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Li, Y.; Hussey, N. E.; and Zhang, Y., Zhang, Y., "Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks" (2016). *Aquatic Biology*, 25, 53-60.

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Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks

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ABSTRACT: Comparative analysis of isotope values from different tissues can capture temporal variation in the trophic and foraging behavior of difficult to study large marine predators, revealing either uniform or variable ecological roles over time. The isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of dermis, and muscle tissue of silky *Carcharhinus falciformis* and blue sharks *Prionace glauca* sampled in the northeast central Pacific were analyzed to quantify ontogenetic inter- and intra-tissue isotopic variation. Consistent differences in $\delta^{15}\text{N}$ values occurred between dermis and muscle tissue for both species ($2.5 \pm 0.4\text{‰}$ and $2.1 \pm 0.3\text{‰}$, respectively), while tissue differences in $\delta^{13}\text{C}$ values were more variable between species ($2.3 \pm 0.6\text{‰}$ and $0.7 \pm 0.6\text{‰}$, respectively), likely a result of tissue composition. The overall $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of dermis and muscle were highly correlated for blue sharks and for silky sharks with the exception of silky shark $\delta^{13}\text{C}$ values. This pattern indicates that dermis isotope values are able to provide a proxy for muscle tissue, similar to that previously reported for fin, accepting dermis-specific diet–tissue discrimination factors. Tissue-specific ontogenetic isotopic variation for the silky shark, and the low regression slope value between dermis and muscle $\delta^{13}\text{C}$ values, however, may suggest that dermis and muscle tissue have different isotopic turnover rates. These data demonstrate that dermis yields valuable isotope data to examine the trophic ecology and feeding/movement behavior of sharks, but further work is required to address dermis-specific turnover rates and diet–tissue discrimination factors.

KEY WORDS: Pelagic shark · Stable isotope · Dermis

INTRODUCTION

In recent years, our understanding of the temporal and spatial variation in trophic roles and foraging dynamics of shark species has improved based on the application of carbon and nitrogen stable isotopes (Matich et al. 2010, 2011, Hussey et al. 2011, Kim et al. 2012a). Compared to the instantaneous ‘snapshot’ of dietary information obtained from gut content analysis, stable isotope analysis (SIA) allows exami-

nation of feeding behaviors integrated over numerous time periods (Martínez del Río et al. 2009, Willis et al. 2013). For example, the isotopic composition of different metabolic tissues with diverse turnover rates can provide dietary information integrated over short (weeks, using plasma; Matich et al. 2011) to long (months to years, using muscle; MacNeil et al. 2005) periods. Consequently, inter-tissue stable isotope comparisons can examine dietary shifts and variation in trophic position of species (MacNeil et al.

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2005, Malpica-Cruz et al. 2013), characterize the degree of dietary specialization at individual and population levels, and identify inter/intra-specific resource partitioning and niche overlap over time (Matich et al. 2010, 2011, Kinney et al. 2011). To date, the most common tissue analyzed for SIA in sharks is white muscle (Estrada et al. 2003, MacNeil et al. 2005), but other tissues include liver (Olin et al. 2011, Malpica-Cruz et al. 2013), blood (Matich et al. 2010, 2011, Malpica-Cruz et al. 2013), fin (Matich et al. 2010, 2011, Hussey et al. 2011), and vertebrae (Estrada et al. 2006, Kim et al. 2012b, Carlisle et al. 2015).

Considering the threatened status of many shark species (Dulvy et al. 2014) and the requirement to limit mortalities, nondestructive or minimally invasive sampling methods are increasingly being adopted in field studies (Hammerschlag & Sulikowski 2011). Such tissue sampling from large sharks often includes blood (plasma and red blood cells), fin, and/or a biopsy sample of skin, connective tissue, and muscle. Fin is considered an attractive tissue to sample, given the easy, quick, and minimally invasive sampling protocol (Matich et al. 2010, Hussey et al. 2011). Skin, or more specifically the dermal collagen fiber layers that underlie the dermal denticles of shark skin (hereafter referred to as dermis), potentially provides another tissue that is easy to sample, and is likely more homogeneous in terms of structural composition compared to fin (Matich et al. 2010, Hussey et al. 2011, Carlisle et al. 2012). To date, the isotopic composition of dermis has received limited attention (but see Carlisle et al. 2012, Jaime-Rivera et al. 2013) despite the above advantages.

In this study, we analyzed the isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of dermis and muscle tissue sampled from silky *Carcharhinus falciformis* and blue sharks *Prionace glauca*, 2 pelagic species that are commonly caught in commercial fisheries. Both species are known to predominantly feed on cephalopods in pelagic waters, but silky sharks are considered to forage both in nearshore and pelagic habitats in contrast to the blue shark (Rabehagaso et al. 2012, Galván-Magaña et al. 2013). Both species, however, undertake large-scale movement patterns between foraging locations (Bonfil 2008, Stevens et al. 2010). The objectives of this study were (1) to quantify inter-tissue isotopic differences and (2) to examine inter- and intra-tissue isotopic variation by age for both pelagic shark species. Overall, the aim was to determine if $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of dermis can provide valuable temporal insights into the trophic ecology and feeding behavior of these impacted pelagic species.

MATERIALS AND METHODS

Sample collection

Tissue samples were obtained from silky and blue sharks caught as bycatch in the Chinese tuna longline fishery operating in the northeast central Pacific (approximately 8° to 10°N, 115° to 125°W) between June and November 2014. A skin tissue sample, including both dermis and dermal denticles, was excised from the anal fin region of each individual to minimize the effects of specific variation in skin thickness, and a small portion (~10 g) of white muscle tissue was excised adjacent to the vertebral column. Tissue samples were immediately placed on ice and frozen within 30 min of sampling. For each individual, precaudal length (PCL) was measured as the straight-line distance from the tip of the snout to the beginning of the caudal fin. The age of all sharks sampled was estimated from PCL data using von Bertalanffy growth parameters for the 2 species from the same sampling region (Oshitani et al. 2003, Nakano & Stevens 2008). The length at 50% maturity was set at 151 to 156 cm PCL and 150 to 159 cm PCL for the silky and blue shark, respectively (Oshitani et al. 2003, Nakano & Stevens 2008).

Stable isotope analysis

Following the removal of dermal denticles and epidermis, isolated dermis tissue was washed and soaked overnight in de-ionized water. Dermis was then freeze-dried at -55°C for 48 h using a Christ Alpha 1-4 LD plus Freeze Dryer (Martin Christ), and each sample was homogenized using a Retsch Mixer Mill MM 400 (Retsch). Sharks maintain urea and trimethylamine oxide in their tissues for osmotic balance, which are considered to be ^{15}N -depleted and may also affect $\delta^{13}\text{C}$ values (Fisk et al. 2002, Li et al. 2016). Consequently, urea was removed from muscle tissue using a standard water washing technique (Kim & Koch 2012, Li et al. 2016). For $\delta^{15}\text{N}$ analysis, all muscle tissue samples were urea-extracted, while for $\delta^{13}\text{C}$, both urea and lipids were removed using combined water washing and standard chloroform-methanol extraction (Li et al. 2016). Tissue samples were then freeze-dried to remove remaining solvent and divided into approximately 1 to 1.5 mg subsamples for stable isotope analysis using an IsoPrime 100 isotope ratio mass spectrometer (IsoPrime) and a vario ISOTOPE cube elemental analyzer (Elementar

Analysensysteme) at Shanghai Ocean University Stable Isotope Laboratory. Reference standards for quantifying ^{13}C and ^{15}N stable isotope values, were USGS 24 ($-16.1 \pm 0.04\%$ V-PDB) and USGS 26 ($53.7 \pm 0.4\%$ V-AIR), respectively. The analytical errors of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were $\pm 0.05\%$ and $\pm 0.06\%$, respectively.

Statistical analysis

To examine the relationship of isotopic values between tissues, paired t -tests and correlation analyses were used to compare individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between dermis and muscle for silky and blue sharks. The mean differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between dermis and muscle were then calculated to examine the isotopic offset between tissues for both species. To examine tissue isotopic differences over ontogeny, least squares linear regressions were fitted to determine the relationship between estimated age and muscle and dermis $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The absolute isotopic differences between dermis and muscle per individual were calculated to further quantify age-based inter- and intra-tissue isotopic variation and to identify whether the observed age-related trends were comparable between species. The above analyses were conducted on all the data grouped by species because an initial examination found no regional differences in isotope values. The normality of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dermis and muscle tissue were examined using a Shapiro-Wilk normality test. All statistical analyses were conducted using R version 3.1.2 (R Development Core Team 2014), and the level of statistical significance was set at $\alpha = 0.05$.

RESULTS

Muscle and dermis from a total of 39 silky and 26 blue sharks ranging in size from 57 to 167 (mean: 107 ± 36) cm and 130 to 205 (mean: 153 ± 21) cm PCL, respectively, were sampled and analyzed (Fig. 1, Table 1). Paired t -tests found significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between dermis and muscle tissue for both silky ($\delta^{13}\text{C}_{\text{muscle-dermis}}$: $t_{38} = 40.2$, $p < 0.01$, $\delta^{15}\text{N}_{\text{muscle-dermis}}$: $t_{38} = -22.9$, $p < 0.01$) and blue sharks ($\delta^{13}\text{C}_{\text{muscle-dermis}}$: $t_{25} = 37.1$, $p < 0.01$, $\delta^{15}\text{N}_{\text{muscle-dermis}}$: $t_{25} = -6.5$, $p < 0.01$). Mean dermis $\delta^{13}\text{C}$ values of both pelagic sharks were consistently higher than those of muscle tissue by $2.5 \pm 0.4\%$ for silky and $2.1 \pm 0.3\%$ for blue sharks. In contrast, the trend of dermis–muscle $\delta^{15}\text{N}$ values was not consistent between species.

Table 1. Mean (\pm SD) tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\%$) of *Carcharhinus falciformis* and *Prionace glauca* sampled from the northeast central Pacific Ocean. PCL: precaudal length in cm; age in yr

	<i>C. falciformis</i> (n = 39)	<i>P. glauca</i> (n = 26)
PCL (range)	107 (57–167)	153 (130–205)
Age (range)	3.3 (0.3–8.2) ^a	4.0 (2.6–7.7) ^b
Carbon		
Muscle	-16.4 ± 0.2	-17.8 ± 0.7
Skin	-13.9 ± 0.4	-15.6 ± 0.6
Difference	-2.5 ± 0.4	-2.1 ± 0.3
Nitrogen		
Muscle	16.0 ± 0.7	16.1 ± 1.3
Skin	13.7 ± 0.9	15.4 ± 1.0
Difference	2.3 ± 0.6	0.7 ± 0.6

^aOshitani et al. (2003)
^bNakano & Stevens (2008)

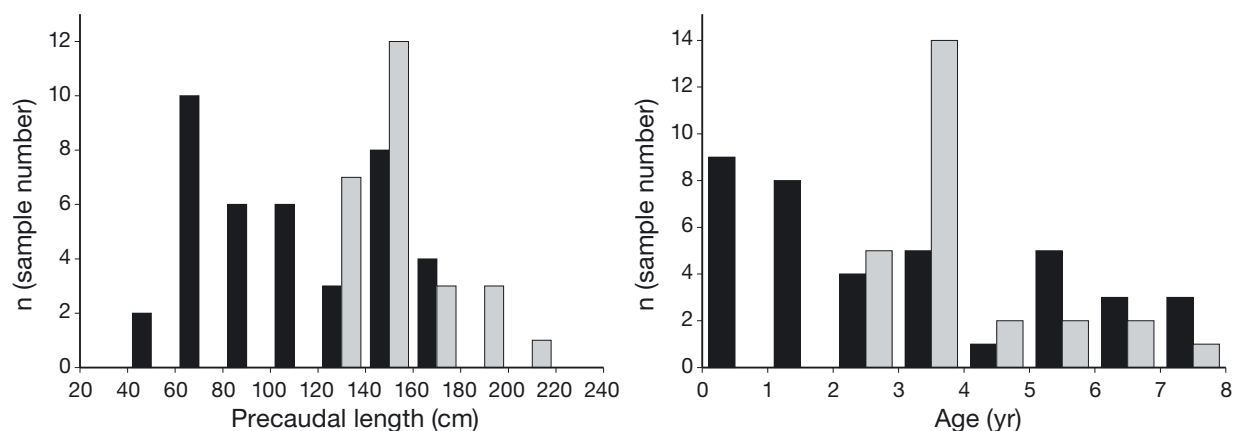


Fig. 1. Size distribution and age of silky sharks *Carcharhinus falciformis* (black bars) and blue sharks *Prionace glauca* (grey bars) sampled from the northeast central Pacific

The mean difference in $\delta^{15}\text{N}$ values between tissues was much larger for silky ($2.3 \pm 0.6\text{‰}$) than blue sharks ($0.7 \pm 0.6\text{‰}$) ($p < 0.01$; Table 1).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dermis and muscle were highly correlated for both pelagic sharks. For silky sharks, the relationship between dermis and muscle $\delta^{13}\text{C}$ values was significant but relatively weak compared to the dermis–muscle $\delta^{15}\text{N}$ relationship (Fig. 2a,c). In contrast, the above $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relationships for blue sharks were both strong with $R^2 > 0.84$ and regression slopes that were not statistically different from 1 (Fig. 2b,d).

Overall, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each tissue exhibited similar trends with increasing age of shark. For silky sharks, the $\delta^{13}\text{C}$ values of both tissues showed no relationship with age (dermis: $R^2 = 0.01$, $p = 0.5$ and muscle: $R^2 = 0.01$, $p = 0.1$; Fig. 3a). Conversely, there was a significant negative relationship between the $\delta^{15}\text{N}$ values of silky shark dermis and age, and a similar but non-significant trend was observed between muscle $\delta^{15}\text{N}$ values and age (Fig. 3c). For blue sharks, $\delta^{13}\text{C}$ values of both tissues increased significantly with increasing age (dermis: $R^2 = 0.2$, $p < 0.05$ and muscle: $R^2 = 0.2$, $p < 0.05$; Fig. 3b). In

contrast, there was no relationship between $\delta^{15}\text{N}$ values and age of blue sharks for either dermis or muscle (dermis: $R^2 = 0.01$, $p = 0.5$ and muscle: $R^2 = 0.01$, $p = 0.1$; Fig. 3d).

The absolute difference between dermis and muscle $\delta^{13}\text{C}$ values for both shark species were variable and showed no relationship with age (silky: $R^2 = 0.1$, $p = 0.06$; blue: $R^2 = 0.1$, $p = 0.2$, Fig. 4). For the silky shark, the absolute difference in dermis–muscle $\delta^{15}\text{N}$ values increased significantly with age ($R^2 = 0.6$, $p < 0.001$), while the absolute differences in $\delta^{15}\text{N}$ values for the blue shark were more variable, and no age relationship was evident ($R^2 = 0.003$, $p = 0.8$).

DISCUSSION

Examination of the carbon and nitrogen isotopic relationships between different tissues sampled from pelagic sharks provides insights into the amenability of these tissues to address ecological questions over the species' trophic roles, foraging behavior, and habitat use. Certain tissues may provide reliable surrogate isotope values for the most commonly ana-

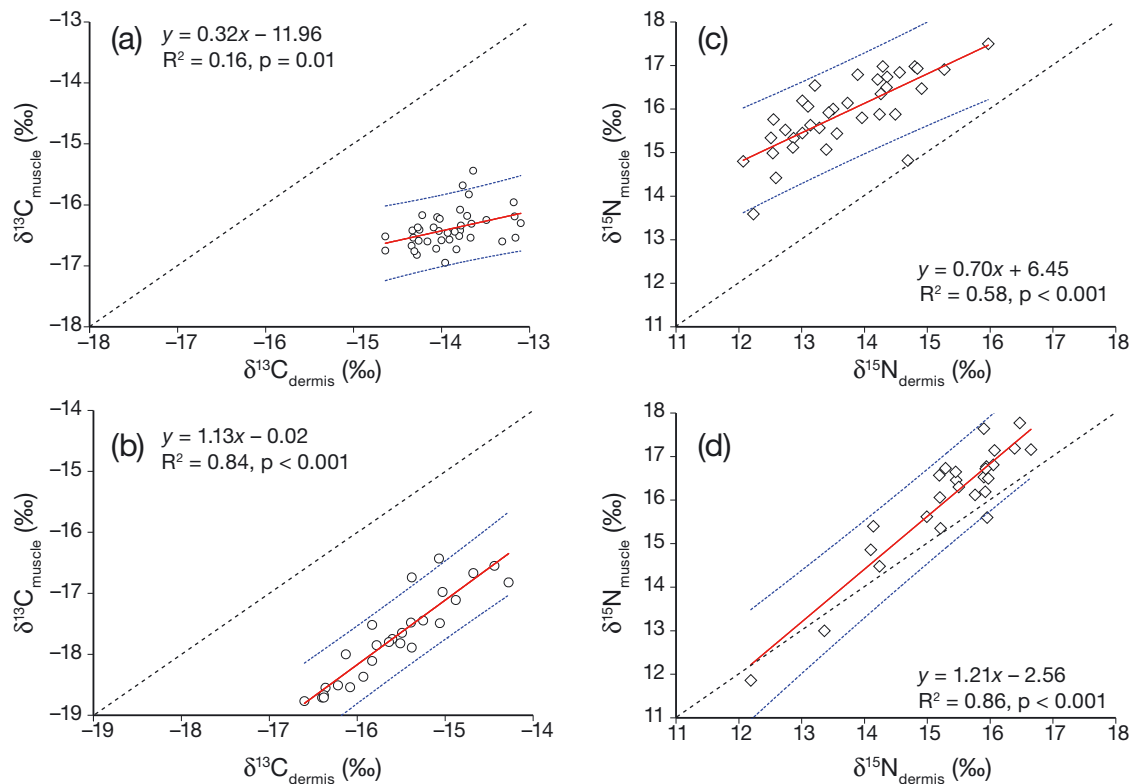


Fig. 2. Comparison of $\delta^{13}\text{C}$ (open circle) and $\delta^{15}\text{N}$ (open diamond) values between skin and muscle tissue of (a,c) silky shark *Carcharhinus falciformis* ($n = 39$) and (b,d) blue shark *Prionace glauca* ($n = 26$). The dashed black line depicts the 1:1 isotopic relationship between the 2 tissues. The solid red lines indicate the regression slope for significant relationships, while the blue dashed lines represent the 95% confidence intervals of the regression analysis

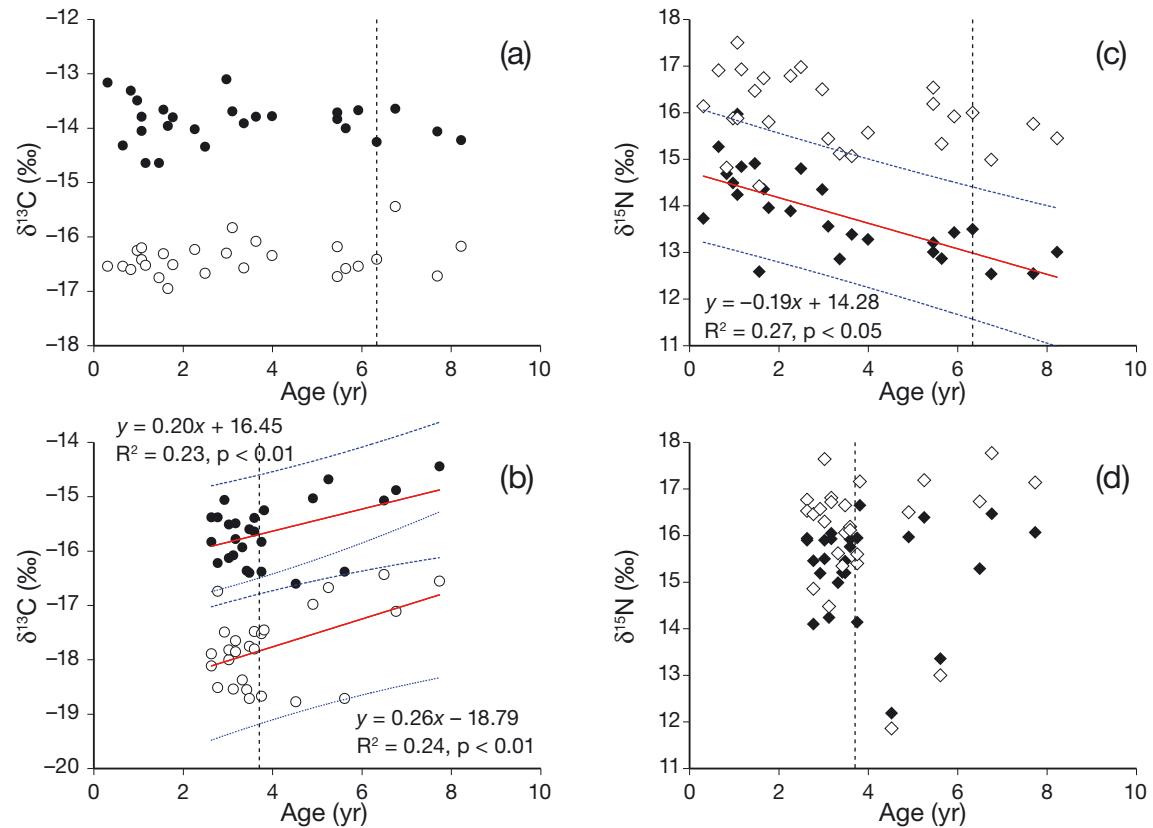


Fig. 3. Relationship of (a,b) $\delta^{13}\text{C}$ and (c,d) $\delta^{15}\text{N}$ values from paired samples of skin (solid circle and diamond) and muscle (open circle and diamond) and age for (a,c) silky shark *Carcharhinus falciformis* ($n = 39$) and (b,d) blue shark *Prionace glauca* ($n = 26$) sampled in northeast central Pacific. Solid red lines indicate the regression slope for significant relationships, while the blue dashed lines represent the 95% confidence intervals of the regression analysis. The vertical black dashed lines indicate age at 50% maturity

lyzed muscle tissue but are less invasive to sample, while multiple tissue sampling allows opportunities to examine temporal variation in diet and feeding interactions. The data present a comparative analysis of the isotope values of dermis and identify that it provides valuable information to further explore the trophic dynamics of these commercially exploited species.

The fact that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of dermis and muscle tissues for both pelagic shark species were significantly correlated and that standard deviations of each tissue were low for both isotopes indicates that dermis isotope values may provide a potential proxy for muscle tissue for specific life stages. These findings are in agreement with those previously reported for fin–muscle tissue isotopic relationships for sharks (Hussey et al. 2011, Matich et al. 2011). There was clear evidence, however, to suggest tissue-specific isotope values. Specifically, the weaker correlations between tissue isotope values for silky sharks compared to blue sharks support this latter point, identifying tissue-specific turnover rates and

isotopic integration periods. For the silky shark, this pattern may be a result of the different life-history stages sampled relative to the blue shark, given age-specific feeding reported among juvenile, sub-adult, and adult life-stages (Cabrera-Chávez-Costa et al. 2010, Malpica-Cruz et al. 2013). Marked ontogenetic dietary shifts have been previously reported for silky sharks, with juveniles feeding predominantly on jumbo squid *Dosidicus gigas*, whereas sub-adults consumed teleost fish *Scomber japonicus* (Cabrera-Chávez-Costa et al. 2010). Variable turnover rates between dermis and muscle tissue coupled with a marked diet shift would result in the observed greater inter-tissue isotopic variation. In contrast, most blue sharks sampled were sub-adult and mature animals (Hsu et al. 2011) that consume epipelagic and mesopelagic cephalopods (Hernández-Aguilar et al. 2015), similar to the diet of juveniles (Nakano & Stevens 2008). This consumption would result in more consistent isotope values between tissues even with different turnover rates, but small sample sizes over ontogeny limit the conclusions that can be made.

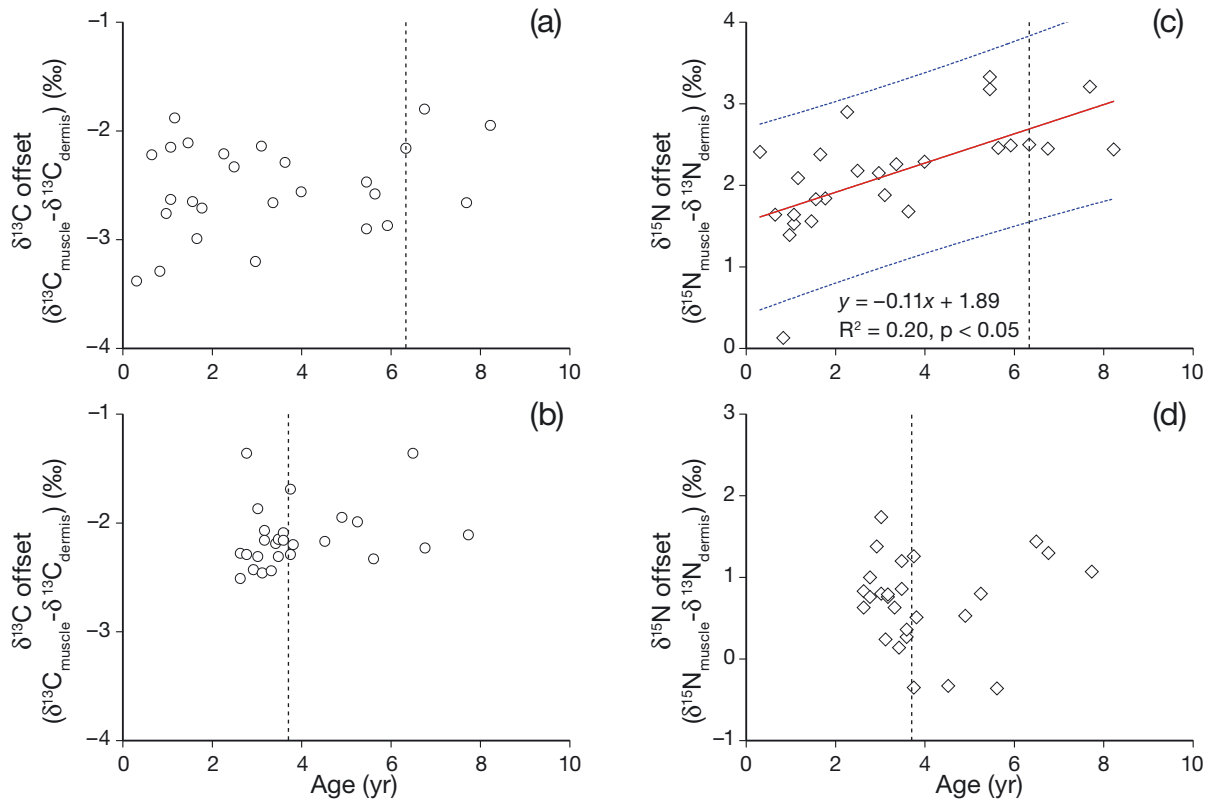


Fig. 4. Difference of (a,b) $\delta^{13}\text{C}$ and (c,d) $\delta^{15}\text{N}$ values between paired skin and muscle tissue samples from (a,c) silky shark *Carcharhinus falciformis* and (b,d) blue shark *Prionace glauca*. Solid red lines indicate the regression slope for significant relationships, while the blue dashed lines represent the 95% confidence intervals of the regression analysis. The vertical black dashed lines indicate age at 50% maturity

Shark placoid scales or dermal denticles form in the dermis and increase in size with increasing body surface or body growth during the lifetime of an animal (reviewed by Meyer & Seegers 2012). The growth of these placoid scales is continuous, with substitution taking place as scales shed, resulting in different growth stages of scales occurring between fully grown dermal denticles (Meyer & Seegers 2012). Consequently, the isotopic incorporation rate of dermis is likely relatively fast, providing insight into short-term diet and feeding behavior when compared with muscle (Carlisle et al. 2012). The low regression slopes between muscle and dermis tissue isotope values of the silky shark may support this point. Previous studies have identified relatively fast isotopic turnover rates in skin tissue of marine mammals; for example, half lives of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were <2 mo for manatees *Trichechus manatus latirostris* and bottlenose dolphins *Tursiops truncatus*, respectively (Alves-Stanley & Worthly 2009, Browning et al. 2014). Although the dermis of sharks is structurally different to that of marine mammals, the fact that skin shedding/replacement occurs across a broad

range of marine species (Alves-Stanley & Worthly 2009, Meyer & Seegers 2012, Browning et al. 2014) would suggest a faster isotopic turnover rate.

For both shark species, dermis $\delta^{13}\text{C}$ values were consistently higher than those of muscle tissue. Collagen and elastic fibers, the primary constituents of dermis (Meyer & Seegers 2012), typically drive enriched ^{13}C values relative to diet (Kim & Koch 2012). This trend was previously reported for white shark dermal $\delta^{13}\text{C}$ values relative to muscle (Carlisle et al. 2012, Jaime-Rivera et al. 2013). It is likely that ^{13}C enrichment of dermis is a reflection of differences in biochemical composition between dermis and muscle, because each amino acid comprising the tissue proteins can vary in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Carlisle et al. 2012, Vander Zanden et al. 2014). Alternatively, tissue-specific diet discrimination factors may provide an explanation for the observed isotopic differences between tissues (Matich et al. 2010, Hussey et al. 2011, 2012, Cherel et al. 2014). It is still likely, however, that variable tissue-turnover rates explain some of the observed variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between dermis and muscle (Hussey et al. 2011).

It is important to note that the thickness of dermis can vary among species and over ontogeny, and females at different stages of reproductive maturity typically have variable dermal thickness related to mating (Motta 1977, Meyer & Seegers 2012). In this study, samples were taken from the anal fin area to minimize potential variation, but given that sex was not recorded, dermal thickness may also account for some of the observed isotopic variation.

Following birth, juvenile silky sharks transition from coastal waters to offshore and pelagic habitat (Dagorn et al. 2007, Bonfil 2008) but continue to use shallow waters near the edge of the continental shelf and over deep-water reefs (Bonfil 1997). The $\delta^{13}\text{C}$ -age relationships for both tissues of silky shark were non-significant, while the $\delta^{15}\text{N}$ values in dermis decreased and the offset between dermis and muscle tissue $\delta^{15}\text{N}$ values increased significantly with age, i.e. there was a large difference in values between tissues of older animals. These $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ relationships indicate that older and larger animals show a marked temporal shift in diet but that animals of all ages consistently occur in the same habitat (Malpica-Cruz et al. 2013).

For blue sharks, there were no obvious tissue-specific isotopic trends with age, but large variation among individuals was observed. This variation may suggest variable diets, but more likely reflects basin-scale movements across spatial gradients in baseline $\delta^{15}\text{N}$ values (Cherel et al. 2007, Polo-Silva et al. 2013, Kiszka et al. 2014). In contrast to the silky shark, the $\delta^{13}\text{C}$ values of both tissues of blue sharks increased with age, possibly reflecting an ontogenetic shift in foraging location or increased consumption of ^{13}C -enriched prey. Such size-related isotopic patterns are consistent with those reported for blue sharks in the Indian Ocean (Rabehagaso et al. 2012, Kiszka et al. 2015).

Pelagic sharks are top predators that couple marine ecosystems over large spatio-temporal scales; consequently, their removal is predicted to destabilize food web interactions (Heithaus et al. 2008). These data demonstrate that dermis can yield valuable isotope data to better understand the role of pelagic sharks, but observed differences between dermis and muscle tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were species-specific and life stage-dependent, which may relate to variable isotopic routing, tissue-specific amino acid composition, and/or different turnover rates between tissues. Our data when compared to published fin and dermis isotope data for sharks suggest a combination of these influencing factors (Hussey et al. 2011, Carlisle et al. 2012), but further work will be required

to quantify dermis turnover rates and discrimination factors in sharks to improve confidence when interpreting multi-tissue stable isotope data.

Acknowledgements. We thank the observers who helped with the collection and processing of isotopic samples. We are grateful to Dr. Michael Heithaus for assistance with the raw data. We also acknowledge the detailed comments of 3 reviewers that improved the overall content of the manuscript. Y.L. was supported by the National Natural Science Foundation of China (#41206124; #41541042) and PhD Programs Foundation of Ministry of Education of China (#20123104120001) and N.E.H. was supported by funding from the Government of Nunavut and the Ocean Tracking Network.

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