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Complete List of Authors:	Carlisle, Aaron; Hopkins Marine Station of Stanford University, Litvin, Steven; Stanford University Madigan, Daniel; Harvard University, Center for the Environment Lyons, Kady; University of Calgary Bigman, Jennifer; Simon Fraser University Ibarra, Melissa; University of California Davis Bizzarro, Joseph; Moss Landing Marine Laboratories
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Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes

Aaron B. Carlisle[1*], Steven Y. Litvin[1], Daniel J. Madigan[2], Kady Lyons[3], Jennifer S. Bigman[4], Melissa Ibarra[5] & Joseph J. Bizzarro[6]

[1] Hopkins Marine Station of Stanford University, 120 Oceanview Blvd, Pacific Grove, CA, USA 93950

[2] Harvard University Center for the Environment, 24 Oxford Street, Cambridge, MA, USA 02138

[3] University of Calgary, 2500 University Dr NW, Calgary, Alberta, Canada T2N 4N1

[4] Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

[5] University of California Davis, One Shields Avenue, Davis, CA, USA 95616

[6] Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039

[*]Corresponding author

Aaron B. Carlisle – aaroncar@stanford.edu
Steven Y. Litvin – litvin@stanford.edu
Daniel J. Madigan – danieljmadigan@fas.harvard.edu
Kady Lyons – kady.lyons@sbcglobal.net
Jennifer S. Bigman – jbigman@sfu.ca
Melissa Ibarra – mibarra08@yahoo.com
Joseph J. Bizzarro – jbizzarro@mlml.calstate.edu

Abstract

Stable isotope analysis (SIA) is becoming a commonly used tool to study the ecology of elasmobranchs. However, the retention of urea by elasmobranchs for osmoregulatory purposes may bias the analysis and interpretation of SIA data. We examined the effects of removing urea and lipid on the stable isotope composition of fourteen species of sharks, skates, and rays from the eastern North Pacific Ocean. While effects were variable across taxa, removal of urea generally increased $\delta^{15}N$ and C:N. Urea removal had less influence on $\delta^{13}C$, whereas extracting urea and lipid generally increased $\delta^{15}N$ and C:N while also increasing $\delta^{13}C$. Because C:N values of non-extracted tissues are often used to infer lipid content and adjust $\delta^{13}C$, shifts in C:N following urea extraction will change the inferred lipid content and bias any mathematical adjustment of $\delta^{13}C$. These results highlight the importance of urea and lipid extraction and demonstrate the confounding effects of these compounds, making it impossible to use C:N of non-urea-extracted samples as a diagnostic tool to estimate and correct for lipid content in elasmobranch tissues.

Keywords: Stable isotopes, urea, lipid, carbon, nitrogen, C:N, elasmobranch, mathematical lipid correction, elasmobranch

Introduction

Stable isotope analysis (SIA) uses the stable isotope composition of organismal tissue to understand a diverse suite of biological and ecological processes. SIA is increasingly being used to investigate the ecology of marine taxa (Peterson and Fry 1987, Michener and Kaufman 2007), including sharks, skates, and rays (elasmobranchs) (Hussey et al. 2012b). Since SIA makes inferences based on the chemical composition of tissues, certain compounds found in specific taxa can interfere with analysis and, therefore, conclusions. Here, we investigate the effects of urea and lipid extraction on tissues from fourteen elasmobranch species and report results that demonstrate the necessity to account for these compounds when using SIA in elasmobranch studies.

The physiology and anatomy of elasmobranchs present unique challenges when applying SIA to study their ecology. In particular, elasmobranchs retain urea ($(NH_2)_2CO$) and trimethylamine oxide (TMAO (C_3H_9NO)) in their tissues for osmoregulatory processes (Ballantyne 1997, Olson 1999, Hazon et al. 2003). This retention of urea can differentially bias stable isotope results depending upon the tissue type examined (Hazon et al. 2008, Kim and Koch 2011, Hussey et al. 2012b, Churchill et al. 2015). As a waste product, urea is expected to have low $\delta^{15}N$ values (Minagawa and Wada 1984, Balter et al. 2006) because ^{14}N is preferentially concentrated in urea by deaminases and transaminases (Gannes et al. 1998). We were unable to find any comparable data on TMAO, but as a waste product it also would be expected to be depleted in ^{15}N. As a result, the relative concentrations of urea and TMAO in a tissue may influence the $\delta^{15}N$ value of that tissue. As urea and TMAO (hereafter referred to together as urea) both contain carbon, they could potentially affect $\delta^{13}C$. Kim and Koch (2011) reported that the carbon in urea is enriched in $\delta^{13}C$ in some terrestrial taxa; however information on the isotopic composition of these waste products, especially in aquatic taxa, remains lacking. Further complicating the effect of urea on SIA is its varying concentration within organisms, which is influenced by a variety of factors including tissue type (Ballantyne 1997), ambient salinity (Hazon et al. 2003, Pillans et al. 2005) and diet (Wood et al. 2010). Information on how to address the effects of urea on SIA results is needed, both in terms of appropriate

sample treatment methodology and data interpretation (Martinez del Rio et al. 2009, Logan and Lutcavage 2010, Kim and Koch 2011, Hussey et al. 2012b, Li et al. 2016).

In addition to the potential effect of urea on the stable isotope composition of elasmobranchs, the presence of lipids is known to influence the $\delta^{13}C$ values of tissues (Post et al. 2007, Martinez del Rio et al. 2009, Hussey et al. 2012a). Because lipids are depleted in ^{13}C relative to protein, the presence of lipid in tissues can bias $\delta^{13}C$ values and increase the tissue carbon-to-nitrogen ratio (C:N) (Pinnegar and Polunin 1999, Post et al. 2007). Tissue samples with high lipid concentrations have lower $\delta^{13}C$ values than samples of the same tissue with lipids removed (Post et al. 2007). To account for variation in lipids across tissue types, researchers either chemically extract or mathematically correct for lipids based on the tissue C:N, which has been used as a proxy for relative lipid content in tissues (Post et al. 2007).

The influence of lipid content on SIA data of elasmobranch tissues has been relatively well studied (Kim and Koch 2011, Hussey et al. 2012a) compared to that of urea (Hussey et al. 2012b). Logan and Lutcavage (2010) and Kim and Koch (2011) directly assess the effects of urea extraction on SIA data of elasmobranchs. Logan and Lutcavage (2010) reported no effect of urea extraction on elasmobranch tissues, whereas Kim and Koch (2011) reported a significant increase in $\delta^{15}N$ in urea-extracted tissues. However, treatment methods differed between studies, with Kim and Koch (2011) using a more extensive deionized water (DIW) extraction, which potentially resulted in more complete urea removal. Given that lipid has a high C:N and urea has low C:N (0.5), removal of these compounds will influence tissue C:N. Several studies examining the effect of lipid extraction on elasmobranch tissue noted increases in $\delta^{15}N$ and C:N following lipid extraction in a manner consistent with the removal of urea, suggesting that lipid extraction may effectively remove urea as well as lipid (Hussey et al. 2010, Kim and Koch 2011, Hussey et al. 2012a, Churchill et al. 2015, Li et al. 2016). However Kim and Koch (2011) reported that elasmobranch tissues should have both urea and lipid-extracted to obtain the most reliable results. Li et al. (2016) recently conducted the most thorough study of the interactive effects of urea and lipid to-date, examining the effects of urea and lipid extraction on six species of pelagic sharks. They reported significant increases in $\delta^{15}N$ and C:N following lipid extraction, urea extraction, and lipid and urea

extraction, with $\delta^{13}C$ also increasing significantly in treatments with lipid extraction. Li et al. (2016) supported the conclusion of Kim and Koch (2011) that both urea and lipid should be removed when analyzing elasmobranch tissues for SIA.

Despite the reported shifts in C:N following urea and lipid extraction and recommendations to make lipid extraction a standard practice when processing elasmobranch tissues for SIA, estimating lipid content based on the C:N of unextracted bulk samples remains a common practice. Specifically, it is typically assumed that tissues of aquatic organisms with C:N values < 3.3 – 3.5 have low lipid content and do not warrant lipid extraction (Post et al. 2007). This is of potential concern since this does not account for the influence of urea on C:N, which is then used to mathematically adjust $\delta^{13}C$ to account for inferred (based on C:N) lipid content. As a result, the $\delta^{15}N$ and potentially $\delta^{13}C$ values of samples processed without urea and/or lipid extraction may be biased, with any resulting analyses or ecological interpretations being potentially based on inaccurate $\delta^{15}N$ and $\delta^{13}C$ values. Given the common use of $\delta^{15}N$ to estimate trophic level and $\delta^{15}N$ and $\delta^{13}C$ to understand habitat use and trophic relationships in elasmobranchs (Fisk et al. 2002, Estrada et al. 2003, Dale et al. 2011, Kim and Koch 2011, Vaudo and Heithaus 2011, Carlisle et al. 2012, Hussey et al. 2012b), these biases may have important effects on the ecological interpretation of SIA data.

To examine how common this issue may be, we surveyed 50 recent scientific publications (2013-present) that used SIA to study the ecology of elasmobranchs (Google scholar; search terms: “stable isotope”, “elasmobranch”, “shark”, “ray”; selected the first 50 pertinent results, Table S1). We found 28% used low C:N values (< 3.5) of tissues containing urea to support not extracting lipid from their samples, another 16% used mathematic corrections to adjust $\delta^{13}C$ based on lipid content estimates inferred from C:N values of tissues containing urea, and 12% did not account for urea or lipid in any manner. Thus, 56% of the surveyed studies potentially had results biased due to not accounting for the combined effects of urea and lipids. While it is not possible to know if the lack of urea or lipid extraction had any meaningful effect on the isotopic results or their interpretation in these studies, it is clear previous

recommendations to make urea and lipid extraction the standard practice when analyzing elasmobranch tissues for SIA (Kim & Koch 2011, Hussey et al. 2012a, Li et al. 2016) have not been fully adopted.

In this study, we expand upon previous work to better understand and account for the interactive effects of urea and lipids on SIA in elasmobranch tissues. The importance of urea and lipid extraction has been demonstrated in leopard sharks (*Triakis semifasciata*, Kim and Koch (2011) and a suite of pelagic sharks (Li et al. 2016), yet published studies commonly do not appropriately account for the potential effects of urea and lipid on elasmobranch tissues. In addition, the effects of applying multiple chemical treatments to remove urea and lipid, and how to determine an appropriate methodological course for individual species or taxa of interest, remain unaddressed for many elasmobranch taxa across broad ranges of tissue compositions and habitats. In particular, while the interactive effects of urea and lipid have been explored in sharks, they have not been investigated in batoids (skates and rays), a group that comprises over 50% of extant elasmobranchs (Dulvy et al. 2014). Finally, it may not always be feasible or desirable to perform lipid extraction (i.e. avoid the cost, chemical waste generated, and time associated with chemical extraction or to preserve information on the movement of lipids through foodwebs). While Li et al. (2016) provides species specific isotopic correction models to account for urea content in lipid extracted samples, there is a lack of specific guidance in the literature on the appropriate development and application of mathematical correction models for $\delta^{13}C$ based on inferred tissue lipid content (C:N) for elasmobranchs that account for urea's effects on C:N, $\delta^{13}C$ and $\delta^{15}N$.

The goals of this study were to 1) assess the relative effects of urea and lipid extraction on the stable isotope composition of muscle tissue from a variety of shark and batoid species, including pelagic, demersal and benthic species, with variable lipid content, 2) address the utility of using C:N as a diagnostic tool to understand and adjust for lipid content in elasmobranch tissue, particularly in the context of the influence of urea on C:N, 3) develop models to mathematically adjust $\delta^{13}C$ of urea-extracted samples to account for lipid content and 4) provide a conceptual framework to understand how urea and lipid interact to influence SIA results in elasmobranchs in order to facilitate proper application of the technique.

Materials and Methods

To examine the effects of urea extraction and lipid extraction on elasmobranch tissues, we collected white muscle samples from fourteen species, including six species of sharks from the families Lamnidae, Carcharhinidae, Squalidae, and Triakidae, and eight species of batoids (skates, rays, and their allies) from the families Arhynchobatidae, Rajidae, Myliobatidae, Gynmnuridae, Dasyatidae, and Urolophidae. All samples were collected in the eastern North Pacific, primarily off California but ranging as far north as the Gulf of Alaska. Samples were collected from juvenile salmon sharks (*Lamna ditropis*) stranded on beaches in California and Oregon as described by Carlisle et al. (2015). Juvenile white sharks (*Carcharodon carcharias*) caught as incidental bycatch in the coastal gillnet fisheries in southern California were sampled as part of the Monterey Bay Aquarium juvenile white shark research program as described in Mull et al. (2012). Samples from shortfin makos (*Isurus oxyrinchus)*, blue sharks (*Prionace glauca*), and pelagic rays (*Pteroplatytrygon violacea*) were collected during the annual National Oceanic and Atmospheric Administration (NOAA) Juvenile Shark Longline Survey off southern California. Round stingray samples (*Urobatis halleri*), leopard shark (*Triakis semifasciata*), butterfly ray (*Gymnura marmorata*), and bat rays (*Myliobatis californica*) were collected in southern California as described by Lyons et al. (2014). Skates (*Bathyraja aleutica, Bathyraja interrupta, Beringraja binoculata, Raja rhina*) were collected from the western Gulf of Alaska as described in Bizzarro et al. (2014). Spotted spiny dogfish (*Squalus suckleyi*) were collected off central California during the National Marine Fisheries Service Northwest Fisheries Science Center West Coast Groundfish Bottom Trawl Survey. All muscle samples were collected from the dorsal musculature and stored frozen (-20°C) until processed and analyzed.

Individual tissue samples were homogenized and subdivided into three parts for analysis, with one as the control sample (Control), one for urea extraction (U), and one for both urea and lipid extraction (UL). Methods to process tissues and extract lipids and urea generally followed Kim and Koch (2011). To extract urea, tissue samples were placed in scintillation vials and rinsed three times in 10 mL of DIW (Kim and Koch 2011). A rinse entailed sonication for 15 minutes and then decanting the supernatant.

Lipids were extracted from all tissues except skate tissues using a 2:1 chloroform:methanol solution (Bligh and Dyer 1959, Logan and Lutcavage 2010) by immersing tissues in the solution for 24 hours in glass scintillation vials (Bligh and Dyer 1959, Logan and Lutcavage 2010). Following both treatments, tissue samples were lyophilized and homogenized using a Spex/CertiPrep 5100 mill.

Skate samples were processed slightly differently than the other species as part of another study. For urea extraction of skate tissue, 10 mL of DIW were added to each homogenized sample, and then the samples were mixed using a vortex mixer (Fisher Scientific). After 30 minutes, the sample was centrifuged and the supernatant was decanted. For samples that were lipid and urea-extracted, lipids were extracted using Petroleum Ether (PE) following Kim and Koch (2011). Briefly, samples were immersed in PE, mixed in a vortex mixer and left uncapped in a fume hood for 8 hours, centrifuged for 10 minutes, and the supernatant decanted. The sample was then rinsed in DIW using the method described for urea extraction of skate tissue. Following urea or urea and lipid extraction samples were dried in an oven at 60˚C for 24 hours.

For each treatment, approximately 500 µg of tissue was weighed into tin boats and analyzed at the Stable Isotope Laboratory at the University of California Santa Cruz (UCSC) using an elemental analyzer coupled to an isotope ratio monitoring mass spectrometer (Delta XP-EA,Thermo- Finnagen IRMS). For skate and dogfish samples, 500 µg of tissue was weighed into tin boats and analyzed at Idaho State University (ISU) using an elemental analyzer coupled to an isotope ratio monitoring mass spectrometer (Elemental Combustion System (ECS) 4010 interfaced with a Delta V Advantage mass spectrometer through the ConFlo IV System). Isotopic composition is expressed using standard δ notation, using Vienna Pee Dee Belemnite limestone as the standard for carbon and AIR for nitrogen. For runs at UCSC, analytical precision, based on an internal lab standard (Pugel), was 0.11‰ for $\delta^{15}N$ and 0.07‰ for $\delta^{13}C$ across multiple runs. For runs at ISU, analytical precision, based on internal lab standards of ISU Peptone, Costech Acetanilide, and DORM-3, was 0.08, 0.04, and 0.04 ‰ for $\delta^{15}N$ respectively and 0.05, 0.05, and 0.04 for $\delta^{13}C$, respectively. Where parametric assumptions were met (assessed with One-Sample Kolmogorov-Smirnov and Levene's Tests and visual inspection of residuals) a single factor

ANOVA, followed by Tukey's post hoc tests, was used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ among treatments for species with sample sizes > 3. When assumptions were not met for $\delta^{13}C$, $\delta^{15}N$, or C:N differences were tested using Mann-Whitney 2-sample tests with sequential Bonferroni adjustments (Rice 1989). To show the magnitude and direction of the effects of treatments U and UL, differences between treatment and control samples (U – Control, UL – Control, UL - U) were calculated.

We considered four previously used lipid correction models (Post et al. 2007, Logan et al. 2008, Reum 2011) to examine the utility of using C:N as a diagnostic tool to understand and adjust for lipid content in urea-extracted elasmobranch tissue. Lipid correction models were used to characterize $\Delta^{13}C$, the difference between lipid and urea (UL) and urea-extracted (U) $\delta^{13}C$ values ($\Delta^{13}C = \delta^{13}C_U - \delta^{13}C_{UL}$) as a function of the C:N of urea-extracted tissue ($C:N_U$). The first (model 1) is a three-parameter model derived by Logan et al. (2008) from McConnaughey and McRoy (1979): $\Delta^{13}C = (aC:N_U + b)(C:N_U + c)^{-1}$, where a, the y-asymptote, corresponds to protein-lipid $\delta^{13}C$ discrimination and $-ba^{-1}$, the x-intercept, is the urea and lipid free C:N value ($C:N_{UL}$), and bc^{-1}, the y-intercept, is the value of $\Delta^{13}C$ at $C:N_U = 0$. The second (model 2) is a two parameter model (Fry 2002): $\Delta^{13}C = P - PF(C:N_U)^{-1}$, where P represents protein-lipid $\delta^{13}C$ discrimination and F is $C:N_{UL}$. The third and fourth are linear models: (model 3, Logan et al. 2008) $\Delta^{13}C = \beta_0 + \beta_1 Ln(C:N_U)$ and (model 4, Post et al. 2007) $\Delta^{13}C = b + aC:N_U$, where $e^{(-\beta_0\beta_1^{-1})}$ and $-ba^{-1}$ are estimates of $C:N_{UL}$, respectfully.

We modeled the relationship between $C:N_U$ and $\Delta^{13}C$ for five groups: all species, batoids, all sharks other than *S. suckleyi*, *S. suckleyi,* and *C. carcharias.* We modeled *S. suckleyi* independently since its lipid content was higher than all other taxa and its urea-extracted samples had the widest range of C:N values (Results). For *C. carcharias,* we wanted to attempt to develop a species-specific relationship and this species had the largest sample size. To compare the performance of potential lipid correction models, the corrected Akaike Information Criterion, AIC_c (Burnham and Anderson 2002), was calculated for each model. The model with the lowest AIC_c is considered the best fit, but any model(s) with AIC_c values within two units of the lowest value have strong support as well (Burnham and Anderson 2002). In

addition for those models that preformed best based on AIC_c, we calculated the mean and standard deviation of the absolute values of the residuals errors, to further evaluate model fit, and compared estimates of protein-lipid $\delta^{13}C$ discrimination and $C:N_{UL}$ (Logan et al. 2008, Reum 2011). All models were fitted with least-squares procedures using R and the libraries nlme and AICcmodavg (www.r-project.org).

Results

The removal of urea and lipid from elasmobranch tissue influenced $\delta^{15}N$, $\delta^{13}C$, and C:N in most species, although the direction and magnitude of the effects varied by species and with the lipid content of the sample (Table 1, Figures 1-2, Figure S1). The C:N of control samples was consistently low for all species, with 13 of the 14 taxa having values < 3.5 (mean ± SD of all species 3.0 ± 0.4). The only exception to this was *S. suckleyi*, which had a high C:N of 4.5 due to higher lipid content of its muscle.

Urea extraction (treatment U) generally increased $\delta^{15}N$ and C:N, but generally did not significantly change $\delta^{13}C$, results that are consistent with the removal of isotopically light nitrogen present in urea (Table 1, Figures 2a and 3a). In seven of the ten species (4 of 5 sharks, 3 of 5 batoids) that were statistically tested, $\delta^{15}N$ increased significantly following urea extraction (mean 0.8‰ ± 0.2). This result was very similar to the overall trend across all taxa, which showed an average increase of 0.7‰ ± 0.2. Three taxa had increases in $\delta^{15}N$ greater than 1‰ (*B. aleutica* 1.1‰, *L. ditropis* 1.1‰ and *I. oxyrinchus* 1.0 ‰). $\delta^{13}C$ only changed significantly in *B. binoculata* (-0.9‰) and *U. halleri* (-0.5‰). Overall, there was a consistent, though generally non-significant, decrease in $\delta^{13}C$ across the batoids that was not evident in sharks (mean -0.4‰ for batoids, 0.0‰ for sharks, and -0.2‰ ± 0.3 for all taxa). C:N increased significantly in nine of the ten taxa statistically tested (4 of 5 sharks, 5 of 5 batoids), with the exception being *S. suckleyi*, which had a high initial C:N that increased from 4.5 to 5.6 (+ 1.1‰) following urea extraction. Overall, C:N increased by an average of 0.7 (± 0.2) across all taxa following urea extraction. C:N values increased to values above 3 (mean C:N of 3.6) in all species, and the C:N of *C. carcharias, L. ditropis, G. marmorata, and U. halleri* increased to values above 3.5.

Extracting urea and lipid (treatment UL) from samples consistently increased $\delta^{15}N$ and C:N in a fashion similar to what was observed with urea extraction only, while also generally increasing $\delta^{13}C$ (Table 1, Figures 2b and 3b). Seven out of the ten taxa tested had significantly higher $\delta^{15}N$ values following urea and lipid extraction, although all taxa showed some increase (mean 0.8‰ ± 0.2). *L. ditropis* showed the largest increase in $\delta^{15}N$ following lipid extraction (1.1‰). Four of the five statistically tested sharks had significantly higher $\delta^{13}C$ following urea and lipid extraction, with dogfish (mean 2.0‰), salmon sharks (1.5‰) and white sharks (1.1‰) having the largest increases. Three batoids showed a decrease in $\delta^{13}C$ following urea and lipid extraction, with *B. binoculata* exhibiting a significant decrease (mean -0.9‰). Except for *S. suckleyi*, all taxa exhibited an increase in C:N (mean increase 0.3 ± 0.3, mean of species C:N 3.3), with nine of ten taxa tested statistically having significant changes. C:N of *S. suckleyi* decreased, though non-significantly, following urea and lipid extraction (control C:N 4:5, UL C:N 3.9).

The differences between the U and UL treatments were more obvious in sharks than in the batoids, in which the differences were relatively small (Table 1). All taxa showed an increase in $\delta^{13}C$ in the UL treatment relative to the U treatment, although only three of the five sharks (*C. carcharias, L. ditropis* and *S. suckleyi*), and one of five batoids (*U. halleri*), had significant increases. There were no significant differences in $\delta^{15}N$ between U and UL treatments in any species examined ($p > 0.05$), indicating that lipid extraction did not affect $\delta^{15}N$. C:N was generally lower in the UL treatment relative to the U treatment, especially in the sharks. In the five shark species tested, all had significant decreases in C:N in UL treatments relative to U treatments, whereas only two of the five tested batoids had significant decreases.

Lipid correction models were created to adjust urea-extracted tissue $\delta^{13}C$ to account for lipid content. Model performance varied across elasmobranch groups (Figure 3, Figure S2), with no single model amongst those exhibiting the lowest AIC_c values across all groups (see supplementary Table S2 for AIC_c Values, r^2 (linear models only) and model parameters). For all species pooled, models 1 and 2 (non-linear models) had the lowest AIC_c, with identical mean ± SD of the absolute values of the residual errors

(MRE, 0.29 ± 0.24) and similar estimates of protein-lipid $\delta^{13}C$ discrimination (5.39 and 6.12) and $C:N_{UL}$ (3.20 and 3.18). All four models for sharks (excluding *S. suckleyi*) had similarly low AIC_c values (i.e. within 2 units), MREs (0.20 – 0.25 ± 0.25 – 0.29) and estimates of $C:N_{UL}$ (3.10 – 3.13). However, estimates of protein-lipid $\delta^{13}C$ discrimination for sharks varied widely between models 1 (1.94) and 2 (7.86, Table S2). The two linear models (3 and 4) performed equally for batoids (Table S2), with identical MRE (0.21 ± 0.16) and estimates of $C:N_{UL}$ (3.27). Model performance varied between the two single species groups. For *S. suckleyi*, which had both the widest range and highest C:N values, model 2 provided the singular best fit (MRE 0.26 ± 0.24) with estimates of protein-lipid $\delta^{13}C$ discrimination and $C:N_{UL}$ of 6.31 and 3.35, respectively (Table S2) . In contrast, for *C. carcharias*, which had only two urea-extracted C:N values > 4, models 2, 3 and 4 had similarly low AIC_c, MREs (0.12 – 0.13 ± 0.12 – 0.13) and estimates of $C:N_{UL}$ (3.05 – 3.14). The estimate of urea extracted protein-lipid $\delta^{13}C$ discrimination for *C. carcharias* was the highest in the study (8.30, Table S2).

Discussion

Our results provide further evidence of the substantial, direct affect that urea can have on $\delta^{15}N$. We also show the important, and often unconsidered, indirect role urea plays in influencing $\delta^{13}C$ values by lowering the C:N, effectively masking lipid content and leading to inaccurate assessments of lipid content and inappropriate mathematical corrections (Figure 4). Our results indicate that urea must be removed to obtain reliable $\delta^{15}N$ and C:N values, and that only with urea-extracted tissues can C:N be used as a diagnostic tool for understanding and mathematically adjusting for lipid content.

Urea extraction resulted in an increase in $\delta^{15}N$ across all taxa, and a significant increase in 7 of 10 statistically tested taxa, ranging from ~0.5 to 1.1‰ (mean 0.7 or 0.8‰ for U and UL respectively), with urea and lipid extraction producing similar changes. When using $\delta^{15}N$ to infer trophic level, this shift is equivalent to an inferred trophic level difference of ~22 – 50% or ~15 – 30% assuming a trophic discrimination factor for nitrogen of 2.3‰ (Hussey et al. 2010) or 3.7‰ (Kim et al. 2012), respectively. This shift is similar to that reported by Hussey et al. (2012a) for elasmobranch tissues following lipid extraction as well as by Li et al. (2016) following both urea extraction and urea and lipid extraction.

Clearly the presence of urea in analyzed tissues will directly bias the use of $\delta^{15}N$ as a tracer in ecological studies, whether it is being used to assess trophic level, reconstruct diet, habitat or migration patterns, or even for simple qualitative comparisons.

The urea effects we describe here also have important implications for the use of C:N as a metric of lipid content of elasmobranch tissue. The C:N of non-extracted elasmobranch tissues are consistently very low (< 3) across studies (Logan and Lutcavage 2010, Matich et al. 2010, Dale et al. 2011, Kim and Koch 2011, Vaudo and Heithaus 2011, Hussey et al. 2012a, Hussey et al. 2012b), and are often much lower than would be expected of pure protein. Frequently, when tissues with C:N values < 3.5 are assumed to have little lipid content (Post et al. 2007), the low C:N values are used to infer that lipid extraction is not warranted. However, results from this study and previous work (Kim and Koch 2011, Hussey et al. 2012a, Li et al. 2016) demonstrate that urea extraction generally increases the C:N as nitrogen is removed (Figures 1 and 2). In this study, extracting urea through DIW rinses caused significant increases in C:N in every species tested, increasing it by as much as 1.2‰ (mean 0.7‰).

Removal of the nitrogen contributed by urea will increase the C:N value of a sample, thereby changing the estimated lipid content that are based on C:N (Figure 4). Following removal of urea, C:N can increase from very low values to values above threshold levels that are used to indicate low lipid content (e.g. 3.5). In effect, the presence of urea and its lowering of C:N has the potential to mask lipid content. In four of the species examined in this study (*C. carcharias, L. ditropis, G. marmorata* and *U. halleri*) the C:N value shifted from values < 3 to values > 3.5, and other species had C:N values of ~3.3 following DIW rinses, which is similar to pure protein values (Post et al. 2007). The interpretation of these urea-extracted samples would then be that lipid extraction is warranted, and in two of these species (*C. carcharias, L. ditropis)*, there was a significant increase in $\delta^{13}C$ following lipid extraction. Importantly, despite having C:N values ~3.3 following urea extraction, most of the species exhibited an increase in $\delta^{13}C$ following lipid extraction. These findings suggest that lipid extraction can significantly affect $\delta^{13}C$ even when C:N is < 3.5. Hence, lipid extraction may be required even in a tissue that is relatively lean, a result that is concordant with the findings of Li et al. (2016). Our results indicate that

failure to lipid extract elasmobranch tissues based on low C:N values of untreated tissue, where urea has not been removed, will result in biased $\delta^{15}N$ values, due to the inclusion of the isotopically light nitrogen of urea, and potentially biased $\delta^{13}C$ values as well, due to inclusion of lipid content that was masked by low C:N values.

An additional important, and generally unrecognized implication of the effect of urea on C:N is that it will bias C:N based arithmetic corrections that are used to adjust $\delta^{13}C$ in lieu of lipid extracting tissues. Any adjustment to $\delta^{13}C$ values that is based on C:N values from non-urea-extracted tissue will be biased, although the magnitude of the effect will vary based on the urea and lipid content of the tissue. Thus, failing to extract urea will not only bias $\delta^{15}N$, but by affecting C:N it will lead to incorrect estimates of inferred lipid content upon which mathematical correction models rely. As our results indicate, however, it is possible to develop models to adjust urea-extracted tissues to account for lipid content when lipid extraction is not feasible. For example, the model we developed for *C. carcharias* demonstrates that even with a relatively small sample size (n = 19), we were able to generate a robust species-specific simple linear model based on urea-extracted tissue C:N (model 4, $r^2 = 0.92$, with no systematic prediction biases based on visual inspection of residuals). Deriving taxa-specific relationships is always desirable, but for sharks (excluding dogfish) all four models examined seem to provide potentially suitable lipid correction models. However, examination of model parameters reveals that for model 1 the estimation of protein-lipid $\delta^{13}C$ discrimination is unrealistically low, ~3 times, or more, lower than other estimates from this study and the generally reported range of 5–8‰ (Fry 2002, Post et al. 2007, Logan et al. 2008, Reum 2011), demonstrating the need to consider other factors beyond AIC_c and fit (r^2 and residual distribution) when determining the suitability of a correction model. Sharks (excluding dogfish) have C:N of urea-extracted tissues below ~4.5 and there appears to be a linear relationship between C:N and $\Delta^{13}C$. This suggests that linear correction models might be most appropriate for sharks with relatively low C:N values (~4.5), which is concordant with the findings of Post et al. (2007) for aquatic organisms over a similar C:N range. This relationship is likely non-linear when tissues span a wide range of C:N values, such as with *S. suckleyi* (Logan et al. 2008, Reum 2011). It is therefore

important to consider the range in lipid content in species and tissues of interest when developing and applying lipid correction models. Batoids had a less clear C:N relationship relative to other taxa in the study. While exhibiting a significant linear relationship between $C:N_u$ and $\Delta^{13}C$, the models explained a relatively low proportion of the variability in $\Delta^{13}C$ (models 3 and 4, $r^2 = 0.56$ and 0.58) and may not provide the same relative correction across all batoids (Figure 3). This emphasizes that the interaction between urea and lipid content may change across disparate elasmobranch taxonomic groups.

Although our results indicate that urea directly influences $\delta^{15}N$ values and potentially indirectly influences the $\delta^{13}C$ of elasmobranch tissues by affecting the C:N and inferred lipid content, the effects are variable and species-dependent (Figures 1, 2). For species that have low lipid content in their muscle, such as batoids (e.g. ~0.2% *in U. halleri*, (Lyons unpublished data), ~1-2% in *B. binoculata* and *R. rhina* (Farrugia et al. 2015)), the effect on $\delta^{13}C$ will be minimal, but $\delta^{15}N$ may change substantially. In species with higher lipid content, such as *L. ditropis*, which can have lipid content as high as 6.5 to 14.6% (mean 9%) in their muscle (data source: https://dec.alaska.gov/eh/vet/fish.htm), the effect on $\delta^{13}C$ and $\delta^{15}N$ will be significant. The relatively low lipid content of batoid muscle compared to shark muscle may underlie the observed general decrease in $\delta^{13}C$ of batoids following urea extraction while urea and lipid extraction showed less overall change in $\delta^{13}C$ relative to the control samples. As described by Kim and Koch (2011), the carbon in urea is enriched in ^{13}C relative to the diet in humans (enriched 3 – 5‰) and cattle (0 – 3.5‰), suggesting that its removal may reduce the $\delta^{13}C$ (Ivlev et al. 1996, Knobbe et al. 2006). In batoids with low lipid content, removal of the ^{13}C enriched urea would reduce $\delta^{13}C$, while lipid extraction, by removing a small amount of ^{13}C depleted lipids, would offset the removal of urea and result in little net change in $\delta^{13}C$. This effect would vary based on both the concentration of urea as well as lipid content of the tissue and again highlights the importance of understanding how these relationships change across taxa. It is possible that other differences in the composition of batoid tissue may play a role as well, such as differences in urea concentration or the presence of ceratotrichia, but this remains unclear.

Although lipid extraction by itself may remove lipids and much of the urea present in tissues (Hussey et al. 2012a, Churchill et al. 2015), we reiterate the recommendations of Kim and Koch (2011)

and Li et al. (2016) to make lipid and urea extraction the standard practice when analyzing elasmobranch tissues for SIA. Since extracting urea is simple and inexpensive, there is no practical reason not to remove it. In addition, our results indicate that $\delta^{13}C$ may change significantly following lipid extraction even in apparently lean tissues with relatively low C:N, suggesting that lipid extraction may be warranted in all situations as suggested by Li et al. (2016). However, in some taxa that are very lean, such as the batoids in this study, lipid extraction may not be required. However, urea would still need to be extracted to evaluate the need for lipid extraction or correction. In instances where it is not feasible or desirable to lipid extract every sample, we demonstrate that it is possible to develop species- or group-specific correction curves to adjust for lipid content in urea-extracted tissues. Though lipid extraction did not affect $\delta^{15}N$ in our study, a potential benefit of using mathematical correction models is the ability to account for the effect of lipid content on $\delta^{13}C$ while avoiding potential effects of chemical extraction on $\delta^{15}N$, which have been reported previously in other taxa (Post et al. 2007). However, our results show that the confounding effects of urea and lipids make it impossible to use C:N of non-urea-extracted samples as a diagnostic tool to determine the proper method of tissue treatment, something that occurs regularly in the literature.

The changes in the stable isotope composition of elasmobranch tissue resulting from urea and lipid extraction will be mediated by the relative concentration of those compounds (Figure 4), which vary across taxa, and their differential effects on $\delta^{13}C$ and $\delta^{15}N$ (Figures 1 & 2). We conclude that at a minimum, urea should be removed to evaluate lipid content and a species or group specific lipid correction relationship created to account for lipid content. The most robust approach to most confidently eliminate bias and to facilitate comparisons across studies will be to apply urea and lipid removal techniques in SIA-based ecological studies of elasmobranchs.

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Literature cited

Ballantyne, J.S. 1997. Jaws: the inside story. The metabolism of elasmobranch fishes. Comp Biochem Physiol B Biochem Mol Biol **118**(4): 703-742.

Balter, V., Simon, L., Fouillet, H., and Lécuyer, C. 2006. Box-modeling of $^{15}N/^{14}N$ in mammals. Oecologia **147**(2): 212-222.

Bizzarro, J.J., Broms, K.M., Logsdon, M.G., Ebert, D.A., Yoklavich, M.M., Kuhnz, L.A., and Summers, A.P. 2014. Spatial segregation in eastern north Pacific skate assemblages.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology **37**: 911-917.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media.

Carlisle, A.B., Kim, S.L., Semmens, B.X., Madigan, D.J., Jorgensen, S.J., Perle, C.R., Anderson, S.D., Chapple, T.K., Kanive, P.E., and Block, B.A. 2012. Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). PLoS ONE **7**(2): e30492.

Carlisle, A.B., Litvin, S.Y., Hazen, E.L., Madigan, D.J., Goldman, K.J., Lea, R.N., and Block, B.A. 2015. Reconstructing habitat use by juvenile salmon sharks links upwelling to strandings in the California Current. Mar Ecol Prog Ser **525**: 217-228.

Churchill, D.A., Heithaus, M.R., and Grubbs, R.D. 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 Research Part II: Topical Studies in Oceanography **115**: 103-108.

Dale, J.J., Wallsgrove, N.J., Popp, B.N., and Holland, K.N. 2011. Nursery habitat use and foraging ecology of the brown stingray Dasyatis lata determined from stomach contents, bulk and amino acid stable isotopes. Mar Ecol Prog Ser **433**: 221-236.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N.K., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J.V., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S., and White, W.T. 2014. Extinction risk and conservation of the world's sharks and rays. eLIFE **3**: e00590.

Estrada, J.A., Rice, A.N., Lutcavage, M.E., and Skomall, G.B. 2003. Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. J Mar Biol Assoc U K **83**(6): 1347-1350.

Farrugia, T.J., Oliveira, A.C., Knue, J.F., and Seitz, A.C. 2015. Nutritional content, mercury, and trace element analyses of two skate (Rajidae) species in the Gulf of Alaska. Journal of Food Composition and Analysis **42**: 152-163.

Fisk, A.T., Tittlemier, S.A., Pranschke, J.L., and Norstrom, R.J. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. Ecology **83**(8): 2162-2172.

Fry, B. 2002. Stable isotopic indicators of habitat use by Mississippi River fish. J North Am Benthol Soc **21**(4): 676-685.

Gannes, L.Z., Martinez Del Rio, C., and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology **119A**: 725-737.

462 Hazon, N., Wells, A., Anderson, G., Good, J., Pillans, R., and Franklin, C. 2008. Osmoregulation in
463 elasmobranch fish-A review. Comparative Biochemistry and Physiology A-Molecular, pp. S87-
464 S87.
465 Hazon, N., Wells, A., Pillans, R.D., Good, J.P., Anderson, W.G., and Franklin, C.E. 2003. Urea based
466 osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity.
467 Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology **136**(4): 685-
468 700.
469 Hussey, H.E., Olin, J.A., Kinney, M.J., McMeans, B.C., and Fisk, A.T. 2012a. Lipid extraction effects on
470 stable isotope values (δ13C and δ15N) of elasmobranch muscle tissue. J Exp Mar Biol Ecol **434-**
471 **435**: 7-15.
472 Hussey, N.E., Brush, J., McCarthy, I.D., and Fisk, A.T. 2010. $\delta^{15}N$ and $\delta^{13}C$ diet-tissue discrimination
473 factors for large sharks under semi-controlled conditions. Comp. Biochem. Physiol. A-Mol.
474 Integr. Physiol. **155**(4): 445-453.
475 Hussey, N.E., MacNeil, M.A., Olin, J.A., McMeans, B.C., Kinney, M.J., Chapman, D.D., and Fisk, A.T.
476 2012b. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J
477 Fish Biol: 1449-1484.
478 Ivlev, A., Knyazev, Y.A., and Logachev, M. 1996. Daily average carbon isotope composition of CO2 of
479 expired air and urine urea in norm and some endocrine pathologies in man. Biofizika **41**(2): 508-
480 516.
481 Kim, S.L., Casper, D.R., Galván-Magaña, F., Ochoa-Díaz, R., Hernández-Aguilar, S.B., and Koch, P.L.
482 2012. Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-
483 term controlled feeding study. Environ Biol Fishes **95**(1): 37-52.
484 Kim, S.L., and Koch, P.L. 2011. Methods to collect, preserve, and prepare elasmobranch tissues for stable
485 isotope analysis. Environ Biol Fishes **95**(1): 53-63.
486 Knobbe, N., Vogl, J., Pritzkow, W., Panne, U., Fry, H., Lochotzke, H., and Preiss-Weigert, A. 2006. C
487 and N stable isotope variation in urine and milk of cattle depending on the diet. Analytical and
488 bioanalytical chemistry **386**(1): 104-108.
489 Li, Y., Zhang, Y., Hussey, N.E., and Dai, X. 2016. Urea and lipid extraction treatment effects on δ15N
490 and δ13C values in pelagic sharks. Rapid Communications in Mass Spectrometry **30**(1): 1-8.
491 Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. 2008. Lipid
492 corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and
493 modelling methods. J Anim Ecol **77**(4): 838-846.
494 Logan, J.M., and Lutcavage, M. 2010. Stable isotope dynamics in elasmobranch fishes. Hydrobiologia
495 **644**: 231-244.
496 Lyons, K., Lavado, R., Schlenk, D., and Lowe, C. 2014. Bioaccumulation of organochlorine contaminants
497 and EROD activity in southern California round stingrays (*Urobatis halleri*) exposed to planar
498 aromatic compounds. Environ Toxicol Chem **33**(6): 1380-1390.
499 Martinez del Rio, C., Wolf, N., Carleton, S.A., and Gannes, L.Z. 2009. Isotopic ecology ten years after a
500 call for more laboratory experiments. Biol Rev **84**: 91-111.
501 Matich, P., Heithaus, M.R., and Layman, C.A. 2010. Size-based variation in intertissue comparisons of
502 stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger
503 sharks (*Galeocerdo cuvier*). Can J Fish Aquat Sci **67**: 877-885.
504 McConnaughey, T., and McRoy, C. 1979. Food-web structure and the fractionation of carbon isotopes in
505 the Bering Sea. Mar Biol **53**(3): 257-262.
506 Michener, R.H., and Kaufman, L. 2007. Stable isotope ratios as tracers in marine food webs: an update. *In*
507 Stable isotopes in ecology and environmental science. *Edited by* R. Michener and K. Lajtha.
508 Blackwell, Boston. pp. 238 - 282.
509 Minagawa, M., and Wada, E. 1984. Stepwise enrichment of 15 N along food chains: further evidence and
510 the relation between δ 15 N and animal age. Geochim Cosmochim Acta **48**(5): 1135-1140.
511 Mull, C.G., Blasius, M.E., O'Sullivan, J.B., and Lowe, C.G. 2012. Heavy metals, trace elements, and
512 organochlorine contaminants in muscle and liver tissue of juvenile White Sharks, Carcharodon

carcharias, from the Southern California Bight. Global perspectives on the biology and life history of the White Shark. CRC Press, Boca Raton, Florida: 59-75.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu Rev Ecol Syst **18**: 293-320.

Pillans, R.D., Good, J.P., Anderson, W.G., Hazon, N., and Franklin, C.E. 2005. Freshwater to seawater acclimation of juvenile bull sharks (*Carcharhinus leucas*): plasma osmolytes and Na+/K+-ATPase activity in gill, rectal gland, kidney and intestine. Journal of Comparative Physiology B **175**(1): 37-44.

Pinnegar, P.K., and Polunin, N.V.C. 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, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montana, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia **152**(1): 179-189.

Reum, J.C.P. 2011. Lipid correction model of carbon stable isotopes for a cosmopolitan predator, spiny dogfish *Squalus acanthias*. J Fish Biol **79**(7): 2060-2066.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution **43**: 223-225.

Vaudo, J.J., and Heithaus, M.R. 2011. Dietary niche overlap in a nearshore elasmobranch mesopredator community. Mar Ecol Prog Ser **425**: 247-260.

Wood, C.M., Walsh, P.J., Kajimura, M., McClelland, G.B., and Chew, S.F. 2010. The influence of feeding and fasting on plasma metabolites in the dogfish shark (*Squalus acanthias*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **155**(4): 435-444.

560 **Tables**
561

SHARKS

Species	ID	Treatment	$\delta^{13}C$ mean	sd	C test	U test	$\delta^{15}N$ mean	sd	C test	U test	C:N mean	sd	C test	U test	n	TL (cm) mean (SD)
Carcharodon carcharias	CC	C	-16.8	0.9	-	-	16.8	0.6	-	-	2.8	0.2	-	-	19	161 (21)
		U	-16.6	0.8	ns	-	17.3	0.7	*	-	3.6	0.3	*	-		
		UL	-15.6	0.5	*	*	17.3	0.7	*	ns	3.2	0.0	*	*		
Isurus oxyrinchus	IO	C	-18.0	0.2	-	-	16.4	0.2	-	-	2.8	0.1	-	-	8	147 (22)
		U	-17.8	0.7	ns	-	17.4	0.3	*	-	3.3	0.1	*	-		
		UL	-17.6	0.2	ns	ns	17.4	0.3	*	ns	3.2	0.1	*	*		
Lamna ditropis	LD	C	-19.4	0.4	-	-	14.4	0.3	-	-	2.9	0.2	-	-	10	106 (9)
		U	-19.0	0.4	ns	-	15.4	0.4	*	-	3.7	0.2	*	-		
		UL	-17.9	0.6	*	*	15.5	0.4	*	ns	3.2	0.1	*	*		
Prionace glauca	PG	C	-18.0	0.2	-	-	16.1	0.6	-	-	2.6	0.1	-	-	10	116 (26)
		U	-18.1	0.3	ns	-	16.9	0.5	*	-	3.2	0.0	*	-		
		UL	-17.8	0.2	*	ns	17.0	0.6	*	ns	3.1	0.0	*	*		
Squalus suckleyi	SS	C	-19.4	1.1	-	-	14.0	0.7	-	-	4.5	1.1	-	-	9	73 (15)
		U	-19.7	1.0	ns	-	14.5	0.6	ns	-	5.6	1.2	ns	-		
		UL	-17.4	0.2	*	*	14.9	0.6	*	ns	3.9	0.1	ns	*		
Triakis semifasciata	TS	C	-16.0	0.3			15.3	0.2			2.9	0.3			2	U
		U	-16.1	0.5			16.0	0.2			3.3	0.0				
		UL	-15.7	0.4			16.0	0.2			3.1	0.0				

BATOIDS

Species	ID	Treatment	$\delta^{13}C$ mean	sd	C test	U test	$\delta^{15}N$ mean	sd	C test	U test	C:N mean	sd	C test	U test	n	TL (cm) mean (SD)
Bathyraja aleutica	BA	C	-16.7	0.5	-	-	15.0	0.5	-	-	2.7	0.0	-	-	7	128 (20)
		U	-16.9	0.4	ns	-	16.1	0.5	*	-	3.4	0.0	*	-		
		UL	-16.7	0.4	ns	ns	15.9	0.5	*	ns	3.4	0.0	*	ns		
Bathyraja interrupta	BI	C	-16.3	0.4			15.7	0.4			2.9	0.0			3	72 (14)
		U	-16.6	0.3			16.2	0.5			3.4	0.0				
		UL	-16.5	0.4			16.1	0.2			3.3	0.0				
Gymnura marmorata	GM	C	-17.0	1.1			16.6	0.9			3.1	0.3			3	U
		U	-17.2	1.5			17.0	0.9			3.7	0.4				
		UL	-16.1	0.9			17.2	0.7			3.2	0.0				
Myliobatis californica	MC	C	-16.4	1.2			16.2	0.4			3.0	0.4			2	U
		U	-16.5	1.2			16.9	0.1			3.3	0.0				
		UL	-16.2	1.2			17.0	0.0			3.1	0.0				
Pteroplatytrygon violacea	PV	C	-18.6	0.5	-	-	13.5	0.8	-	-	2.6	0.1	-	-	7	55 (9)
		U	-18.8	0.5	ns	-	13.9	1.2	ns	-	3.4	0.1	*	-		
		UL	-18.5	0.4	ns	ns	14.1	0.9	ns	ns	3.2	0.1	*	*		
Beringraja binoculata	BB	C	-15.2	0.5	-	-	15.0	0.1	-	-	2.9	0.1	-	-	5	132 (24)
		U	-16.2	0.4	*	-	15.5	0.3	*	-	3.4	0.0	*	-		
		UL	-16.1	0.4	*	ns	15.5	0.3	*	ns	3.4	0.0	*	ns		
Raja rhina	RR	C	-16.2	1.0	-	-	15.7	0.7	-	-	2.9	0.0	-	-	6	104 (11)
		U	-16.8	0.6	ns	-	16.5	0.8	ns	-	3.4	0.0	*	-		
		UL	-16.7	0.6	ns	ns	16.4	0.7	ns	ns	3.4	0.1	*	ns		
Urobatis halleri	UH	C	-15.0	0.3	-	-	15.7	0.3	-	-	2.7	0.1	-	-	11	15 (4)
		U	-15.4	0.3	*	-	16.5	0.3	*	-	3.8	0.1	*	-		
		UL	-14.9	0.3	ns	*	16.5	0.4	*	ns	3.4	0.1	*	*		

562
563 Table 1: Effects of urea and lipid extraction on stable isotope composition of various sharks (top panel)
564 and batoids (bottom panel). Treatments are C (control), U (urea-extracted), UL (urea & lipid-extracted).
565 C test shows results of statistical comparisons between the control (C) and U and UL treatments, whereas
566 U test shows comparison between U and UL (ns = not significant, * = significant, $p < 0.05$). Note that for
567 taxa with low sample sizes (<=3) we did not test for statistical differences.
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Figure Captions

Figure 1: Differences in $\delta^{13}C$, $\delta^{15}N$, and C:N between urea-extracted and control samples (A) and urea- and lipid-extracted and control samples (B) in sharks. Statistically significant differences between control and U and UL treatments are indicated (* p <= 0.05). Species are *Prionace glauca* (PG), *Squalus suckleyi* (SS), *Triakis semifasciata* (TS), *Isurus oxyrinchus* (IO), *Lamna ditropis* (LD), and *Carcharodon carcharias* (CC). We did not test for differences in TS due to low sample size.

Figure 2: Differences in $\delta^{13}C$, $\delta^{15}N$, and C:N between urea-extracted and control samples (A) and urea- and lipid- extracted and control samples (B) in batoids. Statistically significant differences between control and U and UL treatments are indicated (* p <= 0.05). Species are *Bathyraja aleutica* (BA), *Bathyraja interrupta* (BI), *Beringraja binoculata* (BB), *Raja rhina* (RR), *Myliobatis californica* (IO), *Gymnura marmorata* (TS), *Pteroplatytrygon violacea* (PV), and *Urobatis halleri* (UH). We did not test for differences in BI, MC and GM due to low sample sizes.

Figure 3: Relationships between the C:N of urea-extracted tissue (treatment U, $C:N_u$) and changes in $\delta^{13}C$ between the U and UL treatments ($\Delta\delta^{13}C = \delta^{13}C_U - \delta^{13}C_{UL}$). Lines show selected best fit modeled relationship between $C:N_u$ and $\Delta\delta^{13}C$ using best fit models based on AIC_c, which for groups with relatively low lipid content and $C:N_u$ (batoids and all sharks except for dogfish) was a linear model, but for groups with a higher lipid content and $C:N_u$ was based on the two parameter model from Fry (2002).

Figure 4: Conceptual diagram showing relative effects of urea and lipid extraction on elasmobranch muscle tissue. Axes show relative change in $\delta^{13}C$ and $\delta^{15}N$ following different treatments, and color bar shows C:N. The secondary axes show the relative effect of urea and lipid removal on $\delta^{13}C$ and $\delta^{15}N$, with the size of arrows indicating relative magnitude of effect. Urea extraction will generally increase $\delta^{15}N$ and C:N, and potentially also affect $\delta^{13}C$ as ^{13}C enriched urea is removed. Lipid extraction does not influence $\delta^{15}N$, but increases $\delta^{13}C$ and reduces C:N. The degree to which the different treatments affect $\delta^{13}C$, $\delta^{15}N$ (depicted by the magnitude and direction of the "Urea extraction" and "Urea & lipid extraction" arrows) and C:N (depicted by the shading gradient within each treatment arrow) will vary based on the urea and lipid (dashed arrow) content of the tissue.

Figure S1: Changes in $\delta^{13}C$ and $\delta^{15}N$ of urea-extracted (grey) and urea- & lipid-extracted samples (black) relative to control samples in sharks (A) and batoids (B). Dotted lines show relative shift in values between urea-extracted and urea & lipid-extracted samples. Shark species are *Prionace glauca* (PG), *Squalus suckleyi* (SS), *Triakis semifasciata* (TS), *Isurus oxyrinchus* (IO), *Lamna ditropis* (LD), and *Carcharodon carcharias* (CC). Batoid species are *Bathyraja aleutica* (BA), *Bathyraja interrupta* (BI), *Beringraja binoculata* (BB), *Raja rhina* (RR), *Myliobatis californica* (IO), *Gymnura marmorata* (TS), *Pteroplatytrygon violacea* (PV), and *Urobatis halleri* (UH).

Figure S2: Relationship between the C:N of urea extracted tissue (U, $C:N_u$) and changes in $\delta^{13}C$ between the U and UL treatments ($\Delta\delta^{13}C = \delta^{13}CU - \delta^{13}C_{UL}$) for individual species. Inset: magnified view of data with low C:N (< 4.4).

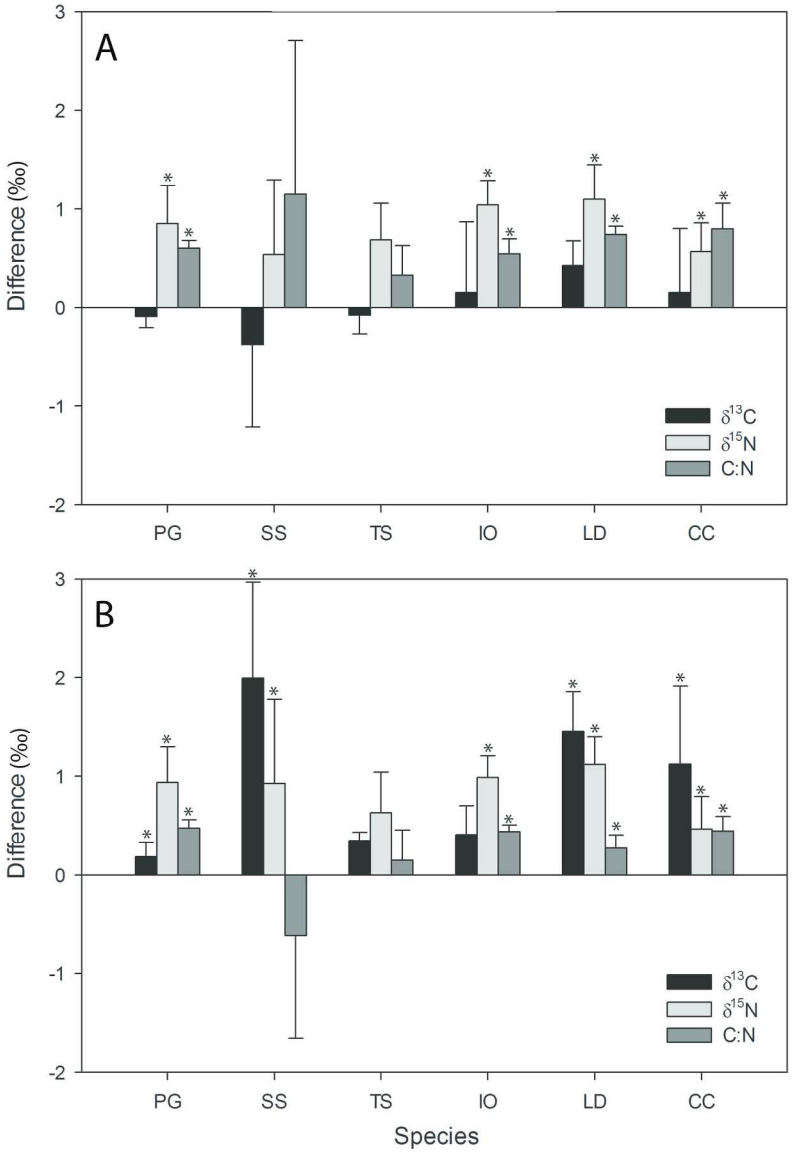

Figure 1: Differences in $\delta^{13}C$, $\delta^{15}N$, and C:N between urea-extracted and control samples (A) and urea- and lipid-extracted and control samples (B) in sharks. Statistically significant differences between control and U and UL treatments are indicated (* p <= 0.05). Species are *Prionace glauca* (PG), *Squalus suckleyi* (SS), *Triakis semifasciata* (TS), *Isurus oxyrinchus* (IO), *Lamna ditropis* (LD), and *Carcharodon carcharias* (CC).We did not test for differences in TS due to low sample size.

205x256mm (300 x 300 DPI)

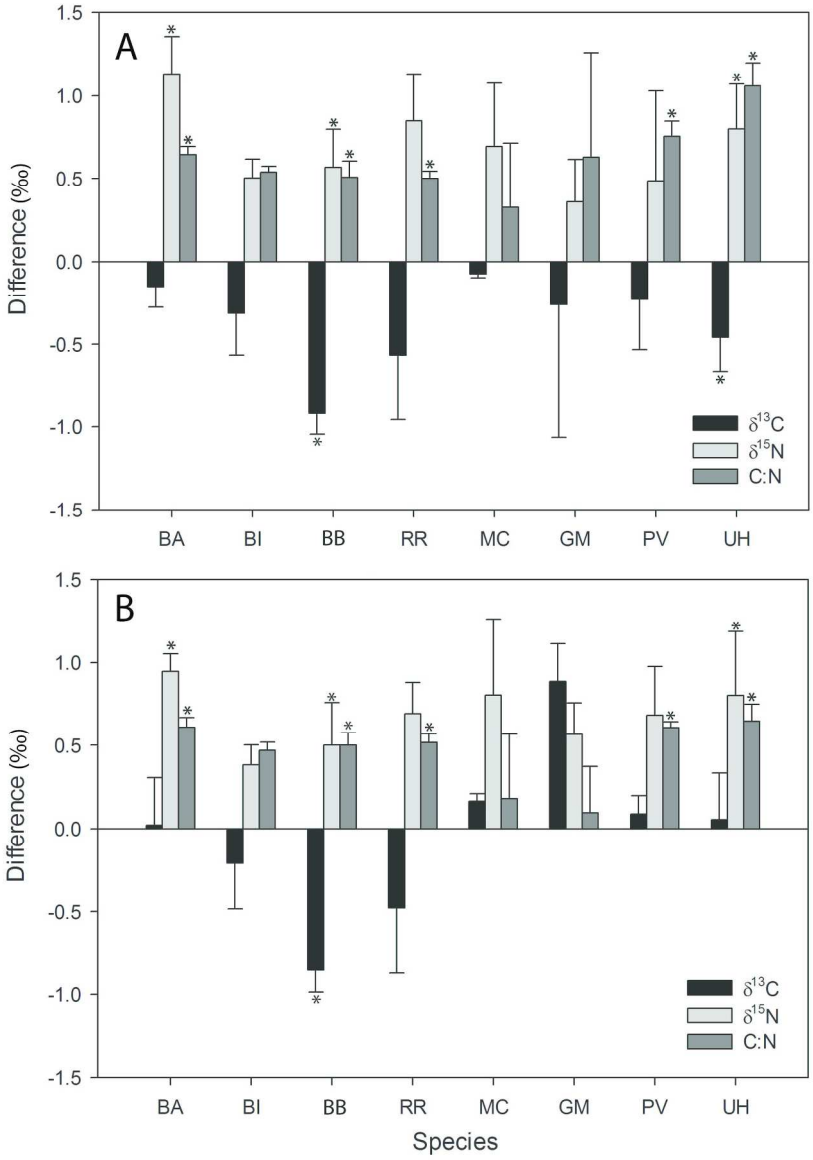

Figure 2: Differences in $\delta^{13}C$, $\delta^{15}N$, and C:N between urea-extracted and control samples (A) and urea- and lipid- extracted and control samples (B) in batoids. Statistically significant differences between control and U and UL treatments are indicated (* p <= 0.05). Species are *Bathyraja aleutica* (BA), *Bathyraja interrupta* (BI), *Beringraja binoculata* (BB),*Raja rhina* (RR), *Myliobatis californica* (IO), *Gymnura marmorata* (TS), *Pteroplatytrygon violacea* (PV), and *Urobatis halleri* (UH). We did not test for differences in BI, MC and GM due to low sample sizes.

207x261mm (300 x 300 DPI)

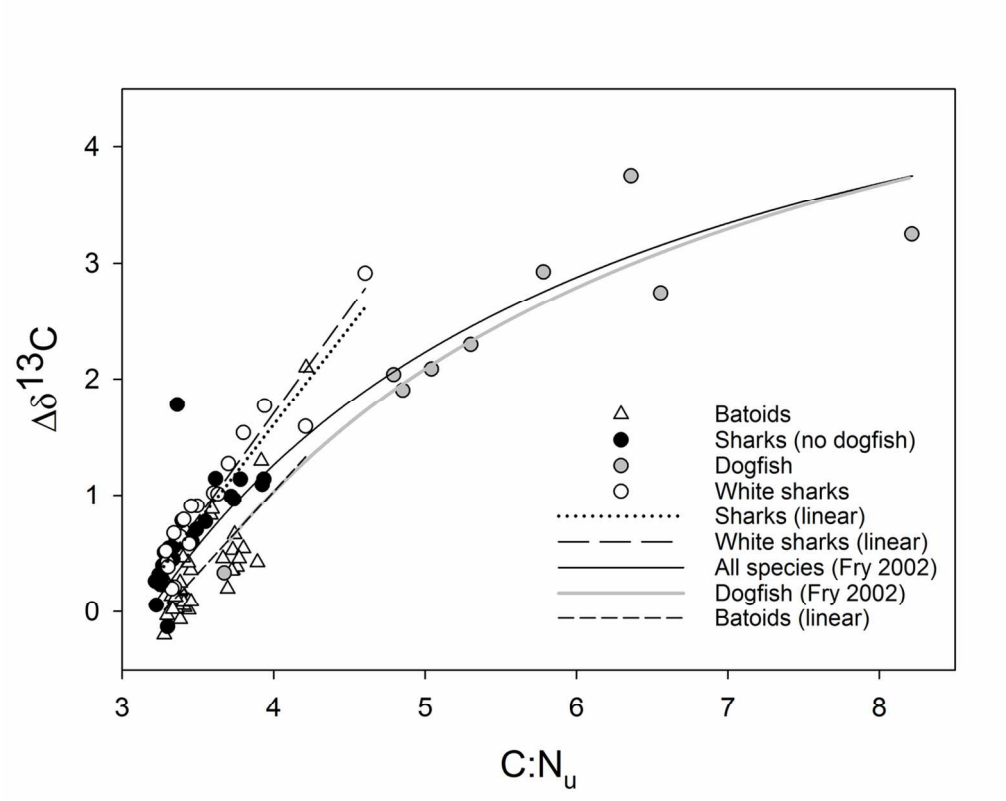

Figure 3: Relationships between the C:N of urea-extracted tissue (treatment U, $C:N_u$) and changes in $\delta^{13}C$ between the U and UL treatments ($\Delta\delta^{13}C = \delta^{13}C_U - \delta^{13}C_{UL}$). Lines show selected best fit modeled relationship between $C:N_u$ and $\Delta\delta^{13}C$ using best fit models based on AIC_c, which for groups with relatively low lipid content and $C:N_u$ (batoids and all sharks except for dogfish) was a linear model, but for groups with a higher lipid content and $C:N_u$ was based on the two parameter model from Fry (2002).

122x98mm (300 x 300 DPI)

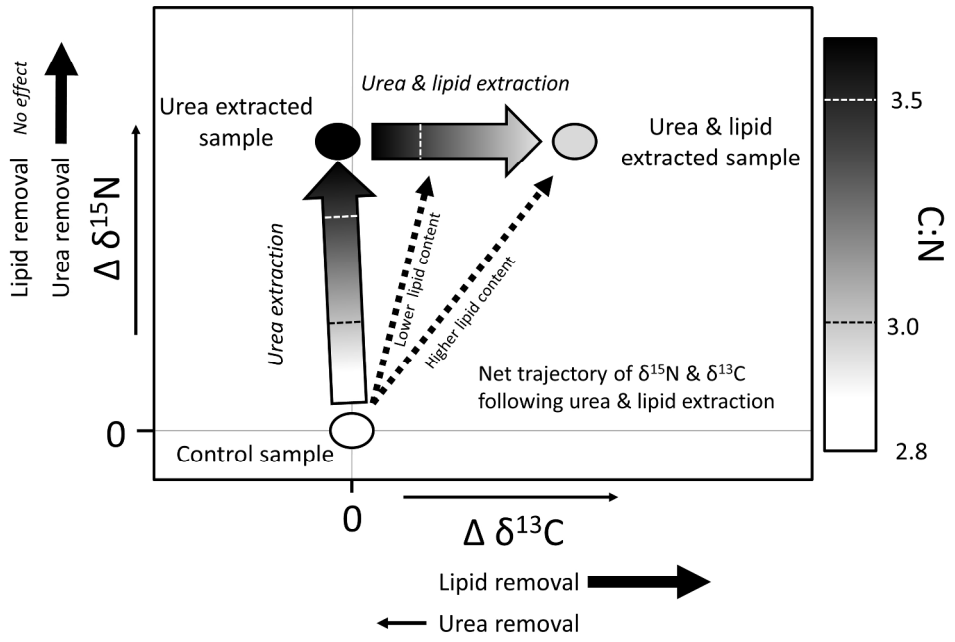

Figure 4: Conceptual diagram showing relative effects of urea and lipid extraction on elasmobranch muscle tissue. Axes show relative change in $\delta^{13}C$ and $\delta^{15}N$ following different treatments, and color bar shows C:N. The secondary axes show the relative effect of urea and lipid removal on $\delta^{13}C$ and $\delta^{15}N$, with the size of arrows indicating relative magnitude of effect. Urea extraction will generally increase $\delta^{15}N$ and C:N, and potentially also affect $\delta^{13}C$ as ^{13}C enriched urea is removed. Lipid extraction does not influence $\delta^{15}N$, but increases $\delta^{13}C$ and reduces C:N. The degree to which the different treatments affect $\delta^{13}C$, $\delta^{15}N$ (depicted by the magnitude and direction of the "Urea extraction" and "Urea & lipid extraction" arrows) and C:N (depicted by the shading gradient within each treatment arrow) will vary based on the urea and lipid (dashed arrow) content of the tissue.

246x171mm (300 x 300 DPI)

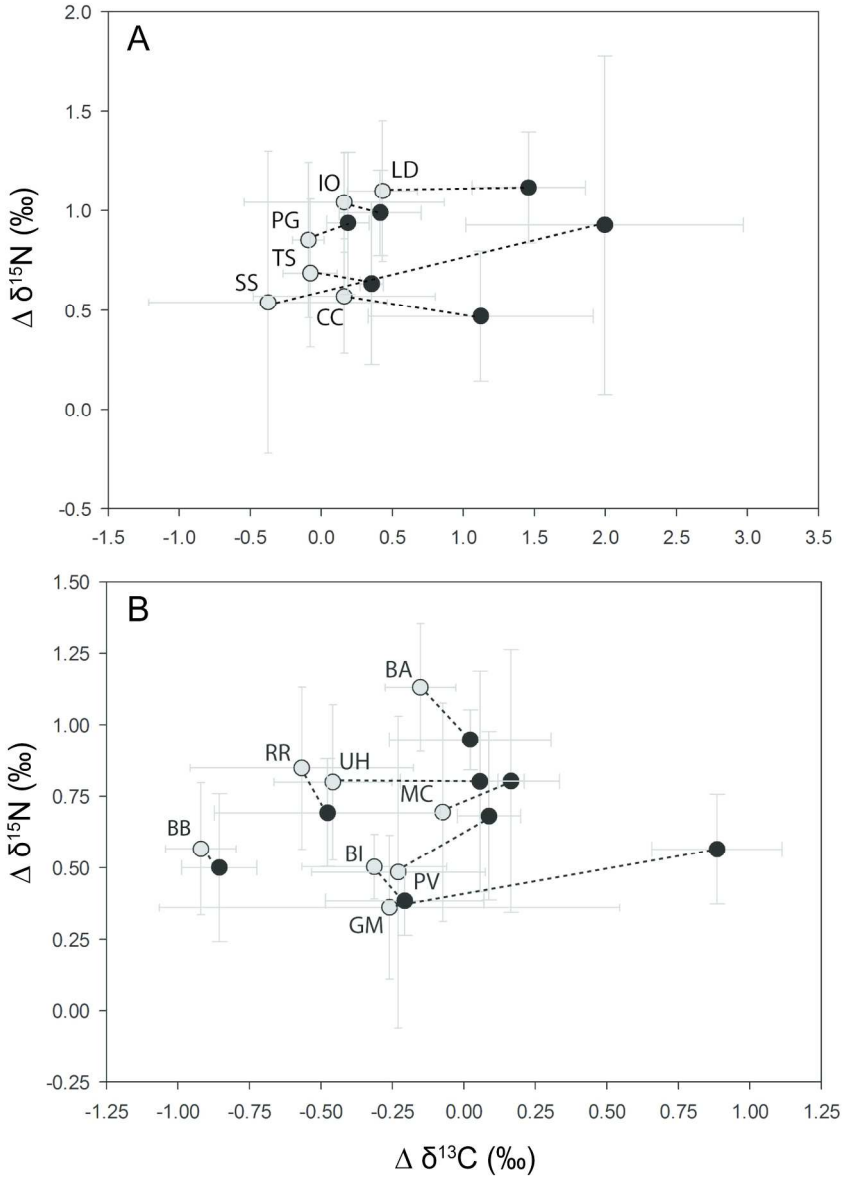

Figure S1: Changes in $\delta^{13}C$ and $\delta^{15}N$ of urea-extracted (grey) and urea- & lipid-extracted samples (black) relative to control samples in sharks (A) and batoids (B). Dotted lines show relative shift in values between urea-extracted and urea & lipid-extracted samples. Shark species are *Prionace glauca* (PG), *Squalus suckleyi* (SS), *Triakis semifasciata* (TS), *Isurus oxyrinchus* (IO), *Lamna ditropis* (LD), and *Carcharodon carcharias* (CC). Batoid species are *Bathyraja aleutica* (BA), *Bathyraja interrupta* (BI), *Beringraja binoculata* (BB), *Raja rhina* (RR), *Myliobatis californica* (IO), *Gymnura marmorata* (TS), *Pteroplatytrygon violacea* (PV), and *Urobatis halleri* (UH).

209x273mm (300 x 300 DPI)

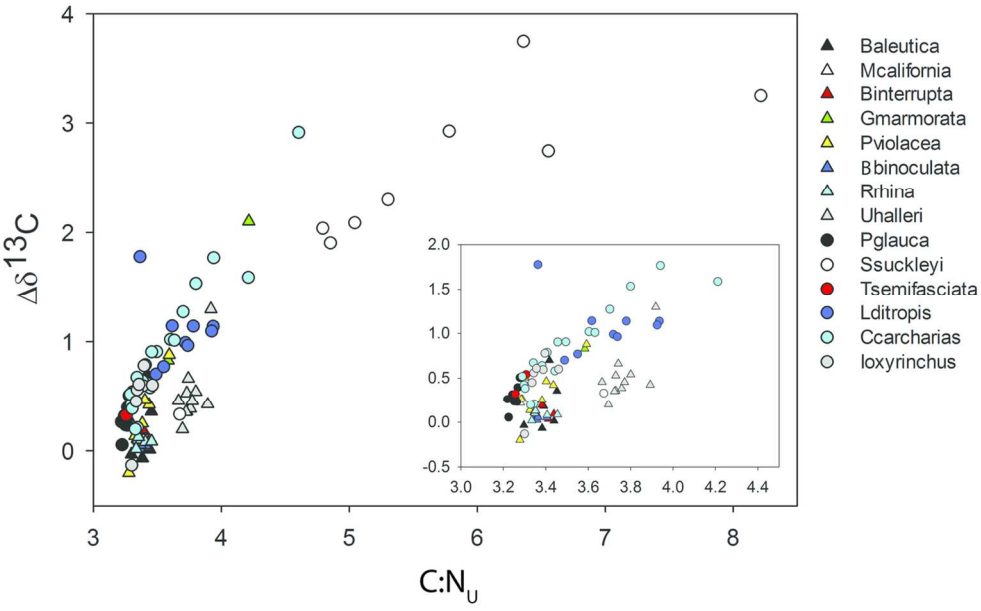

Figure S2: Relationship between the C:N of urea extracted tissue (U, $C:N_U$) and changes in $\delta^{13}C$ between the U and UL treatments ($\Delta\delta^{13}C = \delta^{13}C_U - \delta^{13}C_{UL}$) for individual species. Inset: magnified view of data with low C:N (< 4.4).

123x78mm (300 x 300 DPI)

Study #	C:N metric	Adjustment	Nothing	Lipid ext.	Urea ext.
1	0	1	0	0	0
2	1	0	0	0	0
3	0	0	0	1	0
4	1	0	0	0	0
5	0	0	0	1	0
6	0	0	1	0	0
7	1	0	0	0	0
8	0	0	0	1	1
9	0	0	1	0	0
10	0	0	0	1	0
11	1	0	0	0	0
12	1	0	0	0	0
13	0	0	0	1	0
14	0	0	0	1	0
15	0	0	0	1	0
16	0	0	0	0	1
17	0	0	0	1	0
18	0	0	0	1	1
19	0	0	0	1	0
20	0	0	0	1	0
21	0	1	0	0	0
22	0	0	0	1	0
23	0	0	1	0	0
24	0	0	0	0	1
25	1	0	0	0	0
26	1	0	0	0	0
27	1	0	0	0	0
28	1	0	0	0	0
29	0	1	0	0	0
30	0	1	0	0	0
31	0	1	0	0	0
32	0	0	0	1	1
33	0	0	1	0	0
34	0	1	0	0	0
35	0	0	0	0	1
36	0	0	1	0	0
37	0	0	0	1	0
38	1	0	0	0	0
39	0	1	0	0	0
40	0	0	0	0	1
41	1	0	0	0	0
42	1	0	0	0	0
43	0	0	0	1	0
44	0	0	0	1	0
45	0	0	0	1	0
46	0	0	0	1	0
47	1	1	0	0	0

48	0	0	0	1	0
49	1	0	0	0	0
50	0	0	1	0	0
Number	14	8	6	19	7

Table S2: Survey of 50 recent (2013-present) studies using stable isotope analysis to
"stable isotope", "elasmobranch", "shark", "ray"; and selected the first 50 pertinent
indicates the study used the C:N of bulk, non-extracted samples (i.e. contain urea an
whether lipid extraction was required or not. "Adjustment" indicates the study used

Study

Couturier et al. 2013. Stable Isotope and Signature Fatty Acid Analyses Suggest Reef Manta Rays Feed on Demersa
Matich & Heithaus 2014. Multi -tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in t
Hussey et al. 2014. Rescaling the trophic structure of marine food webs. Ecology Letters, 17(2), pp.239-250.
Daly et al. 2013. Comparative feeding ecology of bull sharks (Carcharhinus leucas) in the coastal waters of the Sout
McMeans et al. 2013. The role of Greenland sharks (Somniosus microcephalus) in an Arctic ecosystem: assessed via
Meneses et al. 2016. Trophic overlap between blue sharks (Prionace glauca) and shortfin makos (Isurus oxyrinchus)
Heithaus et al. 2013. Apparent resource partitioning and trophic structure of large-bodied marine predators in a relat
Shiffman et al. 2014. Feeding ecology of the sandbar shark in south carolina estuaries revealed through δ13C and δ1
De Lecea et al. 2013. Processes controlling the benthic food web of a mesotrophic bight (KwaZulu-Natal, South Afr
Caut et al. 2013. Diet-and tissue-specific incorporation of isotopes in the shark Scyliorhinus stellaris, a North Sea m
Tilley et al. 2013. Diet reconstruction and resource partitioning of a Caribbean marine mesopredator using stable iso
Navarroet al. 2014. Short-and long-term importance of small sharks in the diet of the rare deep-sea shark Dalatias lic
Polo-Silva et al. 2013. Trophic shift in the diet of the pelagic thresher shark based on stomach contents and stable is
Olin et al. 2013. Seasonal variability in stable isotopes of estuarine consumers under different freshwater flow regim
Munroe et al. 2015. Regional movement patterns of a small -bodied shark revealed by stable -isotope analysis. Jo
Li et al. 2014. Trophic ecology of sharks in the mid-east Pacific ocean inferred from stable isotopes. Journal of Ocea
Churchill et al. 2015. Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexic
Kiszka et al. 2015. Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean rev
Espinoza et al. 2015. Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica
McMean et al. 2015. Impacts of food web structure and feeding behavior on mercury exposure in Greenland Sharks
Malpica-Cruz et al. 2013. Tissue -specific stable isotope ratios of shortfin mako (Isurus oxyrinchus) and white (Car
Torres et al. 2014. Trophic ecology and bioindicator potential of the North Atlantic tope shark. Science of The Total
McCauley et al. 2014. Reliance of mobile species on sensitive habitats: a case study of manta rays (Manta alfredi) ar
Papastamatiou et al. 2015. Movements and foraging of predators associated with mesophotic coral reefs and their po
de Moura et al. 2015. Assessment of trace elements, POPs, 210 Po and stable isotopes (15 N and 13 C) in a rare filte
Madigan et al. 2015. Diet shift and site-fidelity of oceanic whitetip sharks Carcharhinus longimanus along the Grea
Speed et al. 2012. Trophic ecology of reef sharks determined using stable isotopes and telemetry. Coral Reefs, 31(2)
Drymon et al. 2012. Trophic plasticity in the Atlantic sharpnose shark (Rhizoprionodon terraenovae) from the north
Fanelli et al. 2013. Trophic webs of deep-sea megafauna on mainland and insular slopes of the NW Mediterranean:
Courtney & Foy, R., 2012. Pacific sleeper shark Somniosus pacificus trophic ecology in the eastern North Pacific O
McCauley et al. 2012. Assessing the effects of large mobile predators on ecosystem connectivity. Ecological Applic
Lyons et al. 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of
Matich et al. 2015. Short-term shifts of stable isotope (δ 13 C, δ 15 N) values in juvenile sharks within nursery areas
Albo-Puigserver et al. 2015. Feeding ecology and trophic position of three sympatric demersal chondrichthyans in th
Rojas et al. 2014. Feeding grounds of juvenile scalloped hammerhead sharks (Sphyrna lewini) in the south-eastern C
Reum & Marshall 2013. Evaluating δ15N–body size relationships across taxonomic levels using hierarchical models
Hussey et al. 2015. Expanded trophic complexity among large sharks. Food Webs, 4, pp.1-7.
Valls et al. 2014. Structure and dynamics of food webs in the water column on shelf and slope grounds of the wester
Teffer et al. 2014. Trophic influences on mercury accumulation in top pelagic predators from offshore New England
Kiszka et al. 2014. Trophic ecology of common elasmobranchs exploited by artisanal shark fisheries off south-weste
Cresson et al. 2014. Mercury in organisms from the Northwestern Mediterranean slope: Importance of food sources.
Barría et al. 2015. Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobra
Lopez et al. 2013. Trophic ecology of the dusky catshark Bythaelurus canescens (Günther, 1878)(Chondrychthyes: S
Freedman et al. 2015. Connectivity and movements of juvenile predatory fishes between discrete restored estuaries i
Shaw et al. 2016. Trophic Ecology of a Predatory Community in a Shallow-Water, High-Salinity Estuary Assessed b
Jaime-Rivera et al. 2014. Feeding and migration habits of white shark Carcharodon carcharias (Lamniformes: Lamn
Torres-Rojas et al. 2015. Diet and trophic level of scalloped hammerhead shark (Sphyrna lewini) from the Gulf of C

Hernández-Aguilar et al. 2015. Trophic ecology of the blue shark (Prionace glauca) based on stable isotopes (δ
Raoult et al. 2015. Not all sawsharks are equal: species of co-existing sawsharks show plasticity in trophic consump
Frisch et al. 2016. Reassessing the trophic role of reef sharks as apex predators on coral reefs. Coral Reefs, pp.1-14.

› study the ecology of elasmobranchs. We conducted the search on Google scholar using the search terms:
results. We assessed each study to determine how they treated samples for urea and lipid. “C:N metric”
ıd lipid) to assess the lipid content of their samples and use that inferred lipid content to inform them
the C:N of non-extracted samples to mathematically adjust δ ^{13}C to account for inferred lipid content.

l Zooplankton. PLoS ONE 8(10): e77152
the trophic interactions of juvenile bull sharks in a coastal estuary. Journal of Animal Ecology, 83(1), pp.199-213

hwest Indian Ocean inferred from stable isotope analysis. PloS one, 8(10), p.e78229.
a stable isotopes and fatty acids. Marine biology, 160(5), pp.1223-1238.
: Trophic linkages between two shark species In the Eastern Pacific Ocean food web. Food Webs.
ively pristine seagrass ecosystem. Mar Ecol Prog Ser, 481, pp.225-237.
5N stable isotope analysis. Marine and Coastal Fisheries, 6(1), pp.156-169.
ica) revealed by stable isotope analysis. Marine Ecology Progress Series, 484, pp.97-114.
esopredator. Marine Ecology. Progress Series, 492.
tope Bayesian modelling. PloS one, 8(11), p.e79560.
cha. Marine biology, 161(7), pp.1697-1707.
otope analyses. Marine Biology Research, 9(10), pp.958-971.
es. Marine Ecology Progress Series, 487, pp.55-69.
urnal of fish biology, 86(5), pp.1567-1586.
n University of China, 13(2), pp.278-282.
o revealed through stable isotope and stomach content analysis. Deep Sea Research Part II: Topical Studies in O
ealed by stable isotope and mercury analyses. Deep Sea Research Part I: Oceanographic Research Papers, 96, pp
inferred from stable isotope and stomach content analyses. Journal of Experimental Marine Biology and Ecology
(Somniosus microcephalus). Science of The Total Environment, 509, pp.216-225.
charodon carcharias) sharks as indicators of size -based differences in foraging habitat and trophic level. Fis
Environment, 481, pp.574-581.
nd lagoons. Marine biology, 161(9), pp.1987-1998.
tential for linking ecological habitats. Marine Ecology Progress Series.
r-feeding shark: The megamouth. Marine pollution bulletin, 95(1), pp.402-406.
t Bahama Bank. Marine Ecology Progress Series, 529, p.185.
, pp.357-367.
central Gulf of Mexico. Environmental biology of fishes, 95(1), pp.21-35.
a comparison by stable isotope analysis. Marine ecology. Progress series, 490, pp.199-221.
cean inferred from nitrogen and carbon stable -isotope ratios and diet. Journal of fish biology, 80(5), pp.1
ations, 22(6), pp.1711-1717.
young of the year lamniform sharks. Marine environmental research, 90, pp.27-38.
suggest rapid shifts in energy pathways. Journal of Experimental Marine Biology and Ecology, 465, pp.83-91.
ne northwestern Mediterranean. Marine Ecology Progress Series, 524, p.255.
Gulf of California. Hydrobiologia, 726(1), pp.81-94.
s. Oecologia, 173(4), pp.1159-1168.

n Mediterranean. Journal of Marine Systems, 138, pp.171-181.
l waters of the northwest Atlantic Ocean. Marine environmental research, 101, pp.124-134.
ern Madagascar inferred from stable isotopes. Aquatic Biology, 23, pp.29-38.
Science of The Total Environment, 497, pp.229-238.
nchs in the western Mediterranean Sea. Marine Ecology Progress Series, 539, p.225.
Scyliorhinidae) in the southeast Pacific Ocean. Journal of Applied Ichthyology, 29(4), pp.751-756.
in southern California. Marine Ecology Progress Series, 520, p.191.
by Stable Isotope Analysis. Marine and Coastal Fisheries, 8(1), pp.46-61.
idae) from Isla Guadalupe inferred by analysis of stable isotopes δ15N and δ13C. Revista de Biolog ía Tr
California and Gulf of Tehuantepec, Mexico. Iranian Journal of Fisheries Sciences, 14(3), pp.767-785.

13 C and δ 15 N) and stomach content. Journal of the Marine Biological Association of the United Kingdom, pp
ɔtion both within and between species. Canadian Journal of Fisheries and Aquatic Sciences, 72(11), pp.1769-177

ceanography, 115, pp.92-102.
.49-58.
y, 470, pp.12-25.

sheries Oceanography, 22(6), pp.429-445.

508-1545.

opical, 62(2), pp.637-647.

.1-8.
5.

data	$\Delta^{13}C = (a\,C{:}N_U + b)(C{:}N_U + c)^{-1}$		$\Delta^{13}C = P - PF(C{:}N_U)^{-1}$		$\Delta^{13}C = \beta_0 + \beta_1 Ln(C{:}N_U)$		$\Delta^{13}C = b + a\,C{:}N_U$	
All species	AIC_c:	94.02	AIC_c:	92.53	AIC_c:	102.33	AIC_c:	121.93
	a =	5.39 ± 0.85	P =	6.12 ± 0.30	β_0 =	-4.89 ± 0.33	a =	0.88 ± 0.06
	b =	-17.25 ± 2.62	F =	3.18 ± 0.03	β_1 =	4.34 ± 0.25	b =	-2.57 ± 0.22
	c =	-0.68 ± 0.75			r^2:	0.76	r^2:	0.70
All sharks	AIC_c:	38.58	AIC_c:	38.97	AIC_c:	39.54	AIC_c:	40.50
(no Dogfish)	a =	1.94 ± 0.23	P =	7.86 ± 0.70	β_0 =	-7.69 ± 0.82	a =	1.81 ± 0.18
	b =	-6.27 ± 0.75	F =	3.15 ± 0.03	β_1 =	6.74 ± 0.66	b =	-5.61 ± 0.63
	c =	-2.98 ± 0.06			r^2:	0.69	r^2:	0.68
All batoids	AIC_c:	*nf*	AIC_c:	15.41	AIC_c:	13.87	AIC_c:	12.26
	a =	-	P =	5.63 ± 0.77	β_0 =	-6.15 ± 0.91	a =	1.45 ± 0.19
	b =	-	F =	3.28 ± 0.04	β_1 =	5.19 ± 0.73	b =	-4.74 ± 0.69
	c =	-			r^2:	0.56	r^2:	0.58
Dogfish	AIC_c:	22.46	AIC_c:	17.30	AIC_c:	20.93	AIC_c:	24.39
S. suckleyi	a =	4.76 ± 1.03	P =	6.31 ± 0.62	β_0 =	-4.08 ± 1.27	a =	0.62 ± 0.16
	b =	-16.96 ± 3.44	F =	3.35 ± 0.20	β_1 =	3.79 ± 0.74	b =	-1.18 ± 0.91
	c =	-1.90 ± 1.03			r^2:	0.76	r^2:	0.65
Great White	AIC_c:	-0.46	AIC_c:	-5.15	AIC_c:	-3.88	AIC_c:	-3.27
C. carcharias	a =	14.18 ± 16.56	P =	8.30 ± 0.52	β_0 =	-7.80 ± 0.61	a =	1.80 ± 0.13
	b =	-43.96 ± 50.48	F =	3.14 ± 0.03	β_1 =	6.90 ± 0.48	b =	-5.49 ± 0.46
	c =	3.22 ± 9.12			r^2:	0.92	r^2:	0.92

Table S1. Parameter estimates (± SE), r^2 (linear models only) and corrected Akaike Information Criteria valeus (AIC_c) for models of Δ13C, the difference between lipid and urea (UL) and urea (U) extracted $\delta^{13}C$ values ($\Delta^{13}C = \delta^{13}C_U - \delta^{13}C_{UL}$), fit to all species, batoids, all sharks other than *S. suckleyi* (dogfish), *S. suckleyi* , and *C. carcharias* . *nf* = model failed to converg.