

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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Abstract: Stable isotopes are important tools 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 $\delta^{13}\text{C}$ than for $\delta^{15}\text{N}$. Paired differences between isotopic values of tissues were relatively small but varied significantly with shark total length, suggesting that 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. Although correlations were relatively strong, results suggest that correction factors should be generated for the desired study species and may only allow coarse-scale comparisons between studies using different tissue types.

Résumé : Les isotopes stables sont des outils précieux pour comprendre les rôles trophiques des élastombranches. Il n'est pas clair, cependant, si les différents tissus fournissent des valeurs compatibles d'isotopes stables chez un même individu. Afin d'aborder ce problème, nous avons mesuré les relations entre les valeurs des isotopes de carbone et d'azote dans le sang, le muscle et les nageoires chez de jeunes requins bouledogues (*Carcharhinus leucas*) et le sang et les nageoires de grands requins tigres (*Galeocerdo cuvier*) récoltés dans deux écosystèmes différents. Nous avons aussi étudié la relation entre la taille des requins et l'importance des différences entre les valeurs isotopiques des divers tissus. Il existe une corrélation significative positive entre les valeurs isotopiques dans toutes les comparaisons appariées de tissus, mais les valeurs de R^2 sont beaucoup plus fortes dans le cas de $\delta^{13}\text{C}$ que dans celui de $\delta^{15}\text{N}$. Les différences appariées entre les valeurs isotopiques des tissus sont relativement petites, mais elles varient significativement en fonction de la longueur totale du requin, ce qui laisse croire que la taille du requin peut être un facteur important qui influence l'ampleur des différences de valeurs isotopiques entre les divers tissus. Dans les études de jeunes requins, il faut faire attention lorsqu'on utilise des tissus à faible taux de remplacement, tels que le muscle ou les nageoires, car ils peuvent retenir une signature maternelle pendant un longue période de temps. Bien que les corrélations soient relativement fortes, nos résultats indiquent qu'il faut calculer des facteurs de correction pour l'espèce qu'on veut étudier; de plus, seules des comparaisons à l'échelle grossière peuvent être possibles entre des études qui utilisent différents types de tissus.

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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 be-

cause 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 (Heithaus 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 draw-

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

backs, 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., Domi et al. 2005; Fisk et al. 2002; 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 (Martínez 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 ($\delta^{15}\text{N}$ in captive *Potamotrygon motoro* (MacNeil et al. 2006); $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in captive *Carcharhinus plumbeus* (Logan and Lutcavage 2010)) compared with 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; McIntyre and Flecker 2006; Perga and Gerdeaux 2005). In addition to understanding turnover rates, it is important to understand the variability of isotopic values for various tissue types within an individual to make full use of stable isotopic data and compare information among studies (e.g., Pinnegar and Polunin 1999; Sweeting et al. 2005; Vander Zanden and Rasmussen 2001).

The purpose of this study was (i) to compare the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 intraspecific values from one tissue are comparable with those of other tissues for each species, and (ii) to 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.

Materials and methods

Muscle, whole blood ("blood" hereafter), and dorsal fin ("fin" hereafter) tissues were collected from 81 juvenile bull sharks (70–162 cm total length, TL) captured on 500 m longlines within the Shark River estuary of Everglades National Park, Florida, USA (for specific details of the study area and capture methods, see Heithaus et al. 2009). We used a biopsy punch to collect a 0.5 cm³ muscle tissue biopsy about 5 cm lateral to the first dorsal fin, scissors to collect a 0.5 cm³ 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 (for study site and sampling details, see Wirsing et al. 2006). 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 (North Miami, Florida; 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 (New Haven, Connecticut; 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-to-N ratios (C:N) 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were $0.13\text{‰} \pm 0.20\text{‰}$ standard error (SE).

We used least squares regression analysis to determine (i) the relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all paired tissues of bull sharks (i.e., blood–muscle, blood–fin, muscle–fin) and tiger sharks (i.e., blood–fin), and (ii) the relationship between shark length and paired differences between tissues. Each paired difference was calculated by taking the absolute difference 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 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 Martínez 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values revealed highly significant positive correlations for all tissue pairs in bull sharks. The slopes of all three bull shark $\delta^{13}\text{C}$ comparisons did not differ from 1:1 and all R^2 values were >0.71 (Figs. 1a, 1c, 1e). On average, blood was $0.57\text{‰} \pm 0.055\text{‰}$ (mean \pm SE) more depleted (i.e., more negative) than muscle and $2.8\text{‰} \pm 0.10\text{‰}$ more depleted than fin, and muscle was $2.1\text{‰} \pm 0.092\text{‰}$ more depleted than fin

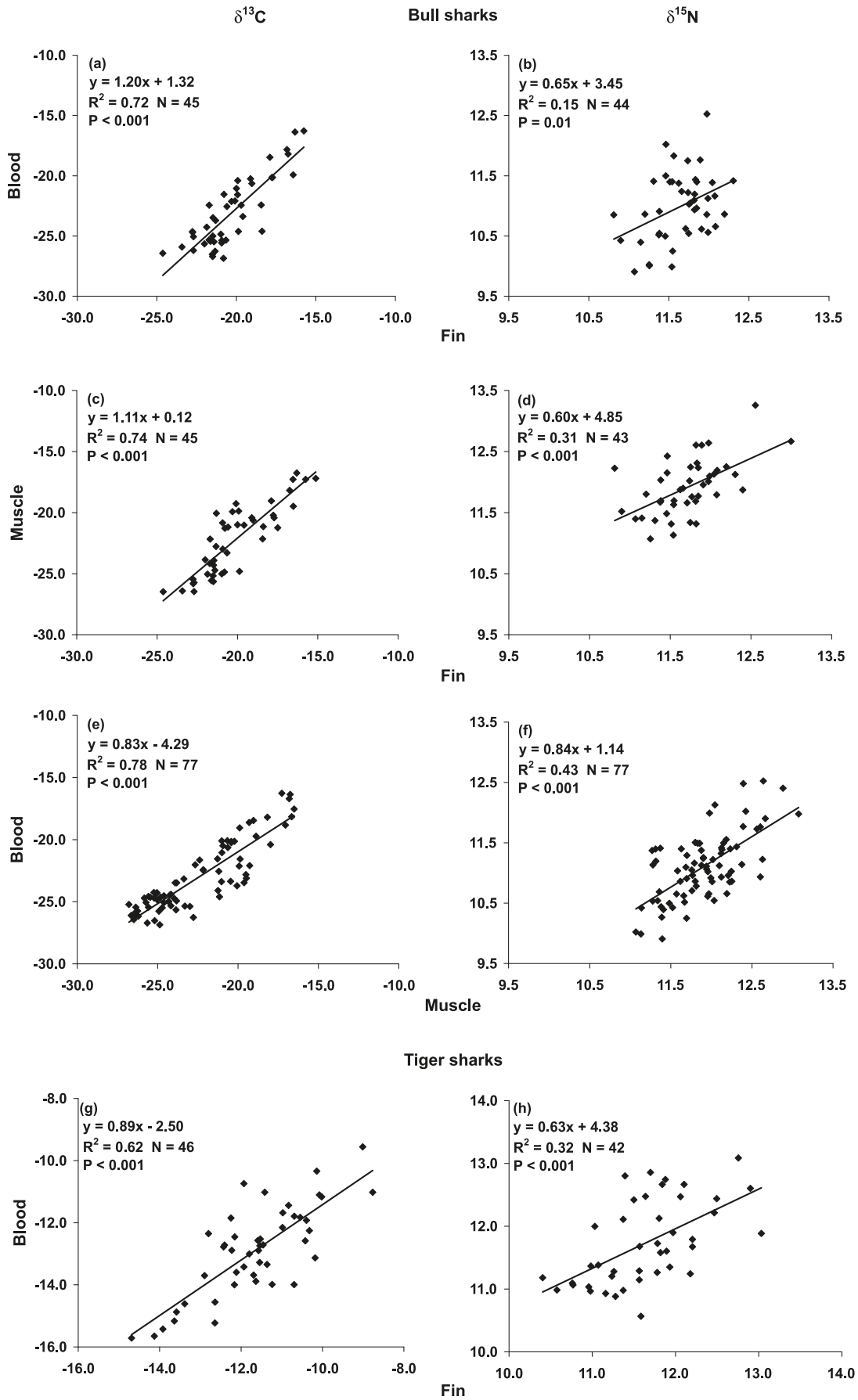


Fig. 2. Comparisons of $\delta^{13}\text{C}$ and total length for (a) fin, (c) blood, and (e) muscle and of $\delta^{15}\text{N}$ and total length for (b) fin, (d) blood, and (f) muscle for bull sharks (*Carcharhinus leucas*), and comparisons of $\delta^{13}\text{C}$ and total length for (g) fin and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

Table 1. Minimum (Min.) and maximum (Max.) values (in ‰) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for blood, muscle, and fin for bull sharks (*Carcharhinus leucas*) and blood and fin for tiger sharks (*Galeocerdo cuvier*).

	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

(Figs. 1a, 1c, 1e). Relationships between $\delta^{15}\text{N}$ values were significant but weaker than those of $\delta^{13}\text{C}$, with R^2 values between 0.15 and 0.43 (Figs. 1b, 1d, 1f). Only the relationship between muscle and fin deviated from a slope of one (slope = 0.6, $t_{[41]} = -7.8$, $p < 0.001$). Mean (\pm SE) differences for bull shark $\delta^{15}\text{N}$ were $0.80\text{‰} \pm 0.064\text{‰}$ for blood and muscle, $0.65\text{‰} \pm 0.16\text{‰}$ for blood and fin, and $0.20\text{‰} \pm 0.15\text{‰}$ for muscle and fin (Figs. 1b, 1d, 1f). The ranges of $\delta^{13}\text{C}$ values were relatively wide for all bull shark tissue types, whereas the ranges of $\delta^{15}\text{N}$ values were relatively narrow (Table 1).

Relationships between tissue types were similar in tiger sharks. Correlations for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of blood and fin were positive and significant, but the relationship was tighter for $\delta^{13}\text{C}$ ($R^2 = 0.62$) than for $\delta^{15}\text{N}$ ($R^2 = 0.32$) (Figs. 1g, 1h). The slope for $\delta^{13}\text{C}$ was not significantly different from one, but the slope for $\delta^{15}\text{N}$ was (slope = 0.63, $t_{[40]} = -10.0$, $p < 0.001$). For tiger sharks, on average, the $\delta^{13}\text{C}$ of blood was $1.2\text{‰} \pm 0.26\text{‰}$ (mean \pm SE) more depleted than fin, whereas the difference in $\delta^{15}\text{N}$ was only $0.09\text{‰} \pm 0.21\text{‰}$ (Figs. 1g, 1h). Similar to the bull sharks, the ranges of $\delta^{13}\text{C}$ values were relatively wider than those of $\delta^{15}\text{N}$ values (Table 1).

Based on the tight relationships in isotopic values of tissues, it is not surprising that most tissue types showed similar relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and shark total length. For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bull sharks, all tissues declined until 110–130 cm TL and then increased (Figs. 2a–2f). All relationships between isotope values and shark total length were significant ($p < 0.05$) for bull sharks. For tiger sharks, $\delta^{13}\text{C}$ of fin and blood slightly increased with size until 250–300 cm TL and then declined (Figs. 2g and 2i), whereas $\delta^{15}\text{N}$ declined with size until 250–300 cm TL and then increased (Figs. 2h and 2j). Only the relationship between blood $\delta^{13}\text{C}$ values and tiger shark total length was significant.

The difference in $\delta^{13}\text{C}$ values between tissue types for bull sharks was influenced by shark total length for all pairings. In all cases for bull sharks, paired differences in $\delta^{13}\text{C}$ values were highest for the smallest individuals and decreased with size. This relationship was strongest for fin and blood ($R^2 = 0.64$) and weakest for fin and muscle ($R^2 =$

0.21) (Figs. 3a, 3c, 3e). The paired difference between muscle and blood dropped rapidly until ~110 cm TL, when the direction of the difference became less predictable. The difference between fin and blood dropped linearly and approached zero at ~165 cm TL, and the difference between fin and muscle showed a relatively weak relationship with shark length. Paired differences for $\delta^{15}\text{N}$ of bull sharks showed a different pattern. There was no significant relationship between shark size and tissue difference in $\delta^{15}\text{N}$ of fin and muscle, whereas somewhat weak, but significant, nonlinear relationships were found for comparisons between blood and muscle ($R^2 = 0.18$) and blood and fin ($R^2 = 0.39$) (Figs. 3b, 3d, 3f). The difference in $\delta^{15}\text{N}$ for these comparisons was relatively low at small total lengths, increased slightly with size, and then declined in the largest individuals.

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

Discussion

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

Body size appears to be one factor that explained the regression slopes for some of the intertissue paired differences for our sample populations. The paired differences in $\delta^{13}\text{C}$ of bull shark tissues were greatest in smaller individuals and decreased with size, indicating that isotopic values of different tissues were more similar for larger individuals. Prior to birth, bull sharks are directly connected to their mothers by an umbilical cord, which serves as a pathway through which nutrients and energy are transferred between mother and fetus. Based on the presence of open umbilical scars, bull sharks in the coastal Everglades are born between 65 and 75 cm TL. Because of their connection to their mothers, pups should have $\delta^{13}\text{C}$ values similar those of their mothers (coastal predators; $\delta^{13}\text{C} \sim -15\text{‰}$ in our study area; Chasar et al. 2005), as seen in cetaceans (e.g., bottlenose dolphins, *Tursiops truncatus* (Knoff et al. 2008); sea lions, *Zalophus californianus* (Porras-Peters et al. 2008)). After birth, juvenile sharks spend several years in low-salinity estuaries and nearshore waters (e.g., Heithaus et al. 2009; Wiley and Simpfendorfer 2007), and therefore $\delta^{13}\text{C}$ values should begin to diverge from their mothers as they adopt a

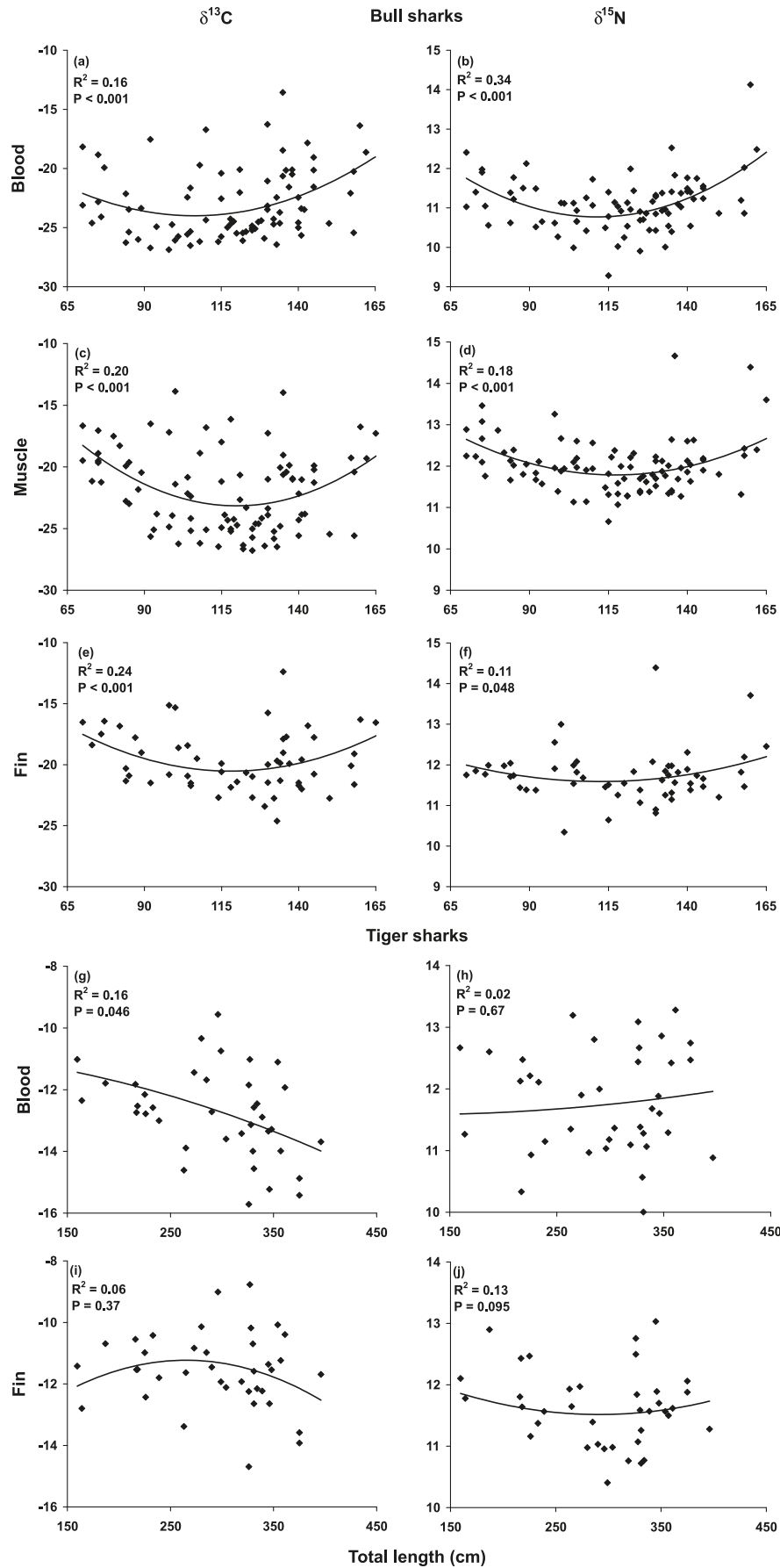


Fig. 3. Paired differences of $\delta^{13}\text{C}$ for (a) blood and fin, (c) muscle and fin, and (e) blood and muscle and of $\delta^{15}\text{N}$ for (b) blood and fin, (d) muscle and fin, and (f) blood and muscle for bull sharks (*Carcharhinus leucas*), and paired differences of (g) $\delta^{13}\text{C}$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

more $\delta^{13}\text{C}$ -depleted estuarine diet (consumer taxa $\delta^{13}\text{C}$ is typically $< -25\text{‰}$ in the Shark River; Williams and Trexler 2006; M. Heithaus, unpublished data). The change in $\delta^{13}\text{C}$ values should occur earlier in tissues that turnover more rapidly. For example, differences between blood and both fin and muscle in the smallest bull sharks suggest that fin tissue largely maintains the maternal signature, likely owing to a slower turnover rate. In contrast, blood reflects the young sharks' diet within two years of birth, likely owing to a faster turnover rate in this tissue type (MacNeil et al. 2006).

The regression model for the paired difference of $\delta^{13}\text{C}$ for muscle and blood appears to reach equilibrium at around 110 cm TL and two years of age (based on growth rates in Branstetter and Stiles (1987) and estimated sizes at birth (Heithaus et al. 2009)). This may indicate the time period when muscle $\delta^{13}\text{C}$ values are no longer influenced by the maternal diet for juveniles and accurately portray that individual's diet over its lifetime. Deviations in isotope values of larger individuals may reflect other underlying ecological patterns, for example, seasonal shifts in diet, which may be displayed more rapidly in blood values than in muscle or fin (P. Matich and M. Heithaus, unpublished data). In contrast to bull sharks, differences in $\delta^{13}\text{C}$ among blood and fin clips increased with size in tiger sharks. This likely reflects a difference in the feeding ecology of the two species, and the increasing difference in $\delta^{13}\text{C}$ of blood and fin may reflect a shift in the diets of tiger sharks as they grow (e.g., Lowe et al. 1996; Simpfendorfer et al. 2001).

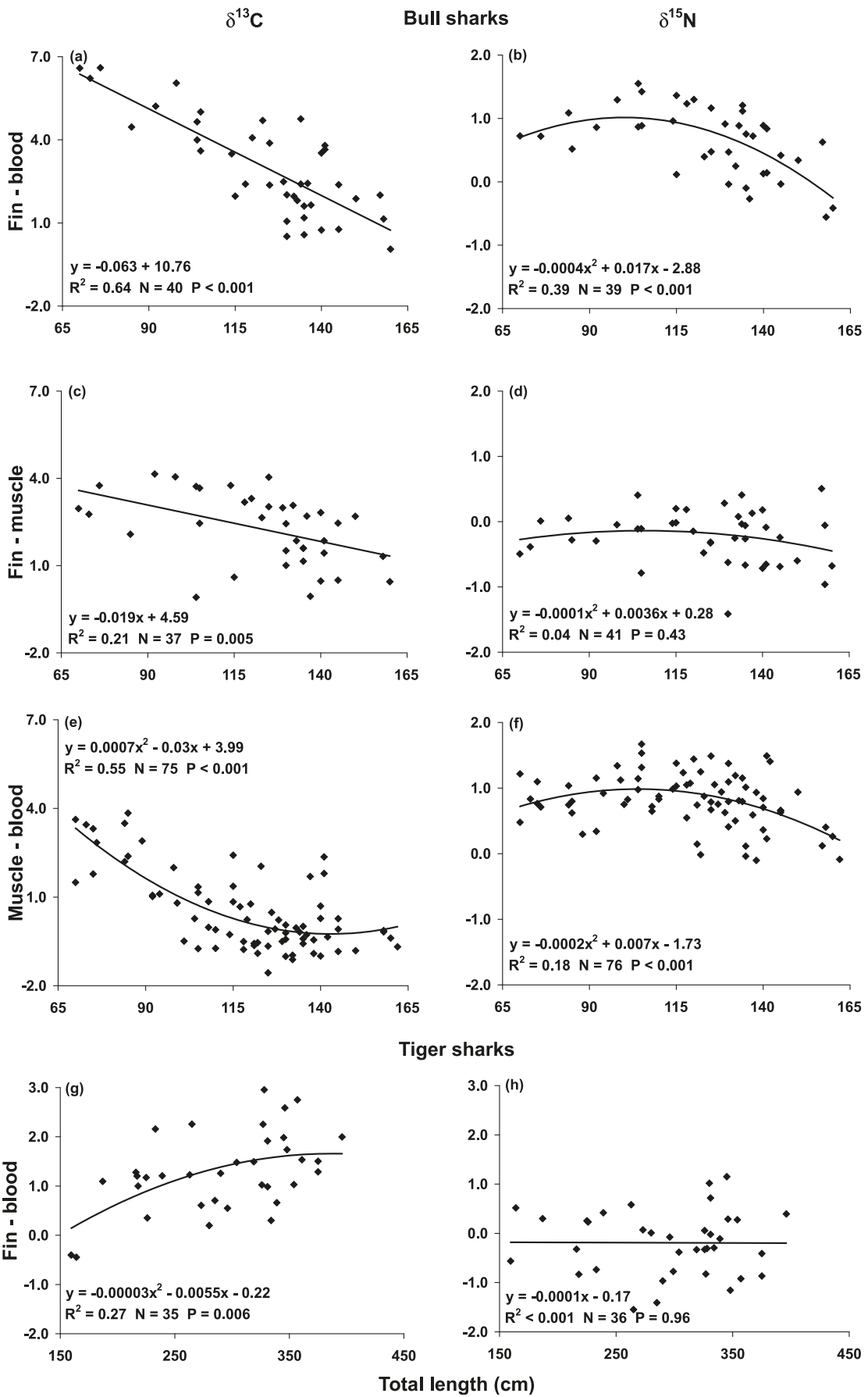
Size-based differences among tissues in stable isotope values are important to consider when investigating the ecological drivers of dietary variation within populations. $\delta^{13}\text{C}$ values support the hypothesis that the maternal influence on isotopic values of juvenile bull sharks is evident for several years, but individual variability in isotopic values makes it difficult to draw conclusions about the precise timing of tissue values equilibrating. Especially for $\delta^{13}\text{C}$ of both species, the range of isotope values was relatively wide, even for sharks of a given size, suggesting that other factors such as habitat use (e.g., Darimont et al. 2009; Quevedo et al. 2009), body condition (e.g., Tinker et al. 2008; Tucker et al. 2009), and (or) seasonal shifts (e.g., Chérel et al. 2007; Inger et al. 2006) may affect the diet patterns for individuals of these two populations.

The strong positive correlations between tissues in $\delta^{13}\text{C}$ for both bull sharks and tiger sharks suggest that for a species, multiple tissues may be compared after applying a correction factor. A strict 1:1 substitution of values among tissues is not recommended, and we suggest that correction factors should be generated for individual populations because ecological differences may lead to variability in isotopic assimilation across individuals of the same taxa (Post 2002). Using correction factors generated for a species in one ecosystem may differ from those generated for the same species collected from a different ecosystem, and therefore it is currently most appropriate to generate correction factors on a per-population basis.

Tissue comparisons may allow for gaps within data sets to be filled and to increase the number of individuals that can be directly compared. Individuals for which isotope values of a particular tissue are not available may have correction factors applied to estimate isotopic value(s) of the uncollected tissue. Yet, it is important to consider potential factors that limit the use of correction factors. Species that experience ontogenetic shifts in diet may experience variability in intertissue relationships between isotope values (e.g., Quillfeldt et al. 2008; Tierney et al. 2008; Young et al. 2010), and therefore correction factors may be more accurate for certain age or size classes of animals. For example, the difference between tissues for bull sharks (paired differences) were largest (7‰ fin–blood) for the smallest individuals sampled and tended to decrease and approach equilibrium (1:1 relationship) as bull shark total length increased. This suggests that correction factors may be more useful for larger individuals, which generally had smaller differences in isotope values for different tissues. Therefore, care must be taken when using correction factors, and variability in factors that affect trophic role (such as body size) must be taken into consideration prior to using estimated isotope values produced by correction factors for diet analysis.

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

Published turnover rates for elasmobranch tissues (MacNeil et al. 2006), combined with the long duration before convergence of $\delta^{13}\text{C}$ values of blood and muscle of bull sharks in our study, suggest that using stable isotopes from these tissues is 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 interpopulation variation in feeding behaviors. Other taxa exhibit considerably faster turnover rates for blood (e.g., ~52 days ($\delta^{13}\text{C}$) and ~46 days ($\delta^{15}\text{N}$) for mice (*Mus musculus*); MacAvoy et al. 2006), muscle (e.g., 4–5 months ($\delta^{15}\text{N}$) for whitefish (*Coregonus lavaretus*); Perga and Gerdeaux 2005), and fin (e.g., ~37 days ($\delta^{15}\text{N}$) 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 predators 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 nonlethally. 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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