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**Polar compounds preclude mathematical lipid correction of carbon stable isotopes
in deep-water sharks**

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Abstract

Lipids affect isotope values in marine fishes, however such effects remain poorly described for many extant shark taxa, especially deep-sea species. Here we report the effects of lipid extraction (LE) on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N values of seven deep-sea sharks, generate novel mathematical normalizations for $\delta^{13}\text{C}$ based on the relationship between bulk and lipid extracted values ($\delta^{13}\text{C}_{\text{Bulk}}$ and $\delta^{13}\text{C}_{\text{LE}}$), and examine whether common normalized correction models provide a robust method for addressing lipid-biasing effects in two species, the Cuban dogfish (*Squalus cubensis*; $n = 20$), and Greenland shark (*Somniosus microcephalus*; $n = 24$). LE generally resulted in an isotopic enrichment of ^{13}C and ^{15}N , but produced variable effects on C:N across all species. Novel mathematical normalizations for $\delta^{13}\text{C}$ were derived from the pooled shark community, and a single species-specific correction was generated for the Cuban dogfish, but could not be determined for the Greenland shark. Four common lipid correction models used for teleosts, failed to accurately predict $\delta^{13}\text{C}$ values statistically similar to $\delta^{13}\text{C}_{\text{LE}}$, in both Cuban dogfish and Greenland sharks, likely due to the confounding effects of lipids and urea on C:N. These observations suggest that chemical lipid extraction should be a mandatory procedure prior to interpreting stable isotope data for deep-sea sharks, at least for those species where lipid effects are large.

Keywords: Stable isotope analysis, chondrichthyan, elasmobranch, deep-sea, The Bahamas, The Canadian Arctic

1. Introduction

Stable isotope analysis (SIA) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is a useful, low-cost approach to examine critical aspects of shark ecology (Hussey et al., 2012a; Shiffman et al., 2012). Thus far, SIA has been used to examine trophic interactions at inter- (e.g. Churchill et al., 2015a; Madigan et al., 2015) and intra-species scales (Estrada et al., 2006; Hussey et al., 2011), foraging breadth and energy flow (McCauley et al., 2012; Trueman et al., 2014), migration (Speed et al., 2012; Carlisle et al., 2012; Papastomatiou et al., 2015), and maternal provisioning (McMeans et al., 2009; Olin et al., 2011) in these taxa. Yet, accurate ecological interpretation of stable isotope data relies on confidence in a number of underpinning assumptions, for example assigning accurate trophic-step fractionation values (i.e. the relative isotopic enrichment of a predator relative to its prey), tissue turnover rates, and accounting for biasing effects of polar compounds (Logan and Lutcavage, 2010; Hussey et al., 2012a; b; Shipley et al., 2017).

The confounding effects of polar compounds, namely lipids and urea/*trimethylamine* N-oxide (TMAO, herein referred to as urea), on isotope values of carbon and nitrogen are relatively well described for sharks (Hussey et al., 2011; Li et al., 2016; Carlisle et al., 2016). Lipids are ^{13}C -depleted relative to proteins, and osmolytes that facilitate cellular osmoconformation (Laxson et al., 2010), such as urea are ^{15}N -depleted relative to proteins, such that higher lipid and urea concentrations result in artificially lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Hussey et al., 2012b; Churchill et al., 2015b; Li et al., 2016). The combined effects of lipids and urea, if not accounted for, have the ability to confound accurate ecological interpretation of isotope data in sharks, and may result in biased conclusions regarding wider community dynamics (Kim and Koch 2011; Hussey et al., 2012b; Li et al., 2016; Carlisle et al., 2016).

Given these confounding factors, researchers must choose a suitable approach to offset the biasing effects of lipids, for which three commonly published approaches are

preferred for sharks: 1. assume a negligible lipid content based on a low C:N ratio (< 3.4), and present bulk data (Pethybridge et al., 2012); 2. perform chemical extraction, which may additionally remove urea (Hussey et al., 2012b; Burgess and Bennett, 2017) or 3. mathematically correct for lipids using models generated for teleost fishes (Reum et al., 2011). Mathematically predicting ‘lipid-free’ $\delta^{13}\text{C}$ relies largely on the C:N of bulk tissue, in addition to the application of pre-determined lipid offsets (i.e. the isotopic depletion of lipid relative to protein, Sweeting et al., 2006), which vary between models e.g. Sweeting et al. (2006) (ca. -7.0%), or McConnaughey and McRoy (1978) (ca. -6.0%). As such, their application to normalize shark isotope data is unreliable, due to urea-effects on C:N_{Bulk} (Carlisle et al., 2016). Thus, the difficulty in generating broadly applicable mathematical normalizations for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in sharks has resulted in a consensus to chemically extract polar compounds prior to SIA (Kim and Koch, 2011; Li et al., 2016; Carlisle et al., 2016). Despite this argument, deep-sea species are largely unrepresented across the current literature base, highlighting a need to examine polar compound dynamics in these data-poor taxa.

In recent years, isotopic approaches have been applied to deep-water shark communities (Pethybridge et al., 2012; Churchill et al., 2015a; Shipley et al., 2017), to help drive novel management and conservation approaches. However, little consideration has been applied to the most appropriate techniques to account for lipid effects on $\delta^{13}\text{C}$ values in these taxa. Pethybridge et al. (2010) observed variable lipid content of deep-sea shark tissues, however those commonly run for SIA, e.g. white muscle, was similarly low and comparable to pelagic and coastal species. However, as such analysis remains limited to a small number of species and locations, a need exists to further understand the biasing effects of lipids on deep-water shark tissue, and to provide a methodological proxy on which to base standardized sample preparation for future isotope studies. This is required as lipid-biasing

effects in deep-sea taxa may be unique, as their physiologies and life-history characteristics diverge from that of coastal and pelagic species (Cotton and Grubbs, 2015).

Here we address the issue of whether commonly used mathematical correction models for $\delta^{13}\text{C}$ are appropriate for standardizing isotope bulk-tissue data for a number of data-poor deep-water sharks. First we quantify the changes (Δ) in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N following lipid extraction for this group. We then examine whether normalized correction models provide a robust method for addressing lipid-biasing effects, and generate novel mathematical normalizations based on the relationship between $\delta^{13}\text{C}_{\text{Bulk}}$ and $\delta^{13}\text{C}_{\text{LE}}$. This is critical given previous recommendations that have been made over the requirement of lipid and urea removal prior to SIA (Hussey et al., 2012b; Li et al., 2016; Carlisle et al., 2016) but there is a lack of observation for this recommendation and a renewed call to address this issue in data-poor deep-water species.

2. Materials and methods

2.1 Tissue sampling and stable isotope analysis

Six species of shark: the Cuban dogfish (*Squalus cubensis*), gulper shark (*Centrophorus* spp.), big-eye sixgill shark (*Hexanchus nakamurai*), dusky smoothhound (*Mustelus canis-insularis*), sharpnose sevengill shark (*Hexanchus perlo*), and blotched catshark (*Syliorhinus meadi*) were sampled from The Exuma Sound, The Bahamas from 2013 – 2014 (Table 1). Capture methods followed Brooks et al. (2015), and white muscle tissue was excised from the dorsal region, anterior to the first dorsal fin. Muscle samples were stored on ice before being frozen ($-20\text{ }^{\circ}\text{C}$) on return to the laboratory. Greenland sharks (*Somniosus microcephalus*) were sampled from Maxwell Bay, Lancaster Sound, The Canadian Arctic, in 2011 (Table 1). Individuals were caught using deep-water ($\sim 200\text{ m}$) bottom-set long-lines (length: $\sim 120\text{ m}$), with 50 hooks baited with beluga meat and set for

~24 h. Sharks that were cannibalized on the line were sampled. White muscle tissue was excised immediately anterior to the first dorsal fin where possible or from posterior to the cranium or anterior to the caudal fin for severely cannibalized animals. Samples were immediately stored frozen (-20°C). Muscle tissues from all sharks were freeze-dried for >72 hours, homogenized and separated into paired bulk and lipid-extracted treatments (referred to as LE herein). Lipid extraction was undertaken using a 2:1 chloroform:methanol approach following Sweeting et al. (2006).

For individuals captured in The Bahamas, stable isotope analysis of carbon and nitrogen was performed using a Sercon INTEGRA2 mass spectrometer (Sercon Ltd, Cheshire, UK) at the University of Exeter, Penryn Campus (UK). All samples were run in duplicate, and internal analytical precision (standard error) was determined by running two alanine standards ($n = 94$, < 0.02) every 8 samples. Analytical precision between consecutive runs was determined using an in-house laboratory standard of blue antimora (*Antimora rostrata*), which were placed at the beginning and end of each run ($n = 14$, < 0.05). For Greenland sharks stable carbon and nitrogen isotope ratios were provided from a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA) at the Chemical Tracers Laboratory - Great Lakes Institute for Environmental Research, University of Windsor, Canada. Precision, assessed by the standard deviation of replicate analyses of four standards (NIST1577c, internal lab standard (tilapia muscle), USGS 40 and urea ($n = 13$ for all), measured $\leq 0.17\text{‰}$ for $\delta^{15}\text{N}$ and $\leq 0.16\text{‰}$ for $\delta^{13}\text{C}$ for all standards. The accuracy, based on the certified values of USGS 40 ($n = 13$ for $\delta^{13}\text{C}$) and urea IVA33802174 ($n = 13$ for $\delta^{15}\text{N}$) analyzed throughout runs and not used to normalize samples showed a difference of -0.16‰ for $\delta^{15}\text{N}$ and -0.08‰ for $\delta^{13}\text{C}$ from the certified value. Instrumentation accuracy checked throughout the period of time that these samples were analyzed was based on NIST

standards 8573 and 8574 for $\delta^{15}\text{N}$ and 8542, 8573, 8574 for $\delta^{13}\text{C}$ ($n=18$ for all). The mean difference from the certified values was -0.14 , and 0.04‰ for $\delta^{15}\text{N}$ and -0.05 , -0.03 and -0.05‰ for $\delta^{13}\text{C}$ respectively.

2.2 Statistical Analysis

To assess the effects of LE on isotope values, differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N between LE and bulk tissue were determined using students t-tests or Wilcoxon signed ranks test based on Shapiro-Wilks normality ($\alpha = 0.05$). For Cuban dogfish (*Squalus cubensis*; $n = 20$) and Greenland sharks (*Somniosus microcephalus*; $n = 24$) least squares linear regression was used to assess whether $\Delta\delta^{13}\text{C}$ could be predicted based on the C:N of bulk tissue; this would provide further evidence of whether lipid or urea effects were more responsible for changes in C:N in these species. Bulk and LE data are presented for five additional deep-water species to show overall isotopic trends between treatments in support of the above two species, but were not rigorously tested due to small sample sizes ($n \geq 6$). Although the sample size for *Centrophorus* spp. met the criteria for analysis, they were excluded as on-going taxonomic revision precluded positive species ID during sampling (see White et al., 2013; Verissimo et al., 2014; Brooks et al., 2015).

Mathematical normalization of $\delta^{13}\text{C}$ was established for species with a sample size >20 through a linear function based on LE and bulk $\delta^{13}\text{C}$ following Li et al. (2016):

$$\delta^{13}\text{C}_{\text{LE}} = a_1 \times \delta^{13}\text{C} + b_1 \quad \text{equation 1}$$

Where a_1 is the slope and b_1 is the intercept.

To test the performance of mathematical lipid correction models on $\delta^{13}\text{C}$, we considered four common mathematical correction approaches. The first correction requires the calculation of the relative proportion of in-tissue lipid content (equation 1), which is then integrated into the final correction (equation 2) following McConnaughey and McRoy

174 (1979):

$$175 \quad L = 93/1 + (0.246 \times (C:N_{\text{Bulk}}) - 0.775^{-1}) \quad \text{equation 2}$$

176

$$177 \quad \delta^{13}\text{C}_{\text{Corrected}} = \delta^{13}\text{C} + D \times (I + (3.90/1 + 287/L)) \quad \text{equation 3}$$

178

179 Where L is the proportion of lipid within a tissue, $\delta^{13}\text{C}_{\text{Corrected}}$ is the corrected value, D is the
180 proportional differences between protein and lipid, and I is a literature derived constant of (-
181 0.207).

182 The second correction followed that of Alexander et al. (1996):

183

$$184 \quad \delta^{13}\text{C}_{\text{Corrected}} = \delta^{13}\text{C} + D \times L / 100 \quad \text{equation 4}$$

185

186 Where D is the assumed lipid-offset of 6‰, and L is calculated from equation 1 (de Lecea
187 and Charmoy 2015).

188 Due to its simplicity, the third correction was a linear function described by Post et al.
189 (2007), and follows:

190

$$191 \quad \delta^{13}\text{C}_{\text{Corrected}} = \delta^{13}\text{C} - 3.32 + 0.99 \times C:N_{\text{Bulk}} \quad \text{equation 5}$$

192

193 Finally, the fourth correction, and most exclusive to deep-water taxa, followed Hoffman
194 and Sutton (2010):

195

$$196 \quad \delta^{13}\text{C}_{\text{Corrected}} = \delta^{13}\text{C}_{\text{Bulk}} + (-6.39\% \times (3.76 - C:N_{\text{Bulk}}))/C:N_{\text{Bulk}} \quad \text{equation 6}$$

197

Generated values were then compared to values of chemically extracted tissues using students t-tests or Wilcoxon signed ranks tests, as outlined above, with the expectation that the correction with the best fit would be most similar to LE results.

3. Results

LE and mathematical lipid correction showed enrichment of both ^{13}C and ^{15}N in all seven deep-sea shark species from six families (Centrophoridae, Hexanchidae, Triakidae, Squalidae, Scyliorhinidae and Somniosidae; Table 1), however, the direction and magnitude of changes in C:N appeared species-specific (Figure 1). Generally, LE resulted in an isotopic enrichment of both ^{13}C and ^{15}N for all species, however, the direction and magnitude of changes to C:N appeared species-specific (Figure 1). All species had C:N ratios consistently < 3.4 for both bulk and LE tissue, with the exception of the Greenland shark, which had the highest C:N_{Bulk} values (Table 1). Sample sizes for two species, Cuban dogfish and the Greenland shark allowed for statistical comparisons between LE and bulk tissue. For the Cuban dogfish, LE resulted in small, but non-significant increases in $\delta^{13}\text{C}$ (0.3‰), $\delta^{15}\text{N}$ (0.1‰), and C:N (0.1) compared to bulk tissue (Table 1). For Greenland sharks all pair wise tests were significant, with marked increases in both $\delta^{13}\text{C}$ (4.8‰) and $\delta^{15}\text{N}$ (0.8‰), and a large decrease in C:N (8.0‰) compared to bulk tissue (Table 2). C:N_{Bulk} only correlated significantly with $\Delta\delta^{13}\text{C}$ for Greenland sharks, and not for Cuban dogfish (Figure 2).

Least squares regression highlighted strong positive relationships between $\delta^{13}\text{C}_{\text{LE}}$ and $\delta^{13}\text{C}_{\text{Bulk}}$ for the whole community, and for Cuban dogfish (Figure 3a), which allowed the generation of normalized mathematical corrections ($p < 0.05$, Table 2). Non-significant relationships, and a low R^2 value for Greenland sharks (0.31, $p > 0.05$) prevented the generation of a robust mathematical normalization for this species, however we still report outputs for comparative purposes (Table 2).

None of the four mathematical corrections predicted statistically similar $\delta^{13}\text{C}_{\text{Corrected}}$ and $\delta^{13}\text{C}_{\text{LE}}$ values for Cuban dogfish and Greenland sharks (Table 3). However, for Greenland sharks, a visual inspection of model outputs (Figure 3) highlighted that the Hoffman and Sutton (2010) model produced $\delta^{13}\text{C}_{\text{Corrected}}$ values closest to that of $\delta^{13}\text{C}_{\text{LE}}$; but results were still significantly different.

4. Discussion

We corroborate confounding effects of lipids on the interpretation of $\delta^{13}\text{C}$ in data-poor deep-water sharks, and present additional effects, and implications of LE on $\delta^{15}\text{N}$ and C:N. Further, we highlight that four commonly used lipid correction models fail to accurately predict lipid-free $\delta^{13}\text{C}$, based on C:N_{Bulk}, providing further argument in favour of LE prior to interpretation of $\delta^{13}\text{C}$ data in deep-water sharks.

Lipid extraction resulted in variable increases in $\delta^{13}\text{C}$ for all species, consistent with findings in other elasmobranch species (Hussey et al., 2012b; Li et al., 2016; Carlisle et al., 2016). The greatest increases in $\delta^{13}\text{C}$ values were observed in Greenland sharks ($4.8 \pm 0.5\%$) suggesting high in-tissue lipid content (Hussey et al., 2012b). For the remaining taxa, $\delta^{13}\text{C}$ increases were markedly low, which supports previous observations of low in-tissue lipid content of white muscle in deep-water sharks (Pethybridge et al., 2010). Positive shifts in $\delta^{15}\text{N}$ were observed for all species after LE, but were greatest in the Blotched Catshark (*Scyliorhinus meadi*; 1.2‰). A positive shift in $\delta^{15}\text{N}$ was also significant for Greenland sharks. These observations further highlight that lipid extraction can remove urea, which is known to deplete $\delta^{15}\text{N}$ values in shark tissue (Hussey et al., 2012a; Lie et al., 2016); however, contention exists over whether extraction with 2:1 chloroform:methanol is sufficient in removing all soluble urea (Hussey et al., 2012b; Churchill et al., 2015b). Therefore, we recommend following the protocols of Kim and Koch (2011), Li et al. (2016) and Carlisle et

al. (2016) and perform an additional deionized-water rinse to remove any remaining urea from shark muscle tissue.

C:N_{Bulk} was low (< 3.4) for all species, and variable changes (both positive and negative shifts) were observed following LE. The low C:N_{Bulk} of deep-water shark tissue suggests that conventional assumptions still being used to determine the necessity of lipid removal (C:N < 3.4 , Pethybridge et al., 2012; Burgess et al., 2016) could be valid, however the increases in $\delta^{15}\text{N}$ and C:N_{LE} with lipid removal observed here and in other studies contradict this point and negates this argument. For these data, adopting a C:N of 3.4 as the ‘cut-off’ point for lipid removal, would have resulted in considerable misrepresentation of $\delta^{13}\text{C}$ ($2.1 \pm 2.2\text{‰}$), and also similar implications for $\delta^{15}\text{N}$ ($0.6 \pm 0.6\text{‰}$). Therefore we strongly advise against the presentation of bulk isotopic data for sharks, even if C:N_{Bulk} is below ‘lipid-free’ considerations (3.5 – 3.0), unless additional lipid and urea data are also presented for a subset of samples, and the effects of lipid and urea are shown to be negligible.

Species-specific corrections are becoming increasingly applied to sharks (e.g. Churchill et al., 2015b; Li et al., 2016) in accordance with the growing literature-base exploring the interactive effects of lipids and urea on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Reum et al., 2011; Hussey et al., 2012b; Churchill et al., 2015b; Li et al., 2016; Carlisle et al., 2016). Here, two linear normalizations were determined from the $\delta^{13}\text{C}_{\text{Bulk}}$ and $\delta^{13}\text{C}_{\text{LE}}$ relationship; 1) a community-wide normalization, and 2) a species-specific normalization for Cuban dogfish. In addition a $\Delta\delta^{13}\text{C}$ vs. C:N_{Bulk} relationship was established for the Greenland shark. This suggests the ability of C:N_{Bulk} to predict both $\delta^{13}\text{C}_{\text{LE}}$, and $\Delta\delta^{13}\text{C}$ is not effective for all species, and the normalized prediction of $\delta^{13}\text{C}$ using simplistic linear functions vary in their effectiveness depending upon the predictor. We therefore conclude it is unlikely that a single cross-species correction can be confidently established (Churchill et al., 2015b), suggesting

the application of chemical extraction still provides the most logical solution to obtain accurate $\delta^{13}\text{C}$ data.

The lipid correction models of McConnaughey and McRoy (1979), Alexander et al. (1996), Post et al. (2007) and Hoffman and Sutton (2010) all failed to accurately predict 'lipid-free' $\delta^{13}\text{C}$ values that were statistically similar to $\delta^{13}\text{C}_{\text{LE}}$. As models are reliant upon C:N_{Bulk} as a predictor for the contribution of lipid relative to pure protein, the interactive depletion of both urea and lipid on C:N can explain the poor performance of lipid correction models. The effectiveness of these models in predicting $\delta^{13}\text{C}_{\text{LE}}$ after removing urea (e.g. Trueman et al., 2014), however, remains undetermined and warrants further study.

We thus provide strong evidence against the use of common lipid correction models reliant upon C:N_{Bulk} to predict $\delta^{13}\text{C}_{\text{LE}}$ in deep-water shark tissues. Although prior removal of urea may improve model performance and is recommended, the variability of in-tissue lipid content between taxa strongly suggests that chemical extraction provides the most reliable method to offset polar compound effects.

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Figure 1. Differences (Δ) between bulk and LE values for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N from

Figure 2. Relationship between $\Delta\delta^{13}\text{C}$ and C:N_{BULK} for Cuban dogfish and Greenland sharks.

Figure 3. Linear regression comparing $\delta^{13}\text{C}_{\text{BULK}}$ and $\delta^{13}\text{C}_{\text{LE}}$ for Cuban dogfish, Greenland shark and the whole deep-water shark community. Note different scales on each graph.

Figure 4. Relationship between mathematical correction/LE and bulk $\delta^{13}\text{C}$ for Cuban dogfish and Greenland sharks (chemical lipid extraction = black points, mathematical correction = light grey squares (Post et al., 2007), dark grey squares (McConnaughey and McRoy, 1979), black squares (Alexander et al., 1996) and white squares (Hoffman and Sutton, 2010).

429 **Table 1.** Summary of the size range and the mean ($\pm$ S.D.) for bulk and LE $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N values for the deep-water sharks ($n \geq 3$) collected
 430 from The Bahamas and Canadian Arctic.

Common Name	Latin name	<i>n</i>	Size Range (TL, cm)	$\delta^{13}\text{C}_{\text{bulk}}$	$\delta^{13}\text{C}_{\text{LE}}$	$\delta^{15}\text{N}_{\text{bulk}}$	$\delta^{15}\text{N}_{\text{LE}}$	C:N _{bulk}	C:N _{LE}
Cuban dogfish	<i>Squalus cubensis</i>	20	39 - 76.8	-14.4 (0.8)	-14.0 (0.7)	9.9 (1.0)	10.0 (0.8)	2.74 (0.2)	2.8 (0.1)
Greenland shark	<i>Somniosus microcephalus</i>	24	-	-22.5 (0.5)	-17.7 (0.5)	17.1 (0.7)	17.9 (0.7)	11.3 (3.0)	3.4 (0.1)
Gulper shark	<i>Centrophorus</i> spp.	9	70 - 103.5	-16.7 (0.5)	-16.1 (0.2)	10.0 (0.3)	10.6 (0.2)	2.8 (0.6)	2.8 (0.1)
Big-eye sixgill shark	<i>Hexanchus nakamurai</i>	4	120 - 151	-14.4 (0.9)	-13.8 (0.2)	10.7 (0.8)	11.2 (0.1)	2.7 (0.2)	2.9 (0.1)
Dusky smoothhound	<i>Mustelus canis- insularis</i>	3	78 - 94	-12.2 (0.8)	-11.5 (0.8)	8.5 (0.5)	9.3 (0.2)	2.8 (0.3)	2.8 (0.1)
Sharpnose sevengill shark	<i>Heptranchias perlo</i>	2	63 - 87	-14.6; -18.6	-16.1; -16.9	9.1; 11.9	12.4; 13.2	3.0; 3.0	2.9; 2.8
Blotched catshark	<i>Scyliorhinus meadi</i>	1	49.5	-14.5	-14.1	6.8	8.0	2.2	2.76

Table 2. Mathematically normalized corrections generated from linear models ($\delta^{13}\text{C}_{\text{LE}}$ vs. $\delta^{13}\text{C}_{\text{Bulk}}$) for deep-water sharks (*significant relationship between x, y).

Species	<i>n</i>	Normalized Correction	R^2	<i>p</i> -value
Cuban dogfish	20	$\delta^{13}\text{C}_{\text{Corrected}} = 0.727 \times \delta^{13}\text{C}_{\text{Bulk}} - 3.595$	0.76	0.02*
Greenland shark	24	$\delta^{13}\text{C}_{\text{Corrected}} = 0.501 \times \delta^{13}\text{C}_{\text{Bulk}} - 6.398$	0.31	0.07
Whole community	63	$\delta^{13}\text{C}_{\text{Corrected}} = 0.468 \times \delta^{13}\text{C}_{\text{Bulk}} - 7.339$	0.88	< 0.001*

Table 3. Results of pairwise tests comparing LE vs bulk $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N and LE vs. corrected $\delta^{13}\text{C}$ for Cuban dogfish and Greenland sharks (*tests proved significant).

Corrections follow: Model 1 = McConnaghey and McRoy (1979), Model 2 = Alexander et al. (1996); Model 3 = Post et al. (2007); Model 4 = Hoffman and Sutton (2010).

Species	Variables (x, y)	Test-statistic	P-value
Cuban dogfish	$\delta^{13}\text{C}_{\text{Bulk}}, \delta^{13}\text{C}_{\text{LE}}$	$t = 1.34$	0.19
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 1}}$	$t = -24.50$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 2}}$	$t = -26.74$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 3}}$	$t = 4.01$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 4}}$	$t = -11.47$	< 0.001*
	$\delta^{15}\text{N}_{\text{Bulk}}, \delta^{15}\text{N}_{\text{LE}}$	$t = 0.49$	0.63
	$\text{C:N}_{\text{Bulk}}, \text{C:N}_{\text{LE}}$	$t = 1.47$	0.15
Greenland Shark	$\delta^{13}\text{C}_{\text{Bulk}}, \delta^{13}\text{C}_{\text{Extracted}}$	$w = 576$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 1}}$	$t = -12.45$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 2}}$	$t = -12.42$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 3}}$	$t = -5.84$	< 0.001*
	$\delta^{13}\text{C}_{\text{LE}}, \delta^{13}\text{C}_{\text{Model 4}}$	$w = 96$	< 0.001*
	$\delta^{15}\text{N}_{\text{Bulk}}, \delta^{15}\text{N}_{\text{LE}}$	$t = 3.88$	< 0.001*
	$\text{C:N}_{\text{Bulk}}, \text{C:N}_{\text{LE}}$	$t = -13.08$	< 0.001*