Size-based variation in inter-tissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks and tiger sharks

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Running head: isotope variation in shark tissues

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Abstract

Stable isotopes are an important tool for understanding the trophic roles of elasmobranchs. However, whether different tissues provide consistent stable isotope values within an individual are largely unknown. To address this, the relationships among carbon and nitrogen isotope values were quantified for blood, muscle, and fin from juvenile bull sharks (*Carcharhinus leucas*), and blood and fin from large tiger sharks (*Galeocerdo cuvier*) collected in two different ecosystems. We also investigated the relationship between shark size and the magnitude of differences in isotopic values between tissues. Isotope values were significantly positively correlated for all paired tissue comparisons, but R^2 values were much higher for $\delta^{13}C$ than $\delta^{15}N$. Paired differences between isotopic values of tissues were relatively small, but varied significantly with shark total length, suggesting shark size can be an important factor influencing the magnitude of differences in isotope values of different tissues. For studies of juvenile sharks, care should be taken in using slow turnover tissues like muscle and fin, because they may retain a maternal signature for an extended time. While correlations were relatively strong, results suggest correction factors should be generated for the desired study species, and may only allow course-scale comparisons between studies using different tissue types.

Key words:

Apex predator, *Carcharhinus leucas*, estuary, food webs, foraging ecology, *Galeocerdo cuvier*, stable isotopes

Introduction

Elasmobranchs (sharks, skates, and rays) play crucial roles in marine ecosystems (Heithaus et al. 2008), but gaps in our knowledge of their trophic interactions hinder understanding of marine community dynamics and ecosystem function. Current studies of trophic interactions of elasmobranchs, especially sharks, are particularly important because populations of many species are declining rapidly worldwide (e.g. Dulvy et al. 2008). These declines already may be causing drastic shifts in food web structure and function (Heithuas et al. 2008).

Most studies of elasmobranch trophic interactions have employed stomach content analysis (see Weatherbee and Cortes 2004 for a review). Although stomach content analysis allows identification of specific prey taxa, it has drawbacks, including the need for large sample sizes and often destructive sampling. Sharks also often have empty stomachs (Weatherbee and Cortes 2004), further limiting information that can be gleaned from this approach. Stable isotope analysis provides an alternative, or complementary, method for gaining insights into the trophic interactions of sharks (e.g. Fisk et al. 2002, Domi et al. 2005, MacNeil et al. 2005), especially because samples can be collected without sacrificing individuals. This method is based on the principle that a consumer's tissues isotopically resemble those of its food (Post 2002), and thus present an extended dietary record (Bearhop et al. 2004). However, stable isotopes are incorporated into different body tissues at different rates, which can affect interpretation of data (Martinez del Rio et al. 2009).

Our understanding of the dynamics of stable isotope values in elasmobranchs lags behind that of other taxa. For example, isotopic turnover rates in tissues of elasmobranchs have only been reported for two species ($\delta^{15}N$ in captive *Potamotrygon motoro*; MacNeil et al. 2006; $\delta^{15}N$

and $\delta^{13}C$ in captive *Carcharhinus plumbeus*; Logan and Lutcavage 2010), compared to numerous studies investigating isotopic turnover rates in mammals (e.g. MacAvoy et al. 2006, Miller et al. 2008), birds (e.g. Hobson and Clark 1992, Haramis et al. 2007), and bony fishes (e.g. Jardine et al. 2004, Perga and Gerdeaux 2005, McIntyre and Flecker 2006). In addition to understanding turnover rates, it is important to understand the variability of isotopic values for various tissue types within an individual in order to make full use of stable isotopic data and compare information among studies (e.g. Pinnegar and Polunin 1999, Vander Zanden and Rasmussen 2001, Sweeting et al. 2005).

The purpose of this study was to (1) compare the $\delta^{13}C$ and $\delta^{15}N$ values of muscle, blood, and dorsal fin tissues from juvenile bull sharks (*Carcharhinus leucas*) and blood and dorsal fin tissues of large (juvenile and adult) tiger sharks (*Galeocerdo cuvier*) to determine if resulting intra-specific values from one tissue are comparable to those of other tissues for each species, and (2) gain insights into how differences among tissues within individuals may vary with shark size. Understanding if stable isotope analysis provides relatively consistent dietary data across tissue types, and if this consistency is similar across size-classes, may allow for less invasive sampling of tissues, and provide insight into ecological drivers of dietary variation.

Methods

Muscle, whole blood ("blood" hereafter), and dorsal fin ("fin") tissues were collected from 81 juvenile bull sharks (70-162 cm total length) captured on 500m longlines within the Shark River estuary of Everglades National Park, Florida, USA (see Heithaus et al. 2009 for specific details of the study area and capture methods). We used a biopsy punch to collect a 0.5 cm^3 muscle tissue biopsy *ca.* 5 cm lateral to the first dorsal fin, scissors to collect a 0.5 cm^3 tissue

clip from the dorsal fin, and an 18 gauge needle to collect 2 ml of blood from the caudal vein. Tissues were placed on ice and frozen upon return to the laboratory. Skin was removed from muscle samples before laboratory preparations. All samples were dried and homogenized. Blood and fin clips were collected from 46 tiger sharks (159-396 cm TL) captured on drumlines during long-term studies in the hypersaline seagrass ecosystem of Shark Bay, Western Australia (see Wirsing et al. 2006 for study site and sampling details). Sample collection, storage, and processing protocols were identical to those for bull sharks.

All samples were analyzed at the Florida International University Stable Isotope Facility (43 *C. leucas* blood samples, 50 *C. leucas* muscle samples, and 26 *C. leucas* fin samples) or the Yale Earth System Center for Stable Isotopic Studies (34 *C. leucas* blood samples, 27 *C. leucas* muscle samples, 19 *C. leucas* fin samples, 46 *G. cuvier* blood samples, and 46 *G. cuvier* fin samples). Lipids were not extracted from any tissues, and C:N ratios indicated that corrections for lipid content were not necessary (Post et al. 2007). To verify analytical consistency, we randomly selected samples to be analyzed at both Florida International University and Yale University, for which the variation between resulting $\delta^{13}C$ $\delta^{15}N$ values were 0.13‰ ± 0.20SE.

We used least squares regression analysis to determine (1) the relationships between $\delta^{13}C$ and $\delta^{15}N$ values for all paired tissues of bull sharks (i.e. blood-muscle, blood-fin, muscle-fin) and tiger sharks (i.e. blood-fin), and (2) the relationship between shark length and paired differences between tissues. Each paired difference was calculated by taking the absolute difference between the $\delta^{13}C$ or $\delta^{15}N$ values of two tissue types for each shark (e.g. if muscle = -13.1‰ and blood = -13.8‰, then the paired difference = 0.7‰). Cook's test was used to identify outliers, each tissue comparison regression model slope was tested to determine if it deviated significantly from a slope of one, and paired difference models were tested as linear and polynomial models to

identify the best fitting model. Because isotope assimilation into body tissues experiences a lag time based on the turnover rate of the specific tissue type (reviewed by Martinez del Rio et al. 2009), and sharks can experience ontogenetic shifts in diet (reviewed by Weatherbee and Cortes 2004), in some cases polynomial models may produce the best fit for determining the relationship between isotope values and shark size.

Results

Comparisons of $\delta^{13}C$ and $\delta^{15}N$ values revealed highly significant positive correlations for all tissue pairs in bull sharks. The slopes of all three bull shark $\delta^{13}C$ comparisons did not differ from 1:1 and all R^2 values were >0.71 (Fig.1a, c, e). Blood was on average 0.57‰ ± 0.055 SE more depleted (i.e. more negative) than muscle and on average 2.8‰ ± 0.10 SE more depleted than fin, and muscle was on average 2.1‰ ± 0.092 SE more depleted than fin (Fig. 1a, c, e). Relationships between $\delta^{15}N$ values were significant, but weaker than those of $\delta^{13}C$, with R^2 values between 0.15-0.43 (Fig. 1b, d, f). Only the relationship between muscle and fin deviated from a slope of one (slope = 0.6, t_{41} = -7.8, p = <0.001). Mean differences for bull shark blood and muscle $\delta^{15}N$ was 0.80‰ ± 0.064 SE, blood and fin was 0.65‰ ± 0.16 SE, and muscle and fin was 0.20‰ ± 0.15 SE (Fig. 1b, d, f). The ranges of $\delta^{13}C$ values were relatively wide for all bull shark tissue types, while the ranges of $\delta^{15}N$ values were relatively narrow (Table 1).

Relationships between tissue types were similar in tiger sharks. Correlations for $\delta^{13}C$ and $\delta^{15}N$ of blood and fin were positive and significant, but the relationship was tighter for $\delta^{13}C$ (R^2 = 0.62) than for $\delta^{15}N$ (R^2 = 0.32) (Fig. 1g, h). The slope for $\delta^{13}C$ was not significantly different from one, but the slope for $\delta^{15}N$ was (slope = 0.63, t_{40} = -10.0, p = <0.001). For tiger sharks, the $\delta^{13}C$ of blood was on average 1.2‰ ± 0.26 SE more depleted than fin while the mean difference

136 in $\delta^{15}N$ was only 0.09‰ ± 0.21 SE (Fig. 1g, h). Similar to the bull sharks, the ranges of $\delta^{13}C$
137 values were relatively wider than those of $\delta^{15}N$ values (Table 1). **[Insert Figure 1 and Table 1]**
138 Based on the tight relationships in isotopic values of tissues, it is not surprising most
139 tissue types showed similar relationships between $\delta^{13}C$ and $\delta^{15}N$ and shark total length. For both
140 $\delta^{13}C$ and $\delta^{15}N$ in bull sharks, all tissues declined until 110-130 cm TL, and then increased (Fig.
141 2a-f). All relationships between isotope values and shark total length were significant ($p < 0.05$)
142 for bull sharks. For tiger sharks, $\delta^{13}C$ of fin and blood slightly increased with size until 250-300
143 cm TL, and then declined (Fig. 2g and i), while $\delta^{15}N$ declined with size until 250-300 cm TL,
144 and then increased (Fig. 2h and j). Only the relationship between blood $\delta^{13}C$ values and tiger
145 shark total length was significant. **[Insert Figure 2]**
146 The difference in $\delta^{13}C$ values between tissue types for bull sharks was influenced by
147 shark total length for all pairings. In all cases for bull sharks, paired differences in $\delta^{13}C$ values
148 were highest for the smallest individuals and decreased with size. This relationship was
149 strongest for fin and blood ($R^2 = 0.64$), and weakest for fin and muscle ($R^2 = 0.21$; Fig. 3a, c, e).
150 The paired difference between muscle and blood dropped rapidly until ~110cm TL, when the
151 direction of the difference became less predictable. The difference between fin and blood
152 dropped linearly and approached zero at approximately 165cm TL, and the difference between
153 fin and muscle showed a relatively weak relationship with shark length. Paired differences for
154 $\delta^{15}N$ of bull sharks showed a different pattern. There was no significant relationship between
155 shark size and tissue difference in $\delta^{15}N$ of fin and muscle, while somewhat weak, but significant,
156 nonlinear relationships were found for comparisons between blood and muscle ($R^2 = 0.18$), and
157 blood and fin ($R^2 = 0.39$; Fig. 3b, d, f). The difference in $\delta^{15}N$ for these comparisons was

158 relatively low at small total lengths, increased slightly with size, and then declined in the largest
159 individuals.

160 For tiger sharks, there was a significant but relatively weak ($R^2 = 0.27$), positive effect of
161 shark size on differences in $\delta^{13}C$ of fin and blood, and shark size explained no variation in
162 differences between $\delta^{15}N$ of fin and blood (Fig. 3g, h). **[Insert Figure 3]**

163

164 **Discussion**

165 Our study of two shark species at different life history stages, and from two different
166 environments, has important implications for using stable isotope data in studies of
167 elasmobranchs. Variability in stable isotope values within and among individuals can be driven
168 by many ecological factors, including environmental conditions, metabolic processes, food
169 quality, or changes in behavior, among many other factors (reviewed by Martinez del Rio et al.
170 2009). Yet, patterns of variability in stable isotope values among individuals can provide
171 important insights into the trophic ecology of individuals within a population, as well as into
172 differences among population and species.

173 Body size appears to be one factor that explained the regression slopes for some of the
174 inter-tissue paired differences for our sample populations (Fig. 3). The paired differences in $\delta^{13}C$
175 of bull shark tissues were greatest in smaller individuals and decreased with size, indicating that
176 isotopic values of different tissues were more similar for larger individuals. Prior to birth, bull
177 sharks are directly connected to their mothers by an umbilical cord, which serves as a pathway
178 through which nutrients and energy are transferred between mother and fetus. Based on the
179 presence of open umbilical scars, bull sharks in the coastal Everglades are born between 65-75
180 cm TL. Because of their connection to their mothers, pups should have $\delta^{13}C$ values similar to

181 their mothers (coastal predators; $\delta^{13}C$ ~-15‰ in our study area; Chasar et al. 2005), as seen in
182 cetaceans (e.g. bottlenose dolphins, *Tursiops truncatus,* Knoff et al. 2008; sea lions, *Zalophus*
183 *californianus,* Porras-Peters et al. 2008). After birth, juvenile sharks spend several years in low-
184 salinity estuaries and nearshore waters (e.g. Wiley and Simpfendorfer 2007, Heithaus et al.
185 2009), and therefore $\delta^{13}C$ values should begin to diverge from their mothers as they adopt a more
186 $\delta^{13}C$-depleted estuarine diet (consumer taxa $\delta^{13}C$ is typically < -25‰ in the Shark River;
187 Williams and Trexler 2006, M. Heithaus *unpublished data;* see also Fig 2). The change in $\delta^{13}C$
188 values should occur earlier in tissues that turnover more rapidly. For example, differences
189 between blood and both fin and muscle in the smallest bull sharks suggests that fin tissue largely
190 maintains the maternal signature, likely due to a slower turnover rate. In contrast, blood reflects
191 the young sharks' diet within two years of birth, likely due to a faster turnover rate in this tissue
192 type (MacNeil et al. 2006).
193 The regression model for the paired difference of $\delta^{13}C$ for muscle and blood appears to
194 reach equilibrium around 110 cm TL and two years of age (based on growth rates in Branstetter
195 and Stiles 1987 and estimated sizes at birth; Heithaus et al. 2009). This may indicate the time
196 period for which muscle $\delta^{13}C$ values are no longer influenced by the maternal diet for juveniles,
197 and accurately portray that individual's diet over its lifetime. Deviations in isotope values of
198 larger individuals may reflect other underlying ecological patterns, for example seasonal shifts in
199 diet, which may be displayed more rapidly in blood values than in muscle or fin (P. Matich et al.
200 *unpublished data*). In contrast to bull sharks, differences in $\delta^{13}C$ among blood and fin clips
201 increased with size in tiger sharks. This likely reflects a difference in the feeding ecology of the
202 two species, and the increasing difference in $\delta^{13}C$ of blood and fin may reflect a shift in the diets
203 of tiger sharks as they grow (e.g. Lowe et al. 1996, Simpfendorfer et al. 2001).

204 Size-based differences among tissues in stable isotope values are important to consider
205 when investigating the ecological drivers of dietary variation within populations. $\delta^{13}C$ values
206 (Fig. 2a, c, e) support the hypothesis that the maternal influence on isotopic values of juvenile
207 bull sharks is evident for several years, but individual variability in isotopic values makes it
208 difficult to draw conclusions about the precise timing of tissue values equilibrating. Especially
209 for $\delta^{13}C$ of both species, the range of isotope values was relatively wide, even for sharks of a
210 given size, suggesting that other factors, like habitat use (e.g. Darimont et al. 2009, Quevedo et
211 al. 2009), body condition (e.g. Tinker et al. 2008, Tucker et al. 2009), and/or seasonal shifts (e.g.
212 Inger et al. 2006, Cherel et al. 2007) may affect the diet patterns for individuals of these two
213 populations.
214 The strong positive correlations between tissues in $\delta^{13}C$ for both bull sharks and tiger
215 sharks (Fig. 1) suggest that for a species, multiple tissues may be compared after applying a
216 correction factor. A strict 1:1 substitution of values among tissues is not recommended, and we
217 suggest correction factors should be generated for individual populations because ecological
218 differences may lead to variability in isotopic assimilation across individuals of the same taxa
219 (Post 2002). Using correction factors generated for a species in one ecosystem may differ from
220 those generated for the same species collected from a different ecosystem, and therefore it is
221 currently most appropriate to generate correction factors on a per-population basis.
222 Tissue comparisons may allow for gaps within data sets to be filled and to increase the
223 number of individuals that can be directly compared. Individuals for which isotope values of a
224 particular tissue are not available may have correction factors applied to estimate isotopic
225 value(s) of the uncollected tissue. Yet, it is important to consider potential factors that limit the
226 use of correction factors. Species that experience ontogenetic shifts in diet may experience

227 variability in inter-tissue relationships between isotope values (e.g. Quillfeldt et al. 2008, Tierney
228 et al. 2008, Young et al. 2010), and therefore correction factors may be more accurate for certain
229 age/size-classes of animals. For example, the difference between tissues for bull sharks (paired
230 differences; Fig. 3) were largest (7‰ fin-blood) for the smallest individuals sampled, and tended
231 to decrease and approach equilibrium (1:1 relationship) as bull shark total length increased. This
232 suggests that correction factors may be more useful for larger individuals, which generally had
233 smaller differences in isotope values for different tissues. Therefore, care must be taken when
234 using correction factors and variability in factors that affect trophic role (such as body size) must
235 be taken into consideration prior to using estimated isotope values produced by correction factors
236 for diet analysis.

237 Relationships among tissues in $\delta^{15}N$ were relatively weak, raising doubts as to whether
238 tissues can be compared reliably. The relatively small range in $\delta^{15}N$ for both species (3.3‰ and
239 3.4‰ for tiger sharks and bull sharks, respectively), however, could be responsible for these
240 patterns, and the question of interest may determine the magnitude of potential error when
241 substituting $\delta^{15}N$ values for different tissue types when using correction factors. The paired
242 differences in $\delta^{15}N$ for bull sharks ($R^2 = 0.04$ to 0.39) and tiger sharks ($R^2 < 0.01$) were relatively
243 weak, suggesting that combining data sets with multiple tissue types may be problematic for
244 $\delta^{15}N$. Because we found the $\delta^{15}N$ relationships to be relatively weak, we suggest that further
245 ecological and physiological studies are needed to elucidate the factor(s) affecting inter-tissue
246 differences in $\delta^{15}N$.

247 Published turnover rates for elasmobranch tissues (MacNeil et al. 2006), combined with
248 the long duration before convergence of $\delta^{13}C$ values of blood and muscle of bull sharks in our
249 study, suggest that using stable isotopes from these tissues are most appropriate for elucidating

long-term dietary patterns. Such long-term information may be useful for investigating questions such as the degree of specialization within populations, how changes in environmental factors may influence consumer diets, and what ecological factors influence inter-population variation in feeding behaviors. Other taxa exhibit considerably faster turnover rates for blood (e.g. ~52 days ($\delta^{13}C$) and ~46 days ($\delta^{15}N$) for mice (*Mus musculus*) MacAvoy et al. 2006), muscle (e.g. 4-5 months ($\delta^{15}N$) for whitefish (*Coregonus lavaretus*) Perga and Gerdeaux 2005), and fin (e.g. ~37 days ($\delta^{15}N$) for armored catfish (*Ancistrus triradiatus*) McIntyre and Flecker 2006) tissues, allowing for more fine-scale diet studies. Therefore, stomach content analysis remains an important complimentary method for studying elasmobranch trophic ecology, especially when investigating short-term variability in diets.

Our understanding and application of stable isotopes in elasmobranchs is still in its infancy. Sharks and rays are important top and mesopredators in multiple ecosystems (Heithaus et al. 2010). With many populations jeopardized worldwide, stable isotope analysis provides an important tool for studying their trophic ecology non-lethally. Yet, further studies in the field and laboratory, and across a variety of taxa, environments, and life history stages, are needed to better understand how stable isotopes can be best applied and interpreted for studies of their trophic ecology.

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References

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., and MacLeod, H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol. **73**: 1007-1012.

Branstetter, S. and Stiles, R. 1987. Age and growth estimates of the bull shark, *Carcharhinus leucas*, from the northern Gulf of Mexico. Environ. Biol. Fishes **20**: 169-181.

Chasar, L.C., Chanton, J.P., Koenig, C.C., and Coleman, F.C. 2005. Evaluating the effect of environmental disturbance on the trophic structure of Florida Bay, U.S.A.: Multiple stable isotope analyses of contemporary and historical specimens. Limnol. Oceanogr. **50**: 1059-1072.

Cherel, Y., Hobson, K.A., Guinet, C., and Vanpe, C. 2007. Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predators from the Southern Ocean. J. Anim. Ecol. **76**: 826-836.

Darimont, C.T., Paquet, P.C., and Reimchen, T.E. 2009. Landscape heterogeneity and marine subsidy generate extensive intrapopulation niche diversity in a large terrestrial vertebrate. J. Anim. Ecol. **78**: 126-133.

296 Domi, N., Bouquegneau, J.M., and Das, K. 2005. Feeding ecology of five commercial shark
297 species of the Celtic Sea through stable isotope and trace metal analysis. Mar. Environ.
298 Res. **60**: 551-569.
299 Dulvy, N.K., Baum, J.K., Clarke, S., Compagno, L.J.V., Cortes, E., Domingo, A., Fordham, S.,
300 Fowler, S., Francis, M.P., Gibson, C., Martinez, J., Musick, J.A., Soldo, A., Stevens, J.D.,
301 and Valenti, S. 2008 You can swim but you can't hide: the global status and conservation
302 of oceanic pelagic sharks and rays. Aquat. Conserv.: Mar. Freshw. Ecosyst. **18**: 459-482.
303 Fisk, A.T., Tittlemier, S.A., Pranschke, J.L., and Norstrom, R.J. 2002. Using anthropogenic
304 contaminants and stable isotopes to assess the feeding ecology of Greenland sharks.
305 Ecology **83**: 2162-2172.
306 Haramis, G.M., Link, W.A., Osenton, P.C., Carter, D.B., Weber, R.G., Clark, N.A., Teece, M.A.,
307 and Mizrahi, D.S. 2007. Stable isotope and pen feeding trial studies confirm the value of
308 horseshoe crab *Limulus polyphemus* eggs to spring migrant shorebirds in Delaware Bay.
309 J. Avian Biol. **38**: 367-376.
310 Heithaus, M.R, Frid, A., Wirsing, A.J., and Worm, B. 2008. Predicting ecological consequences
311 of marine top predator declines. Trends Ecol. Evol. **23**: 202-210.
312 Heithaus, M.R., Delius, B.K., Wirsing, A.J., and Dunphy-Daly, M.M. 2009. Physical factors
313 influencing the distribution of a top predator in a subtropical oligotrophic estuary.
314 Limnol. Oceanogr. **54**: 472-482.
315 Heithaus M.R., Frid A., Vaudo J.J., Worm B., and Wirsing A.J. 2010. Unraveling the ecological
316 importance of elasmobranchs. Pp 608-633 In: Carrier, J.C., Musick, J.A., and Heithaus,
317 M.R. (eds) Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and
318 Conservation. CRC Press, Boca Raton, FL.

319 Hobson, K.A. and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: turnover of
320 13C in tissues. Condor **94**: 181-188.
321 Inger, R., Ruxton, G.D., Newton, J., Colhoun, K., Robinson, JA, Jackson, A.L., and Bearhop, S.
322 2006. Temporal and intrapopulation variation in prey choice of wintering geese
323 determined by stable isotope analysis. J. Anim. Ecol. **75**: 1190-1200.
324 Jardine, T.D., MacLatchy, D.L., Fairchild, W.L., Cunjak, R.A., and Brown, S.B. 2004. Rapid
325 carbon turnover during growth of Atlantic salmon (*Salmo salar*) smolts in sea water, and
326 evidence for reduced food consumption by growth-stunts. Hydrobiologia **527**: 63-75.
327 Knoff, A., Hohn, A., and Macko, S. 2008. Ontogenetic diet changes in bottlenose dolphins
328 (*Tursiops truncatus*) reflected through stable isotopes. Mar. Mamm. Sci. **24**: 128-137.
329 Logan, J.M. and Lutcavage, ME. Stable isotope dynamics in elasmobranch fishes. Hydrobiologia
330 DOI 10.1007/s10750-010-0120-3.
331 Lowe, C.G., Wetherbee, B.M., Crow, G.L., and Tester, A.L. 1996. Ontogenetic dietary shifts and
332 feeding behavior of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. Environ.
333 Biol. Fishes **47**: 203-211.
334 MacAvoy, S.E., Arneson, L.S., and Bassett, E. 2006. Correlation of metabolism with tissue
335 carbon and nitrogen turnover rate in small mammals. Oecologia **150**: 190-201.
336 MacNeil, M.A., Skomal, G.B., and Fisk, A.T. 2005. Stable isotopes from multiple tissues reveal
337 diet switching in sharks. Mar. Ecol. Prog. Ser. **302**: 199-206.
338 MacNeil, M.A., Drouillard, K.G., and Fisk, A.T. 2006. Variable uptake and elimination of stable
339 nitrogen isotopes between tissues in fish. Can. J. Fish. Aquat. Sci. **63**: 345-353.
340 Martinez del Rio, C., Wolf, N., Carleton, S.A., and Gannes, L.Z. 2009. Isotopic ecology ten
341 years after a call for more laboratory experiments. Biol. Rev. **84**: 91-111.

McIntyre, P.B. and Flecker, A.S. 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia **148**: 12-21.

Miller, J.F., Millar, J.S., and Longstaffe, F.J. 2008. Carbon- and nitrogen-isotope tissue-diet discrimination and turnover rates in deer mice, *Peromyscus maniculatus*. Can. J. Zoo. **86**: 685-691.

Perga, M.E. and Gerdeaux, D. 2005. 'Are fish what they eat' all year round? Oecologia **144**: 598-606.

Pinnegar, J.K. and Polunin, N.V.C. 1999. Differential fraction of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct. Ecol. **13**: 225-231.

Porras-Peters, H., Aurioles-Gamboa, D., Cruz-Escalona, V.H., and Koch, P.L. 2008. Trophic level and overlap of sea lions (*Zalophus californianus*) in the Gulf of California, Mexico. Mar. Mamm. Sci. **24**: 554-576.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**: 703-718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Montaña, C.G., and Quattrochi, J. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia **152**: 179-189.

Quevedo, M., Svanback, R., and Eklov, P. 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology **90**: 2263-2274.

Quillfeldt, P., Bugoni, L., McGill, R.A.R., Masello, J.F., and Furness, R.W. 2008. Differences in stable isotopes in blood and feathers of seabirds are consistent across species, age, and latitude: implications for food web studies. Mar. Biol. **155**: 593-598.

Simpfendorfer, C.A., Goodreid, A.B., and McAuley, R.B. 2001. Size, sex, and geographic variation in the diet of tiger sharks, *Galeocerdo cuvier*, from Western Australian waters. Environ. Biol. Fishes **61**: 37-46.

Sweeting, C.J., Jennings, S., and Polunin, N.V.C. 2005. Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Funct. Ecol. **19**: 777-784.

Tierney, M., Southwell, C., Emmerson, L.M., and Hindell, M.A. 2008. Evaluating and using stable-isotope analysis to infer diet composition and foraging ecology of Adelie penguins *Pygoscelis adeliae*. Mar. Ecol. Prog. Ser. **355**: 297-307.

Tinker, M.T., Bentall, G., and Estes, J.A. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. PNAS **105**: 560-565.

Tucker, S., Bowen, W.D., Iverson, S.J., Blanchard, W., and Stenson, G.B. 2009. Sources of variation in the diets of harp and hooded seals estimated from quantitative fatty acid signature analysis (QFASA). Mar. Ecol. Prog. Ser. **384**: 287-302.

Vander Zanden, M. J. and Rasmussen, J.B. 2001. Variation in δ15N and δ 13C trophic fractionation: implications for aquatic food web studies. Limnol. Oceanogr. **48**:2061-2066.

Weatherbee, B.M. and Cortes, E. 2004. Food consumption and feeding habits. *In*: Carrier, JC, JA Musick and MR Heithaus (eds) Biology of sharks and their relatives. Boca Raton, FL. CRC Press, pp 225-246.

Wiley, T.R. and Simpfendorfer, C.A. 2007. The ecology of elasmobranches occurring in the Everglades National Park, Florida: implications for conservation and management. Bull. Mar. Sci. **80**: 171-189.

386 Williams, A.J. and Trexler, J.C. 2006. A preliminary analysis of the correlation of food-web
387 characteristics with hydrology and nutrient gradients in the southern Everglades.
388 Hydrobiologia **569**:493–504.
389 Wirsing, A.J., Heithaus, M.R., and Dill, L.M. 2006. Tiger shark (*Galeocerdo cuvier*) abundance
390 and growth rates in a subtropical embayment: evidence from seven years of standardized
391 fishing efforts. Mar. Biol. **4**: 961-968.
392 Young, B.G., Loseto, L.L., and Ferguson, S.H. 2010. Diet differences among age classes of
393 Arctic seals: evidence from stable isotope and mercury biomarkers. Pol. Biol. **33**: 153-
394 162.

Table and Figure Legends

Table 1: Minimum and maximum values for $\delta^{13}C$ and $\delta^{15}N$ values for blood, muscle, and fin for *Carcharhinus leucas* and blood and fin for *Galeocerdo cuvier* in ‰.

Figure 1: Comparisons of $\delta^{13}C$ for blood and fin (a), muscle and fin (c), and blood and muscle (e), and comparisons of $\delta^{15}N$ for blood and fin (b), muscle and fin (d), and blood and muscle (f) for *Carcharhinus leucas*, and $\delta^{13}C$ for blood and fin (g), and $\delta^{15}N$ for blood and fin (h) for *Galeocerdo cuvier.*

Figure 2: Comparisons of $\delta^{13}C$ and shark total length for fin (a), blood (c), and muscle (e), and comparisons of $\delta^{15}N$ and shark total length for fin (b), blood (d), and muscle (f) for *Carcharhinus leucas*, and $\delta^{13}C$ and shark total length for fin (g) and blood (i), and $\delta^{15}N$ and shark total length for fin (h) and blood (j) for *Galeocerdo cuvier.*

Figure 3: Paired differences of $\delta^{13}C$ for blood and fin (a), muscle and fin (c), and blood and muscle (e), and of $\delta^{15}N$ for blood and fin (b), muscle and fin (d), and blood and muscle (f) for *Carcharhinus leucas*, and $\delta^{13}C$ for blood and fin (g), and $\delta^{15}N$ for blood and fin (h) for *Galeocerdo cuvier.*

Table 1: Ranges of $\delta^{13}C$ and $\delta^{15}N$ in bull sharks and tiger sharks in ‰.

		Min $\delta^{13}C$	Max $\delta^{13}C$	Min $\delta^{15}N$	Max $\delta^{15}N$
Bull Sharks	Blood	-26.86	-16.27	9.91	12.53
	Muscle	-26.79	-16.51	11.07	13.26
	Fin	-24.62	-15.13	10.81	13.00
Tiger Sharks	Blood	-15.72	-9.56	10.57	13.09
	Fin	-14.69	-8.77	10.41	13.03

Figure 1

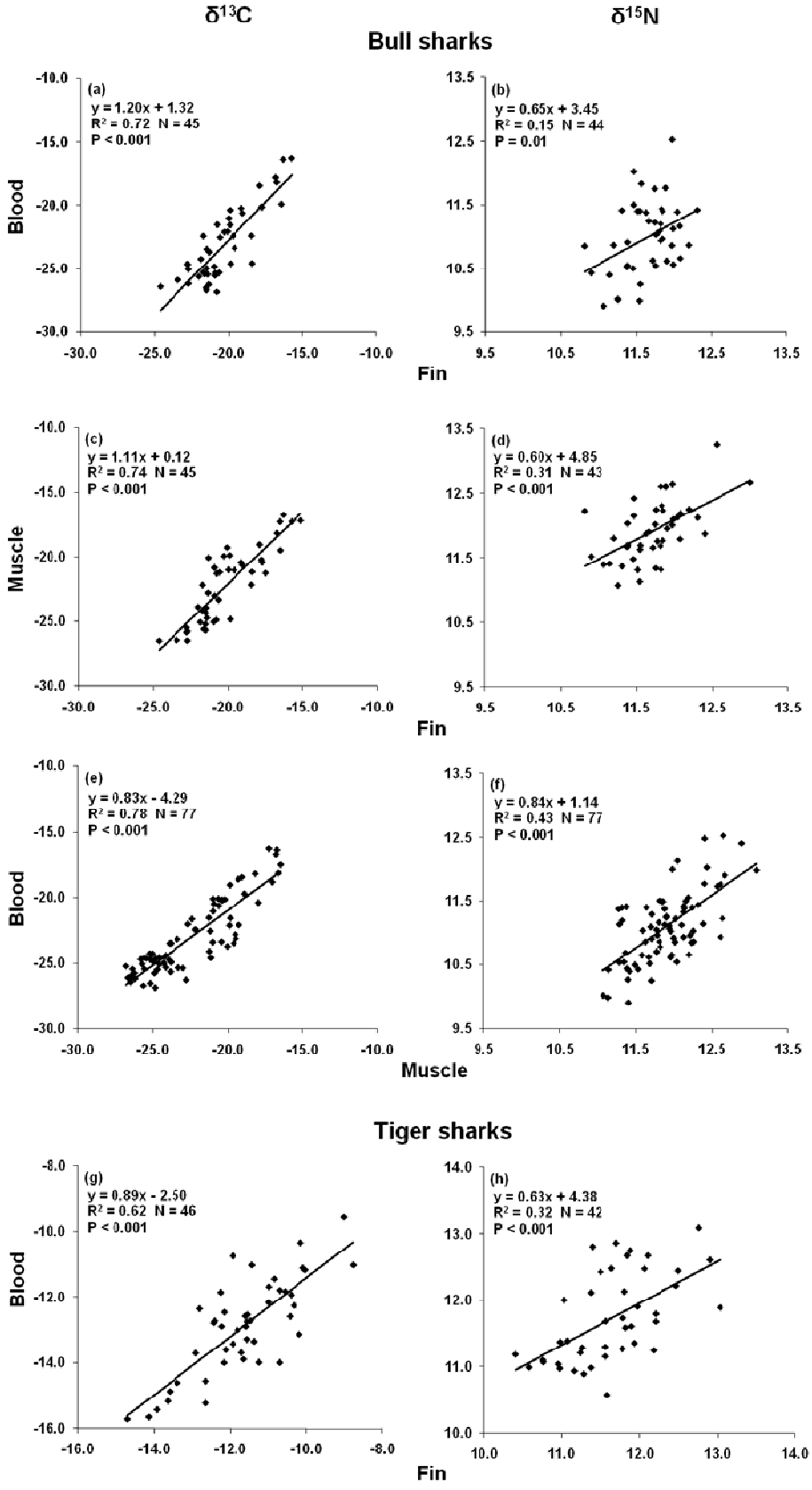

δ13C
δ15N
Bull sharks
(a)
y = 1.20x + 1.32
R² = 0.72 N = 45
P < 0.001
(b)
y = 0.65x + 3.45
R² = 0.15 N = 44
P = 0.01
Blood
Fin
(c)
y = 1.11x + 0.12
R² = 0.74 N = 45
P < 0.001
(d)
y = 0.60x + 4.85
R² = 0.31 N = 43
P < 0.001
Muscle
Fin
(e)
y = 0.83x - 4.29
R² = 0.78 N = 77
P < 0.001
(f)
y = 0.84x + 1.14
R² = 0.43 N = 77
P < 0.001
Blood
Muscle
Tiger sharks
(g)
y = 0.89x - 2.50
R² = 0.62 N = 46
P < 0.001
(h)
y = 0.63x + 4.38
R² = 0.32 N = 42
P < 0.001
Blood
Fin

Figure 2

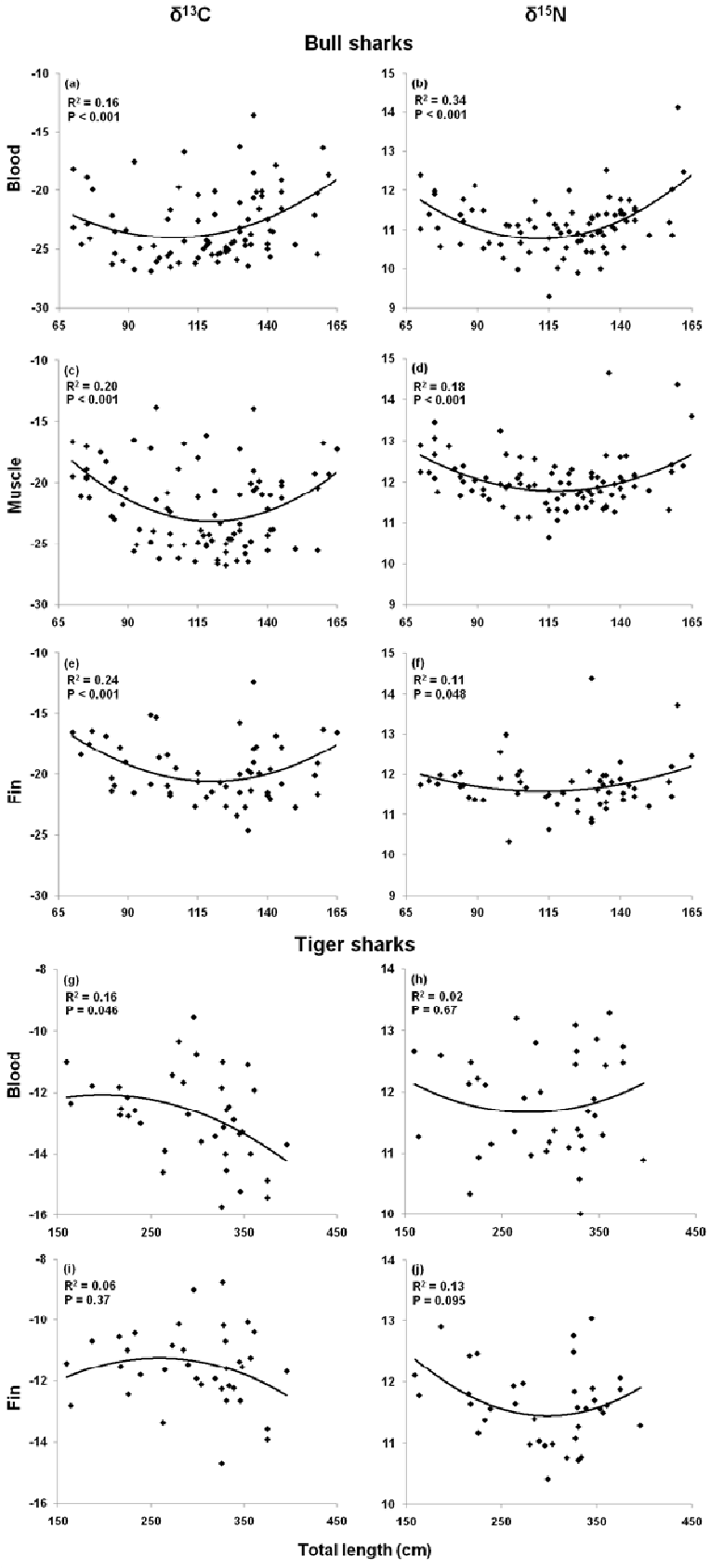
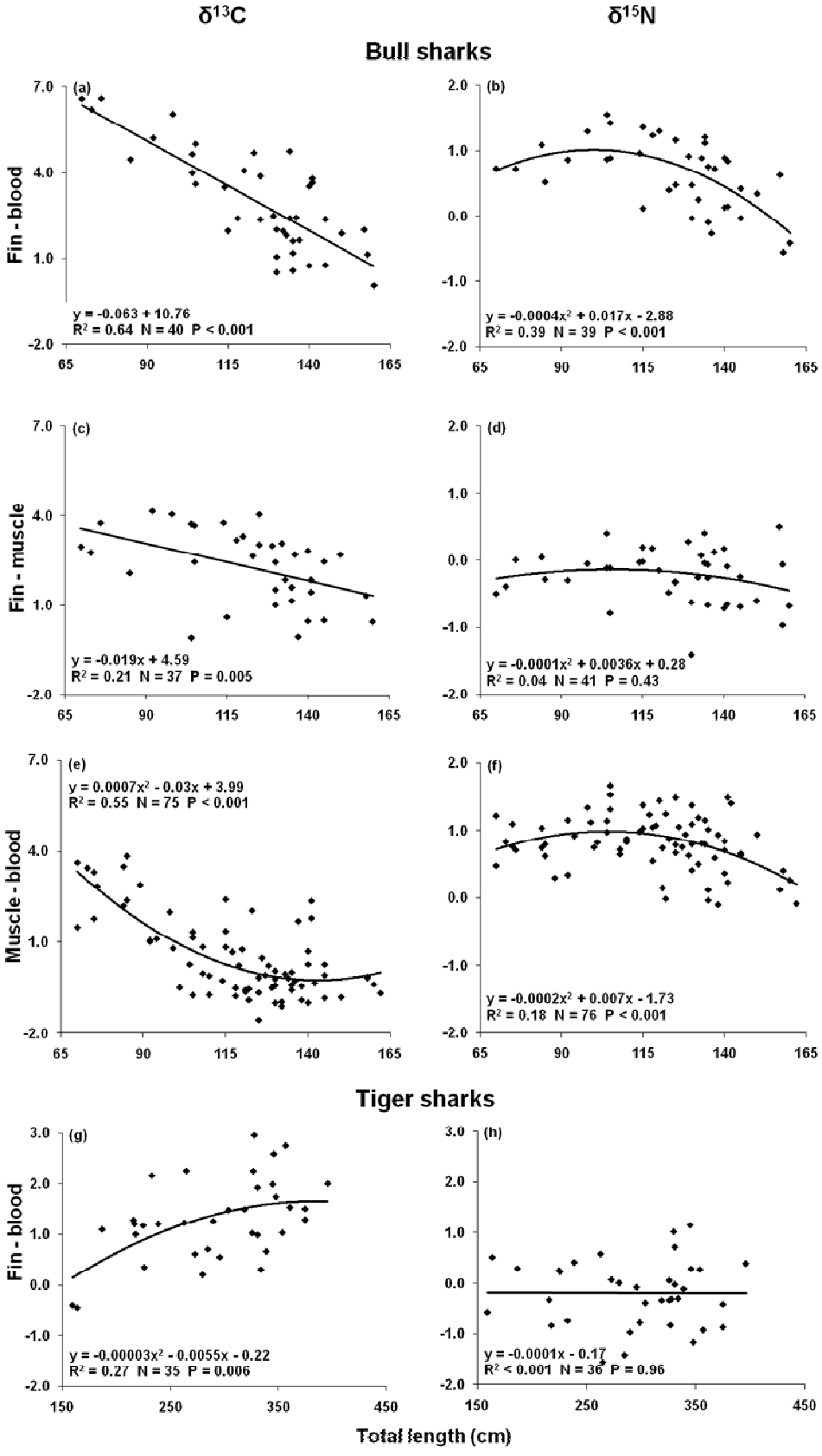

δ13C
δ15N
Bull sharks
Blood
Muscle
Fin
(a) R² = 0.16 P < 0.001
(b) R² = 0.34 P < 0.001
(c) R² = 0.20 P < 0.001
(d) R² = 0.18 P < 0.001
(e) R² = 0.24 P < 0.001
(f) R² = 0.11 P = 0.048
Tiger sharks
(g) R² = 0.16 P = 0.046
(h) R² = 0.02 P = 0.67
(i) R² = 0.06 P = 0.37
(j) R² = 0.13 P = 0.095
Total length (cm)

Figure 3


δ13C
δ15N
Bull sharks
(a)
(b)
(c)
(d)
(e)
(f)
Fin - blood
Fin - muscle
Muscle - blood
y = -0.063 + 10.76
R² = 0.64 N = 40 P < 0.001
y = -0.0004x² + 0.017x - 2.88
R² = 0.39 N = 39 P < 0.001
y = -0.019x + 4.59
R² = 0.21 N = 37 P = 0.005
y = -0.0001x² + 0.0036x + 0.28
R² = 0.04 N = 41 P = 0.43
y = 0.0007x² - 0.03x + 3.99
R² = 0.55 N = 75 P < 0.001
y = -0.0002x² + 0.007x - 1.73
R² = 0.18 N = 76 P < 0.001
Tiger sharks
(g)
(h)
y = -0.00003x² - 0.0055x - 0.22
R² = 0.27 N = 35 P = 0.006
y = -0.0001x - 0.17
R² < 0.001 N = 36 P = 0.96
Total length (cm)