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

2 **predators**

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

10 Marine Sciences Program

11 Florida International University

12 3000 NE 151st

13 North Miami, FL 33181, USA

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19 Running head: specialization in top marine predators

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

22 (305) 919-4030 fax

Summary

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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.

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

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

Introduction

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

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

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   Ravigne, Dieckmann & Olivieri 2009).

Methods

Coastal Everglades, Florida

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

Bull sharks were captured from 2005-2009 on ~500m longlines fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to ~2m of 400kg 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 cm^3 muscle tissue biopsy *ca.* 5 cm lateral to the first dorsal fin, and an 18 gauge needle to collect 4ml of blood from the caudal vein. From the blood, 3ml was placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and separated into its components, including plasma, using a centrifuge spun for one minute at 3000rpm. The remaining 1ml 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

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 $\delta^{13}C$ 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 cm^2 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 $\delta^{13}C$ 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 δ^{13}C values of shark muscle

164   (Hussey *et al.* 2010). Samples from producers and invertebrates with carbonate shells were

165   acidified for δ^{13}C values (δ^{15}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   δ^{13}C values and δ^{15}N values were 0.13‰ ± 0.20SE. The standard deviations of standards run for

170   Yale were 0.14‰ for δ^{13}C and 0.22‰ for δ^{15}N, and 0.29‰ for δ^{13}C and 0.24‰ for δ^{15}N for

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 $\delta^{13}C$ 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 δ^{13}C values).

214   Therefore, to normalize isotope data for bull sharks and tiger sharks, we converted δ^{13}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 δ^{13}C values: the Shark River has a "freshwater/estuarine food

217   web" (mean δ^{13}C = -29.7‰ ± 0.7SE) and a "marine food web" (-14.5‰ ± 0.3SE), and Shark

218   Bay has a "benthic food web" (-8.5‰ ± 0.3SE) and a "pelagic food web" (-16.1‰ ± 0.8SE).

219   Therefore, "p-values" were calculated based on mean δ^{13}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 δ^{13}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
$$E(SS_B) = \frac{n \sum_{i=1}^{m} (\bar{Y}_i - \bar{Y})^2}{n(m-1)} \qquad (1)$$

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
$$E(SS_W) = \frac{\left[\sum_{i=1}^{m} \sum_{j=1}^{n} (Y_{ij} - Y_i)^2 \right]}{m(n-1)} \qquad (2)$$

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

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

285
$$WIC = E[Var(x_j|i)] \tag{5}$$

286
$$BIC = Var[E(x_j|i)] \tag{6}$$

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

288

289   **Results**

290   *Characterization of food webs*

291   The δ^{13}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 δ^{13}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 δ^{13}C values between -11‰ and -19‰. Although consumers with

296   intermediate δ^{13}C values (-19‰ to -25‰) are found in the Shark River Estuary (e.g. snook,

297   *Centropomus undecimalis*, δ^{13}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 δ^{13}C values relatively close to freshwater/estuarine consumers

300   (e.g. snook mean δ^{13}C = -25.0‰ ± 0.6SE, M. Heithaus *unpublished data*). Several species are

301   found in both the freshwater/estuarine and marine habitats, and often have a δ^{13}C value of the

302   habitat where they were captured (e.g. blue crabs, *Callinectes sapidus,* Florida Bay δ^{13}C = -

303   14.3‰; Shark River mid-estuary δ^{13}C = -27.8‰ ± 0.3SE; gray snapper, *Lutjanus griseus*, Florida

304   Bay δ^{13}C = -13.4‰ ± 1.2SE; Shark River δ^{13}C = -28.4‰ ± 0.4SE; Chaser *et al.* 2005; C. McIvor

305   *et al.* personal communication).

306   Consumers of Shark Bay, Australia were not as separated in δ^{13}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 δ^{13}C values lower than -16‰,

309   while those from the benthic food web had δ^{13}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 δ^{15}N value) of taxa increased, taxa mean δ^{13}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 δ^{13}C of bull sharks were: -

317   22.8‰ ± 0.4SE (muscle), -22.9‰ ± 0.4SE (whole blood), and -21.5‰ ± 0.7SE (plasma). Mean

318   δ^{13}C values, however, masked considerable variability, i.e. a δ^{13}C range of 12.7‰, which was

319   60% of the δ^{13}C range of producers and consumers in the Shark River Estuary and surrounding

320   coastal waters of Florida Bay (~22‰). Nineteen bull sharks fell within the range of isotope

15

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 δ^{13}C values that fell between these two food webs

323   (Fig. 3a). Tiger sharks had a narrower δ^{13}C range (5.9‰) that was 42% of the entire δ^{13}C range

324   of producers and consumers in Shark Bay (14‰), and all individuals, except for one, had δ^{13}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   vales 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: $T_{samples} = 0.96$; $p < 0.01$). Similarly, the three-tissue

365   individual variance analysis showed that tiger sharks were less specialized than bull sharks

366   (Kolmogorov-Smirnov test: $T_{samples} = 1$; $p < 0.01$; 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 $\delta^{13}C$ 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 $\delta^{15}N$ more than $\delta^{13}C$ (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　$\delta^{13}C$ 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 δ^{13}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 abitoic conditions (e.g.

418 temperature) can modify isotope discrimination and routing, and lead to variability in δ^{13}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: 15-33°C;

422 Heithaus *et al*. 2009; Shark Bay: 13-28°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 loger 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; Montevecchi *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 δ^{13}C values suggest bull shark individuals derive carbon from multiple

491   food webs, but the δ^{13}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 δ^{13}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, δ^{13}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

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

550 Research Project.

551

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750   specialization in diet by a generalist marine predator reflects specialization in foraging

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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 (± 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 (± 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

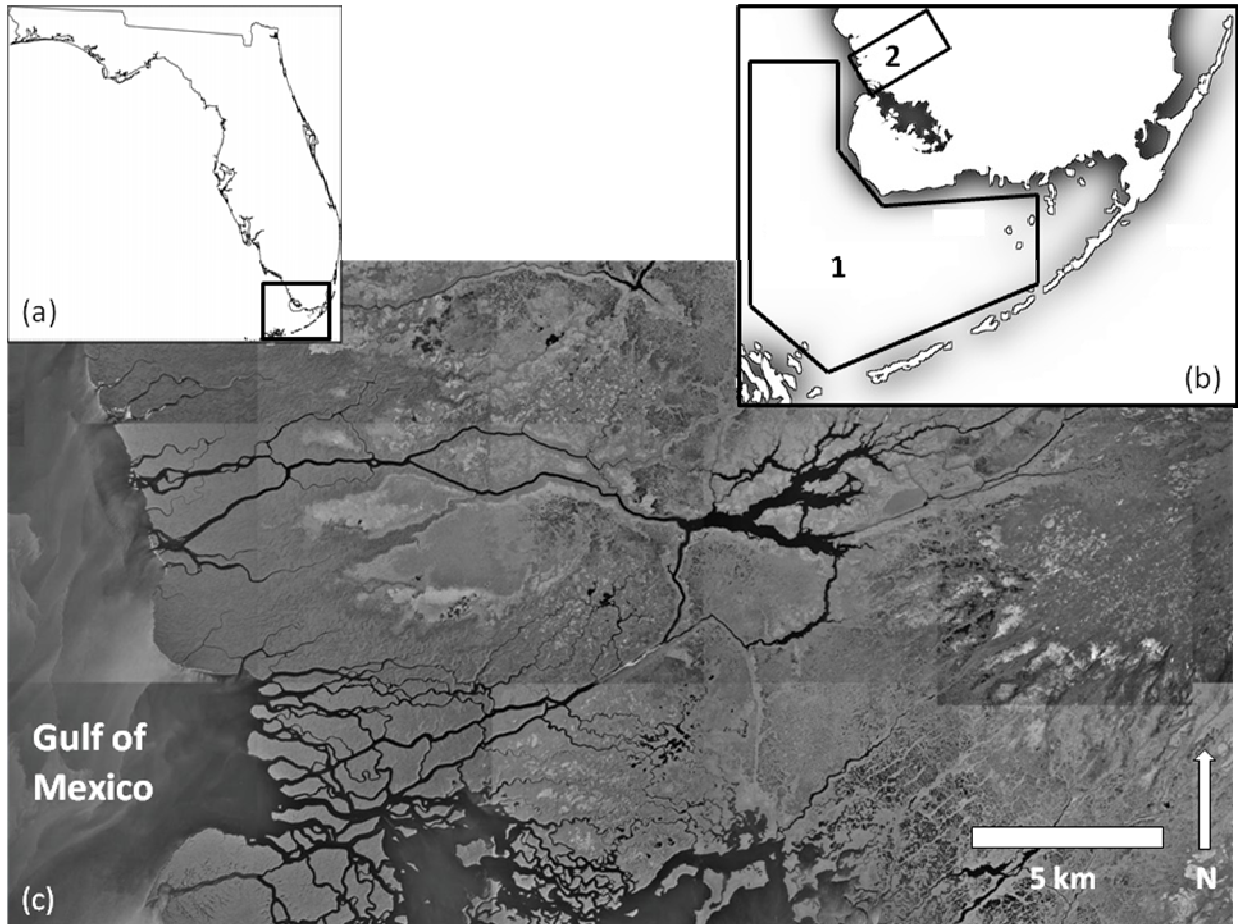792   individual specialization index values of tiger sharks separated by d) sex, f) capture season, and
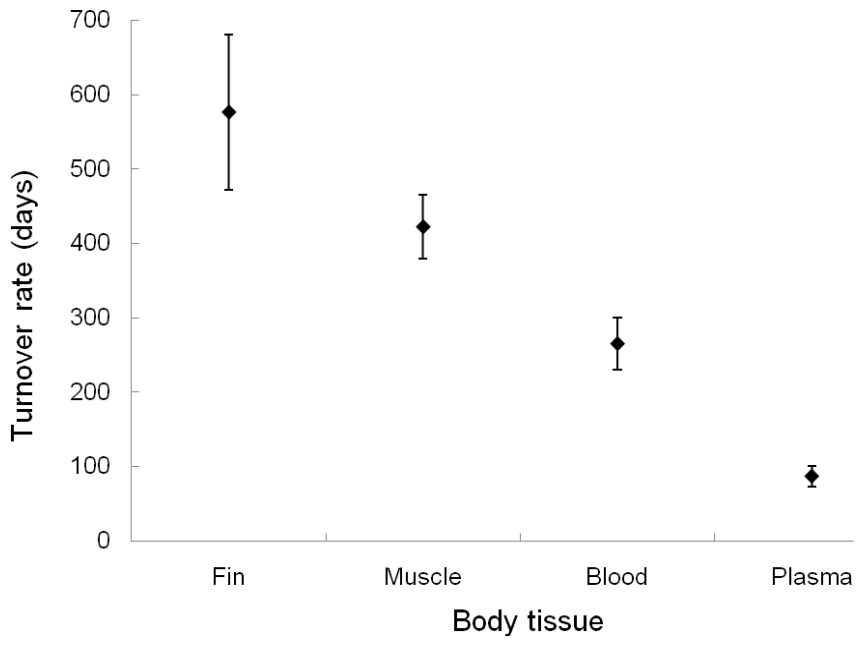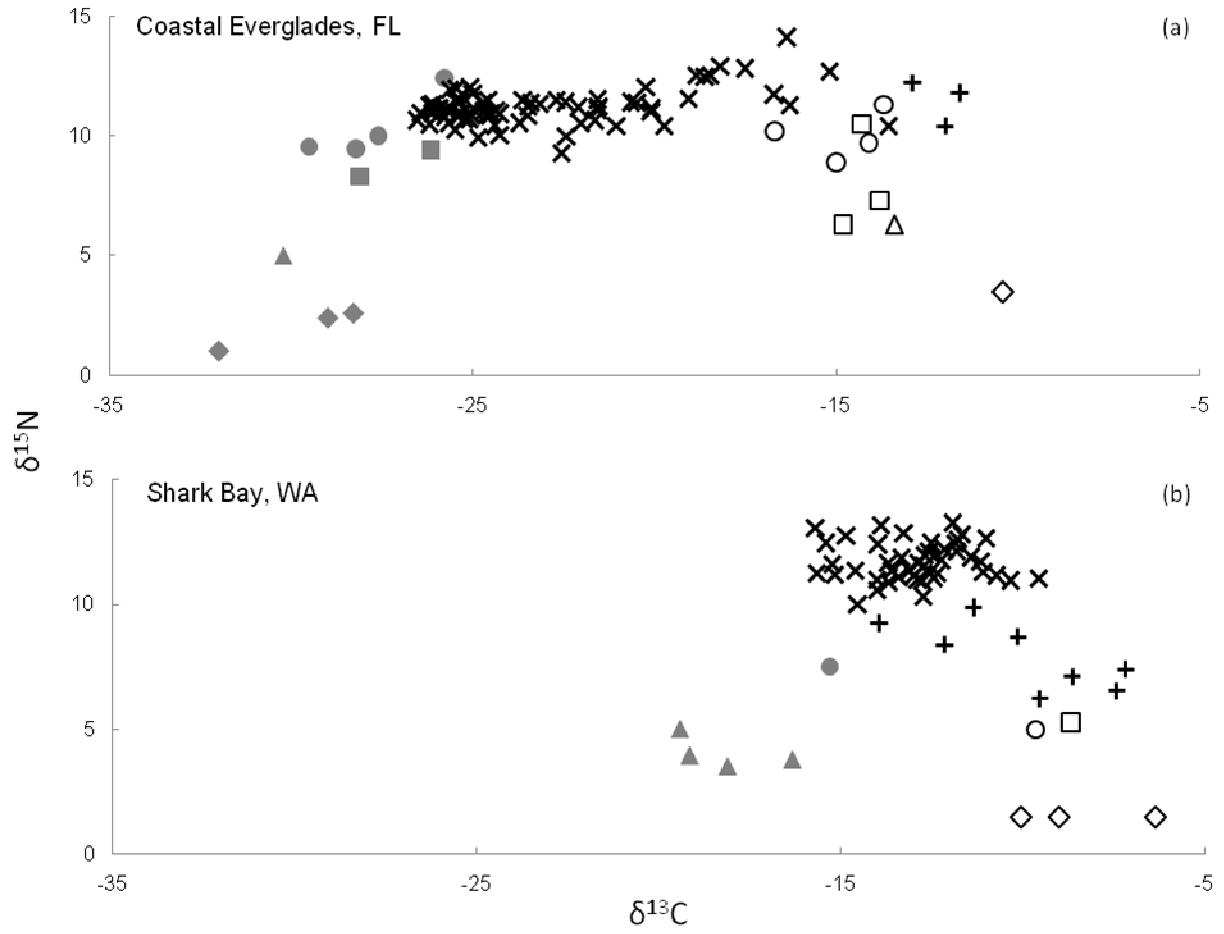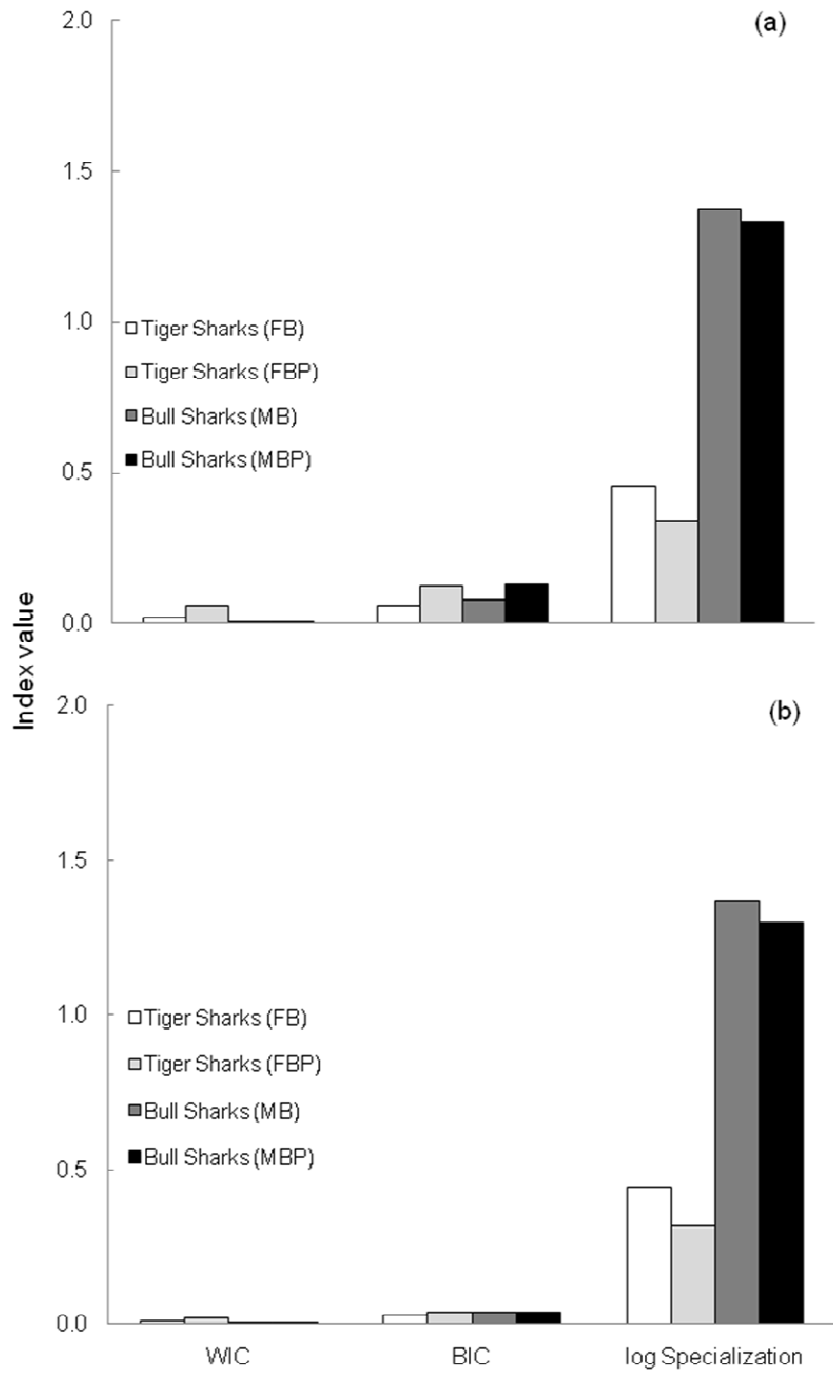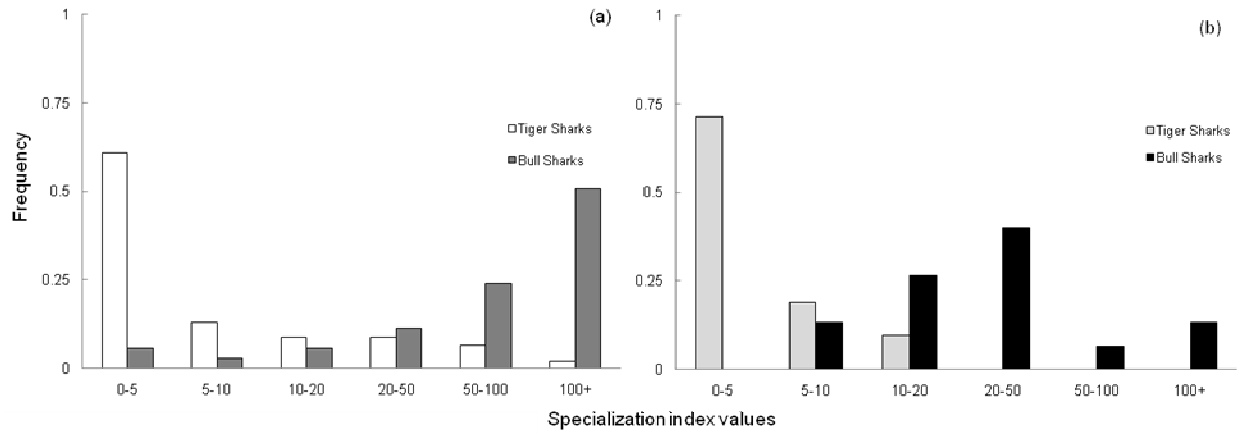
793   h) capture year.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6