

2010

Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*)

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Recommended Citation

Matich, P., M.R. Heithaus, C.A. Layman. 2010. Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). *Canadian Journal of Fisheries and Aquatic Sciences* 67: 877-885.

This material is based upon work supported by the National Science Foundation through the Florida Coastal Everglades Long-Term Ecological Research program under Cooperative Agreements #DBI-0620409 and #DEB-9910514. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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

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Running head: isotope variation in shark tissues

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24 **Abstract**

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

40

41 **Key words:**

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

44 **Introduction**

45 Elasmobranchs (sharks, skates, and rays) play crucial roles in marine ecosystems
46 (Heithaus et al. 2008), but gaps in our knowledge of their trophic interactions hinder
47 understanding of marine community dynamics and ecosystem function. Current studies of
48 trophic interactions of elasmobranchs, especially sharks, are particularly important because
49 populations of many species are declining rapidly worldwide (e.g. Dulvy et al. 2008). These
50 declines already may be causing drastic shifts in food web structure and function (Heithaus et al.
51 2008).

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

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

67 and $\delta^{13}\text{C}$ in captive *Carcharhinus plumbeus*; Logan and Lutcavage 2010), compared to numerous
68 studies investigating isotopic turnover rates in mammals (e.g. MacAvoy et al. 2006, Miller et al.
69 2008), birds (e.g. Hobson and Clark 1992, Haramis et al. 2007), and bony fishes (e.g. Jardine et
70 al. 2004, Perga and Gerdeaux 2005, McIntyre and Flecker 2006). In addition to understanding
71 turnover rates, it is important to understand the variability of isotopic values for various tissue
72 types within an individual in order to make full use of stable isotopic data and compare
73 information among studies (e.g. Pinnegar and Polunin 1999, Vander Zanden and Rasmussen
74 2001, Sweeting et al. 2005).

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

83

84 **Methods**

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

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

97 All samples were analyzed at the Florida International University Stable Isotope Facility
98 (43 *C. leucas* blood samples, 50 *C. leucas* muscle samples, and 26 *C. leucas* fin samples) or the
99 Yale Earth System Center for Stable Isotopic Studies (34 *C. leucas* blood samples, 27 *C. leucas*
100 muscle samples, 19 *C. leucas* fin samples, 46 *G. cuvier* blood samples, and 46 *G. cuvier* fin
101 samples). Lipids were not extracted from any tissues, and C:N ratios indicated that corrections
102 for lipid content were not necessary (Post et al. 2007). To verify analytical consistency, we
103 randomly selected samples to be analyzed at both Florida International University and Yale
104 University, for which the variation between resulting $\delta^{13}\text{C}$ $\delta^{15}\text{N}$ values were $0.13\text{‰} \pm 0.20\text{SE}$.

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

113 identify the best fitting model. Because isotope assimilation into body tissues experiences a lag
114 time based on the turnover rate of the specific tissue type (reviewed by Martinez del Rio et al.
115 2009), and sharks can experience ontogenetic shifts in diet (reviewed by Weatherbee and Cortes
116 2004), in some cases polynomial models may produce the best fit for determining the
117 relationship between isotope values and shark size.

118

119 **Results**

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

131 Relationships between tissue types were similar in tiger sharks. Correlations for $\delta^{13}\text{C}$ and
132 $\delta^{15}\text{N}$ of blood and fin were positive and significant, but the relationship was tighter for $\delta^{13}\text{C}$ ($R^2 =$
133 0.62) than for $\delta^{15}\text{N}$ ($R^2 = 0.32$) (Fig. 1g, h). The slope for $\delta^{13}\text{C}$ was not significantly different
134 from one, but the slope for $\delta^{15}\text{N}$ was (slope = 0.63, $t_{40} = -10.0$, $p = <0.001$). For tiger sharks, the
135 $\delta^{13}\text{C}$ of blood was on average $1.2\text{‰} \pm 0.26$ SE more depleted than fin while the mean difference

136 in $\delta^{15}\text{N}$ was only $0.09\text{‰} \pm 0.21$ SE (Fig. 1g, h). Similar to the bull sharks, the ranges of $\delta^{13}\text{C}$
137 values were relatively wider than those of $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and shark total length. For both
140 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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, $\delta^{13}\text{C}$ of fin and blood slightly increased with size until 250-300
143 cm TL, and then declined (Fig. 2g and i), while $\delta^{15}\text{N}$ declined with size until 250-300 cm TL,
144 and then increased (Fig. 2h and j). Only the relationship between blood $\delta^{13}\text{C}$ values and tiger
145 shark total length was significant. **[Insert Figure 2]**

146 The difference in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ of bull sharks showed a different pattern. There was no significant relationship between
155 shark size and tissue difference in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ of fin and blood, and shark size explained no variation in
162 differences between $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$
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 $\delta^{13}\text{C}$ values similar to

181 their mothers (coastal predators; $\delta^{13}\text{C}$ \sim -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 $\delta^{13}\text{C}$ values should begin to diverge from their mothers as they adopt a more
186 $\delta^{13}\text{C}$ -depleted estuarine diet (consumer taxa $\delta^{13}\text{C}$ is typically $<$ -25‰ in the Shark River;
187 Williams and Trexler 2006, M. Heithaus *unpublished data*; see also Fig 2). The change in $\delta^{13}\text{C}$
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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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. $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ were relatively weak, raising doubts as to whether
238 tissues can be compared reliably. The relatively small range in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values for different tissue types when using correction factors. The paired
242 differences in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$. Because we found the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$.

247 Published turnover rates for elasmobranch tissues (MacNeil et al. 2006), combined with
248 the long duration before convergence of $\delta^{13}\text{C}$ 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

250 long-term dietary patterns. Such long-term information may be useful for investigating
251 questions such as the degree of specialization within populations, how changes in environmental
252 factors may influence consumer diets, and what ecological factors influence inter-population
253 variation in feeding behaviors. Other taxa exhibit considerably faster turnover rates for blood
254 (e.g. ~52 days ($\delta^{13}\text{C}$) and ~46 days ($\delta^{15}\text{N}$) for mice (*Mus musculus*) MacAvoy et al. 2006),
255 muscle (e.g. 4-5 months ($\delta^{15}\text{N}$) for whitefish (*Coregonus lavaretus*) Perga and Gerdeaux 2005),
256 and fin (e.g. ~37 days ($\delta^{15}\text{N}$) for armored catfish (*Ancistrus triradiatus*) McIntyre and Flecker
257 2006) tissues, allowing for more fine-scale diet studies. Therefore, stomach content analysis
258 remains an important complimentary method for studying elasmobranch trophic ecology,
259 especially when investigating short-term variability in diets.

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

267

268 **Acknowledgements**

269 Funding for this project was provided by the National Science Foundation (DBI0620409,
270 DEB9910514, OCE0526065, OCE0746164) and Florida International University's Marine
271 Sciences Program. We thank the many volunteers who assisted with shark fishing and
272 processing stable isotope samples, especially Derek Burkholder, Richard Chang, Bryan Delius,

273 Meagan Dunphy-Daly, Kirk Gastrich, and Aaron Wirsing. Thanks also to Joel Trexler and the
274 FCE LTER for providing funding and logistical support for this project. Research was conducted
275 under Everglades National Park permits EVER-2009-SCI-0024, EVER-2007-SCI-0025, and
276 EVER-2005-SCI-0030, authorizations from the Department of Environment and Conservation,
277 Western Australia and Fisheries WA and with Florida International University IACUC approval.
278 This is publication 41 of the Shark Bay Ecosystem Research Project.

279

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395 **Table and Figure Legends**

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

398

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

403

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

408

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

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

		Min $\delta^{13}\text{C}$	Max $\delta^{13}\text{C}$	Min $\delta^{15}\text{N}$	Max $\delta^{15}\text{N}$
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

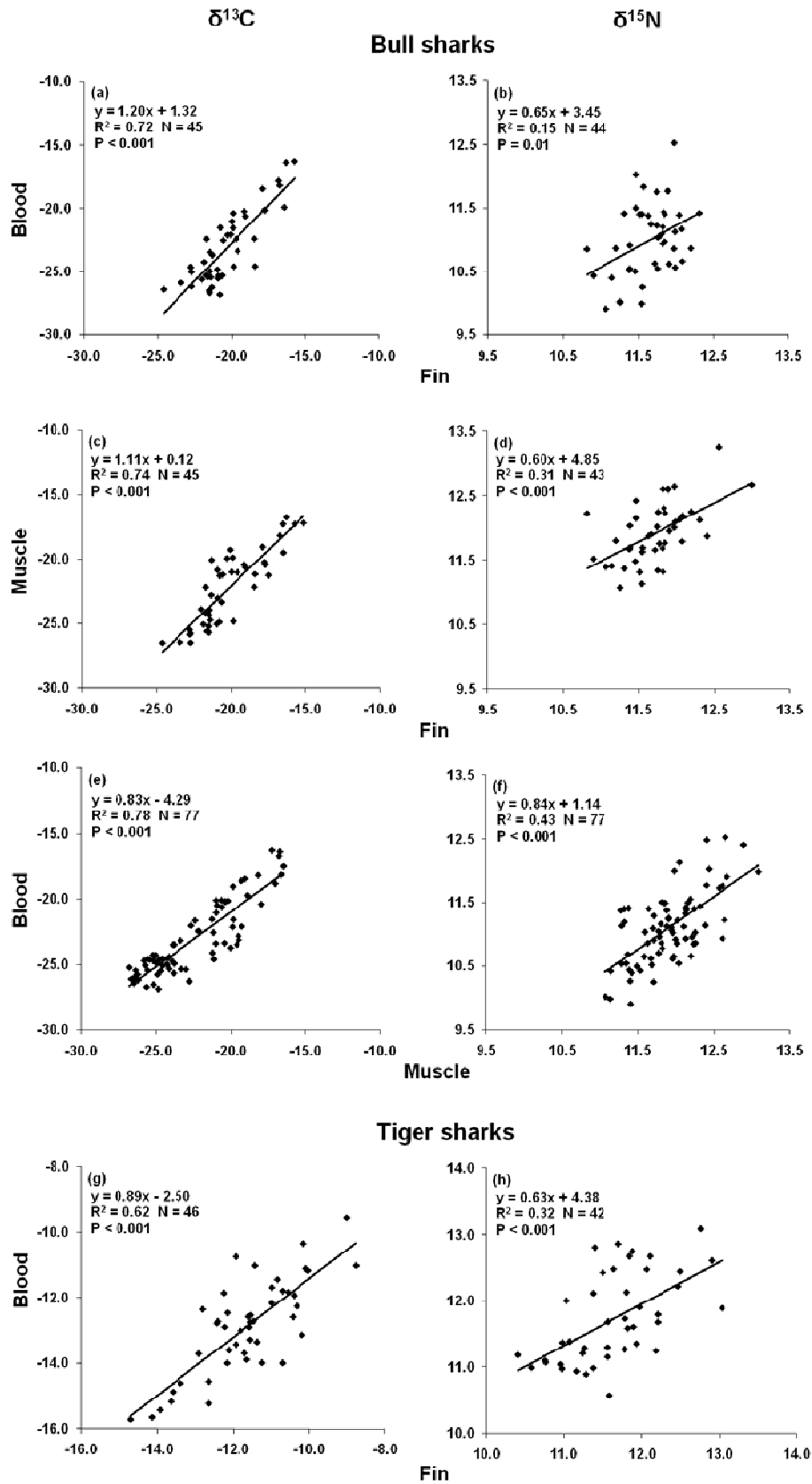


Figure 2

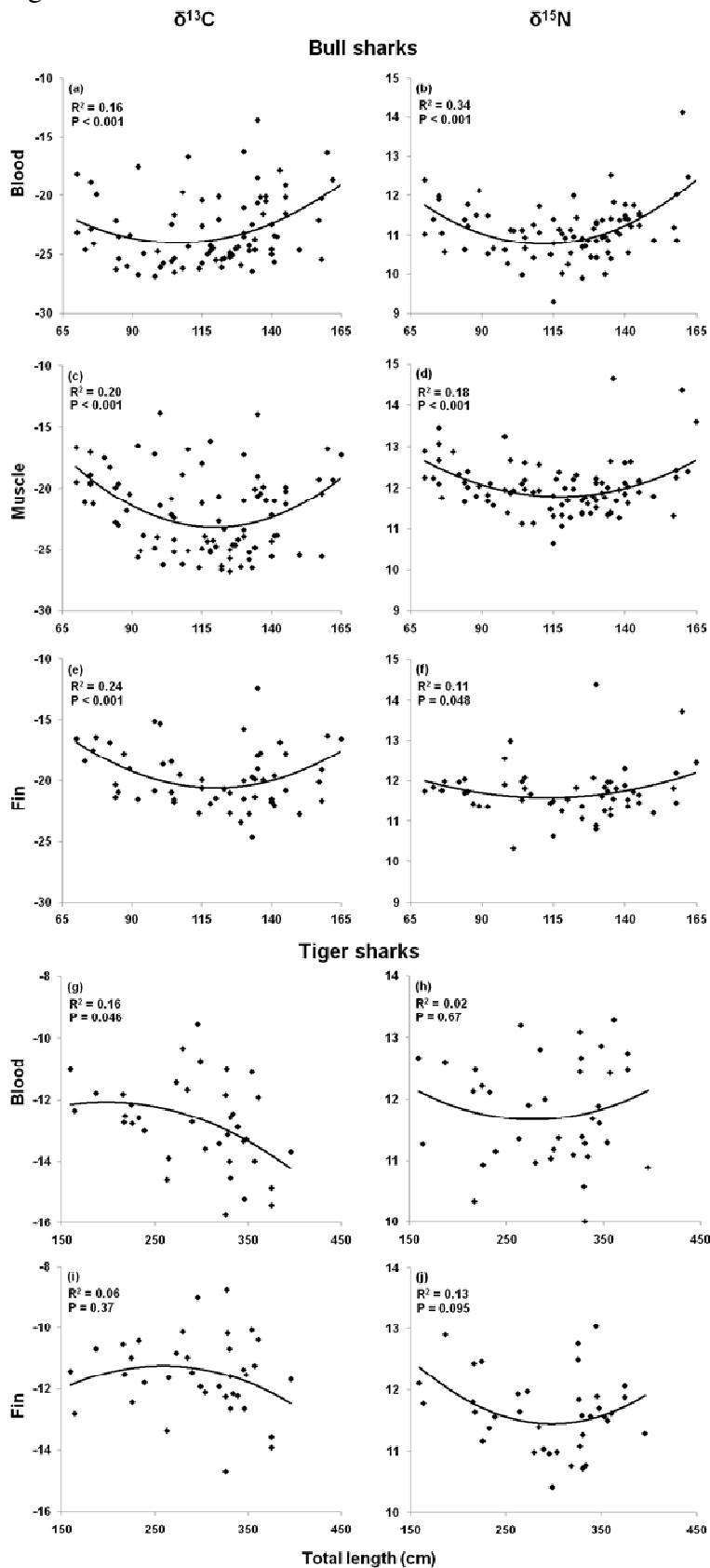


Figure 3

