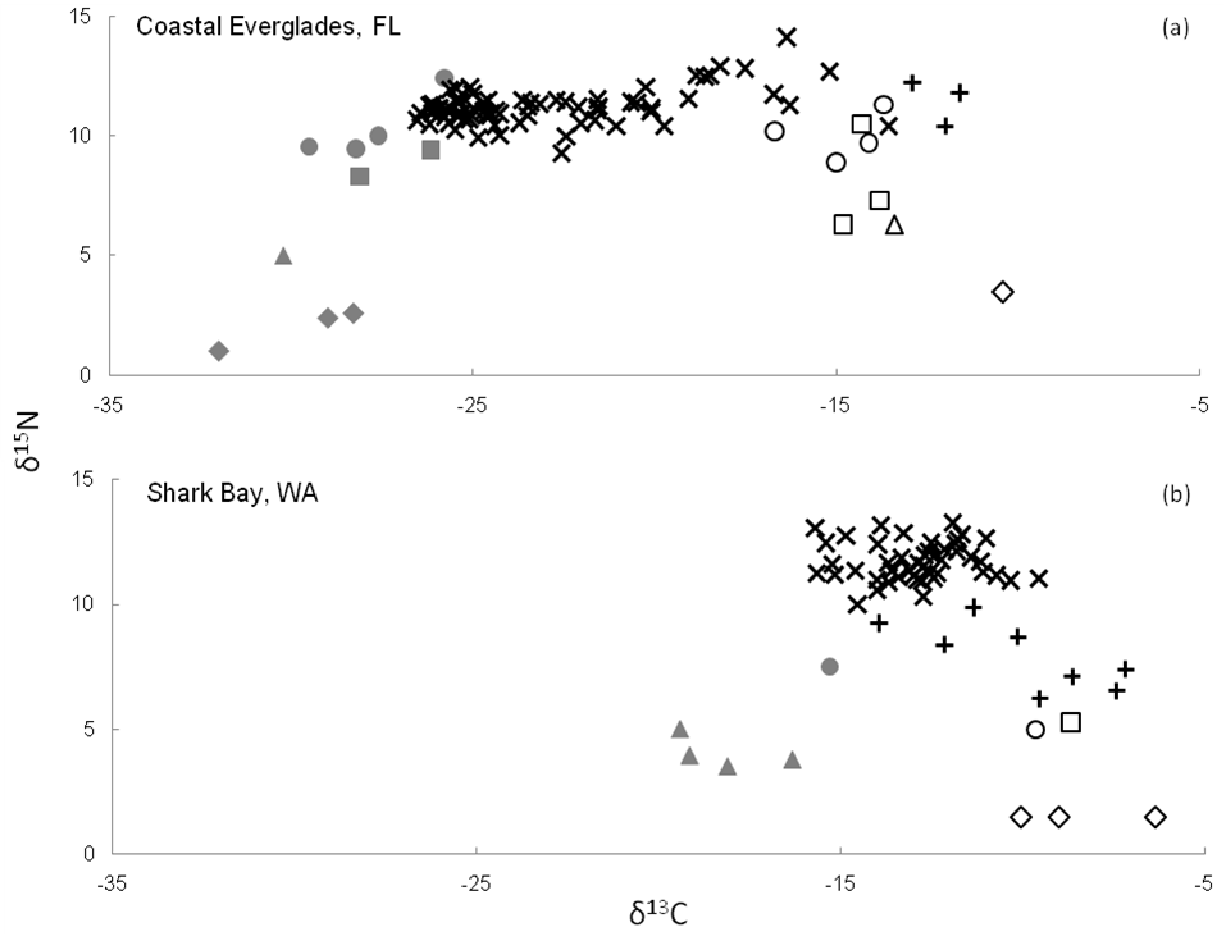


Figure 4

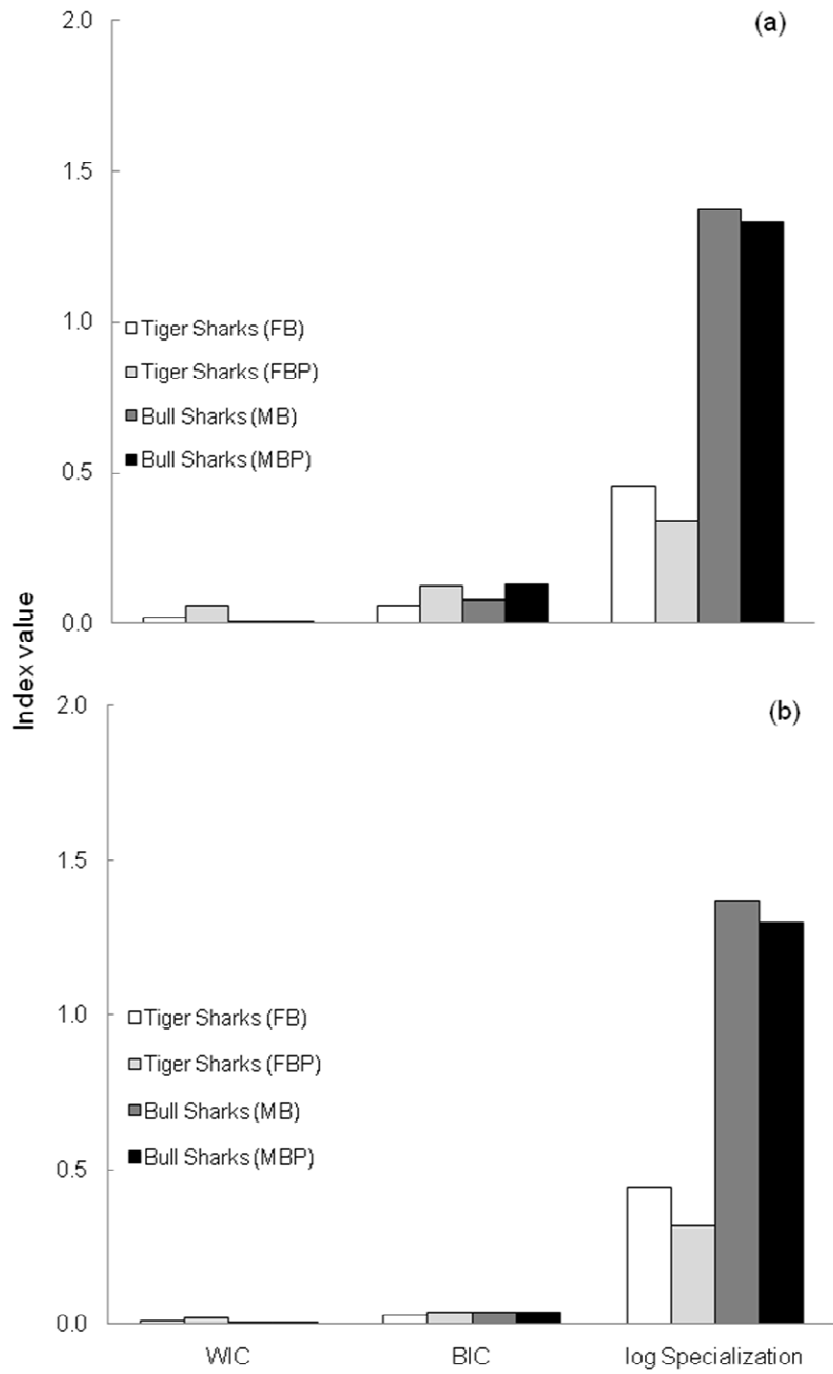


Figure 5

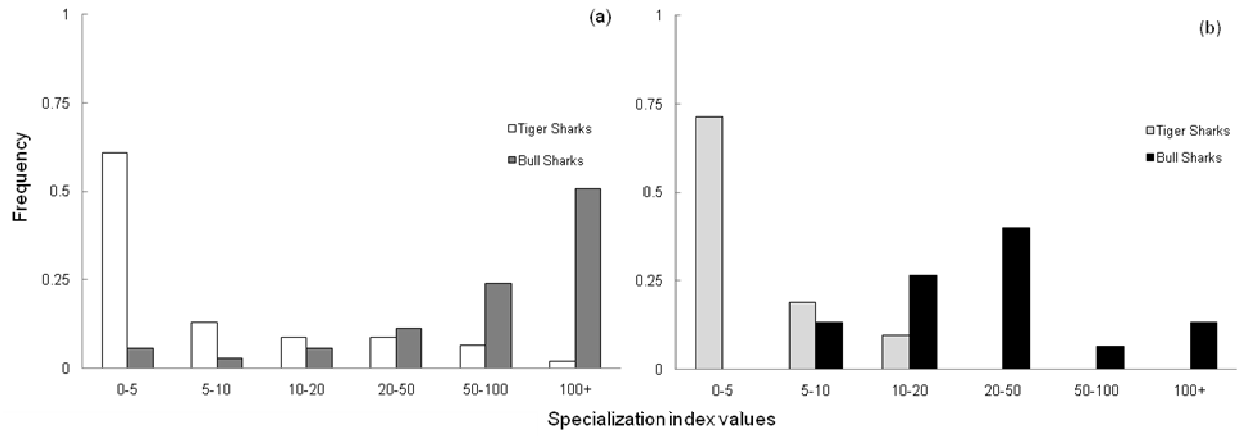


Figure 6

