2-2020

Effects of Lipid Extraction on δ13C and δ15N Values and Use of Lipid-Correction Models Across Tissues, Taxa and Trophic Groups

Carl S. Cloyed

Kayla P. DaCosta

Matthew R. Hodanbosi

Ruth H. Carmichael

Received: 23 November 2019 | Accepted: 25 February 2020

DOI: 10.1111/2041-210X.13386

RESEARCH ARTICLE

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

The effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values and use of lipid-correction models across tissues, taxa and trophic groups

Carl S. Cloyed[1] | Kayla P. DaCosta[1,2] | Matthew R. Hodanbosi[1,2] | Ruth H. Carmichael[1,2]

[1]University Programs, Dauphin Island Sea Lab, Dauphin Island, AL, USA

[2]Department of Marine Sciences, University of South Alabama, Mobile, AL, USA

Correspondence
Carl S. Cloyed
Email: ccloyed@disl.org

Funding information
National Fisheries and Wildlife Foundation, Grant/Award Number: 45720; Ocean Leadership, Grant/Award Number: 18-12-003; Gulf of Mexico Research Initiative; National Marine Mammal Foundation (NMMF); University of South Alabama

Handling Editor: Carolyn Kurle

Abstract

1. Lipid-rich animal tissues have low $\delta^{13}C$ values, which can lead to inaccurate ecological inferences. Chemical lipid extraction (LE) or correction models account for this depletion, but the need for LE or correction is tissue- and species-specific. Also, LE can alter $\delta^{15}N$ values, increasing labour and costs because bulk samples must be analysed for $\delta^{15}N$ values separately.
2. We studied the effects of LE on $\delta^{13}C$ and $\delta^{15}N$ values in liver, muscle and skin of common bottlenose dolphins *Tursiops truncatus* and West Indian manatees *Trichechus manatus*, two ecologically important species that occupy different trophic levels. We fit lipid-correction models to each species. We also performed a meta-analysis to more broadly determine the effects of LE across taxa, tissues and trophic groups (carnivores, omnivores and herbivores) and to fit lipid-correction models to different taxonomic and trophic groups.
3. Lipid extraction increased the $\delta^{13}C$ values in dolphin tissues but had little effect on manatee tissues and no effect on the $\delta^{15}N$ values in either species. A mass balance lipid-correction model best fit the data from all dolphin tissues, and a linear model best fit data for manatee liver while null models best fit data from manatee muscle and skin. Across 128 terrestrial and aquatic species, the effects of LE varied among tissues and were lower for herbivores compared to carnivores. The best-fitting lipid-correction models varied among tissue, taxa and trophic groups. Finally, the $\delta^{15}N$ values from muscle and liver were affected by LE.
4. Our results strengthen the growing body of evidence that the need for LE is tissue- and species-specific, without a reliable C:N ratio predictive threshold. The prediction errors of lipid-correction models generally decreased with taxonomic and trophic specificity. The smaller effects of LE in herbivores may be due to differences in diet composition or the physiology of lipid synthesis in members of this trophic group. These results suggest that researchers should use the most species-, tissue- and trophic group-specific information on LE available and, if not available, perform LE on a subset of samples prior to analysis to determine effects.

KEYWORDS
bottlenose dolphins, chemical lipid extraction, lipid extraction, lipid-correction models, meta-analysis, stable isotopes, trophic discrimination, West Indian manatees

1 | INTRODUCTION

Stable isotopes are an invaluable tool for ecologists to quantify resource and habitat use and determine trophic positions of consumers (Layman, Arrington, Montaña, & Post, 2007; Post, 2002). Despite the predictable variation in isotopic patterns across and within ecosystems, important caveats must be considered to make accurate ecological inferences from isotopic values. One important caveat is that tissues rich in lipids can be depleted in the heavier ^{13}C isotope (DeNiro & Epstein, 1977; Newsome, Chivers, & Berman Kowalewski, 2018; Post et al., 2007). The ^{13}C isotopes are discriminated against during the pyruvate dehydrogenase reaction when pyruvate is turned into acetyl-CoA during lipid synthesis (DeNiro & Epstein, 1977). Failing to account for depleted carbon isotope values in lipid-rich tissues can lead to erroneous conclusions, particularly when quantifying diet with mixing models (Perkins et al., 2013; Tarroux et al., 2010). This problem not only yields flawed understanding of ecological relationships but can also adversely impact applied projects and ecosystem management (Phillips et al., 2014). To manage the effects of lipids on stable carbon isotope values, ecologists either chemically extract lipids from consumer tissues before analysing their isotopic values or use lipid-correction models (Logan & Lutcavage, 2008; Sweeting, Polunin, & Jennings, 2006).

When to extract lipids from tissues or use corrective models is an ongoing debate in stable isotope ecology (Logan et al., 2008; Newsome et al., 2018; Patterson & Carmichael, 2016). Despite the widespread use of stable isotopes in ecology and the apparently ubiquitous effect of lipid synthesis on stable isotope values, little is known about the general patterns of lipid extraction (LE) on stable isotope values. Many researchers only lipid-extract tissues when carbon-to-nitrogen (C:N) ratios are >3.5 because such tissues are generally lipid rich (Cloyed & Eason, 2016; Post et al., 2007). For example, liver is rich in lipids, has high C:N ratios and almost always requires LE (Logan et al., 2008; Papiol, Fanelli, Cartes, Rumolo, & López-Pérez, 2017; Sardenne et al., 2015). Lipid content (type and quantity) and the need for LE of other tissues frequently used in stable isotope analyses vary among species (Njinkoué, Barnathan, Miralles, Gaydou, & Samb, 2002; Papiol et al., 2017; Patterson & Carmichael, 2016), and the C:N ratio threshold of 3.5 for LE can be arbitrary or tissue- and species-specific (Fagan, Koops, Arts, & Power, 2011; Patterson & Carmichael, 2016; Wilson, Chanton, Balmer, & Nowacek, 2014). Some researchers lipid extract all tissues, regardless of the C:N ratio (e.g. Hooker, Iverson, Ostrom, & Smith, 2001; MacAvoy, Cortese, Cybulski, Hohn, & Macko, 2017; O'Donovan, Budge, Hobson, Kelly, & Derocher, 2018). However, blanket LEs can create unnecessary work and expense if LE is unnecessary or techniques alter nitrogen values, as is common in tissues containing many proteins and polar nitrogenous compounds (Logan et al., 2008; Logan & Lutcavage, 2008; Sweeting et al., 2006), forcing researchers to analyse each sample twice, once on lipid-extracted tissues for carbon values and again on bulk tissues for nitrogen. There is demand, therefore, to better define indicators of when LE is necessary or to provide robust lipid-correction models.

Taxonomy and trophic level may account for some apparent species-specific variation. While tissue-specific trophic discrimination is responsible for the depletion of ^{13}C in lipid-rich tissues, taxonomy and diet affect the degree of discrimination among trophic levels that occurs in all tissues (Caut, Angulo, & Courchamp, 2009). Discrimination typically increases with trophic level (Post, 2002), but carnivores fed diets with greater protein, that is, low C:N ratios, have less discrimination (Ankjærø, Christensen, & Grønkjær, 2012; Bloomfield, Elsdon, Walther, Gier, & Gillanders, 2011; Robbins, Felicetti, & Sponheimer, 2005). Natural diets vary widely in quantity and quality of lipids (Lawson, Magalhães, & Miller, 1998; Patterson & Carmichael, 2016), with carnivorous diets typically rich in lipids and proteins, whereas herbivorous diets are often depleted in those molecules. As a result, herbivores must synthesize lipids in vivo, yielding both proteins and lipids depleted in ^{13}C (DeNiro & Epstein, 1977; Newsome, Fogel, Kelly, & Rio, 2011). Some species have high C:N ratios but low lipid content if energy reserves are stored as glycogen rather than lipids (Patterson & Carmichael, 2016), disrupting the relationship between C:N ratios and ^{13}C. While researchers have determined that trophic level affects trophic discrimination in general, it is unknown if trophic level affects the processes that drive discrimination during lipid synthesis and ultimately affect $\delta^{13}C$ values in consumer tissues. To the best of our knowledge, no research has tested whether the effects of LE or the need for mathematical lipid correction vary by trophic niche.

Here, we compared the effects of LE and lipid-correction models across species and trophic groups (carnivore, omnivore, herbivore). We compared lipid-extracted and bulk stable carbon and nitrogen isotope values in liver, muscle and skin from common bottlenose dolphins *Tursiops truncatus*, and West Indian manatees *Trichechus manatus*, to determine the effects of LE on $\delta^{13}C$ and $\delta^{15}N$ values among these tissues and whether trophic niche contributes to variation in discrimination against ^{13}C during lipid synthesis. Additionally, we fit lipid-correction models to each species and tissue to determine if models were a viable alternative to LE. These large, aquatic mammals occupy different trophic levels. Dolphins are carnivorous, feeding on lipid- and protein-rich fish (Wilson et al., 2017). Manatees are herbivorous, feeding on lipid- and protein-poor seagrasses and aquatic vegetation (Mignucci-Giannoni & Beck, 1998). To determine if the results we found for dolphins and manatees were generalizable across taxa and trophic groups (carnivores, omnivores, herbivores),

we performed a meta-analysis from available literature that included both terrestrial and aquatic organisms to determine the magnitude of differences between lipid-extracted and bulk tissues and fit lipid-correction models to different taxonomic and trophic groups. We had enough data from three taxonomic groups, birds (muscle), fish (liver, muscle) and mammals (liver, muscle, skin), to determine if the effects of LE for each group were different than the average instrument error, because differences greater than the instrument error can be attributed to LE.

2 | MATERIALS AND METHODS

2.1 | Sample collection and preparation

Tissues used in this study were obtained from dolphins that stranded dead along the Alabama coast in 2011 and 2018 and from manatees that stranded dead along the north-central Gulf of Mexico in 2010–2018, from Mississippi to the Florida panhandle. All stranded animals used in this study had undergone little decomposition (i.e. stranding codes of 2 or early 3; Geraci & Lounsbury, 2005; Payo-Payo, Ruiz, Cardona, & Borrell, 2013). Tissues were stored at −20°C prior to sample processing. We randomly selected 10 adult dolphins, six males and four females, for which we had fresh liver (n = 9), muscle (n = 10) and skin samples (n = 10; total sample n = 29). We selected 10 manatees, seven males and three females, five of which died from cold stress and five that died from boat collisions or other causes during the warm season. We had usable muscle samples from all 10 manatee individuals, skin from nine individuals and liver from seven (total sample n = 26). Tissues were thawed, rinsed with ultra-pure (UP) water and ~10 g of each tissue was dissected. Fat was removed from the skin samples, and connective tissues were removed from muscle samples. All tissues were re-rinsed with UP water and split into two subsamples. The first subsamples, hereafter called bulk samples, were dried at 60°C for 24–48 hr and ground into a fine powder with a mortar and pestle. The second subsamples were lipid extracted, hereafter referred to as LE samples, prior to being dried.

2.2 | Lipid extraction

We used a modified Folch method for LE (Sweeting et al., 2006). The samples to be lipid extracted were placed in 15 ml vials with ~2 ml of UP water and homogenized using a handheld rotor–stator (Waverly H100, Waverly Scientific) until the tissues were finely macerated. Six millilitres of 2:1 chloroform:methanol solution was added to the vials, which were sonicated for 5 min and centrifuged for 10 min at 3,353 *g*. The supernatant was removed, and the process was repeated two to four times until the supernatant was clear (Sweeting et al., 2006). The LE samples were then dried at 60°C for 24–48 hr and reground with a mortar and pestle, if necessary.

2.3 | Stable isotope analysis

Both LE and bulk samples were weighed to 1 mg (±0.2) in tin capsules and sent to the Stable Isotope Facility at the University of California, Davis. The $\delta^{13}C$ and $\delta^{15}N$ values were measured using a PDZ Europa ANCA-GSL elemental analyser interfaced with a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd.). Isotopic values were expressed using delta notation (δ) in parts per thousand (‰), where $\delta X = (R_{sample}/R_{standard} - 1) \times 1{,}000$, with R_{sample} and $R_{standard}$ representing the molar ratios of C^{13}/C^{12} and N^{15}/N^{14} of the sample and standard reference material respectively. The reference material was Vienna-Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Repeated analysis of in-house reference materials (bovine liver, glutamic acid, enriched alanine and nylon 6) showed that precision (*SD*) for $\delta^{13}C$ and $\delta^{15}N$ values were ±0.08‰ and ±0.07‰ for carbon and nitrogen respectively. Repeated analysis of individual samples was slightly higher for $\delta^{13}C$ values, 0.18‰, but similar for $\delta^{15}N$ values, 0.05‰.

2.4 | Meta-analysis of lipid-extracted versus bulk tissues

We performed a meta-analysis to determine if the effects of LE on $\delta^{13}C$ and $\delta^{15}N$ values in liver, muscle and skin are widespread among various taxa and to fit lipid-correction models within and across taxa and trophic groups. Using the search terms "LE", "stable isotopes", "$\delta^{13}C$", "$\delta^{15}N$", "liver", "muscle", and "skin", we conducted literature searches on Google Scholar and Web of Science to find papers where the authors compared $\delta^{13}C$, $\delta^{15}N$ and C:N ratios between LE and bulk tissues. From these papers, we recorded the difference between LE and bulk samples ($\Delta^{13}C_{LE\text{-}B}$ and $\Delta^{15}N_{LE\text{-}B}$) and the $C{:}N_{Bulk}$ and $C{:}N_{LE}$ ratios for liver, muscle and skin, or any subset of those tissues (Table S2). Additionally, we recorded the instrument error reported in each study (Table S2).

2.5 | Statistical analysis and lipid-correction models

We calculated concordance correlation coefficients (ρ), which determine if a treatment affects a sample, to test the similarity of LE and bulk tissues for $\delta^{13}C$ values ($\rho < 0.9$ indicates low concordance between paired values, $0.9 \leq \rho \leq 0.95$ moderate concordance and $\rho > 0.95$ high concordance; Lawrence & Lin, 1989). We used two-sided Welch's t tests to determine if the $\Delta^{13}C_{LE\text{-}B}$ and $\Delta^{15}N_{LE\text{-}B}$ values were different from the instrument error averaged from the studies in the meta-analysis and paired-sample Welch's t tests to determine if $C{:}N_{LE}$ and $C{:}N_{Bulk}$ ratios were different from one another. For dolphins and manatees, we used an ANCOVA to determine if each tissue had separate slopes between $\Delta^{13}C_{LE\text{-}B}$ values and $C{:}N_{Bulk}$ ratios. If the slopes differed, we fit lipid-correction models to each tissue separately, and if slopes did not differ, we fit models to all tissues. We fitted three lipid-correction models

(Fry, 2002; Logan et al., 2008; Post et al., 2007). The first was a mass balance model (Fry, 2002),

$$\Delta^{13}C = p - \frac{p \times f}{C{:}N_{Bulk}}, \quad (1)$$

where f is an estimation of $C{:}N_{LE}$ ratios and p represents protein–lipid discrimination. The second model, which we refer to as the log model, was derived by Logan et al. (2008),

$$\Delta^{13}C = \beta_0 + \beta_1 \times \ln(C{:}N_{Bulk}), \quad (2)$$

where $C{:}N_{LE}$ ratio is estimated from $e^{-\beta_0/\beta_1}$. Finally, we fit a linear model (Post et al., 2007),

$$\Delta^{13}C = a + b \times C{:}N_{Bulk}. \quad (3)$$

We estimated the parameters p, f, β_0 and β_1 using the NLS2 package in R (Grothendieck & Grothendieck, 2013). We used Akaike information criterion (AIC) values and weights to determine the best-fitting model (Burnham & Anderson, 2002) and while we reported the model with the lowest AIC values and highest weights as the best-fitting model in the main text, we discussed other models that had relatively high weights (>0.1). To assess the prediction error, we calculated the mean squared error, mean absolute error, the proportion of samples where the predicted $\Delta^{13}C_{LE\text{-}B}$ values were within 0.5‰ of the observed $\Delta^{13}C_{LE\text{-}B}$ values. For the meta-analysis data, we used two-sided Welch's t tests to determine if the $\Delta^{13}C_{LE\text{-}B}$ and $\Delta^{15}N_{LE\text{-}B}$ values differed from the averaged instrument error for all species as well as for taxonomic (birds, fish, mammals) and trophic (carnivore, omnivore, herbivore) groups. We used an ANOVA to determine differences in $\Delta^{13}C_{LE\text{-}B}$ values among trophic groups and tissues. Additionally, we fit the above lipid-correction models to each tissue for all species, within trophic groups and within taxonomic and trophic groups.

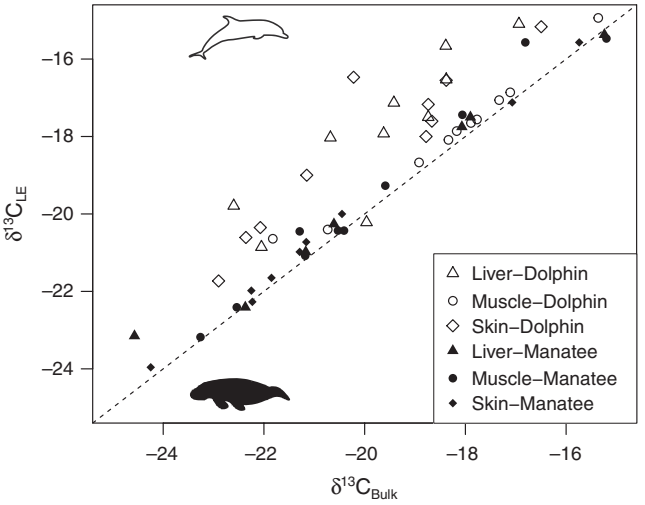

FIGURE 1 Relationship between $\delta^{13}C_{LE}$ and $\delta^{13}C_{bulk}$ values for dolphins (open symbols) and manatees (solid symbols). Samples along the dashed, one-to-one line were not affected by lipid extraction. Samples above the dashed line were affected by lipid extraction. See Table 1 for concordance correlation statistics

3 | RESULTS

3.1 | Dolphins and manatees

For dolphins, ρ values were low for liver and skin and high for muscle (Figure 1; Table 1). The $\Delta^{13}C_{LE\text{-}B}$ values for liver, muscle and skin were all significantly greater than the average instrument error for $\delta^{13}C$ (0.15 ± 0.04 *SD*; Figure 2a; Table 1). LE significantly decreased C:N in all dolphin tissues (Table 1). While the $\Delta^{13}C_{LE\text{-}B}$ values increased with $C{:}N_{Bulk}$ ratios ($F_{5,24}$ = 12.1, p < 0.001, R^2 = 0.66), the slopes did not differ among dolphin tissues (Liver: t = 1.58, p = 0.13; muscle: t = 0.61, p = 0.55; skin: t = 0.47, p = 0.64), and we fit lipid-correction models to all dolphin tissues combined. The best-fitting lipid-correction model was the mass balance (Figure 2c; Table 2). The prediction errors for this model were relatively low and the AIC weights indicated that the log and linear models also fit the data fairly well. The $\Delta^{15}N_{LE\text{-}B}$ values were not significantly different than the average instrument error (0.13 ± 0.05 *SD*) for any tissues (Figure 2b; Table 1).

For manatees, ρ values were high for all tissues (Figure 1; Table 1). The $\Delta^{13}C_{B\text{-}LE}$ values were not different than the averaged instrument error in any tissue (Figure 2d; Table 1). LE decreased the C:N ratios in liver and skin but not muscle (Table 1). The $\Delta^{13}C_{LE\text{-}B}$ values increased with $C{:}N_{Bulk}$ ratios ($F_{5,20}$ = 3.392, p = 0.022, R^2 = 0.324) and varied among tissues (Liver: t = −2.485, p = 0.03; muscle: t = 0.36, p = 0.73; skin: t = 5.13, p < 0.001), and we fit lipid-correction models to each tissue. For liver, the best-fitting model was linear, but, for muscle and skin, the null model best fit the data, highlighting the small effect of LE on manatee tissues (Figure 2f; Table 2). LE did not affect $\Delta^{15}N_{LE\text{-}B}$ values (Figure 2e; Table 1).

3.2 | Meta-analysis

We found LE data on liver, muscle or skin for 128 terrestrial and aquatic species from 27 publications (Table S1). The $\Delta^{13}C_{LE\text{-}B}$ values were greater than the average instrument error for all three tissues (Figure 3a; Table 3). The $\Delta^{13}C_{LE\text{-}B}$ values also differed among trophic groups (Figure 4a; $F_{2,124}$ = 8.17, p < 0.001) and tissue (Figure 3; $F_{2,124}$ = 3.18, p = 0.045). Using a Tukey's honestly significant differences test, we found difference in the $\Delta^{13}C_{LE\text{-}B}$ values between carnivores and herbivores (p < 0.001) and marginally different values between muscle and liver (p = 0.051). For all species, the best-fitting lipid-correction models were mass balance for liver and skin and linear for muscle, but the prediction error was quite high for these models (large differences between the predicted and observed $\Delta^{13}C_{LE\text{-}B}$ values that suggest low predictive power) and the null model was also a good fit for liver (Figure 3c; Table 2; Table S2). For all carnivores, the best-fitting models were the null model

TABLE 1 Mean ± *SD* of $\delta^{13}C$, $\delta^{15}N$ and C:N in lipid-extracted and bulk tissues of dolphins and manatees. *t* Test on $\delta^{13}C$ and $\delta^{15}N$ compared the difference between lipid-extracted and bulk values to the average instrument precision from the meta-analysis ($\delta^{13}C$ = 0.15 and $\delta^{15}N$ = 0.13). For C:N, paired *t* tests compared lipid-extracted and bulk values. Concordance correlation coefficient (ρ) for bulk and LE $\delta^{13}C$ tested the agreement between lipid-extracted and bulk samples, with ρ < 0.9 indicating poor agreement between the samples and ρ > 0.95 indicating strong agreement. C_b values measure deviation from a 1:1 line, and C_b = 1 indicates no deviation from a 1:1 line

					t Test statistics			Correlation statistics	
	Tissue	Ave. extracted	Ave. bulk	Ave. difference	*t*	*df*	*p*	ρ	C_b
Dolphin									
$\delta^{13}C$	Liver	−17.61 ± 1.84	−19.64 ± 1.84	2.03 ± 0.62	9.130	8	<0.001	0.56	0.59
	Muscle	−17.97 ± 1.70	−18.34 ± 1.83	0.37 ± 0.29	2.390	9	0.043	0.96	0.97
	Skin	−18.27 ± 2.10	−19.98 ± 2.09	1.71 ± 0.82	5.970	9	<0.001	0.67	0.73
$\delta^{15}N$	Liver	15.63 ± 1.72	15.94 ± 1.81	−0.31 ± 0.59	−2.250	8	0.054	—	—
	Muscle	14.58 ± 1.81	14.30 ± 1.87	0.27 ± 0.30	1.530	9	0.164	—	—
	Skin	14.79 ± 1.61	14.66 ± 1.73	0.12 ± 0.34	−0.048	9	0.960	—	—
C:N	Liver	3.26 ± 0.15	4.48 ± 0.43	−1.22 ± 0.28	−13.000	8	<0.001	—	—
	Muscle	3.17 ± 0.07	3.36 ± 0.15	−0.18 ± 0.15	−3.852	9	0.004	—	—
	Skin	3.11 ± 0.05	4.40 ± 0.83	−1.28 ± 0.83	−4.900	9	<0.001	—	—
Manatee									
$\delta^{13}C$	Liver	−19.63 ± 2.85	−19.99 ± 3.14	0.36 ± 0.51	1.355	6	0.224	0.98	0.99
	Muscle	−19.57 ± 2.62	−19.88 ± 2.53	0.31 ± 0.45	1.491	9	0.170	0.98	0.99
	Skin	−20.47 ± 2.62	−20.70 ± 2.67	0.23 ± 0.18	2.154	7	0.063	0.99	1.00
$\delta^{15}N$	Liver	8.54 ± 1.28	8.79 ± 1.20	−0.24 ± 0.41	−2.399	6	0.053	—	—
	Muscle	7.66 ± 1.02	7.24 ± 1.37	0.42 ± 0.60	1.508	9	0.166	—	—
	Skin	8.59 ± 1.04	8.51 ± 1.09	0.07 ± 0.17	−1.027	7	0.335	—	—
C:N	Liver	3.42 ± 0.10	4.24 ± 0.35	−0.83 ± 0.28	−7.913	6	<0.001	—	—
	Muscle	3.25 ± 0.05	3.46 ± 0.40	−0.21 ± 0.38	−1.767	9	0.111	—	—
	Skin	3.34 ± 0.23	3.74 ± 0.21	−0.40 ± 0.10	−11.427	7	<0.001	—	—

Abbreviation: LE, lipid extraction.

for liver, linear for muscle and mass balance for skin, but predictive errors were quite high for all tissues (Figure 4b; Table 2). For all herbivores, the best-fitting model was the mass balance for muscle (we did not have enough liver or skin samples for analysis) and the prediction errors were low (Figure 4c; Table 2). The $\Delta^{15}N_{LE\text{-}B}$ values for muscle were different than the average instrument error, but values for liver and skin were not (Figure 3b; Table 3).

We had enough data from three taxonomic groups, birds (muscle; *N* = 28), fish (liver and muscle; *N* = 68) and mammals (liver, muscle, skin; *N* = 37) to test if the $\Delta^{13}C_{LE\text{-}B}$ and $\Delta^{15}N_{LE\text{-}B}$ values differed from the average instrument error and to fit lipid-correction models. The $\Delta^{13}C_{LE\text{-}B}$ values were different than the average instrument error for muscle in carnivorous birds, carnivorous fish and herbivorous mammals, for liver in omnivorous fish and carnivorous mammals and for skin in carnivorous mammals (Figure 5; Table 3). The best-fitting model for muscle in carnivorous birds was the mass balance (relatively low prediction errors), and in herbivorous birds, it was the null model (Figure 6a,b; Table 2). For liver and muscle in carnivorous fish, the best-fitting models were null and linear respectively (relatively low prediction errors for muscle; Figure 6e; Table 2). For carnivorous mammals, the best-fitting models included the null for liver, linear for muscle (low prediction errors) and mass balance for skin (high prediction errors; Figure 6c; Table 2). For muscle in herbivorous mammals, the best-fitting model was mass balance (low prediction errors; Figure 6d). Our models included both aquatic and terrestrial organisms and the inclusion of organisms from both habitats appear to have little effect on the curves (Figure 6). The $\Delta^{15}N_{LE\text{-}B}$ values for muscle in herbivorous birds and carnivorous and omnivorous fish, as well as liver in carnivorous mammals, were significantly different than the instrument error (Figure 5; Table 3).

4 | DISCUSSION

4.1 | Effects of LE on $\delta^{13}C$ values

The effects of LE on $\delta^{13}C$ values varied between dolphins and manatees. For dolphins, LE had large effects on liver and skin and smaller, although significant, effects on muscle, indicating lipid is necessary for each of these tissues. Other studies on dolphins, which focused solely on skin because it is easily obtainable from live dolphins, found lower $\Delta^{13}C_{LE\text{-}B}$ values compared to this study

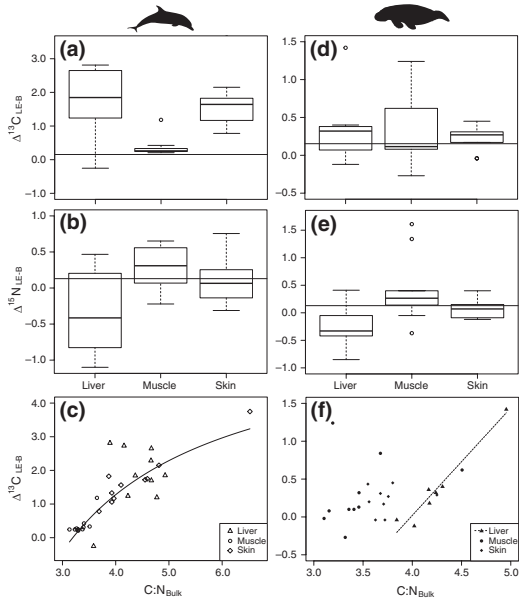

FIGURE 2 Effects of lipid extraction on each tissue type and the relationship between $\Delta^{13}C_{LE\text{-}B}$ and $C{:}N_{bulk}$ values for dolphins (left) and manatees (right). $\Delta^{13}C_{LE\text{-}B}$ values in each tissue from (a) dolphins and (d) manatees. $\Delta^{15}N_{LE\text{-}B}$ in each tissue from (b) dolphins and (e) manatees. Solid, horizontal lines represent the average instrument error for carbon (0.10) and nitrogen (0.13) across studies. $\Delta^{13}C_{LE\text{-}B}$ values versus $C{:}N_{bulk}$ values for (c) dolphins and (f) manatees

but also concluded LE was necessary (~1.0‰ and 0.2‰ compared to 1.71‰ in our study; Giménez et al., 2017; Wilson et al., 2014). We are the first to determine LE effects on muscle and liver in dolphins, tissues that are important for isotopic analysis of stranded animals (Murray, Carmichael, Collins, Russell, & Deming, 2019). For manatees, LE was unnecessary for all tissues. Similarly, although meta-analysis showed significant LE effects on all tissues across species, these effects were smaller in herbivores in general as well as in mammalian and avian herbivores in particular (including manatees). These findings suggest that LE effects not only differ between species but may specifically differ with trophic niche.

Manatees and other herbivores may not store a substantial quantity of fats in the liver, skin and muscle that are depleted in $\delta^{13}C$ values. Similar to our findings that $\Delta^{13}C_{LE\text{-}B}$ values are small in herbivores, general trophic discrimination values are lower in herbivores compared to carnivores (Vander Zanden & Rasmussen, 2001). Herbivore diets are low in lipids (Post et al., 2007), and herbivores must synthesize them. Vander Zanden and Rasmussen (2001) suggested that the small trophic discrimination in herbivores is caused by lipid synthesis. If this was the case, however, removing lipids from herbivore tissues should still alter the $\delta^{13}C$ values, which it does not in manatees. An alternative explanation, which explains both the small trophic discrimination and lack of LE effects in herbivores, is that the demand to synthesize large quantities of lipids may require utilizing all available carbon, resulting

TABLE 2 Best-fitting lipid-correction models including all species for all trophic groups, all carnivores and herbivores, bird and mammal carnivores and herbivores, fish carnivores and omnivores, dolphins and manatees. *N* refers to number of species included in the model, except for dolphin and manatee models, in which is refer to number of individuals. Table S1 contains information regarding the species and data included in the models

Taxa	Trophic group	Tissue	Best-fitting model	*N*	Equation	AIC weight	*MSE*	*MAE*	Pred < 0.5‰ (%)
All	All	Liver	Mass balance	22	$\Delta^{13}C = 3.04 - 8/C{:}N_{Bulk}$	0.427	0.79	0.89	0.5
All	All	Muscle	Linear	99	$\Delta^{13}C = -1.42 + 0.58 \times C{:}N_{Bulk}$	0.956	0.22	0.47	0.63
All	All	Skin	Mass balance	13	$\Delta^{13}C = 5.32 - 15.85/C{:}N_{Bulk}$	0.679	0.23	0.48	0.69
All	Carnivore	Liver	Null	20	$\Delta^{13}C = 1 + error$	0.362			
All	Carnivore	Muscle	Linear	79	$\Delta^{13}C = -1.24 + 0.56 \times C{:}N_{Bulk}$	0.859	0.22	0.47	0.62
All	Carnivore	Skin	Mass balance	11	$\Delta^{13}C = 5.26 - 15.33/C{:}N_{Bulk}$	0.623	0.21	0.45	0.64
All	Herbivore	Muscle	Mass balance	18	$\Delta^{13}C = 2.82 - 9.31/C{:}N_{Bulk}$	0.439	0.08	0.28	0.83
Mammals	Carnivore	Liver	Null	15	$\Delta^{13}C = 1 + error$	0.741			
Mammals	Carnivores	Muscle	Linear	15	$\Delta^{13}C = -2.93 + 0.9 \times C{:}N_{Bulk}$	0.448	0.04	0.2	0.87
Mammals	Carnivores	Skin	Mass balance	11	$\Delta^{13}C = 5.26 - 15.31/C{:}N_{Bulk}$	0.623	0.21	0.45	0.64
Mammals	Herbivores	Muscle	Mass balance	11	$\Delta^{13}C = 2.81 - 9.16/C{:}N_{Bulk}$	0.345	0.08	0.28	0.82
Birds	Carnivores	Muscle	Mass balance	9	$\Delta^{13}C = 3.5 - 11.38/C{:}N_{Bulk}$	0.373	0.11	0.33	0.78
Birds	Herbivores	Muscle	Null	7	$\Delta^{13}C = 1 + error$	0.540			
Fish	Carnivores	Liver	Null	7	$\Delta^{13}C = 1 + error$	0.846			
Fish	Carnivores	Muscle	Linear	53	$\Delta^{13}C = -0.77 + 0.49 \times C{:}N_{Bulk}$	0.914	0.45	0.2	0.7
Dolphin	Carnivore	All	Mass balance	29	$\Delta^{13}C = 6.43 - 20.25/C{:}N_{Bulk}$	0.478	0.14	0.38	0.8
Manatee	Herbivore	Liver	Linear	7	$\Delta^{13}C = -5.66 + 1.42 \times C{:}N_{Bulk}$	0.582	0.01	0.09	1
Manatee	Herbivore	Muscle	Null	10	$\Delta^{13}C = 1 + error$	0.641			
Manatee	Herbivore	Skin	Null	9	$\Delta^{13}C = 1 + error$	0.770			

Abbreviations: AIC, Akaike information criterion; MAE, mean absolute error; MSE, mean squared error.

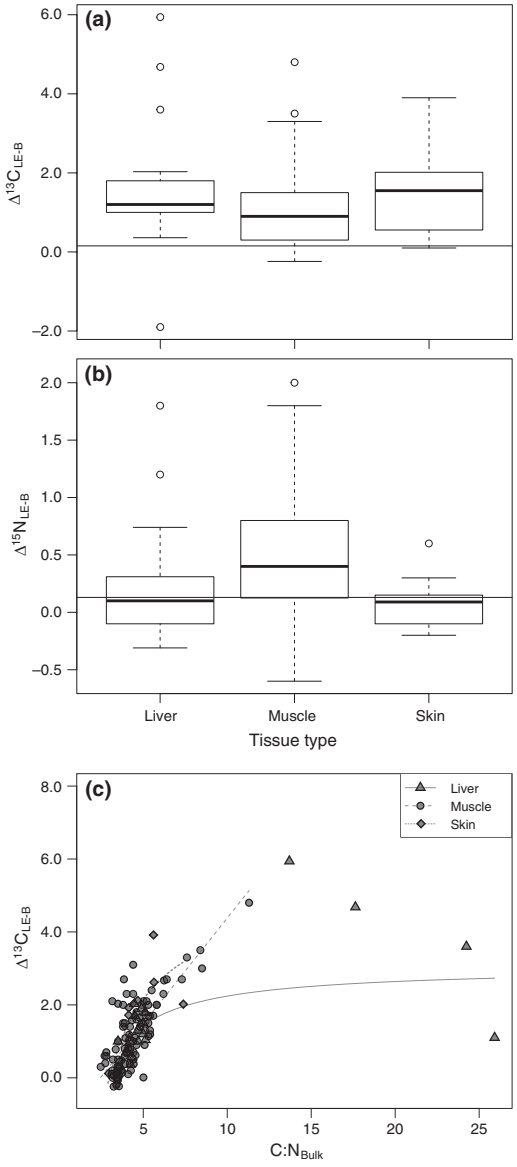

FIGURE 3 Effects of lipid extraction on each tissue type and the relationship between $\Delta^{13}C_{LE-B}$ and C:N_{bulk} values for the meta-analysis. (a) Boxplot of $\Delta^{13}C_{LE-B}$ values of each tissue, (b) boxplot of $\Delta^{15}N_{LE-B}$ values of each tissue and (c) $\Delta^{13}C_{LE-B}$ values versus C:N_{Bulk} values. The linear regression equations are provided in the legend and the presence on a line indicates statistically significant regressions. See Figure 2 legend for information on solid, horizontal lines

in little to no discrimination against ^{13}C. A similar pattern arises in fast-growing crickets, which discriminate against carbon isotopes less than slow-growing crickets because the former must utilize more available carbon to meet the demands of faster growth (Cloyed, Eason, & Dell, 2018). Hence, little isotopic discrimination may occur during natural lipid synthesis in herbivores. Future research on forecasting when to lipid extract should consider trophic group.

In contrast to previous recommendations to lipid extract only when C:N_{Bulk} ratios >3.5, we found this threshold did not provide a good reference for when to lipid extract. The C:N_{Bulk} ratios in dolphin muscle were <3.5 yet required LE, while the C:N_{Bulk} ratios in manatee liver and skin were >3.5 but did not require LE. A growing body of evidence suggests that relying on C:N_{Bulk} ratios to determine the necessity of LE is overly simplistic (Fagan et al., 2011; Patterson & Carmichael, 2016; Wilson et al., 2014). Direct tests have demonstrated that C:N_{Bulk} ratios are not always a good predictor of lipid content (Fagan et al., 2011). Furthermore, many organisms store carbon in forms other than lipids; oysters, for example, store carbon mostly as glycogen, which has a high C:N_{Bulk} ratio but is not depleted in ^{13}C and does not require LE (Patterson & Carmichael, 2016). While C:N_{Bulk} ratios provide valuable information regarding the elemental composition of tissues, explicitly relying on them to determine when to lipid extract should be avoided (Fagan et al., 2011). Overall, our data emphasize that generalized relationships between the C:N ratios and lipid content are not necessarily applicable to determining a species- and study-specific need for LE.

TABLE 3 *t* Test statistics for the comparison between $\Delta^{13}C_{LE-B}$, $\Delta^{15}N_{LE-B}$ and instrument error for all species as well as among taxonomic and trophic groups from the meta-analysis

Isotope	Tissue	Trophic group	*t*	*df*	*p*
All					
$\delta^{13}C$	Liver	—	4.15	20	<0.001
	Muscle	—	11.10	127	<0.001
	Skin	—	4.93	15	<0.001
$\delta^{15}N$	Liver	—	1.27	21	0.220
	Muscle	—	5.52	98	<0.001
	Skin	—	−1.40	15	0.180
Bird					
$\delta^{13}C$	Muscle	Carnivore	5.30	19	<0.001
	Muscle	Herbivore	1.51	7	0.180
$\delta^{15}N$	Muscle	Carnivore	−1.51	19	0.150
	Muscle	Herbivore	−2.63	7	0.034
Mammals					
$\delta^{13}C$	Liver	Carnivore	7.34	8	<0.001
	Muscle	Carnivore	1.50	16	0.150
	Muscle	Herbivore	2.29	10	0.045
	Skin	Carnivore	5.61	13	<0.001
$\delta^{15}N$	Liver	Carnivore	−2.42	8	0.042
	Muscle	Carnivore	0.69	16	0.500
	Muscle	Herbivore	−1.31	10	0.220
	Skin	Carnivore	−1.17	13	0.260
Fish					
$\delta^{13}C$	Liver	Carnivore	3.73	6	0.010
	Muscle	Carnivore	10.50	61	<0.001
	Muscle	Omnivore	5.71	5	0.002
$\delta^{15}N$	Liver	Carnivore	1.62	6	0.160
	Muscle	Carnivore	5.63	61	<0.001
	Muscle	Omnivore	2.85	5	0.036

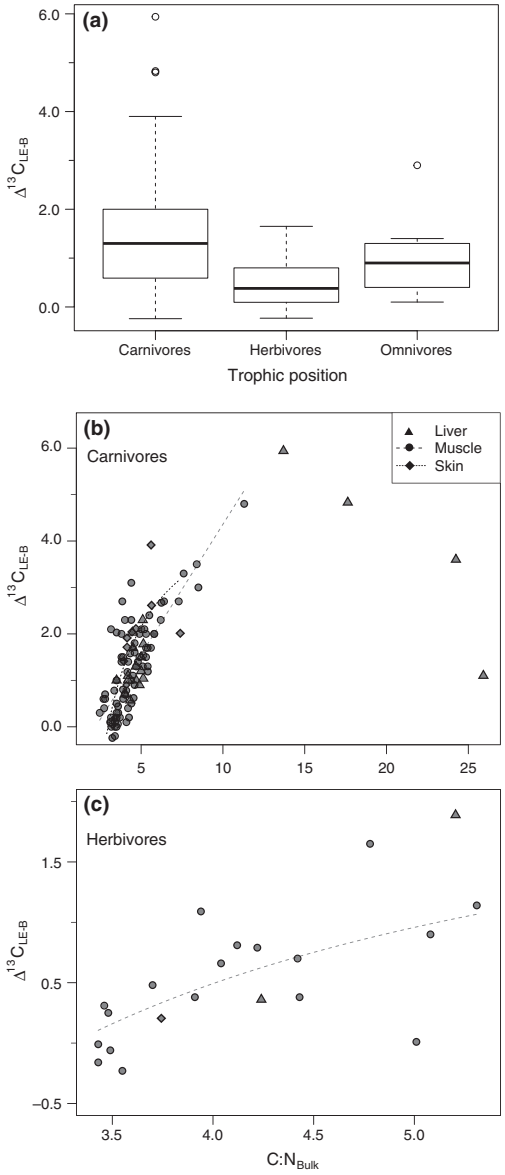

FIGURE 4 (a–c) $\Delta^{13}C_{LE-B}$ for different trophic groups based on data from all tissues. See Figure 2 legend for information on solid, horizontal lines

4.2 | Lipid-correction models

The best-fitting lipid-correction models varied among tissue, taxa and trophic group, with correction models applied to all species generally having lower fits and higher prediction errors. Most studies on lipid correction that found strong predictive fits were performed on fish muscle (Abrantes, Semmens, Lyle, & Nichols, 2012; Hoffman & Sutton, 2010; Logan et al., 2008), and we also found that muscle was a good tissue for lipid correction. A mixture of linear and mass balance models best fit muscle data among different taxa and trophic groups for our study, suggesting, along with the work of others, that best corrective model for muscle can vary among taxonomic and trophic groups (Doucette, Wissel, & Somers, 2010; Ehrich et al., 2011; Yurkowski, Hussey, Semeniuk, Ferguson, & Fisk, 2015). Data from skin were also a good tissue for lipid correction because they were almost exclusively fit by mass balance models, which was also true with a species-specific model on Pacific walruses (Clark, Horstmann, & Misarti, 2019). The only time skin was fit by another model in our results was the null model for manatees, likely due to the minimal effect of LE on $\delta^{13}C$ values in manatee skin (and muscle). These findings also explain the low prediction errors for the non-null models for manatee tissues (Table S2), making correction models for these manatee tissues of little utility. Liver data were frequently fit by null models and overall had high prediction errors, a pattern that occurs within species as well (Clark et al., 2019), suggesting it is not a good tissue on which to use lipid-correction models. Finally, in general, models on herbivore tissues seem to have little utility because LE has little effect on $\delta^{13}C$ values. Overall, the models with the lowest predictive errors occurred when applied to specific taxonomic and trophic groups (i.e. carnivorous and herbivorous birds and mammals and carnivorous fish) as well as to specific species (dolphins and manatees). Our data are consistent with previous studies that the precision of lipid-correction models, and thus their utility, increases with taxonomic and trophic specificity (Clark et al., 2019; Hoffman & Sutton, 2010; Logan et al., 2008). We included both aquatic and terrestrial organisms because previous work that has analysed organisms from each habitat separately produced nearly identical models (Ehrich et al., 2011; Post et al., 2007), and we found that trophic group had a larger effect on these models. Studies applying species-specific models have obtained even better fits with lower predictive models (Clark et al., 2019; Hoffman & Sutton, 2010). Thus, our analyses support using the most taxonomically specific models available as the best way to decrease error from lipid corrective models.

4.3 | Effects of LE on $\delta^{15}N$ values

While LE had smaller effects on $\delta^{15}N$ values compared to $\delta^{13}C$ values, these differences were significant in some instances. LE generally had the greatest effect on $\delta^{15}N$ values in muscle (Logan & Lutcavage, 2008; Sweeting et al., 2006), which was the case for all species in our study. However, as was the case for carbon, general patterns among species were not always true within species. For example, the $\delta^{15}N$ values in muscle of dolphin and manatee were largely unaffected by LE. Thus, our data indicated that researchers performing stable isotope analysis on tissues from dolphins do not need to separately analyse tissues for $\delta^{15}N$ values, and those performing research on manatees do not need to lipid extract, cutting down on labour and costs. The patterns of LE on the $\delta^{15}N$ values across species in our meta-analysis, however, support the expected trend that LE can affect the $\delta^{15}N$ values in muscle via the effects of the polar solvent on the polar, nitrogenous compounds common in muscle (Logan & Lutcavage, 2008; Sotiropoulos, Tonn, & Wassenaar, 2004; Sweeting et al., 2006). Overall, these analyses indicate that if LE affects $\delta^{15}N$ values, it is likely to affect muscle more than other tissues.

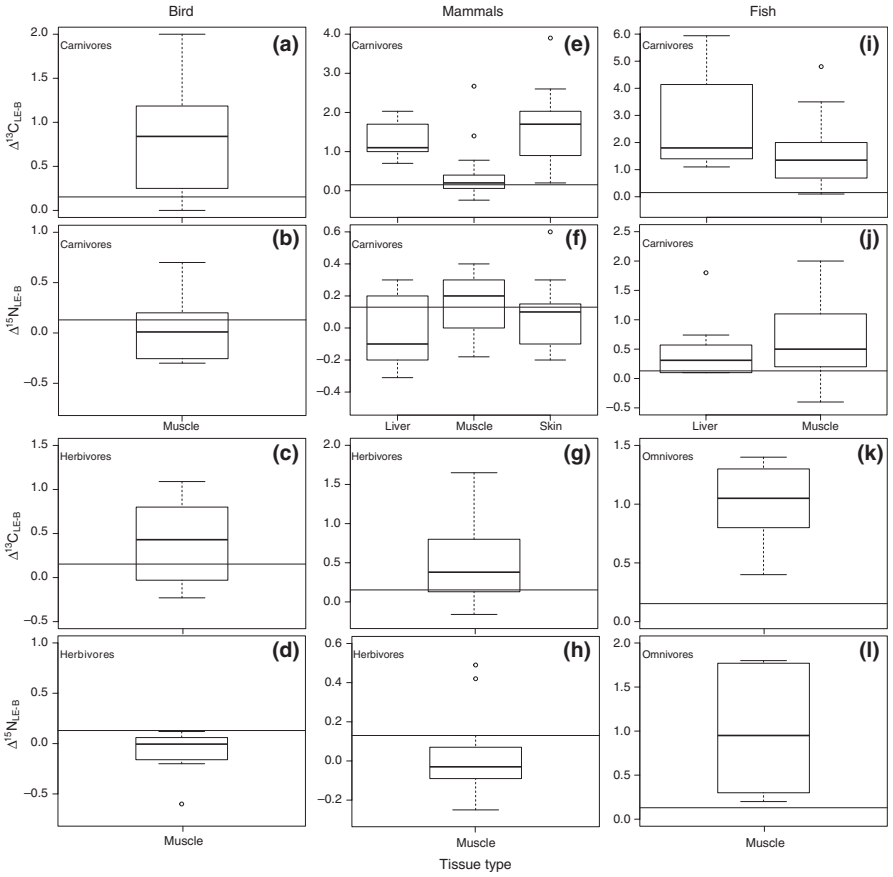

FIGURE 5 $\Delta^{13}C_{LE-B}$ (top) and $\Delta^{15}N_{LE-B}$ (bottom) values of each tissue for different taxonomic groups: birds (left), fish (middle) and mammals (right). (a) $\Delta^{13}C_{LE-B}$ and (b) $\Delta^{15}N_{LE-B}$ values for carnivorous birds, (c) $\Delta^{13}C_{LE-B}$ and (d) $\Delta^{15}N_{LE-B}$ values for herbivorous birds, (e) $\Delta^{13}C_{LE-B}$ and (f) $\Delta^{15}N_{LE-B}$ values for carnivorous mammals, (g) $\Delta^{13}C_{LE-B}$ and (h) $\Delta^{15}N_{LE-B}$ for herbivorous mammals, (i) $\Delta^{13}C_{LE-B}$ and (j) $\Delta^{15}N_{LE-B}$ for carnivorous fish and (k) $\Delta^{13}C_{LE-B}$ and (l) $\Delta^{15}N_{LE-B}$ for omnivorous fish. See Figure 2 legend for information on solid, horizontal lines

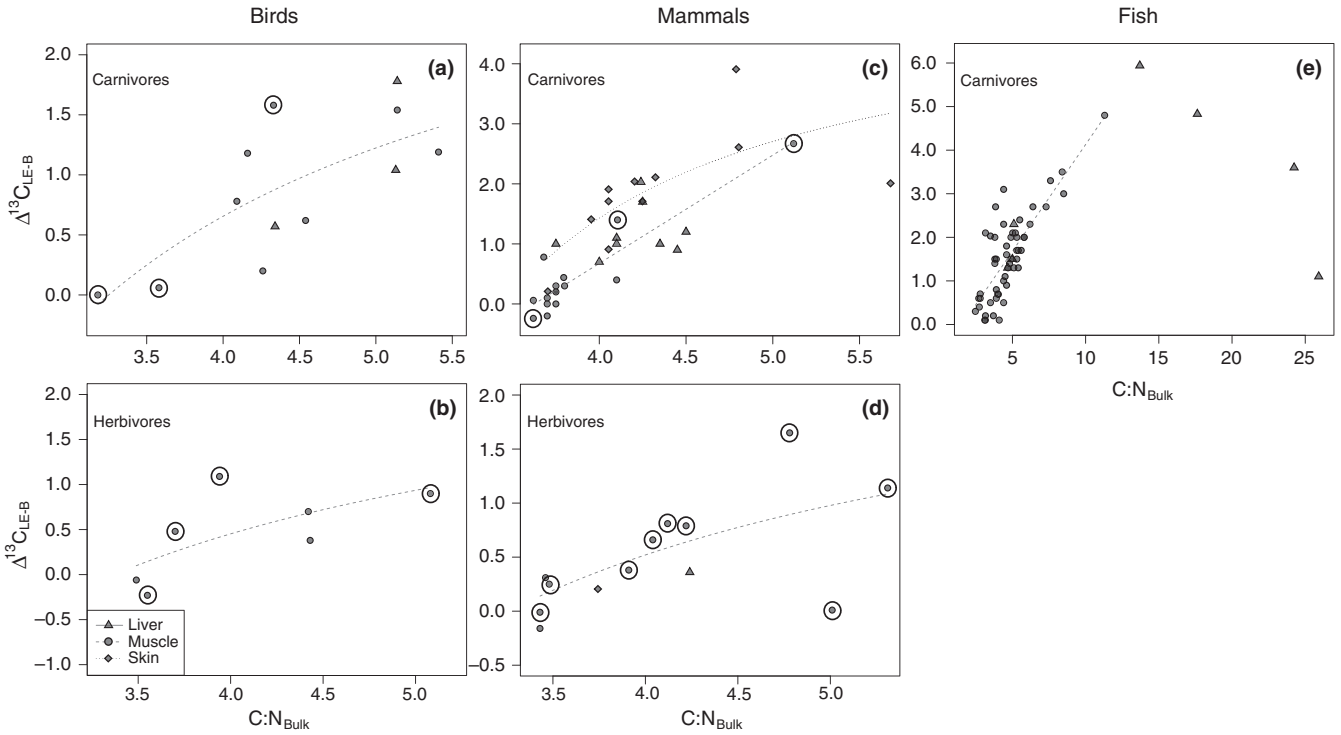

FIGURE 6 Lipid-correction models of different taxonomic and trophic groups. (a) Carnivorous and (b) herbivorous birds, (c) carnivorous and (d) herbivorous mammals and (e) carnivorous fish. The absence of a line for a particular tissue within these groups indicates that the null mode best fit the data for that tissue. Circled symbols indicate terrestrial species

5 | CONCLUSIONS AND RECOMMENDATIONS

- We found that while commonly held assumptions about the necessity and effects of LE were reliable for a broad range of species considered in our meta-analysis, important species-specific differences like those we found between dolphins and manatees should not be overlooked. Thus, researchers should use the most species-specific information available for their organism of interest when deciding whether LE is needed.
- The effects of LE were greater for carnivores than herbivores, and, in the case of manatees, LE had no effect on the $\delta^{13}C$ values and could be avoided. Further work is needed to clearly determine why these patterns persist among trophic groups and some species are more affected than others.
- Muscle and skin were good tissues on which to apply lipid-correction models, whereas liver was not. Increased taxonomic and trophic specificity increased model fit and decreased predictive errors. Researchers should use the most specific model available and develop new models when needed rather than applying poorly fitting models from other studies.
- Testing the effects of LE on newly studied species and tissue types prior to full analysis will provide valuable new data to better understand isotopic discrimination among species and tissues and will save resources.

ACKNOWLEDGEMENTS

This research was made possible in part by a grant from the Gulf of Mexico Research Initiative with a subaward to R.H.C. and C.S.C. in collaboration with the National Marine Mammal Foundation (NMMF) under Grant Agreement No. SA 18-12-003 with Ocean Leadership. K.P.D. and M.R.H. were funded by the Dauphin Island Sea Lab, University of South Alabama and the National Fish and Wildlife Foundation (NFWF #45720). The statements, findings, conclusions, recommendations, opinions and policies are those of the authors and do not necessarily reflect the views of Ocean Leadership, NMMF or NFWF. No authors have any conflicts of interest to report. Nothing contained herein constitutes an endorsement in any respect by NFWF. We would like to thank C. Nelson-Seely, N. Wingers, M. Russell, A. Aven, E. Hieb and ALMMSN/MSN staff, students and volunteers for assistance in collection of carcasses and sample inventory, and C. Zubrick for assistance in sample processing. We would also like to thank an anonymous reviewer whose comments greatly improved the quality of the manuscript.

AUTHORS' CONTRIBUTIONS

C.S.C., K.P.D. and R.H.C. conceived the ideas and methodology of the project; K.P.D. and M.R.H. assisted in the collection and preparation of tissues; C.S.C. analysed the data and led writing the manuscript. All authors contributed critically to the discussion of the results and editing of the manuscript, and all authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data used in this article are available at https://doi.org/10.5061/dryad.tht76hdw8 (Cloyed, DaCosta, Hodanbosi, & Carmichael, 2020).

ORCID


Carl S. Cloyed https://orcid.org/0000-0001-6321-5808

REFERENCES

Abrantes, K. G., Semmens, J. M., Lyle, J. M., & Nichols, P. D. (2012). Normalisation models for accounting for fat content in stable isotope measurements in salmonid muscle tissue. *Marine Biology*, *159*, 57–64. https://doi.org/10.1007/s00227-011-1789-1

Ankjærø, T., Christensen, J. T., & Grønkjær, P. (2012). Tissue-specific turnover rates and trophic enrichment of stable N and C isotopes in juvenile Atlantic cod *Gadus morhua* fed three different diets. *Marine Ecology Progress Series*, *461*, 197–209. https://doi.org/10.3354/meps09871

Bloomfield, A. L., Elsdon, T. S., Walther, B. D., Gier, E. J., & Gillanders, B. M. (2011). Temperature and diet affect carbon and nitrogen isotopes of fish muscle: Can amino acid nitrogen isotopes explain effects? *Journal of Experimental Marine Biology and Ecology*, *399*, 48–59. https://doi.org/10.1016/j.jembe.2011.01.015

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). New York, NY: Springer.

Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, *46*, 443–453. https://doi.org/10.1111/j.1365-2664.2009.01620.x

Clark, C. T., Horstmann, L., & Misarti, N. (2019). Lipid normalization and stable isotope discrimination in Pacific walrus tissues. *Scientific Reports*, *9*, 5843. https://doi.org/10.1038/s41598-019-42095-z

Cloyed, C. S., DaCosta, K. P., Hodanbosi, M. R., & Carmichael, R. H. (2020). Data from: The effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values and use of lipid-correction models across tissues, taxa and trophic groups. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.tht76hdw8

Cloyed, C. S., & Eason, P. K. (2016). Different ecological conditions support individual specialization in closely related, ecologically similar species. *Evolutionary Ecology*, *30*, 379–400. https://doi.org/10.1007/s10682-016-9825-8

Cloyed, C. S., Eason, P. K., & Dell, A. I. (2018). The thermal dependence of carbon stable isotope incorporation and trophic discrimination in the domestic cricket, *Acheta domesticus*. *Journal of Insect Physiology*, *107*, 34–40. https://doi.org/10.1016/j.jinsphys.2018.02.003

DeNiro, M. J., & Epstein, S. (1977). Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, *197*, 261–263. https://doi.org/10.1126/science.327543

Doucette, J. L., Wissel, B., & Somers, C. M. (2010). Effects of lipid extraction and lipid normalization on stable carbon and nitrogen isotope ratios in double-crested cormorants: Implications for food web studies. *Waterbirds*, 273–284. https://doi.org/10.1675/063.033.0302

Ehrich, D., Tarroux, A., Stien, J., Lecomte, N., Killengreen, S., Berteaux, D., & Yoccoz, N. G. (2011). Stable isotope analysis: Modelling lipid normalization for muscle and eggs from arctic mammals and birds. *Methods in Ecology and Evolution*, *2*, 66–76. https://doi.org/10.1111/j.2041-210X.2010.00047.x

Fagan, K.-A., Koops, M. A., Arts, M. T., & Power, M. (2011). Assessing the utility of C:N ratios for predicting lipid content in fishes. *Canadian*

Journal of Fisheries and Aquatic Sciences, *68*, 374–385. https://doi.org/10.1139/F10-119

Fry, B. (2002). Stable isotopic indicators of habitat use by Mississippi River fish. *Journal of the North American Benthological Society*, *21*, 676–685. https://doi.org/10.2307/1468438

Geraci, J. R., & Lounsbury, V. J. (2005). *Marine mammals ashore: A field guide for strandings*. Baltimore, MD: National Aquarium.

Giménez, J., Ramírez, F., Forero, M. G., Almunia, J., de Stephanis, R., & Navarro, J. (2017). Lipid effects on isotopic values in bottlenose dolphins (*Tursiops truncatus*) and their prey with implications for diet assessment. *Marine Biology*, *164*, 122. https://doi.org/10.1007/s00227-017-3154-5

Grothendieck, G., & Grothendieck, M. G. (2013). *Package 'nls2'*. R package version 0.2. Citeseer. Retrieved from https://CRAN.Rproject.org/package=nls2

Hoffman, J. C., & Sutton, T. T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*, 956–964. https://doi.org/10.1016/j.dsr.2010.05.003

Hooker, S. K., Iverson, S. J., Ostrom, P., & Smith, S. C. (2001). Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Canadian Journal of Zoology*, *79*, 1442–1454. https://doi.org/10.1139/z01-096

Lawrence, I., & Lin, K. (1989). A concordance correlation coefficient to evaluate reproducibility. *Biometrics*, *45*, 255–268. https://doi.org/10.2307/2532051

Lawson, J. W., Magalhães, A. M., & Miller, E. H. (1998). Important prey species of marine vertebrate predators in the northwest Atlantic: Proximate composition and energy density. *Marine Ecology Progress Series*, *164*, 13–20. https://doi.org/10.3354/meps164013

Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, *88*, 42–48. https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, *77*, 838–846. https://doi.org/10.1111/j.1365-2656.2008.01394.x

Logan, J. M., & Lutcavage, M. E. (2008). A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. *Rapid Communications in Mass Spectrometry*, *22*, 1081–1086. https://doi.org/10.1002/rcm.3471

MacAvoy, S. E., Cortese, N., Cybulski, J., Hohn, A. A., & Macko, S. A. (2017). Sources of stable isotope variation among stranded Western Atlantic dolphins (*Tursiops truncatus*) in North Carolina. *Marine Mammal Science*, *33*, 1224–1234. https://doi.org/10.1111/mms.12425

Mignucci-Giannoni, A. A., & Beck, C. A. (1998). The diet of the manatee (*Trichechus manatus*) in Puerto Rico. *Marine Mammal Science*, *14*, 394–397. https://doi.org/10.1111/j.1748-7692.1998.tb00733.x

Murray, R. E., Carmichael, R. H., Collins, M. K., Russell, M. L., & Deming, A. C. (2019). Dead or alive: Use of elemental analysis to determine status of stranded perinate bottlenose dolphins (*Tursiops truncatus*). *Gulf and Caribbean Research*, *30*, SC28–SC32. https://doi.org/10.18785/gcr.3001.12

Newsome, S. D., Chivers, S. J., & Berman Kowalewski, M. (2018). The influence of lipid-extraction and long-term DMSO preservation on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in cetacean skin. *Marine Mammal Science*, *34*, 277–293. https://doi.org/10.1111/mms.12454

Newsome, S. D., Fogel, M. L., Kelly, L., & del Rio, C. M. (2011). Contributions of direct incorporation from diet and microbial amino acids to protein synthesis in Nile tilapia. *Functional Ecology*, *25*, 1051–1062. https://doi.org/10.1111/j.1365-2435.2011.01866.x

Njinkoué, J.-M., Barnathan, G., Miralles, J., Gaydou, E.-M., & Samb, A. (2002). Lipids and fatty acids in muscle, liver and skin of three edible fish from the Senegalese coast: *Sardinella maderensis*, *Sardinella aurita* and *Cephalopholis taeniops*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *131*, 395–402. https://doi.org/10.1016/S1096-4959(01)00506-1

O'Donovan, S. A., Budge, S. M., Hobson, K. A., Kelly, A. P., & Derocher, A. E. (2018). Intrapopulation variability in wolf diet revealed using a combined stable isotope and fatty acid approach. *Ecosphere*, *9*, e02420. https://doi.org/10.1002/ecs2.2420

Papiol, V., Fanelli, E., Cartes, J. E., Rumolo, P., & López-Pérez, C. (2017). A multi-tissue approach to assess the effects of lipid extraction on the isotopic composition of deep-sea fauna. *Journal of Experimental Marine Biology and Ecology*, *497*, 230–242. https://doi.org/10.1016/j.jembe.2017.10.001

Patterson, H., & Carmichael, R. (2016). The effect of lipid extraction on carbon and nitrogen stable isotope ratios in oyster tissues: Implications for glycogen-rich species. *Rapid Communications in Mass Spectrometry*, *30*, 2594–2600. https://doi.org/10.1002/rcm.7759

Payo-Payo, A., Ruiz, B., Cardona, L., & Borrell, A. (2013). Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*. *Aquatic Biology*, *18*, 141–147. https://doi.org/10.3354/ab00497

Perkins, M. J., McDonald, R. A., Veen, F., Kelly, S. D., Rees, G., & Bearhop, S. (2013). Important impacts of tissue selection and lipid extraction on ecological parameters derived from stable isotope ratios. *Methods in Ecology and Evolution*, *4*, 944–953. https://doi.org/10.1111/2041-210X.12098

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., … Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, *92*, 823–835. https://doi.org/10.1139/cjz-2014-0127

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*, 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & 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*, 179–189. https://doi.org/10.1007/s00442-006-0630-x

Robbins, C. T., Felicetti, L. A., & Sponheimer, M. (2005). The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, *144*, 534–540. https://doi.org/10.1007/s00442-005-0021-8

Sardenne, F., Ménard, F., Degroote, M., Fouché, E., Guillou, G., Lebreton, B., … Bodin, N. (2015). Methods of lipid-normalization for multi-tissue stable isotope analyses in tropical tuna. *Rapid Communications in Mass Spectrometry*, *29*, 1253–1267. https://doi.org/10.1002/rcm.7215

Sotiropoulos, M., Tonn, W., & Wassenaar, L. (2004). Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: Potential consequences for food web studies. *Ecology of Freshwater Fish*, *13*, 155–160. https://doi.org/10.1111/j.1600-0633.2004.00056.x

Sweeting, C., Polunin, N., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, *20*, 595–601. https://doi.org/10.1002/rcm.2347

Tarroux, A., Ehrich, D., Lecomte, N., Jardine, T. D., Bêty, J., & Berteaux, D. (2010). Sensitivity of stable isotope mixing models to variation in isotopic ratios: Evaluating consequences of lipid extraction. *Methods in Ecology and Evolution*, *1*, 231–241. https://doi.org/10.1111/j.2041-210X.2010.00033.x

Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*, 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Wilson, R. M., Chanton, J. P., Balmer, B. C., & Nowacek, D. P. (2014). An evaluation of lipid extraction techniques for interpretation of carbon and nitrogen isotope values in bottlenose dolphin (*Tursiops truncatus*) skin tissue. *Marine Mammal Science*, *30*, 85–103. https://doi.org/10.1111/mms.12018

Wilson, R. M., Tyson, R. B., Nelson, J. A., Balmer, B. C., Chanton, J. P., & Nowacek, D. P. (2017). Niche differentiation and prey selectivity among common bottlenose dolphins (*Tursiops truncatus*) sighted in St. George Sound, Gulf of Mexico. *Frontiers in Marine Science*, *4*, 235. https://doi.org/10.3389/fmars.2017.00235

Yurkowski, D. J., Hussey, N. E., Semeniuk, C., Ferguson, S. H., & Fisk, A. T. (2015). Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. *Polar Biology*, *38*, 131–143. https://doi.org/10.1007/s00300-014-1571-1

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Cloyed CS, DaCosta KP, Hodanbosi MR, Carmichael RH. The effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values and use of lipid-correction models across tissues, taxa and trophic groups. *Methods Ecol Evol*. 2020;11:751–762. https://doi.org/10.1111/2041-210X.13386