

## Fin-icky samples: an assessment of shark fin as a source material for stable isotope analysis

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### Abstract

Analyzing stable isotopes (SI:  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in a new tissue requires rigorous testing before its general application in examining aspects of animal ecology. Shark fin provides a novel, minor invasive source material, which is important considering the conservation status of many large sharks. Fin, however, is not a single tissue but composed of multiple tissues, primarily skin and cartilage. This may complicate the interpretation of SI, as fin can be sampled from multiple fins and different regions of a fin from an individual. Here, we examined the variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  with sample location on the anal fin of Caribbean reef sharks (*Carcharhinus perezi*). Values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were highly correlated across sampling locations indicating that mean population or size class fin SI data would be reliable. At the individual level, large variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  between anal fin sampling locations indicates that the varying proportional contributions of tissues would complicate individual level analyses. For three pelagic shark species, dorsal fin  $\delta^{13}\text{C}$  values were consistently higher than  $\delta^{13}\text{C}$  muscle tissue values, identifying tissue-specific diet discrimination factors. This would confound multiple tissue studies that assume that SI values across tissues will be equal if the animal is in equilibrium with its diet. Proposed sampling protocols for fin material will negate many of these issues, but caution is warranted for comparisons of SI data between shark fin and other tissues or across species until the isotope dynamics of fin have been experimentally validated.

There is growing interest in the ecological role of sharks, since many species are believed to regulate top-down processes in marine systems (Heithaus et al. 2008; Baum and Worm 2009), and regional shark stocks have been depleted by overfishing (Myers and Worm 2003; Baum et al. 2005). Relative to the teleost fishes, little is known about the role of sharks in coastal and pelagic food webs because predation events are rarely observed directly, and stomach content

analyses require large sample sizes to accurately quantify long-term feeding patterns (Wetherbee and Cortés 2004). As a result, stable isotope analysis (SIA) of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) in multiple tissues is increasingly being applied to examine aspects of shark ecology. Recent studies have used SIA to examine interspecific diet and resource overlap (Domi et al. 2005; MacNeil et al. 2005; McMeans et al. 2010; Papatamatiou et al. 2010; Kinney et al., 2011), to estimate trophic position (Fisk et al. 2002; Estrada et al. 2003 and 2006; Revill et al. 2009; Hussey et al. in press-a and in press-b), to characterize individual feeding behavior (Matich et al. 2010), and to describe the effects of maternal provisioning on the isotope profiles of young sharks (McMeans et al. 2009, Olin et al. 2011). Accurate interpretation of SIA rests on careful consideration of the underlying dynamics of these tracers, which are driven by often unknown variables such as tissue-specific diet discrimination factors and turnover rates (Caut et al. 2009; Martinez del Rio et al. 2009). With the continued application of SIA in the study of shark ecology in the near future, research questions addressed will become both more refined and more sensitive to assumptions about these variables.

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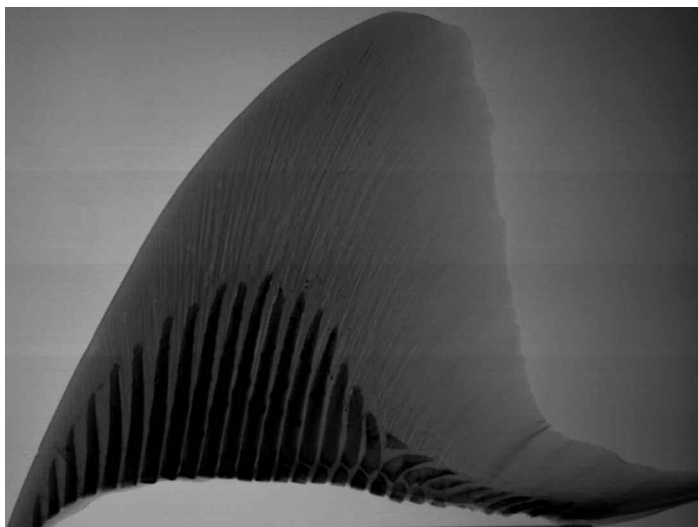
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To date, tissue samples used for SIA of sharks include white muscle (for example, Estrada et al. 2003; MacNeil et al. 2005; Matich et al. 2010; Papastamatiou et al. 2010; Hussey et al. in press-b), liver (Fisk et al. 2002; Olin et al. 2011), blood (plasma, red blood cells, and/or whole blood) (MacNeil et al. 2006; Matich et al. 2010), cartilage (usually from vertebrae) (Estrada et al. 2006), and most recently, fin (Matich et al. 2010). A fin clip or punch is an attractive tissue to sample as it is relatively easy to obtain nonlethally from a live shark (Matich et al. 2010). Nondestructive sampling is of particular importance considering the threatened status of many shark species (Dulvy et al. 2008; Hoffman et al. 2010) and the requirement for limiting destructive sampling (Heupel and Simpfendorfer 2010; Hammerschlag and Sulikowski 2011). A similar interest in nondestructive fin sampling of teleost fish has recently occurred (Kelly and Hagar 2006; Sanderson et al. 2009; German and Miles 2010). In addition, archived fin sample libraries already exist from animals sampled for genetic analyses (e.g., Chapman et al. 2009), enabling immediate access to large sample numbers, and permitting retrospective analysis.

Before SIA of shark fin, however, there is one complication that must be considered: fin is an appendage not a tissue. A shark's fins consist of a mixture of tissues including skin (and dermal denticles) and cartilage [basal cartilage and fin rays (ceratotrichia)] and to a lesser extent connective tissue, blood, and muscle: tissues that are likely to have different diet-tissue dynamics and stable isotope turnover rates (Pinnegar and Polunin 1999; MacNeil et al. 2006; Hussey et al. 2010; Logan and Lutcavage 2010) (Fig. 1). It is possible that the relative propor-



**Fig. 1.** X-ray of the first dorsal fin of a dusky shark (*Carcharhinus obscurus*), showing the differential distribution of basal cartilage and fin rays (ceratotrichia). This image illustrates how samples taken from different locations on the fin may have different proportional contributions of the main structural component tissues (e.g., skin, basal cartilage, and ceratotrichia).

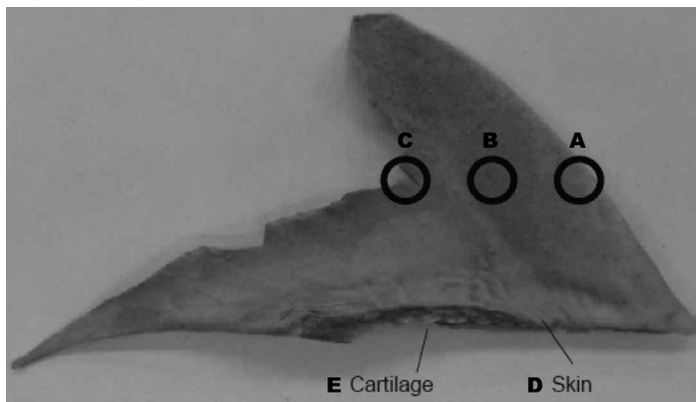
tions of these components vary with location on the shark's fin, which may in turn introduce variation in isotopic values within the same fin that exceed measurement error. This is an important consideration when undertaking inter/intra species comparisons and, if significant, would indicate a need for standardization of field sampling protocols and caution in using archived fin clip samples where sampling protocols may not be standardized. In addition, because shark fin contains cartilage, shark fin  $\delta^{13}\text{C}$  values would be expected to be consistently higher than muscle tissue [i.e., muscle has a lower diet-tissue discrimination factor (MacNeil et al. 2005; Hussey et al. 2010)]. This could complicate multiple tissue analyses that assume diet-tissue discrimination factors are equal across tissues (e.g., MacNeil et al. 2005, Matich et al. 2010) and tissue-specific methods for calculating trophic position.

Here, we present SIA of shark fins that aimed to assess the potential biases associated with using this type of sample. Our first experiment involved testing for differences in nitrogen and carbon stable isotope profiles on different parts of the same fin (leading edge, middle, trailing edge, isolated basal cartilage, and excised skin). This experiment used one type of fin (anal) from a single species (Caribbean reef shark, *Carcharhinus perezi*) from the same location (Turneffe Atoll, Belize). In our second experiment, we tested for differences between fin (trailing edge of the first dorsal) and white muscle from the same individual in three species of pelagic shark [blue (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), and common thresher (*Alopias vulpinus*)] sampled from the Northwest Atlantic. For this experiment, we predicted that carbon isotopes of fin would be consistently higher than muscle and that the level of increase would not be significantly different between species.

## Materials and procedures

### Sample collection

Sharks were sampled from (i) artisanal fisheries catches in Belize (Caribbean reef sharks) and (ii) recreational catches in the Northwest Atlantic (blue, common thresher, and shortfin mako sharks). For the first experiment, individual anal fins from Caribbean reef sharks were sub-sampled at three locations on the fin representative of the most probable field sampling locations based on archived genetic sample collections. These were the front edge of the fin (A), the central region of the fin [i.e., common location for a biopsy punch (B)], and the trailing edge of the fin (C) (Fig. 2). A ~2 g sample of fin was excised from the predetermined anal fin sampling locations. In addition, we isolated ~1 g samples of skin tissue (D) and fin cartilage (E) from the base of each anal fin sampled to enable a comparison between fin [multiple tissues but composed principally of skin and cartilage] to the skin and cartilage itself (Fig. 2). In the second experiment, we collected samples from the trailing edge of the dorsal fin (C) and white muscle tissue from the base of the dorsal fin of blue, common thresher, and shortfin mako sharks. For each shark, we sampled a ~3 g mus-



**Fig. 2.** The three sample locations on the anal fin of Caribbean reef sharks (A – front edge of the fin, B – central section of fin, and C – trailing edge of fin) and isolated skin tissue (D) and basal fin cartilage (E).

cle tissue plug from the base of the dorsal fin and a ~2 g fin sample was excised from the central region of the trailing edge of the dorsal fin. All samples were excised using a scalpel blade.

#### Sample preparation and stable isotope analysis

All fin (anal and dorsal) and skin, cartilage and muscle tissue samples were washed in milli-Q water, dried in an oven at 60°C and ground to a fine powder using scissors and hand-operated polypropylene pellet pestles. Between 1350–1550 µg of fin and 400–600 µg of muscle tissue were weighed into tin capsules and stable carbon and nitrogen isotope ratios were generated from a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan MAT Delta<sup>plus</sup>, Thermo Finnigan). Lipid extraction was not undertaken on fin on the premise of low lipid content, which was verified by C:N values of (mean ±SD) 2.9 ± 0.2. Muscle tissue was lipid extracted following a modified Bligh and Dyer (1959) method due to variable lipid content in sharks and the possible effects of soluble urea on δ<sup>15</sup>N (Kim and Koch 2011).

Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

where X is <sup>15</sup>N or <sup>13</sup>C and R is the ratio <sup>15</sup>N/<sup>14</sup>N or <sup>13</sup>C/<sup>12</sup>C. The standard reference material was atmospheric nitrogen for N<sub>2</sub> and Pee Dee Belemnite carbonate for CO<sub>2</sub>. The analytical precision (standard deviation) based on two standards (*n* = 59 for each standard), NIST 8414 and internal lab fish muscle for δ<sup>15</sup>N were 0.11‰ and 0.19‰, respectively, and for δ<sup>13</sup>C were 0.05‰ and 0.06‰, respectively.

#### Statistical analyses

For experiment one, Pearson correlation coefficients were used to examine the relationship between the δ<sup>13</sup>C and δ<sup>15</sup>N values of Caribbean reef shark anal fin samples from combinations of the three sampling locations (A versus B, A versus

C, and B versus C). Paired differences in δ<sup>15</sup>N and δ<sup>13</sup>C (for example for A versus B: δ<sup>15</sup>N<sub>DIFF</sub> = δ<sup>15</sup>N<sub>A</sub> – δ<sup>15</sup>N<sub>B</sub> and δ<sup>13</sup>C<sub>DIFF</sub> = δ<sup>13</sup>C<sub>A</sub> – δ<sup>13</sup>C<sub>B</sub>) between the three sampling locations (A versus B, A versus C, and B versus C) per individual Caribbean reef shark were then calculated to examine the actual difference in stable isotope values. Paired *t* tests were undertaken to test if the differences in δ<sup>15</sup>N and δ<sup>13</sup>C values between sampling locations (A versus B, A versus C, and B versus C) were significant. Least squares linear regression was used to examine whether there was an effect of size of shark on δ<sup>15</sup>N<sub>DIFF</sub> and δ<sup>13</sup>C<sub>DIFF</sub> for each of the three sample location combinations. Finally, a one factor ANOVA was used to test the difference in δ<sup>15</sup>N and δ<sup>13</sup>C between all anal fin sample locations (A, B, and C) and skin and basal cartilage tissue (D and E).

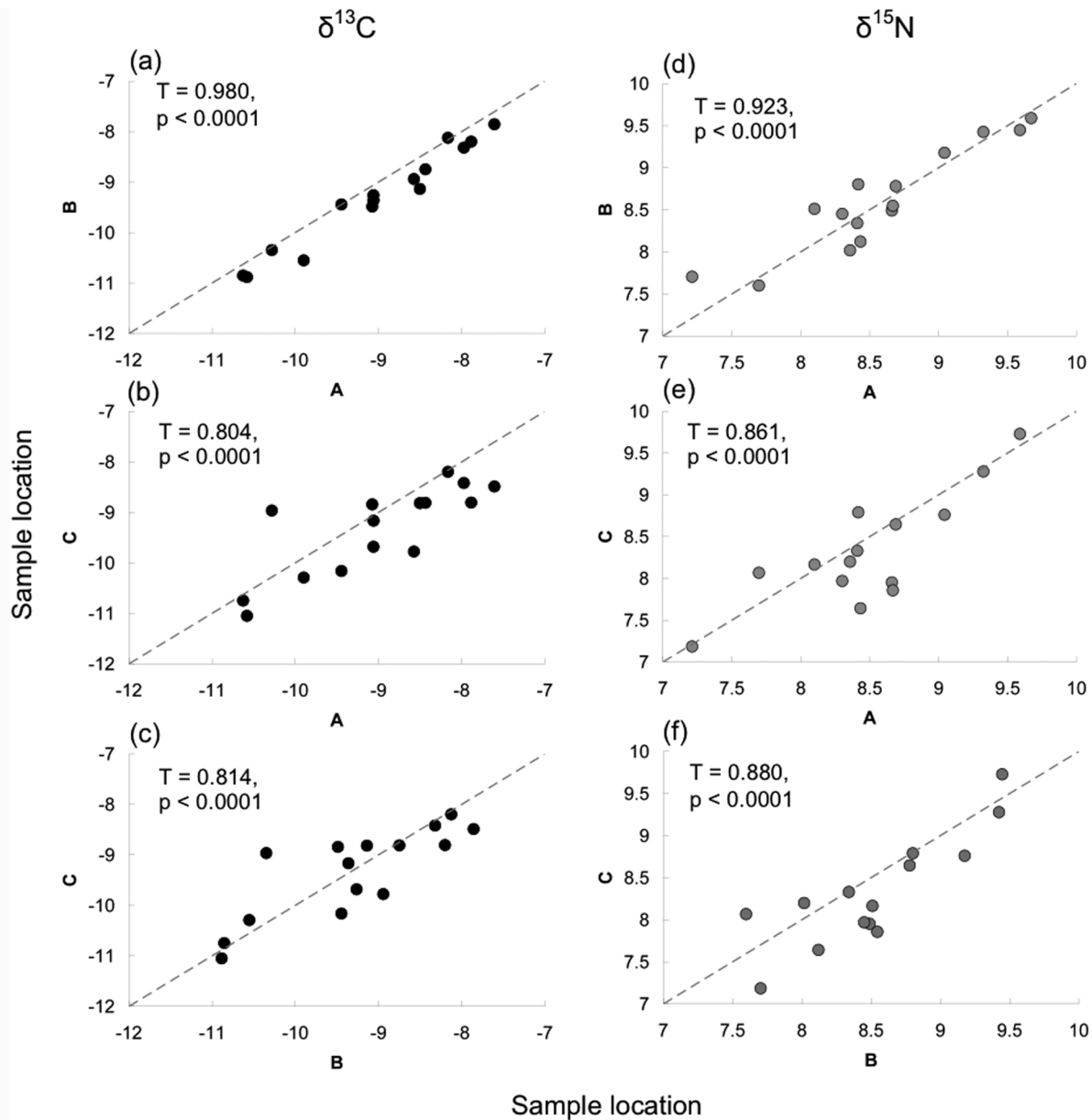
For experiment two, a two factor ANOVA, with δ<sup>15</sup>N and δ<sup>13</sup>C values as the response and tissue type (dorsal fin and muscle tissue), species (shortfin mako, thresher, and blue), and associated interaction as factors was employed. Paired *t* tests were then used to test for differences in dorsal fin and muscle tissue δ<sup>13</sup>C and δ<sup>15</sup>N values for each of the three species. All data were normally distributed and equal in variance. A criterion of *P* < 0.05 was used for all statistical tests.

#### Assessment

##### Variability with sampling location on anal fin

To test whether δ<sup>13</sup>C and δ<sup>15</sup>N values were variable with sampling location on the anal fin a total of 15 individual Caribbean reef shark fins were sampled [total length (TL): 0.68 – 1.68 m]. The δ<sup>13</sup>C and δ<sup>15</sup>N values for the front edge of the fin (A), central region of the fin (B), and trailing edge of the fin (C) were highly correlated, but δ<sup>13</sup>C values for sample locations B and C were generally lower than location A (Fig. 3). The mean δ<sup>13</sup>C<sub>DIFF</sub> and δ<sup>15</sup>N<sub>DIFF</sub> values for the three sample location combinations were similar (Fig. 4). We conclude that combining δ<sup>13</sup>C and δ<sup>15</sup>N values from sharks sampled at different locations on the anal fin would provide reliable mean population or size class estimate data.

The δ<sup>13</sup>C<sub>DIFF</sub> of individual sharks between fin sample locations A versus B, A versus C, and B versus C, however, were highly variable with a minimum and maximum difference of 0.65‰ and –0.04‰ (A versus B), 1.22‰ and –1.32‰ (A versus C), and 0.72‰ and –1.38‰ (B versus C), respectively (Fig. 4a). Similarly, for δ<sup>15</sup>N<sub>DIFF</sub> of individual sharks, the minimum and maximum difference between sample locations were 0.34‰ and –0.49‰ (A versus B), 0.81‰ and –0.37‰ (A versus C), and 0.69‰ and –0.47‰ (B versus C), respectively (Fig. 4b). Most individual shark δ<sup>13</sup>C<sub>DIFF</sub> and δ<sup>15</sup>N<sub>DIFF</sub> values were well above analytical error (Fig. 4). For δ<sup>13</sup>C, paired *t* tests between sampling locations A versus B, and A versus C were significant; no difference in δ<sup>15</sup>N paired *t* tests between the three sampling locations on the fin were found (Table 1). As a result, individual variability in δ<sup>13</sup>C and δ<sup>15</sup>N with sampling location on the anal fin could confound individual level isotope analyses within a single study population if samples are taken from



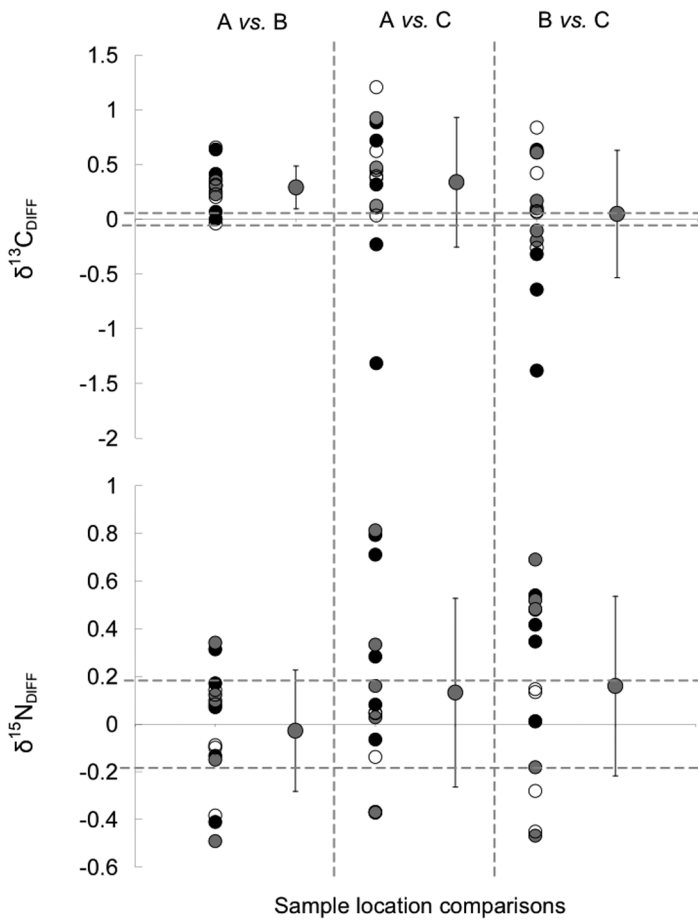
**Fig. 3.**  $\delta^{13}\text{C}$  versus  $\delta^{13}\text{C}$  biplots (a, b, and c) and  $\delta^{15}\text{N}$  versus  $\delta^{15}\text{N}$  biplots (d, e, and f) for the three Caribbean reef shark anal fin sampling locations (A – front edge of fin, B – central section of fin, and C – trailing edge of fin). Correlation statistics and level of significance are shown for each sample location comparison. Gray dashed line shows 1:1 relationship (no effect of sample location).

multiple fin locations. This is particularly problematic for niche width and specialist versus generalist studies (Bearhop et al. 2004; Layman et al. 2007; Matich et al. 2010) and to examine seasonal diet shifts (MacNeil et al. 2005; Phillips and Eldridge 2006), where multiple tissues are compared.

There was no effect of shark size on the  $\delta^{13}\text{C}_{\text{DIFF}}$  and  $\delta^{15}\text{N}_{\text{DIFF}}$  for the three sample location combinations (Linear regression;  $P > 0.05$ ). Schielke and Post (2011) reported that  $\delta^{15}\text{N}$  values of muscle plugs from small bluegill (*Lepomis macrochirus*) were not representative of whole body isotope signatures. This was based on higher  $\delta^{15}\text{N}$  values in the plug when compared with

the whole fish because the plug did not account for the larger ratio of bone to muscle. Shark anal fins are relatively small and uniform, in terms of thickness across a transect of the fin, when compared with the first dorsal fin, and therefore, we would not anticipate large mean isotope differences between anal fin sample locations from different size animals.

The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for sample locations A, B, and C were generally between those of isolated skin (location D) and basal fin cartilage (location E) (Fig. 5), but were not statistically different ( $\delta^{13}\text{C}$ :  $F_{4,68} = 0.62$ ,  $P = 0.65$ ,  $\delta^{15}\text{N}$ :  $F_{4,68} = 1.22$ ,  $P = 0.31$ ). This confirms that sample locations A, B, and C are



**Fig. 4.** The calculated difference in (a)  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{DIFF}}$ ) and (b)  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{DIFF}}$ ) between (i) individual Caribbean reef shark anal fin sample locations and (ii) the calculated mean difference ( $\pm$ SD) for the three anal fin sample location comparisons: A versus B, A versus C, and B versus C. Small sharks (solid white; TL: 0.68–0.75 m), medium-sized sharks (solid dark gray; TL: 0.94–0.97 m), and large sharks (solid black; TL: 1.28–1.68 m). Dashed gray lines are levels of analytical error for each isotope (see “Materials and procedures”).

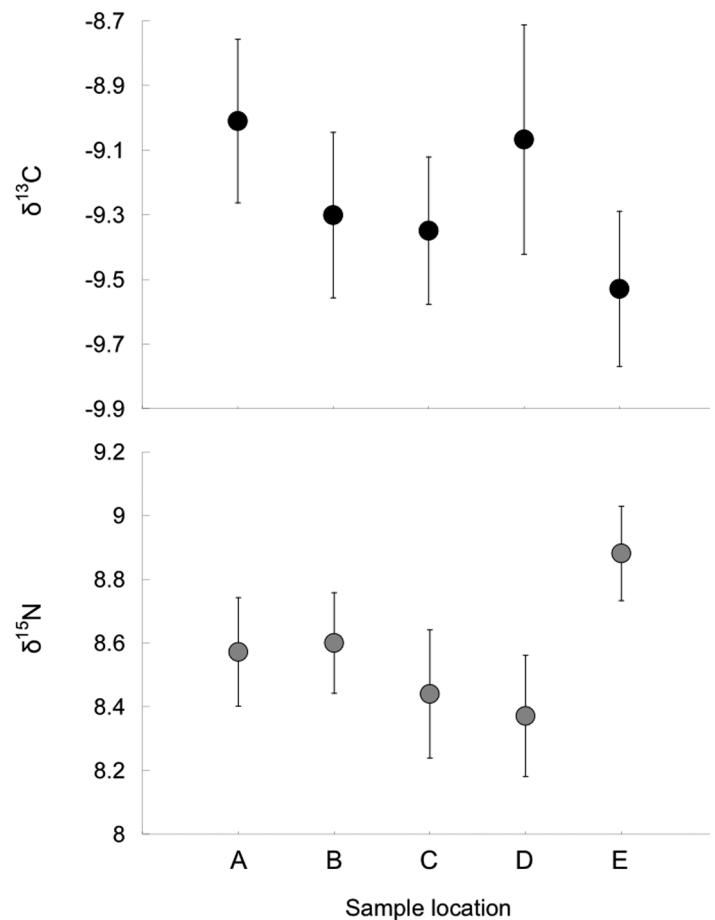
**Table 1.** Paired *t* test results comparing individual shark  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the three sampling locations on the dorsal fin of Caribbean reef sharks: (A) front edge of the fin, (B), central section of the fin, and (C) trailing edge of the fin.

Sample location	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
A versus B	$T_{15} = 5.76, P < 0.0001$	$T_{15} = -0.42, P = 0.68$
A versus C	$T_{15} = 2.21, P < 0.044$	$T_{15} = 1.29, P = 0.218$
B versus C	$T_{15} = 0.32, P = 0.76$	$T_{15} = 1.64, P = 0.123$

composed of predominantly skin and cartilage and highlights the complexities of using fin which is not a single tissue, but composed of multiple structural tissue components.

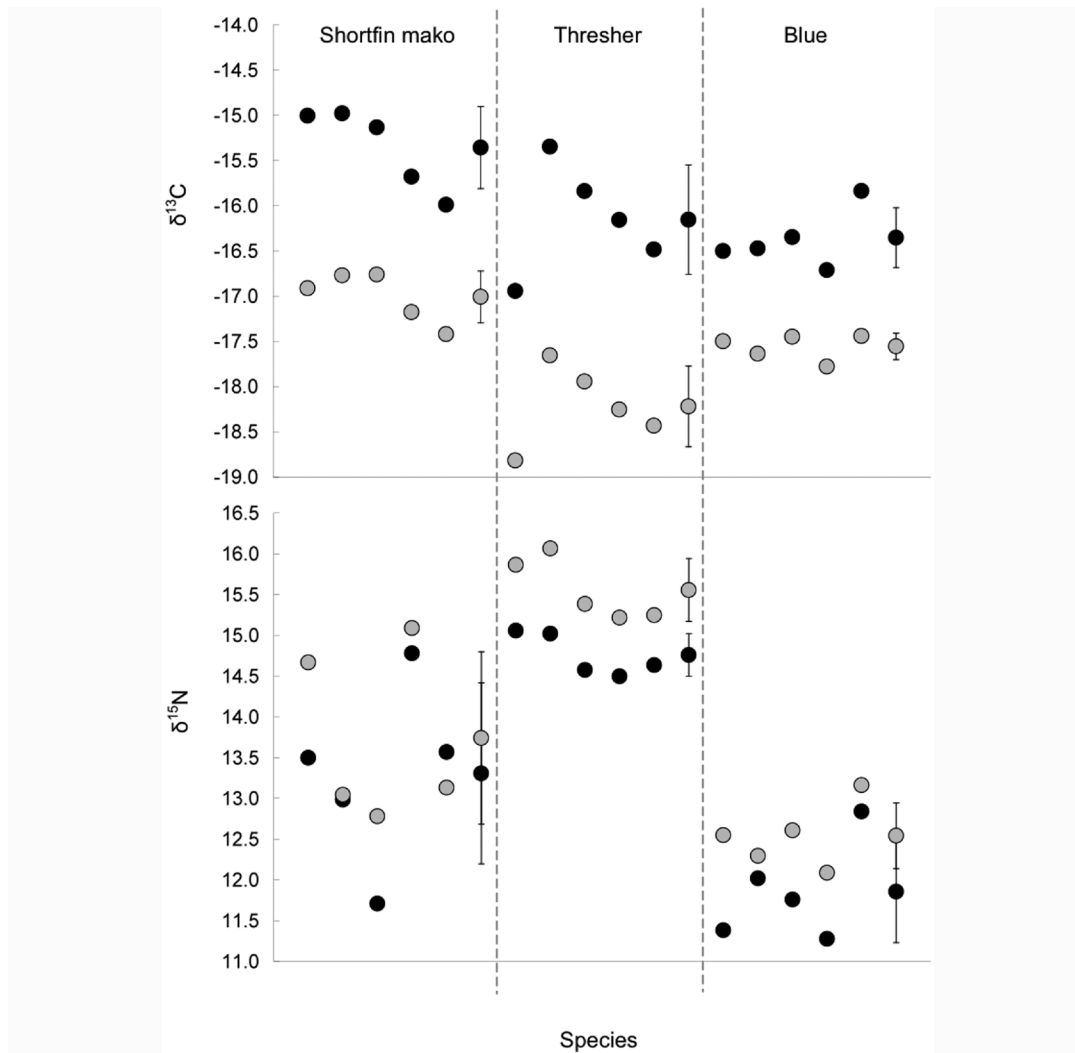
**Tissue-specific diet-tissue discrimination factors: Fin  $\delta^{13}\text{C}$  values consistently higher than muscle tissue**

To determine whether dorsal fin  $\delta^{13}\text{C}$  values were higher



**Fig. 5.** Mean ( $\pm$  SE) (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values for the three Caribbean reef shark anal fin sampling locations (A, B, and C) and for isolated skin (D) and basal fin cartilage (E) sampled from the same individual fins.

than muscle tissue and that the measured increase was consistent across species, we sampled a total of 15 pelagic sharks; 5 shortfin mako (TL: 2.2–2.8 m), 5 thresher (TL: 2.5–3.1 m) and 5 blue (TL: 2.5–2.9 m). Dorsal fin  $\delta^{13}\text{C}$  values of shortfin mako, thresher, and blue sharks were consistently higher than muscle tissue by  $1.65\text{‰} \pm 0.20$ ,  $2.07\text{‰} \pm 0.17$ , and  $1.00\text{‰} \pm 0.53$ , respectively (Fig. 6a; Table 2). For  $\delta^{13}\text{C}$ , a two-factor ANOVA found that sample type (fin and muscle tissue), species and the sample type\*species interaction were significant (Table 2). For  $\delta^{15}\text{N}$ , the difference between dorsal fin and muscle tissue values for the three shark species was less prominent, but in general, muscle tissue  $\delta^{15}\text{N}$  values were higher than fin (Fig. 6b; Table 2). For both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , paired *t* tests between fin and muscle tissue for the three species were significant with the exception of shortfin mako  $\delta^{15}\text{N}$  (Table 3). For sharks held under semi-controlled conditions on a constant diet, Hussey et al. (2010) reported that  $\delta^{13}\text{C}$  of shark vertebrae (cartilage) was enriched by  $\sim 2.9\text{‰}$  relative to muscle tissue; diet-tissue discrimination factors were  $3.75\text{‰} \pm 0.44$  and  $0.90\text{‰} \pm 0.33$ , for cartilage and muscle, respectively. Diet-tissue discrimina-



**Fig. 6.** The (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values for dorsal fin (black dot) and muscle tissue (gray dot) of individual shortfin mako, thresher, and blue sharks ( $n = 5$  each) with mean ( $\pm$ SD) presented for each species.

**Table 2.** Two factor ANOVA testing for the effect of tissue type (fin or muscle tissue), shark species (shortfin mako, thresher, and blue), and associated interaction on derived  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values.

	df	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		
		MS	F	df	MS	F
Tissue type (T)	1	18.54	88.02*	2	3.06	5.89†
Species (S)	2	2.62	12.42*	1	21.97	42.24*
T × S	2	1.44	3.42†	2	0.09	0.17
Residual	24	0.21		24	0.52	
Adjust $r^2$			79.8%			74.7%

Level of significance: **Q2**\* $P < .001$  **Q3** and † $P < .05$ .

tion factors are reported to vary with tissue type across a range of taxa (Pinnegar and Polunin 1999; Caut et al. 2009; DeMots et al. 2010). Consequently, diet-tissue discrimination factors for shark fins would appear to be intermediate to cartilage and

muscle. This complicates multiple tissue analyses, which assume that isotope values in different tissues will be equal if the animal is equilibrium with its diet (MacNeil et al. 2005; Matich et al. 2010).

**Table 3.** Paired *t* test results comparing individual shark  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of muscle tissue and dorsal fin for shortfin mako, thresher, and blue sharks.

Species	Species	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Shortfin mako	Fin versus muscle	$T_5 = 18.56, P < 0.0001$	$T_5 = -1.42, P = 0.23$
Thresher	Fin versus muscle	$T_5 = 27.79, P < 0.0001$	$T_5 = -11.00, P < 0.0001$
Blue	Fin versus muscle	$T_5 = 4.27, P = 0.013$	$T_5 = -4.04, P = 0.016$

## Discussion

Whereas our understanding of SIA or stable isotope dynamics in commonly used tissues, for example muscle tissue, has improved considerably over recent years (Martinez del Rio et al. 2009; Wolf et al. 2009), SIA analyses of new material requires rigorous testing before its broad application in examining aspects of animal ecology. This article undertook a detailed assessment of shark fin, a potentially important tissue for SIA analyses, and provides practical guidelines for its application by identifying the limitations of using a structure, which is composed of multiple tissue components.

Caribbean reef shark  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were highly correlated between the three sample locations on the anal fin indicating that calculated mean population and/or size class  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, derived from multiple anal fin locations, would provide reliable data. In addition, the mean  $\delta^{15}\text{N}_{\text{DIFF}}$  and  $\delta^{13}\text{C}_{\text{DIFF}}$  anal fin values for each sample location comparison did not vary with size of shark suggesting that changes in the size, particularly the thickness of the anal fin with increasing body size, did not bias the overall mean stable isotope results. It is important to note, however, that for shark species that grow to a larger body size, for example the white shark (*Carcharodon carcharias*), the change in fin size with increasing body length is more prominent and thus proportional tissue component differences may be more exaggerated. Moreover, the thickness and also the relative proportion of tissue components in larger fins such as the first dorsal fin will vary more than anal fins with increasing animal size.

On close examination, individual shark  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were variable between sampling locations on the anal fin, with the variability well above analytical error in most instances. Hussey et al. (2010) reported both shark muscle and liver tissue did not vary with sampling location along the length of individual shark body/lobe, respectively. Similarly, Todd et al. (2010) found that Steller sea lion (*Eumetopias jubatus*), California sea lion (*Zalophus californianus*), and harbor seal (*Phoca vitulina*) muscle and skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were homogeneous along the length of individual animals with variation averaging  $<0.5\%$  for both isotope ratios. However, these studies examined variation in a single tissue type, whereas the proportion of tissue types varies across a fin. Using fin sampled from multiple fin locations has the potential to confound studies which examine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data at the individual level. In most instances, this should not be problematic as field sampling can be standardized at the out-

set, but studies using archived samples should exert caution.

As one would expect, Caribbean reef shark anal fin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from sample locations A, B, and C were intermediate to that of skin (D) and cartilage (E). Furthermore,  $\delta^{13}\text{C}$  values of dorsal fin were consistently higher than muscle tissue for all three pelagic sharks sampled. A higher  $\delta^{13}\text{C}$  tissue-specific diet discrimination factor for cartilage compared with muscle is to be expected considering previous work by MacNeil et al. (2005) and Hussey et al. (2010). We cannot rule out, however, that a proportion of the observed difference in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between fin and muscle may be a result of different tissue turnover rates and therefore variables rates of dietary assimilation (MacNeil et al. 2005, 2006). Graham et al. (2009) reported shifts in  $\delta^{15}\text{N}$  between coastal and open ocean foraging zones in the Northwest Atlantic whereas  $\delta^{13}\text{C}$  baseline shifts were less prominent. The  $\delta^{15}\text{N}$  variability may therefore suggest different turnover rates and different equilibration states for muscle and skin with coastal and open ocean feeding grounds. In addition, the measure of increase in  $\delta^{13}\text{C}$  varied up to 2-fold between these species, indicating that it would not be prudent to assume a constant increase in  $\delta^{13}\text{C}$  between muscle and fin for inter-specific studies.

## Comments and recommendations

Knowledge of variation in tissue-specific  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (across multiple sampling sites of an individual animal) is important for interpreting stable isotope data. Our data demonstrate that shark fin samples provide reasonable  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data to examine mean population or size class trends, but individual variability accountable to sampling location on the fin (i.e., tissue composition) may confound more complex individual level and multiple tissue comparison studies. Importantly, the results of this work highlight the need to standardize sampling location on shark fins (within a study and between studies), consider species differences, and exercise caution when using archived fin samples. We recommend that investigators sample material from the trailing edge of fin and sample the same location and fin type across individuals. Where possible, fin samples should be broken into individual components (i.e., skin or cartilage) and these components analyzed separately for carbon and nitrogen stable isotopes. With caution, fin material will be particularly useful for isotopic studies examining endangered or highly threatened species.

The fact that fin  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were intermediate to skin and cartilage poses questions over turnover rates, i.e., skin

(dermal denticles) would be expected to turn over more rapidly than cartilage, and this needs to be considered in the interpretation of fin isotope data, particularly for studies that examine species or populations that undergo seasonal or ontogenetic shifts in diet. In addition, the higher  $\delta^{13}\text{C}$  values in fin relative to muscle highlight the importance of tissue-specific diet discrimination factors and caution in SIA assumptions when comparing multiple tissues from the same animal. Controlled experimental studies on stable isotopes in shark fin are required to (i) determine fin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  turnover rates in conjunction with the turnover rates of individual structural tissues and (ii)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variability amongst multiple fins (anal, pectoral, dorsal, etc.) sampled from the same individuals from animals of different sizes.

## References

- Baum, J. K., D. G. Kehler, and R. A. Myers. 2005. Robust estimates of decline for pelagic shark populations in the Northwest Atlantic and Gulf of Mexico. *Fisheries* 30:26-28.
- , and B. Worm. 2009. Cascading top-down effects of changing oceanic predator abundances. *J. Anim. Ecol.* 78:699-714 [doi:10.1111/j.1365-2656.2009.01531.x].
- Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73:1007-1012 [doi:10.1111/j.0021-8790.2004.00861.x].
- Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Physiol. Pharm.* 37:911-917 [doi:10.1139/y59-099].
- Chapman, D. D., and others. 2009. Long-term natal site fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Mol. Ecol.* 18:3500-3507 [doi:10.1111/j.1365-294X.2009.04289.x].
- Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors ( $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$ ): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* 46:443-453 [doi:10.1111/j.1365-2664.2009.01620.x].
- DeMots, R. L., J. M. Novak, K. F. Gaines, A. J. Gregor, C. S. Romanek, and D. A. Soluk. 2010. Tissue-diet discrimination factors and turnover of stable carbon and nitrogen isotopes in white-footed mice (*Peromyscus leucopus*). *Can. J. Zool.* 88:961-967 [doi:10.1139/Z10-063].
- Domi, N., J. M. Bouqueneau, and K. Das. 2005. Feeding ecology of five commercial shark species of the Celtic Sea through stable isotope and trace metal analysis. *Mar. Environ. Res.* 60:551-569 [doi:10.1016/j.marenvres.2005.03.001].
- Dulvy, N. K., and others. 2008. You can swim but you can't hide: the global status and conservation of oceanic and pelagic sharks and rays. *Aquat. Conserv.* 18:459-482 [doi:10.1002/aqc.975].
- Estrada, J. A., A. N. Rice, M. E. Lutcavage, and G. B. Skomal. 2003. Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotopes. *J. Mar. Biol. Assoc. UK* 83:1347-1350 [doi:10.1017/S0025315403008798].
- , A. N. Rice, L. J. Natanson, and G. B. Skomal. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* 87:829-834 [doi:10.1890/0012-9658(2006)87[829:UOIAOV]2.0.CO;2].
- Fisk, A. T., S. A. Tittlemier, J. L. Pranschke, and R. J. Norstrom. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology* 83:2162-2172 [doi:10.1890/0012-9658(2002)083[2162:UACASI]2.0.CO;2].
- German, D. P., and R. D. Miles. 2010. Stable carbon and nitrogen incorporation in blood and fin tissue of catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). *Environ. Biol. Fish.* 89:117-133 [doi:10.1007/s10641-010-9703-0].
- Graham, B. S., P. Koch, S. Newsome, K. W. McMahon, and D. Aurioles. 2009. Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. *In* J. B. West, G. J. Bowen, T. E. Dawson, and K. P. Tu [eds.], *Isoscapes: understanding movement, pattern and processes on Earth through isotope mapping*. Springer.
- Hammerschlag, N., and J. Sulikowski. 2011. Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. *Endang. Species Res.* 14:135-140 [doi:10.3354/esr00354].
- Heithaus, M. R., A. Frid, A. J. Wirsing, and B. Worm. 2008. Predicting ecological consequences of marine top predator declines. *Trends Ecol. Evol.* 4:202-210 [doi:10.1016/j.tree.2008.01.003].
- Heupel, M. R., and C. A. Simpfendorfer. 2010. Science or slaughter: Need for lethal sampling of sharks. *Conserv. Biol.* 5:1212-1218 [doi:10.1111/j.1523-1739.2010.01491.x].
- Hoffman, M., and others. 2010. The impact of conservation on the status of the world's vertebrates. *Science* 330:1503-1509.
- Hussey, N. E., J. Brush, I. D. McCarthy, and A. T. Fisk. 2010.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  diet-tissue discrimination factors for large sharks under semi-controlled conditions. *Comp. Biochem. Phys. A* 155:445-453 [doi:10.1016/j.cbpa.2009.09.023].
- , H. McCann, G. Cliff, S. F. J. Dudley, S. P. Wintner, and A. T. Fisk. In press-a. Size-based analysis of the diet and trophic position of the white shark (*Carcharodon carcharias*) in South African waters. *In* M. L. Domeier [ed.], *Global perspectives on the biology and life history of the great white shark*. CRC Press.
- , S. F. J. Dudley, I. D. McCarthy, G. Cliff, and A. T. Fisk. In press-b. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet and movement in sharks? *Can. J. Fish. Aquat. Sci.*
- Kelly, M. H., and W. G. Hagar. 2006. Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: how scale and fin tissue compare with muscle tissue. *N. Am. J. Fish. Manage.* 26:921-925 [doi:10.1577/M05-084.1].
- Kim, S. L., and P.L. Koch. 2011. Methods to collect, preserve,



- and prepare elasmobranch tissues for stable isotope analysis. *Environ. Biol. Fish.* [doi:10.1007/s10641-011-9860-9].
- Kinney, M. J., N. E. Hussey, A. T. Fisk, A. J. Tobin, and C. A. Simpfendorfer. 2011. Communal or competitive: Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. *Mar. Ecol. Prog. Ser.* 439:263-276. [doi:10.3354/meps09327].
- Layman, C. A., D. A. Arrington, C. G. Montana, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88:42-48 [doi:10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2].
- Logan, J. M., and M. E. Lutcavage. 2010. Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia* 644:231-244 [doi:10.1007/s10750-010-0120-3].
- MacNeil, M. A., G. B. Skomal, and A. T. Fisk. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. *Mar. Ecol. Prog. Ser.* 302:199-206 [doi:10.3354/meps302199].
- , K. G. Drouillard, and A. T. Fisk. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Can. J. Fish. Aquat. Sci.* 63:345-353 [doi:10.1139/f05-219].
- Martinez del Rio, C., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* 84:91-111 [doi:10.1111/j.1469-185X.2008.00064.x].
- Matich, P., M. R. Heithaus, and C. A. Layman. 2010. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *J. Anim. Ecol.* 80:294-305 [doi:10.1111/j.1365-2656.2010.01753.x].
- McMeans, B. C., J. A. Olin, and G. W. Benz. 2009. Stable isotope comparisons between embryos and mothers of a placental shark species. *J. Fish Biol.* 4:1061-1063.
- , J. Svavarsson, and A. T. Fisk. 2010. Diet and resource use among Greenland sharks (*Somniosus microcephalus*) and teleosts sampled in Icelandic waters using  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and mercury. *Can. J. Fish. Aquat. Sci.* 67:1428-1438 [doi:10.1139/F10-072].
- Myers, R. A., and B. Worm. 2003. Rapid worldwide depletion of predatory fish communities. *Nature* 423:280-283 [doi:10.1038/nature01610].
- Olin, J. A., N. E. Hussey, M. Fritts, M. R. Heupel, C. A. Simpfendorfer, G. R. Poulakis, and A. T. Fisk. 2011. Maternal meddling in neonatal sharks: implication for interpreting stable isotopes in young animals. *Rap. Commun. Mass Spectrom.* 25:1008-1016 [doi:10.1002/rcm.4946].
- Papastamatiou, Y. P., A. M. Freidlander, J. E. Caselle, and C. G. Lowe. 2010. Long term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. *J. Exp. Mar. Biol. Ecol.* 386:94-102 [doi:10.1016/j.jembe.2010.02.009].
- Phillips, D. L., and P. M. Eldridge. 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* 147:195-203 [doi:10.1007/s00442-005-0292-0].
- Pinnegar, J. K., and P. V. C. Polunin. 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Funct. Ecol.* 13:225-231 [doi:10.1046/j.1365-2435.1999.00301.x].
- Revill, A. T., J. W. Young, and M. Lansdell. 2009. Stable isotopic evidence for trophic groupings and bioregionalisation of predators and their prey in oceanic waters off Eastern Australia. *Mar. Biol.* 156:1241-1253 [doi:10.1007/s00227-009-1166-5].
- Sanderson, B. L., C. D. Tran, H. J. Coe, V. Pelekis, E. A. Steel, and W. L. Reichert. 2009. Nonlethal sampling of fish caudal fins yields valuable stable isotope data for threatened and endangered fishes. *T. Am. Fish. Soc.* 138:1166-1177 [doi:10.1577/T08-086.1].
- Schielke, E. G., and D. M. Post. 2011. Size matters: comparing stable isotope ratios of tissue plugs and whole organisms. *Limnol. Oceanogr. Methods* 8:348-351 [doi:10.4319/lom.2010.8.348].
- Todd, S. K., B. Holm, D. A. S. Rosen, and D. J. Tollit. 2010. Stable isotope signal homogeneity and differences between and within pinniped muscle and skin. *Mar. Mammal Sci.* 26:176-185 [doi:10.1111/j.1748-7692.2009.00345.x].
- Wetherbee, B. M., and E. Cortés. 2004. Food consumption and feeding habits, pp. 225-246. *In* J. C. Carrier, J. A. Musick, and M. R. Heithaus [eds.], *Biology of sharks and their relatives*. CRC Marine Biology Series [doi:10.1201/9780203491317.ch8].
- Wolf, N., S. A. Carleton, and C. Martinez del Rio. 2009. Ten years of experimental animal isotopic ecology. *Funct. Ecol.* 23:17-26 [doi:10.1111/j.1365-2435.2009.01529.x].

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