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NOTE

Low lipid and urea effects and inter-tissue comparisons of stable isotope signatures in three nearshore elasmobranchs

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ABSTRACT: Stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a common tool used to examine aspects of elasmobranch biology and ecology; however, accurate ecological interpretation of stable isotope values requires knowledge of lipid and urea dynamics, and the variable turnover rates of different tissue types. Here we examined lipid and urea dynamics and inter-tissue comparisons of stable isotope values in 3 nearshore elasmobranch species, the nurse shark *Ginglymostoma cirratum*, southern stingray *Hypanus americanus*, and the Atlantic chupare stingray *Styracura schmardae*. Chemical extraction had no significant effect on $\delta^{13}C$ and $\delta^{15}N$ values of nurse shark muscle, and southern and chupare stingray fin, suggesting negligible lipid and urea components associated with these tissues. For nurse sharks, $\delta^{13}C$ values were higher in muscle compared to dermis and $\delta^{15}N$ was lower. The causes of this variability are underpinned by the metabolic variability between tissue types, the physiological function of which remains undetermined. Finally, we observed a significant relationship between muscle and dermis $\delta^{13}C$ and $\delta^{15}N$, providing the first inter-tissue isotopic correction for nurse sharks. The results provide insight into lipid and urea dynamics, and aid sample preparation and ecological interpretation of stable isotope data in these taxa.

KEY WORDS: Stable isotope analysis · $\delta^{13}C$ · $\delta^{15}N$ · Elasmobranch · Shark · Stingray · Polar compounds

INTRODUCTION

Stable isotope analysis is a common tool used to examine aspects of elasmobranch biology, and has been widely applied in all major marine biomes (Logan & Lutcavage 2010, Hussey et al. 2012a, Shiffman et al. 2012, Shipley et al. 2017a). Although a useful, low-cost, and in many cases non-lethal approach, many aspects of elasmobranch stable isotope dynamics are poorly understood. For example, few studies have published accurate diet–tissue discrimination factors, or compared the degree of homogeneity in isotope data generated from different tissues. Failure to acknowledge or account for these limitations may introduce bias, and thus limit or compromise the interpretation of stable isotope data (Shipley et al.

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2017a). A further understanding of stable isotope dynamics in elasmobranchs is therefore pertinent to evolve field sampling protocols, laboratory sample preparation, and ecological interpretation of stable isotope data for these species.

Lipids and urea/trimethylamine n-oxide (TMAO, herein referred to as urea) bias $\delta^{13}C$ and $\delta^{15}N$ isotope values of elasmobranch species (Hussey et al. 2011, Li et al. 2016a, Carlisle et al. 2017, Shipley et al. 2017b). Lipids are ^{13}C-depleted relative to pure protein and will therefore reduce $\delta^{13}C$ values as the lipid to protein ratio increases (Sweeting et al. 2006, Hussey et al. 2012b). Similarly, high levels of urea, which facilitates cellular osmoregulation, will reduce $\delta^{15}N$ values relative to pure protein (Kim & Koch 2012, Churchill et al. 2015). Some debate remains over whether these compounds display biasing effects homogenously across species and tissue types, as isotopic data are lacking for a large number of species. To resolve the biasing effects of lipids and urea on elasmobranch stable isotope values, studies have developed normalized corrections (e.g. Reum 2011, Churchill et al. 2015, Li et al. 2016a, Carlisle et al. 2017), as those commonly used for teleost fish and invertebrates (e.g. Post et al. 2007, Hoffman & Sutton 2010) fail to account for the additional effects of urea (Carlisle et al. 2017, Shipley et al. 2017b). Such corrections are often species-specific (Li et al. 2016a), and in some cases cannot be generated (Churchill et al. 2015). Chemical extractions, which remove both lipids and urea, are the most commonly adopted approach in elasmobranch studies (Li et al. 2016a, Carlisle et al. 2017), but are often labor intensive. Furthermore, lipid and urea concentrations are known to vary markedly between tissues (based on metabolic function) and species (driven by life history), which raises the question of whether lipids and urea bias isotope values in all situations. Improved documentation of lipid and urea effects on isotope values, on a species- and tissue-specific basis, may provide clarity on this issue.

In addition to the confounding effects of lipids and polar compounds, stable isotope values can vary between tissues, an artifact resulting from variable tissue metabolism coupled with ontogenetic and/or seasonal shifts in diet (Pinnegar & Polunin 1999, Hussey et al. 2012a). Tissues associated with slow metabolism, such as muscle, reflect diet over longer durations (e.g. ca. 422 d for ocellate river stingrays *Potamotrygon motoro*, MacNeil et al. 2006; ca. >300 d for sandbar sharks *Carcharhinus plumbeus*, Logan & Lutcavage 2010). Tissues associated with faster metabolism, such as plasma (ca. 70–200 d for leopard sharks *Triakis semifasciata*, Kim et al. 2012) or liver (ca. 166 d for *P. motoro*, MacNeil et al. 2006) reflect more recent foraging. Although inter-tissue comparisons may reveal temporal variability in elasmobranch diet, comparing relationships between tissues of similar turnover rate remain relatively unexplored. These observations are of particular importance when comparing isotope values within and between species for which values are generated from different tissues. Such situations can arise when sampling time or the invasiveness of sampling is limited. For example, if the target species displays high vulnerability to capture and must be released promptly, or if sampling is limited to a certain tissue type by ethical protocols (e.g. Huntingford et al. 2006).

Here we examined the cumulative effects of lipids and urea on $\delta^{13}C$ and $\delta^{15}N$ isotope values generated from the tissues of 3 elasmobranch species defined as Data-Deficient by the International Union for the Conservation of Nature (IUCN) red list: the nurse shark *Ginglymostoma cirratum*, southern stingray *Hypanus americanus*, and Atlantic chupare stingray *Styracura schmardae*. We also provide the first muscle–dermis isotope correction for nurse sharks. Such observations will likely streamline sample preparation of tissues, and aid the ecological interpretation of stable isotope data in these taxa.

MATERIALS AND METHODS

Sample collection and analyses

Elasmobranchs were collected from the waters surrounding south Eleuthera, The Bahamas (24.84° N, 76.34° W), between 2013 and 2016. Nurse sharks were captured via stationary mid-water longlines (Brooks et al. 2013). White dorsal muscle and dermis tissue were excised from the base of the dorsal fin using a sterilized scalpel. Stingrays were opportunistically captured with spot seines (O'Shea et al. 2017), and a pelvic fin clip was taken using surgical scissors. All samples were frozen at –20°C.

Samples were oven-dried for 72 h at 60°C and homogenized using a mortar and pestle, before separation into bulk and extracted treatments. In the extracted treatment, lipids were extracted using 2:1 chloroform:methanol (adapted from Folch et al. 1957), and nitrogenous polar compounds via a triple di-ionized water rinse (Carlisle et al. 2017). All tissues were then weighed into 5 × 3.5 mm tin capsules (Elemental Microanalysis) and analyzed on a Thermo-Finnigan Delta Plus continuous flow isotope

Table 1. Summary information for benthic elasmobranchs captured from The Bahamas, highlighting size ranges and bulk and extracted mean (± SD) $\delta^{13}C$, $\delta^{15}N$, and C:N ratios. Size range of nurse sharks = total length; southern and Atlantic chupare stingrays = disc width. (*) Indicates statistically significant differences ($\alpha = 0.05$) between bulk and extracted treatments

Size range (cm)	Tissue	n	$\delta^{13}C_{Bulk}$	$\delta^{13}C_{Extracted}$	$\delta^{15}N_{Bulk}$	$\delta^{15}N_{Extracted}$	$C:N_{Bulk}$	$C:N_{Extracted}$
***Ginglymostoma cirratum* Nurse shark**								
113–246	Muscle	20	−7.64 (2.09)	−8.01 (2.05)	9.03 (1.09)	8.96 (1.01)	2.58 (0.05)*	2.78 (1.01)*
	Dermis	17	–	−7.71 (1.82)	–	8.47 (0.93)	–	2.84 (0.24)
***Hypanus americanus* Southern stingray**								
45–102	Fin	20	−8.11 (0.72)	−8.06 (0.72)	7.04 (1.06)	7.25 (0.81)	2.67 (0.19)*	2.97 (0.81)*
***Styracura schmardae* Atlantic chupare stingray**								
25–120	Fin	18	−9.64 (1.3)	−9.24 (1.12)	4.88 (1.19)	4.95 (1.14)	2.81 (0.23)	2.85 (1.14)

mass spectrometer coupled to a 4010 Elemental Analyzer (Costech International) at the University of Waterloo Environmental Isotope Laboratory, Ontario, Canada. Machine analytical precision was ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and was determined by repeat analysis of duplicates (1 in 10). All resulting measurements were expressed using standard delta (δ) notation as parts per thousand differences (‰) with respect to the international reference standards, Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ (Craig 1957) and atmospheric nitrogen gas (N_2) for $\delta^{15}N$ (Mariotti 1983). Analytical accuracy was validated against internal laboratory standards cross-calibrated against the International Atomic Energy Agency standards CH_6 for carbon and N1 and N2 for nitrogen, and error for $\delta^{13}C$ and $\delta^{15}N$, respectively, during any given sample run did not exceed 0.2 and 0.3‰.

Statistical analyses

Statistical analyses were performed in Rstudio (version 1.0.136, R Development Core Team), and the level of statistical significance (α) was set at 0.05. Data were assessed for normality using Shapiro-Wilk tests. For each species, statistically significant differences in bulk and extracted mean $\delta^{13}C$, $\delta^{15}N$, and C:N ratio were assessed using Student's *t*-tests and Wilcoxon signed ranks tests.

For nurse sharks, we used the same pairwise comparisons to examine differences in $\delta^{13}C$, $\delta^{15}N$, and C:N ratio between muscle and dermis tissue. Using chemically extracted tissues, we explored the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of muscle and dermis tissue using least squares regression.

RESULTS

For nurse sharks and southern stingrays, no statistically significant differences were observed between mean $\delta^{13}C_{Bulk}$ and $\delta^{13}C_{Extracted}$ ($W = 234$, $p = 0.37$; $W = 199$, $p = 0.99$, respectively), and mean $\delta^{15}N_{Bulk}$ and $\delta^{15}N_{Extracted}$ ($W = 201$, $p = 0.99$; $t = -0.71$, $df = 35.48$, $p = 0.48$, respectively) (Table 1, Fig. 1). For the same species, statistically significant differences were observed between mean $C:N_{Bulk}$ and $C:N_{Extracted}$ ratios ($W = 40$, $p < 0.001$, $W = 23.5$, $p < 0.001$, respectively). For chupare stingrays, no statistically significant differences were observed between mean $\delta^{13}C_{Bulk}$ and $\delta^{13}C_{Extracted}$ ($t = -0.10$, $df = 33.28$, $p = 0.33$), $\delta^{15}N_{Bulk}$ and $\delta^{15}N_{Extracted}$ ($W = 167.5$, $p = 0.87$), or $C:N_{Bulk}$ and $C:N_{Extracted}$ ratios ($W = 114.5$, $p = 0.14$; Fig. 1).

For nurse sharks, a statistically significant difference between dermis and muscle mean $\delta^{15}N$ values was observed ($W = 208.5$, $p < 0.03$), but there were no significant differences observed for $\delta^{13}C$ ($W = 130$, $p = 0.63$) or C:N ratio ($W = 90.5$, $p = 0.06$). Least-squares regression indicated significant relationships between chemically extracted muscle and dermis tissue for both $\delta^{13}C$ ($F = 40.03$, $r^2 = 0.73$, $p < 0.05$) and $\delta^{15}N$ ($F = 12.05$, $r^2 = 0.45$, $p < 0.05$; Fig. 2).

DISCUSSION

This study provides insight into lipid and urea dynamics of nurse sharks, southern stingrays, and Atlantic chupare stingrays. For nurse shark muscle tissue, and for southern and Atlantic chupare ray fin tissues, we observed no significant differences in $\delta^{13}C$ or $\delta^{15}N$ between bulk and extracted treatments, suggesting that lipid and urea content in these tis-

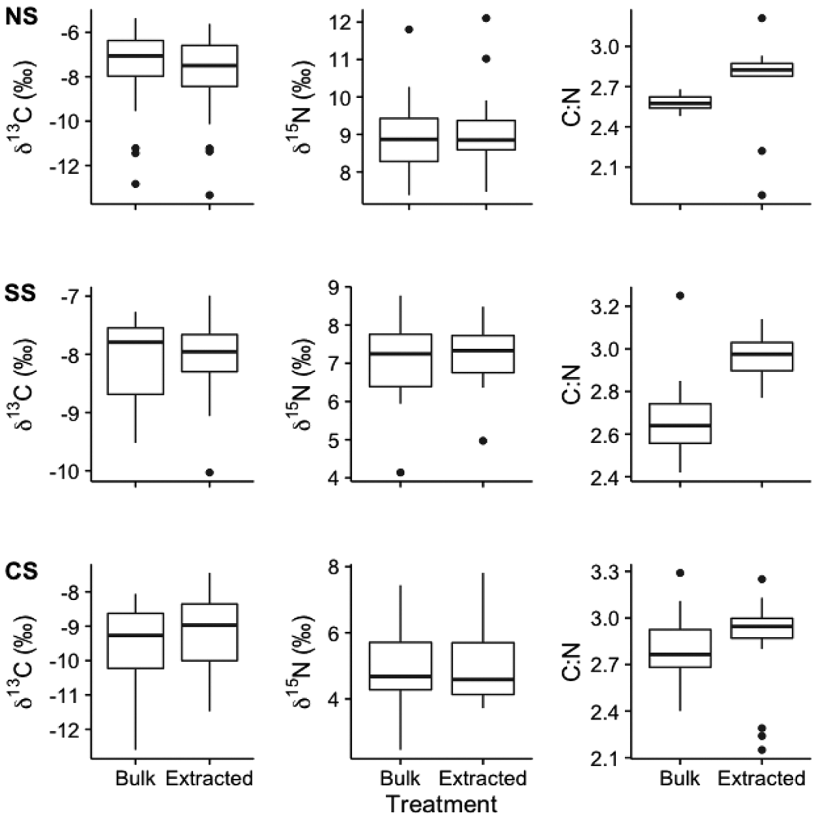

Fig. 1. Boxplots of bulk and extracted $\delta^{13}C$, $\delta^{15}N$, and C:N values of tissues gathered from 3 species of sub-tropical benthic elasmobranchs. NS: nurse shark *Ginglymostoma cirratum* (muscle); SS: southern stingray *Hypanus americanus* (fin); CS: Atlantic chupare stingray *Styracura schmardae* (fin). Mid-line: median; box limits: 1st and 3rd quartiles; whiskers: 1.5 times the interquartile range; points: outliers

sues is negligible. The lipid content of shark tissues is highly variable, and is dependent upon species and tissue metabolic function. Nevertheless, it is generally accepted that both muscle and fin tissue exhibit lower concentrations of lipids compared to more metabolically active tissues such as liver (Pethybridge et al. 2010). It is therefore unsurprising that lipids do not confound the ecological interpretation of bulk $\delta^{13}C$ data for nurse shark muscle tissue, and southern and chupare stingray fin tissue. It remains poorly documented whether the benthic life-history strategy of these species contributes to the low lipid content of the sampled tissues; however, significant shifts in $\delta^{13}C$ towards higher values following lipid extraction have been observed in coastal, pelagic (Li et al. 2016a, Carlisle et al. 2017), and deep-water species (Shipley et al. 2017b). This facet should be further explored in a wider range of species across a variety of habitat types to determine what effects life-history strategy may have on elasmobranch tissue lipid dynamics.

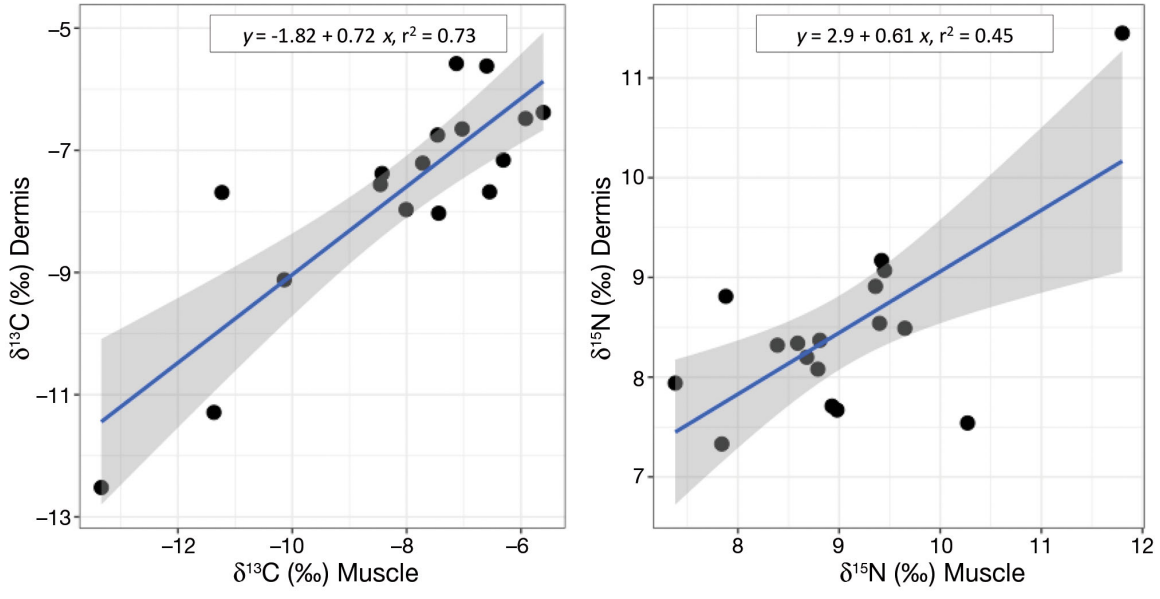

Fig. 2. Linear regressions for stable isotope values generated from chemically extracted dermis and muscle tissue for nurse sharks *Ginglymostoma cirratum* (n = 17). Grey shaded area represents standard error, blue line represents line of best fit

The lack of significant differences between bulk and extracted $\delta^{15}N$ values was unexpected, especially for nurse shark muscle tissue. Elasmobranchs exhibit elevated, ^{15}N-depleted urea concentrations which facilitate osmoconformation (Smith 1931, Goldstein et al. 1968). $\delta^{15}N$ values generally increase following chemical urea extraction, as the ^{15}N-depleted component of tissue is removed, and the effects of urea removal on $\delta^{15}N$ values across species and habitat types remain largely homogenous (Churchill et al. 2015, Li et al. 2016a, Carlisle et al. 2017, Shipley et al. 2017b). Here, all 3 species exhibited concentrations of urea low enough to have negligible effects on bulk $\delta^{15}N$. However, these conclusions are cautionary and cannot be reliably applied to other tissue types, or to other species without further study. For southern and chupare stingrays, observations may pertain to the metabolically inert function of fin tissue, which is comprised of multiple tissue types (e.g. skin, cartilage, connective tissue, muscle, etc.), and has little known physiological function (MacNeil et al. 2006, Hussey et al. 2012a,b). Statistically significant differences in C:N ratio were observed for nurse sharks and southern stingrays, which increased following chemical extraction (Table 1). Despite becoming more elevated following chemical extraction, changes to the percentage contribution of carbon relative to nitrogen did not significantly change $\delta^{13}C$ or $\delta^{15}N$ values in these species. These observations again suggest that small concentrations of lipid and urea may be present and are not significant enough to bias $\delta^{13}C$ or $\delta^{15}N$ values. For nurse sharks and southern stingrays, we were unable to explain the reason for a number of individuals exhibiting extremely low C:N values; however, this could be driven by a small fraction of urea remaining after chemical extraction. We therefore recommend that, at least for this location, chemical extraction of lipids and urea are not necessary for nurse shark white muscle, and southern and chupare stingray fin tissue.

The lack of statistically significant differences between $\delta^{13}C$ values of dermis and muscle tissue implies that dermis may serve as a reliable proxy for muscle (Li et al. 2016b). However, statistically significant differences were observed between muscle and dermis $\delta^{15}N$, and may have routes in variability in amino acid metabolism between these 2 tissues. Regardless of whether significant differences were observed in isotope values between the 2 tissue types, the significant linear relationships between isotope values of muscle and dermis provide novel correction factors. Mathematical corrections are of importance when comparing isotope values across species and tissue types, and could occur in cases where ethical sampling protocols dictate collection of specific tissue types for some species, but not for others. Although inter-tissue comparisons are lacking across the literature, such relationships have been observed in bull sharks *Carcharhinus leucas* (Matich et al. 2011), silky sharks *C. falciformis*, and blue sharks *Prionace glauca* (Li et al. 2016b) but cannot be broadly assumed to occur in other species without further study. Nurse sharks exhibit high site fidelity, with limited evidence to suggest broad-scale migrations (Chapman et al. 2005) or seasonal diet switches (Castro 2000). Although such behaviors cannot be discounted, as animals were sampled over a variety of seasons, the variable turnover rate of different tissues does not appear to significantly confound relationships in $\delta^{13}C$ and $\delta^{15}N$, that may be observed in highly migratory animals (Hussey et al. 2011) or in individuals that exhibit seasonal diet shifts (Madigan et al. 2015). Although statistically significant, these relationships deviate from a slope of 1, and it must be noted that the $\delta^{15}N$ relationship is lacking in elevated values (10.5–12‰). Applying inter-tissue corrections, especially for $\delta^{15}N$, therefore warrants some degree of caution; however, based on the muscle–dermis relationships observed for other elasmobranch species (Li et al. 2016b), we postulate that dermis tissue still serves as a viable proxy for muscle $\delta^{13}C$ and $\delta^{15}N$ values.

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LITERATURE CITED

Brooks EJ, Sims DW, Danylchuk AJ, Sloman KA (2013) Seasonal abundance, philopatry and demographic structure of Caribbean reef shark (*Carcharhinus perezi*) assemblages in the north-east Exuma Sound, The Bahamas. Mar Biol 160:2535–2546

Carlisle AB, Litvin SY, Madigan DJ, Lyons K, Bigman JS, Ibarra M, Bizzarro JJ (2017) Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes. Can J Fish Aquat Sci 74:419–428

Castro JI (2000) The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. Environ Biol Fish 58:1–22

Chapman DD, Pikitch EK, Babcock E, Shivji MS (2005) Marine reserve design and evaluation using automated acoustic telemetry: a case-study involving coral reef-associated sharks in the Mesoamerican Caribbean. Mar Technol Soc J 39:42–55

Churchill DA, Heithaus MR, Grubbs RD (2015) Effects of

lipid and urea extraction on $\delta^{15}N$ values of deep-sea sharks and hagfish: Can mathematical correction factors be generated? Deep Sea Res II 115:103–108

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Goldstein L, Oppelt WW, Maren TH (1968) Osmotic regulation and urea metabolism in the lemon shark *Negaprion brevirostris*. Am J Physiol 215:1493–1497

Hoffman JC, Sutton TT (2010) Lipid correction for carbon stable isotope analysis of deep-sea fishes. Deep Sea Res I 57:956–964

Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandoe P, Turnbull JF (2006) Current issues in fish welfare. J Fish Biol 68:332–372

Hussey NE, Chapman DD, Donnelly E, Abercrombie DL, Fisk AT (2011) Fin-icky samples: an assessment of shark fin as a source material for stable isotope analysis. Limnol Oceanogr Methods 9:524–532

Hussey NE, MacNeil MA, Olin JA, McMeans BC, Kinney MJ, Chapman DD, Fisk AT (2012a) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol 80:1449–1484

Hussey NE, Olin JA, Kinney MJ, McMeans BC, Fisk AT (2012b) Lipid extraction effects on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of elasmobranch muscle tissue. J Exp Mar Biol Ecol 434-435:7–15

Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fish 95:53–63

Kim SL, del Rio CM, Casper D, Koch PL (2012) Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exp Mar Biol Ecol 215:2495–2500

Li Y, Zhang Y, Hussey NE, Dai X (2016a) Urea and lipid extraction treatment effects on $\delta^{15}N$ and $\delta^{13}C$ values in pelagic sharks. Rapid Commun Mass Spectrom 30:1–8

Li Y, Hussey NE, Zhang Y (2016b) Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks. Aquat Biol 25:53–60

Logan JM, Lutcavage ME (2010) Stable isotope dynamics in elasmobranch fishes. Hydrobiologia 644:231–244

MacNeil MA, Drouillard KG, Fisk AT (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can J Fish Aquat Sci 63:345–353

Madigan DJ, Brooks EJ, Bond ME, Gelsleichter J and others (2015) Diet shift and site-fidelity of oceanic whitetip sharks *Carcharhinus longimanus* along the Great Bahama Bank. Mar Ecol Prog Ser 529:185–197

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685–687

Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80:294–305

O'Shea OR, Ward C, Brooks EJ (2017) Range extension in *Styracura* (= *Himantura*) *schmardae* (Caribbean whiptail stingray) from The Bahamas. Caribb Nat 38:1–8

Pethybridge H, Daley R, Virtue P, Nichols P (2010) Lipid composition and partitioning of deepwater chondrichthyans: inferences of feeding ecology and distribution. Mar Biol 157:1367–1384

Pinnegar JK, Polunin NVC (1999) Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Reum JCP (2011) Lipid correction model of carbon stable isotopes for a cosmopolitan predator, spiny dogfish *Squalus acanthias*. J Fish Biol 79:2060–2066

Shiffman DS, Gallagher AJ, Boyle MD, Hammerschlag-Peyer CM, Hammerschlag N (2012) Stable isotope analysis as a tool for elasmobranch conservation research: a primer for non-specialists. Mar Freshw Res 63:635–643

Shipley ON, Brooks EJ, Madigan DJ, Sweeting CJ, Grubbs RD (2017a) Stable isotope analysis in deep-sea chondrichthyans: recent challenges, ecological insights, and future directions. Rev Fish Biol Fish 27:481–497

Shipley ON, Olin JA, Polunin NV, Sweeting CJ and others (2017b) Polar compounds preclude mathematical lipid correction of carbon stable isotopes in deep-water sharks. J Exp Mar Biol Ecol 494:69–74

Smith HW (1931) The absorption and excretion of water and salts by the elasmobranch fishes. Am J Physiol 98:279–295

Sweeting CJ, Polunin NVC, Jennings S (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Commun Mass Spectrom 20:595–601

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