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Lipid correction for carbon stable isotope analysis of deep-sea fishes

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ABSTRACT

Stable isotope analysis of fish tissue can aid studies of deep-sea food webs because sampling difficulties severely limit sample sizes of fish for traditional diet studies. The carbon stable isotope ratio ($\delta^{13}\text{C}$) is widely used in food web studies, but it must be corrected to remove variability associated with varying lipid content in the tissue. A lipid correction has not been determined for any deep-sea fish. These fishes are ideal for studying lipid correction because lipid content varies widely among species. Our objective was to evaluate an application of a mass balance $\delta^{13}\text{C}$ correction to a taxonomically diverse group of deep-sea fishes by determining the effect of lipid extraction on the stable isotope ratios, examining the quality of the model parameters derived for the mass balance correction, and comparing the correction to published results. We measured the lipid extraction effect on the nitrogen stable isotope ratio ($\delta^{15}\text{N}$) and $\delta^{13}\text{C}$ of muscle tissue from 30 North Atlantic species. Lipid extraction significantly increased tissue $\delta^{15}\text{N}$ (+0.66‰) and $\delta^{13}\text{C}$ values, but the treatment effect on $\delta^{13}\text{C}$ was dependent on C:N, a proxy for lipid content. We compared the lipid-extracted $\delta^{13}\text{C}$ to the $\delta^{13}\text{C}$ predicted by the mass balance correction using model variables estimated from either all individuals (pooled) or species-by-species or using published values from other species. The correction using the species-by-species approach performed best; however, all three approaches produced corrected values that were generally within 0.5‰ of the measured lipid-free $\delta^{13}\text{C}$ and that had a small over-all bias (< 0.5‰). We conclude that a generalized mass balance correction works well for correcting $\delta^{13}\text{C}$ in deep-sea fishes, is similar to that developed for other fishes, and recommend caution when applying a generalized correction to fish with high lipid content (C:N > 8).

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1. Introduction

Stable isotopes can be used as food web markers to delineate organic matter flows in aquatic food webs and characterize predator–prey relationships of fishes (Michener and Schell, 1994). Stable isotope analysis is used in food web studies because the stable isotope ratio of a tissue reflects the time-integrated diet of the consumer (Tieszen et al., 1983; Hesslein et al., 1993; Hoffman et al., 2007). The carbon (C) stable isotope ratio, $\delta^{13}\text{C}$, of fish muscle tissue is, on average, about +0.5‰ more enriched than the fish's diet, whereas its nitrogen (N) stable isotope ratio, $\delta^{15}\text{N}$, is on an average +3.4‰ more enriched than the diet (Vander Zanden and Rasmussen, 2001). Thus, dual stable isotope studies are common because the $\delta^{13}\text{C}$ reflects the energy source of the organism and the $\delta^{15}\text{N}$ the trophic level. The trophic discrimination – the isotopic difference between a consumer and its diet – will vary, however, among tissues and in relation to food quality

(e.g., France and Peters, 1997; Pinnegar and Polunin, 1999; McCutchan et al., 2003). Comparability among organisms and studies, therefore, requires consistency in the tissues analyzed (blood, liver, muscle and whole organism studies are common) and in the tissue processing methods (e.g., Smyntek et al., 2007).

Lipid extraction is increasingly used during tissue processing to remove variability in $\delta^{13}\text{C}$ associated with varying lipid content. Lipids are isotopically depleted relative to proteins and carbohydrates (DeNiro and Epstein, 1977). Tissue with a higher lipid content, therefore, will have a lower $\delta^{13}\text{C}$ value compared to tissue with a lower lipid content independent of diet, potentially confounding comparisons among different tissues or among the same tissue if the lipid content is variable (e.g., McConnaughey and McRoy, 1979). For aquatic organisms, the lipid depletion factor ($\Delta\delta^{13}\text{C}_{\text{lipid}}$; the isotopic depletion between the protein and lipid components) ranges from –6‰ to –7‰ (Schlechtriem et al., 2003; Kiljunen et al., 2006; Logan et al., 2008). The change in $\delta^{13}\text{C}$ between the bulk (i.e., untreated) and lipid-extracted sample, therefore is related to the tissue's lipid content; the greater the lipid content the greater the depletion in $\delta^{13}\text{C}$ (McConnaughey and McRoy, 1979).

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In general, lipid extraction will enrich the $\delta^{15}\text{N}$ of the sample, as well. This effect is undesirable and unintended, ranging from no detectable effect (Pinnegar and Polunin, 1999; Logan et al., 2008) to an effect of +0.25‰ to +1.6‰ (Murry et al., 2006; Post et al., 2007). At the higher range, the value is about half that associated with one trophic level enrichment, and thus lipid extraction potentially affects the trophic analysis. For this reason, some investigators have recommended running samples in duplicate, untreated and lipid-extracted, so that accurate $\delta^{15}\text{N}$ data are obtained (e.g., Sotiropoulos et al., 2004). Two methods are most commonly used for lipid extraction of fish tissue (Folch et al., 1957; Bligh and Dyer, 1959), both of which involve introducing a polar solvent to the tissue, generally a mix of chloroform and methanol. The lipid extraction efficiency depends on the solvent's polarity (Schlechtriem et al., 2003).

To reduce the burden of running samples in duplicate (untreated and extracted), investigators have used normalization approaches based on lipid content (McConnaughey and McRoy, 1979; Mintenbeck et al., 2008), arithmetic mass balance using C:N as a proxy for lipid content (Alexander et al., 1996; Fry et al., 2003), and regressions based on either C:N or lipid content (Post et al., 2007; Bodin et al., 2007) to correct for the effect of lipids on untreated tissue $\delta^{13}\text{C}$ using data from lipid-extracted samples. In a comparative analysis of correction methods, Logan et al. (2008) found that the model choice was less important than the level of specificity of the data from which the correction was derived (taxa and tissue type). The advantage of a C:N-based approach, such as a mass balance model, is that it does not require quantitative lipid techniques to determine lipid content (Sweeting et al., 2006). The mass balance correction method requires an estimate of two parameters from the data—C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$. The correction, however, can be sensitive to these parameters (Sweeting et al., 2006; Logan et al., 2008). Thus, a generalized correction applied to a diverse group of fishes spanning a broad range of lipid content is needed to determine the accuracy and precision of the correction, depending on whether the model is based on species or a higher-order grouping.

Our objective was to evaluate an application of a mass balance correction to a taxonomically diverse group of deep-sea fish species by determining the effect of lipid extraction on the stable isotope ratios, examining the quality of the parameters derived (C:N_{protein}, $\Delta\delta^{13}\text{C}_{\text{lipid}}$), and comparing the performance of the parameters to published values. Stable isotope analysis is a useful tool for studies of marine food webs, particularly deep-sea environments, where direct observation of organisms is difficult and traditional diet studies are lacking because organisms often are captured with empty stomachs (Iken et al., 2001; Petursdottir et al., 2008; Drazen et al., 2008). A lipid correction, however, has not been determined for any deep-sea fish. Moreover these fishes are ideal for studying lipid correction because many species have high lipid content. We used a recent fish collection from the northern Mid-Atlantic Ridge as part of MAR-ECO, an international field project of the Census of Marine Life (Bergstad and Godø, 2003; Bergstad et al., 2008a), to determine the lipid correction for 30 mesopelagic, bathypelagic and bathydemersal species.

2. Material and methods

2.1. Tissue analysis

Mesopelagic, bathypelagic and bathydemersal fishes captured during the 2004 RV *G.O. Sars* MAR-ECO expedition (Bergstad et al., 2008b; Sutton et al., 2008) and the 2007 RSS *James Cook* ECOMAR expedition near or over the northern Mid-Atlantic Ridge in the Northeast Atlantic Ocean were sampled for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Fishes

from the *G.O. Sars* expedition were captured using various gears in the region from Iceland (60°N) to the Azores (40°N), from the surface to 3600+ m (Bergstad et al., 2008b; Sutton et al., 2008; Wenneck et al., 2008). Fishes from the *James Cook* expedition were captured using a rectangular mid-water trawl (mouth area 8 m², 4.5 mm mesh) in regions north and south of the Charlie-Gibbs Fracture Zone (55–48°N), from 53 to 361 m. Fishes were identified to species in the field, frozen at sea (either –80 or –20° C), and returned to the laboratory, where they remained frozen. In the laboratory, we targeted ten individuals from each of a total of 48 fish species collected for analysis. Individuals were thawed and dorsal white muscle tissue from the region between the head and dorsal fin extracted. White muscle tissue was used owing to its low isotopic variability relative to other tissues (Pinnegar and Polunin, 1999). The tissue samples were rinsed thoroughly in deionized water, dried (at least 24 h at 45 °C), and homogenized by grinding. A sub-sample of ground tissue was treated directly with concentrated hydrochloric acid to determine whether inorganic carbonates were present. In all cases, samples were carbonate-free (visual verification that the sample did not bubble after acidification), and thus were not acidified prior to stable isotope analysis.

Stable isotope ratios are reported in δ notation, $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$, where X is the C or N stable isotope, R is the ratio of heavy: light stable isotopes, and Pee Dee Belemnite and air are standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The C:N is reported as a molar ratio. Initially, about 1000 μg of bulk sample (not lipid-extracted) was analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using an ANCA GSL elemental analyzer (EA) and Europa Hydra 20-20 IRMS (University of California-Davis Stable Isotope Facility). Laboratory standards (NIST 1577 bovine liver, working standard) were calibrated against National Institute of Standards and Technology (NIST) reference materials (IAEA-N1, IAEA-N2, IAEA-N3, IAEA-CH7, and NBS-22; Table 1). The analytical error, the standard deviation (SD) of replicate reference material, was $< \pm 0.1\%$ $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Of the 48 fish species analyzed, 30 species had a C:N of ≥ 4.0 , indicating at least low lipid content (Table 2). When possible, three samples (generally individuals, but we used composite samples when necessary to obtain sufficient mass) for lipid extraction and subsequent stable isotope analysis were obtained from each of the 30 species (*Benthosema glaciale* from two different depths were included, as well; 86 samples in total). Lipid extraction was done by the Colorado Plateau Stable Isotope Laboratory (CPSIL) at Northern Arizona University using a modified Folch et al. (1957) technique, following Sweeting et al. (2006). About 10–20 mg of ground tissue was soaked in a 2:1 chloroform: methanol (by volume) solvent mixture and the material suspended by stirring. After 15 min, the sample was centrifuged (3000 rpm for 5 min), the supernatant discarded

Table 1

Stable isotope calibration standards for the Colorado Plateau Stable Isotope Laboratory (CPSIL) and laboratory standards for the University of California-Davis Stable Isotope Facility (UCD SIF), comparing the expected to the average measured value (\pm one standard deviation [SD] from sample replicates). The working standard used by UCD SIF is a mixture of sucrose and ammonium sulfate.

Laboratory	Calibration	Standard	Expected	Measured	SD
CPSIL	$\delta^{13}\text{C}$ (‰)	IAEA CH6	–10.4	–10.3	0.04
		IAEA CH7	–31.8	–31.9	0.06
	$\delta^{15}\text{N}$ (‰)	IAEA N1	0.4	0.3	0.05
		IAEA N2	20.3	20.2	0.05
UCD SIF	$\delta^{13}\text{C}$ (‰)	NIST 1577	–21.7	–21.7	0.04
		working	–24.4	–24.5	0.04
	$\delta^{15}\text{N}$ (‰)	NIST 1577	7.7	7.7	0.12
		working	1.3	1.3	0.12

Table 2
Average $\delta^{13}\text{C}$ (‰), $\delta^{15}\text{N}$ (‰), and C:N (molar ratio) for un-extracted (“bulk”) and lipid-extracted muscle tissue sampled from mesopelagic and bathypelagic fishes captured in the MARECO and ECOMAR studies of the Mid-Atlantic Ridge in the northeast Atlantic Ocean, as well as the number of individuals or composite samples analyzed (n) and the average lipid depletion factor, $\Delta\delta^{13}\text{C}_{\text{lipid}}$ (‰; standard deviation is in parentheses). *Benthosema glaciale* were captured at depths of 1972–1959 m (A) and 1000–1500 m (B).

Family	Species	$\delta^{13}\text{C}_{\text{bulk}}$	$\delta^{15}\text{N}_{\text{bulk}}$	C:N _{bulk}	$\delta^{13}\text{C}_{\text{extracted}}$	$\delta^{15}\text{N}_{\text{extracted}}$	C:N _{extracted}	$\Delta\delta^{13}\text{C}_{\text{lipid}}$	n
Mesopelagic spp.									
Myctophidae	<i>Lobianchia doleini</i>	−19.9 (0.1)	9.1 (0.3)	4.5 (0.0)	−18.8 (0.6)	11.1 (2.6)	3.8 (0.1)	−6.9 (2.5)	2
	<i>Lobianchia gemellarii</i>	−19.8 (0.2)	9.5 (0.6)	4.6 (0.3)	−18.9 (0.2)	9.8 (0.6)	3.8 (0.0)	−5.4 (0.5)	3
	<i>Benthosema glaciale</i> (A)	−20.3 (0.9)	6.9 (0.2)	5.3 (0.2)	−18.6 (0.7)	7.1 (0.0)	3.9 (0.1)	−6.2 (0.2)	2
	<i>Protomyctophum arcticum</i>	−22.8 (0.5)	8.2 (0.4)	5.5 (0.4)	−20.8 (0.3)	8.7 (0.5)	3.8 (0.0)	−6.7 (0.9)	3
	<i>Notoscopelus kroeyeri</i>	−21.0 (0.6)	10.6 (0.7)	5.8 (0.7)	−19.0 (0.2)	10.9 (0.7)	3.7 (0.0)	−5.7 (0.6)	3
	<i>Lampanyctus macdonaldi</i>	−20.9 (0.5)	11.2 (0.6)	5.8 (0.3)	−18.9 (0.2)	11.3 (1.7)	3.8 (0.1)	−5.9 (0.9)	3
	<i>Myctophum punctatum</i>	−21.9 (0.2)	8.5 (0.7)	6.4 (0.6)	−19.2 (0.2)	9.0 (0.9)	3.8 (0.1)	−6.7 (0.6)	3
	<i>Benthosema glaciale</i> (B)	−23.3 (1.2)	7.3 (0.2)	6.9 (1.7)	−20.0 (0.2)	8.3 (0.1)	3.8 (0.0)	−7.7 (0.3)	3
	<i>Lampadena speculigera</i>	−21.9 (0.2)	12.1 (0.3)	8.5 (1.0)	−17.9 (1.3)	13.2 (1.3)	3.7 (0.0)	−7.2 (1.7)	3
	<i>Arctozenus rissoi</i>	−22.4 (0.8)	9.5 (0.5)	7.6 (1.8)	−19.1 (0.2)	10.1 (0.5)	3.8 (0.0)	−7.0 (0.3)	3
Paralepididae	<i>Vinciguerria poveriae</i>	−19.6 (−)	7.3 (−)	4.1 (−)	−19.3 (−)	7.7 (−)	3.9 (−)	−6.6 (−)	1
Phosichthyidae	<i>Argyropelecus hemigymnus</i>	−19.8 (−)	9.5 (−)	4.3 (−)	−19.4 (−)	9.9 (−)	3.9 (−)	−5.0 (−)	1
Sternoptychidae	<i>Maurollicus muelleri</i>	−20.7 (1.2)	8.4 (0.8)	4.4 (0.5)	−20.2 (0.7)	8.8 (0.9)	3.9 (0.0)	−3.5 (2.7)	3
	<i>Stomias boa</i>	−19.5 (0.4)	9.4 (0.2)	4.4 (0.5)	−18.5 (0.2)	10.2 (0.2)	3.7 (0.0)	−6.5 (0.6)	3
Stomiidae	<i>Chauliodus sloani</i>	−19.3 (1.3)	10.4 (1.4)	4.7 (0.6)	−18.0 (0.8)	11.2 (1.5)	3.7 (0.0)	−7.0 (1.5)	3
	<i>Malacosteus niger</i>	−20.5 (0.9)	10.2 (0.4)	5.7 (1.0)	−18.2 (0.2)	11.2 (0.6)	3.8 (0.0)	−7.0 (0.5)	3
	<i>Borostomias antarcticus</i>	−20.7 (1.1)	12.1 (0.4)	6.2 (1.3)	−18.4 (0.3)	12.3 (0.5)	3.7 (0.0)	−5.9 (0.5)	3
Meso/Bathypelagic									
Gonostomatidae	<i>Cyclothone microdon</i>	−20.3 (0.5)	11.4 (0.7)	5.1 (0.3)	−19.0 (0.3)	11.3 (0.9)	3.8 (0.0)	−5.6 (0.3)	3
	<i>Sigmops bathyphilum</i>	−21.7 (0.3)	12.3 (0.9)	8.4 (0.9)	−18.2 (0.1)	12.7 (1.0)	3.8 (0.1)	−6.4 (0.1)	3
Melamphidae	<i>Scopelogadus m. mizolepis</i>	−19.0 (0.2)	9.5 (0.3)	4.0 (0.1)	−18.8 (0.4)	10.0 (0.3)	3.7 (0.0)	−3.3 (2.6)	3
	<i>Scopeloberyx robustus</i>	−20.3 (0.7)	12.2 (0.4)	4.9 (0.6)	−18.8 (0.5)	12.9 (0.5)	3.8 (0.0)	−6.2 (0.5)	3
	<i>Melamphaes</i> sp.	−20.8 (0.3)	12.7 (0.4)	5.4 (0.2)	−19.1 (0.2)	13.1 (0.1)	3.7 (0.0)	−5.6 (0.7)	3
	<i>Poromitra crassiceps</i>	−20.7 (0.8)	10.9 (1.0)	5.6 (0.8)	−19.0 (0.4)	11.9 (0.9)	3.7 (0.0)	−5.3 (0.3)	3
Platyroctidae	<i>Maulisia microlepis</i>	−18.1 (0.6)	12.1 (0.5)	4.1 (0.3)	−18.0 (0.8)	12.4 (0.1)	3.7 (0.0)	−1.8 (2.6)	3
	<i>Holtbyrnia anomala</i>	−19.7 (0.7)	10.6 (0.4)	4.6 (0.3)	−18.1 (0.3)	11.7 (0.3)	3.7 (0.0)	−9.0 (1.0)	3
Bathydemersal spp.									
Alepocephalidae	<i>Narces stomias</i>	−19.2 (0.6)	13.3 (0.5)	5.2 (0.5)	−17.1 (0.1)	14.5 (0.3)	3.7 (0.0)	−7.1 (0.7)	3
Halosauridae	<i>Halosauropsis macrochir</i>	−18.8 (1.6)	13.5 (1.2)	4.8 (1.0)	−17.4 (0.8)	13.9 (0.7)	3.7 (0.0)	−6.6 (0.8)	3
	<i>Aldrovandia phalacra</i>	−20.3 (0.5)	12.3 (1.2)	5.3 (1.1)	−18.8 (0.6)	12.5 (0.8)	3.7 (0.0)	−6.2 (2.5)	3
Synaphobranchidae	<i>Synaphobranchus kaupii</i>	−19.7 (1.0)	12.0 (0.6)	5.5 (0.8)	−17.3 (0.0)	13.5 (0.4)	3.7 (0.0)	−7.3 (1.1)	3
Trachichthyidae	<i>Hoplostethus atlanticus</i>	−19.9 (0.9)	14.7 (0.4)	7.3 (2.3)	−17.2 (0.1)	15.8 (0.6)	3.7 (0.0)	−5.9 (0.3)	3
Trichiuridae	<i>Aphanopus carbo</i>	−19.1 (1.2)	11.9 (1.3)	4.6 (1.2)	−17.3 (0.5)	13.6 (0.8)	3.6 (0.1)	−12.5 (8.0)	2

(i.e., the analysis was not quantitative for lipids), and the pellet re-suspended in the solvent mixture. These steps were repeated at least three times or until the solvent ran clear. Finally, the pellet was dried (60 °C) and ground. About 1000 μg of the sample was analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a Costech ECS4010 EA and DELTA plus Advantage IRMS (CPSIL). An IRMS was calibrated with NIST reference materials (Table 1). The analytical error based on standard reference material was $< \pm 0.1\%$ $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Homogenized fish muscle tissue from 10 individuals were sent to both labs prior to the analysis and similar results were obtained for non-extracted tissue (average \pm SD for UCD SIF: $\delta^{13}\text{C} = -31.8 \pm 0.3\%$, $\delta^{15}\text{N} = 11.9 \pm 0.5\%$; CPSIL: $\delta^{13}\text{C} = -31.6 \pm 0.4\%$, $\delta^{15}\text{N} = 12.3 \pm 0.4\%$). These results were not significantly different (t -test; $\delta^{13}\text{C}$: $t=0.96$, $df=17$, $p=0.35$; $\delta^{15}\text{N}$: $t=1.91$, $df=17$, $p=0.08$).

2.2. Treatment effect

We examined the change in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N due to lipid extraction using linear regression analysis of the bulk versus lipid-extracted sample (e.g., $\delta^{15}\text{N}_{\text{bulk}}$ versus $\delta^{15}\text{N}_{\text{extracted}}$). We expected a slope equal to 1.0 for $\delta^{15}\text{N}$ (no bias should occur), whereas we expected a slope < 1.0 for $\delta^{13}\text{C}$ because, all things being equal, the samples with more negative $\delta^{13}\text{C}$ values should undergo a larger enrichment from lipid extraction. We expected no relationship for C:N (i.e., regression not significant) because all extracted samples should have similar C:N. In the former two regressions, we tested the slope against a value of 1.0 using a t -test (Zar, 1999). If the slope was not different from 1.0, we tested

if there was a difference between bulk (untreated) and lipid-extracted samples using a paired t -test (SYSTAT 11). In the case of composite samples, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the bulk sample are mass weighted averages of the individuals comprising the sample. For all statistical analyses, we tested for significance at $\alpha=0.05$. We also used linear regression to test the relationship between the estimate of the lipid depletion factor, $\Delta\delta^{13}\text{C}_{\text{lipid}}$, and $\Delta\text{C:N}$. We expected the relationship to be nil, indicating that the depletion factor is not related to the lipid content.

2.3. Lipid correction

We chose to use a mass balance correction because the model is intuitive, it has a simple mathematical form, and can provide a better correction for species-based corrections than other methods (Logan et al., 2008). The mass balance method attributes the bulk $\delta^{13}\text{C}$ signature to the two primary components of muscle – lipid and protein – and assumes that their respective fractions (f) sum to 1:

$$\delta^{13}\text{C}_{\text{bulk}} = \delta^{13}\text{C}_{\text{protein}} \times f_{\text{protein}} + \delta^{13}\text{C}_{\text{lipid}} \times f_{\text{lipid}} \quad (1)$$

An alternative formulation that does not require quantitative lipid estimates is based on C:N. The mass balance applies because lipids do not contain N, therefore the C:N ratios reflect the C mass balance (Fry et al., 2003):

$$\delta^{13}\text{C}_{\text{bulk}} = \delta^{13}\text{C}_{\text{protein}} \times (\text{C:N}_{\text{protein}}/\text{C:N}_{\text{bulk}}) + \delta^{13}\text{C}_{\text{lipid}} \times [(\text{C:N}_{\text{bulk}} - \text{C:N}_{\text{protein}})/\text{C:N}_{\text{bulk}}] \quad (2)$$

The equation is used to normalize the data to a C:N indicative of a lipid-free sample. A generalized correction may then be derived from a subset of samples, avoiding the need to analyze every sample in duplicate (e.g., Logan et al., 2008).

We examined the difference between the predicted $\delta^{13}\text{C}_{\text{protein}}$ based on the mass balance equation and the $\delta^{13}\text{C}_{\text{extracted}}$ from an extracted tissue, a direct estimate of $\delta^{13}\text{C}_{\text{protein}}$. The mass balance equation can be applied to data from bulk tissue to estimate $\delta^{13}\text{C}_{\text{protein}}$ if two parameters are known: $\text{C:N}_{\text{protein}}$ and the isotopic depletion factor for lipid, $\Delta\delta^{13}\text{C}_{\text{lipid}}$. The former is determined based on %C and %N data from an IRMS for the lipid-extracted samples, assuming the extracted fraction is only protein (C:N can be reported as either a weight or molar ratio, but must be consistent). For fish muscle, the value is about 3.7 (Sweeting et al., 2006). The isotopic depletion factor can be estimated using the bulk $\delta^{13}\text{C}$ for a given individual organism ($\delta^{13}\text{C}_{\text{bulk}}$) and the lipid-extracted $\delta^{13}\text{C}$ from the same individual ($\delta^{13}\text{C}_{\text{extracted}}$) if $\delta^{13}\text{C}_{\text{extracted}} = \delta^{13}\text{C}_{\text{protein}}$:

$$\delta^{13}\text{C}_{\text{lipid}} = ((\delta^{13}\text{C}_{\text{bulk}} \times \text{C:N}_{\text{bulk}}) - (\delta^{13}\text{C}_{\text{extracted}} \times \text{C:N}_{\text{extracted}})) / (\text{C:N}_{\text{bulk}} - \text{C:N}_{\text{extracted}}) \quad (3)$$

$$\Delta\delta^{13}\text{C}_{\text{lipid}} = \delta^{13}\text{C}_{\text{extracted}} - \delta^{13}\text{C}_{\text{lipid}} \quad (4)$$

For untreated samples, the $\delta^{13}\text{C}$ can then be standardized to protein (i.e., lipid-free) by substituting the definition $\delta^{13}\text{C}_{\text{lipid}} = \delta^{13}\text{C}_{\text{protein}} + \Delta\delta^{13}\text{C}_{\text{lipid}}$ into Eq. (1) and re-arranging:

$$\delta^{13}\text{C}_{\text{protein}} = \delta^{13}\text{C}_{\text{bulk}} + (\Delta\delta^{13}\text{C}_{\text{lipid}} \times (\text{C:N}_{\text{protein}} - \text{C:N}_{\text{bulk}})) / \text{C:N}_{\text{bulk}} \quad (5)$$

To investigate parameter sensitivity to taxonomic resolution, we estimated $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ in two ways: first, as an average among all individuals (“model 1”) and, second, on a species-basis by averaging individuals within a species (“model 2”). The results using these parameters were compared to those reported by Sweeting et al. (2006), $\text{C:N}_{\text{protein}} = 3.7$ and $\Delta\delta^{13}\text{C}_{\text{lipid}} = -7\text{‰}$, because their extraction methods and modeling approach were nearly identical to ours (“model 3”). For all three models, the mass balance correction was applied to individual samples. For models 1 and 3, the same $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ were used for all individuals. For model 2, we used the species-specific $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ for individuals of that species. To compare the various models and quantify potential errors associated with application, we estimated the residual sum of squares (RSS), the mean ($\pm 95\%$ confidence interval) and range of the residual errors, and the mean bias. For an individual sample, the residual error is calculated as the absolute difference between its measured value, $\delta^{13}\text{C}_{\text{extracted}}$, and the model prediction, $\delta^{13}\text{C}_{\text{protein}}$ (Eq. (5)). Bias is simply the difference between $\delta^{13}\text{C}_{\text{extracted}}$ and $\delta^{13}\text{C}_{\text{protein}}$ for a sample.

3. Results

3.1. Treatment effect

The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of lipid-extracted samples were linear with respect to the bulk samples (Fig. 1). The $\delta^{15}\text{N}$ regression was highly correlated and had a slope that was not significantly different than 1.0, indicating that the change in $\delta^{15}\text{N}$ was not dependent on C:N_{bulk} (Table 2). Lipid extraction resulted in a significant enrichment in $\delta^{15}\text{N}$ of fish muscle tissue (+0.66‰), within the range of published values. The average $\delta^{15}\text{N} \pm$ one standard deviation (SD) of the bulk sample was $10.8 \pm 2.0\text{‰}$, compared to an average of $11.4 \pm 2.1\text{‰}$ of the lipid-extracted

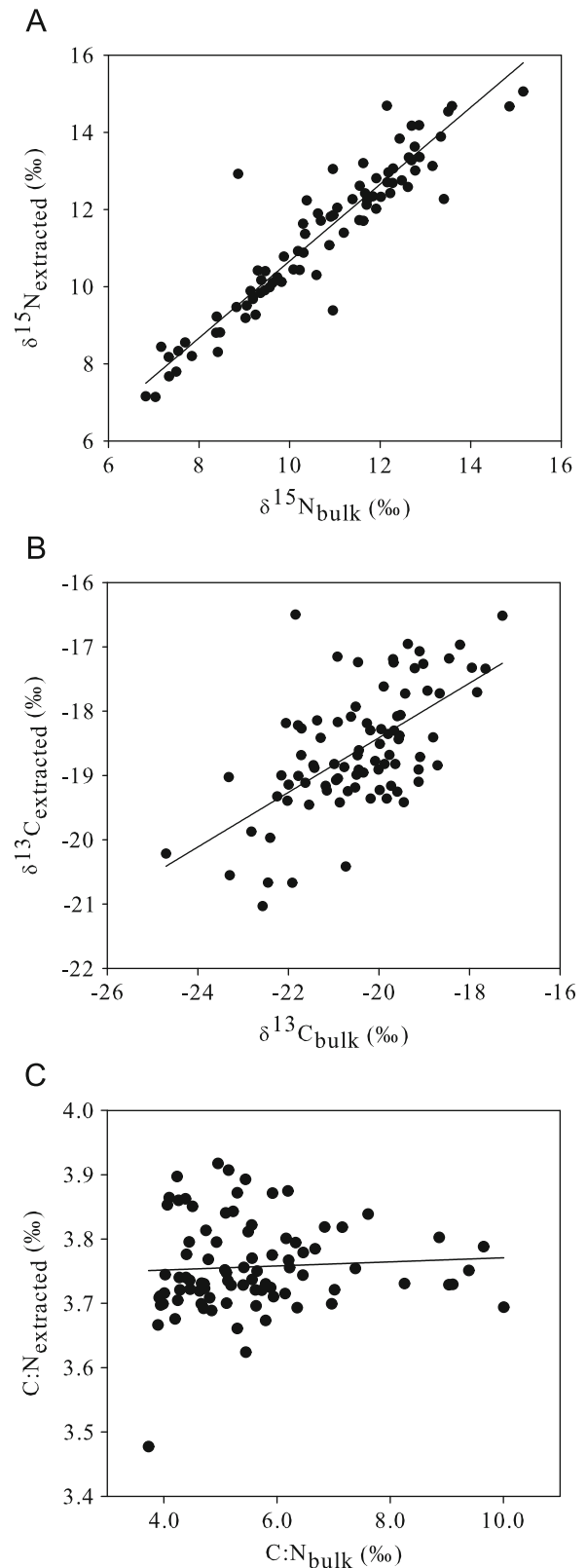


Fig. 1. Linear regression between the $\delta^{15}\text{N}$ (A), $\delta^{13}\text{C}$ (B), and C:N (C) of the bulk muscle sample (untreated) and the lipid-extracted sample.

sample ($t = 8.661$, $df = 85$, $p < 0.001$). The $\delta^{13}\text{C}$ regression was moderately correlated and significantly different from both 0 and 1.0 (Table 3). There was no significant relationship between the C:N of the bulk and lipid-extracted samples (Table 3), indicating

Table 3

Linear regression equations for the various combinations of independent (X) and dependent (Y) variables, along with associated statistics. For all regressions, all individuals analyzed were included ($n=86$).

Variables (X, Y)	Intercept	Slope	p	R ²	slope test		
					H ₀	t	p
$\delta^{15}\text{N}_{\text{bulk}}, \delta^{15}\text{N}_{\text{extracted}}$	0.70	1.00	< 0.0001	0.89	1.00	0.102	0.919
$\delta^{13}\text{C}_{\text{bulk}}, \delta^{13}\text{C}_{\text{extracted}}$	-9.92	0.42	< 0.0001	0.38	1.00	9.635	0.000
C:N _{bulk} , C:N _{extracted}	3.74	0.00	0.56	0.00			
$\Delta\text{C:N}, \Delta\delta^{13}\text{C}_{\text{lipid}}$	-5.91	-0.19	0.25	0.02			

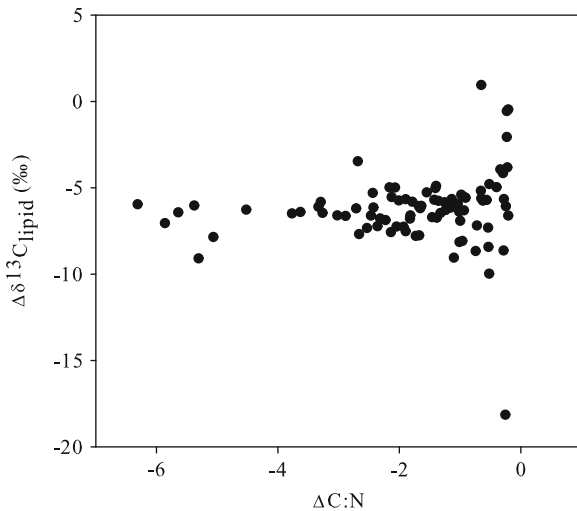


Fig. 2. The change in the lipid depletion factor ($\Delta\delta^{13}\text{C}_{\text{lipid}}$ —difference between protein and lipid components) as a function of the change in the C:N due to lipid extraction, a proxy for lipid content ($\Delta\text{C:N}$ —difference between bulk and lipid-extracted samples).

that the extraction efficiency was not significantly lower for fish with high lipid content (i.e., high C:N_{bulk}). The lipid extraction decreased the average C:N and the variation among individuals. The average C:N \pm SD of the bulk samples was 5.54 ± 1.42 , compared to the lipid-extracted samples, 3.76 ± 0.07 .

3.2. Lipid correction

Averaging all individuals, the mean \pm SD of C:N_{extracted} was 3.76 (0.07) and the mean \pm SD of the lipid depletion factor, $\Delta\delta^{13}\text{C}_{\text{lipid}}$, was $-6.11 \pm 1.73\text{‰}$, excluding a single outlier (-18.1 ; one *Aphanopus carbo*). The $\Delta\delta^{13}\text{C}_{\text{lipid}}$ standard deviation was high (CV=28.3%) because four specimens had estimates of $\Delta\delta^{13}\text{C}_{\text{lipid}}$ that were $> -2.0\text{‰}$ (two *Maulisia microlepis* individuals and one each of *Scopelogadus m. mizolepis* and *Maurolucus muelleri*). Excluding these four data points yielded a normal distribution (Shapiro–Wilk’s test, $p=0.25$) with a slightly higher average \pm SD, $-6.39 \pm 1.21\text{‰}$. The greater variance occurred in those individuals with relatively small $\Delta\text{C:N}$, between 0 and -1.0 (or C:N < 5.0 ; Fig. 2). In essence, those individuals for which we estimate $\Delta\delta^{13}\text{C}_{\text{lipid}} > -2.0\text{‰}$ demonstrated a small $\Delta\delta^{13}\text{C}$ given the $\Delta\text{C:N}$, whereas the extreme outlier, -18.1‰ , demonstrated a large $\Delta\delta^{13}\text{C}$ given the $\Delta\text{C:N}$. The regression between $\Delta\delta^{13}\text{C}_{\text{lipid}}$ and $\Delta\text{C:N}$ was not significant (Table 3).

Averaging by species, we obtain similar estimates of C:N_{extracted} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ (mean \pm SD = 3.76 ± 0.01 and $-6.30 \pm 1.78\text{‰}$, respec-

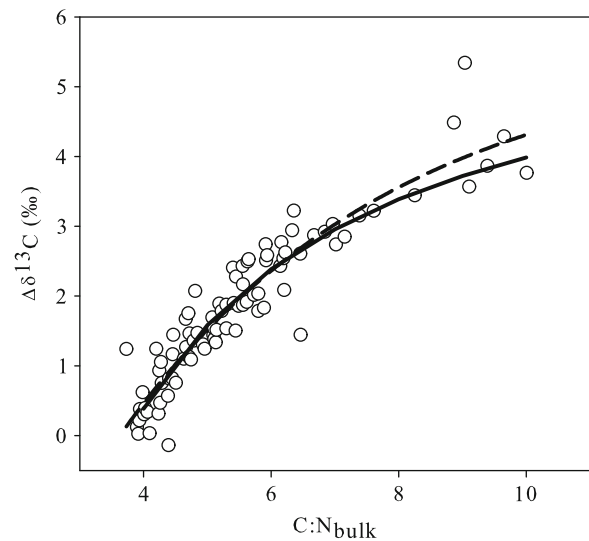


Fig. 3. The change in the muscle $\delta^{13}\text{C}$ due to lipid extraction ($\Delta\delta^{13}\text{C}$ —difference between bulk and lipid-extracted samples) as a function of the bulk sample C:N (untreated) for all individuals analyzed ($n=86$). The dashed line is the best-fit exponential function. The solid line is the mass balance correction from this study derived from the individual samples (Eq. (5); $\Delta\delta^{13}\text{C}_{\text{lipid}}=6.11\text{‰}$, C:N_{protein}=3.76).

tively) as averaging all individuals. By species, the C:N_{extracted} values ranged 3.55–3.89 and the $\Delta\delta^{13}\text{C}_{\text{lipid}}$ values from -12.47‰ to -1.76‰ (Table 2). The average C:N of the lipid-extracted samples, 3.76, was similar to the published values for muscle protein (ca. 3.6–3.7; Fry et al., 2003; Sweeting et al., 2006; Logan et al., 2008) and the distribution had a small variation (range 3.48–3.92; coefficient of variation [CV]=1.9%). We therefore interpret the C:N data to indicate that the lipid extraction successfully removed most or all of the lipids in the tissue samples and that the C:N_{extracted} could be used to represent C:N_{protein} in the mass balance equation.

The $\delta^{13}\text{C}$ correction, $\Delta\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{bulk}} - \delta^{13}\text{C}_{\text{extracted}}$), was generally more than 2‰ for fish with C:N_{bulk} > 6 (Fig. 3). In accordance with the mass balance model (Eq. (5)), $\Delta\delta^{13}\text{C}$ is curvilinear with respect to C:N_{bulk} (Fig. 3). An exponential function provides a significant fit to the data derived from individuals (Fig. 3; $\Delta\delta^{13}\text{C}=5.59 - 12.96e^{-0.23} \text{C:N}_{\text{bulk}}$; $p < 0.0001$, $r^2=0.87$). The mass balance correction based on the individual data ($\Delta\delta^{13}\text{C}_{\text{lipid}}=-6.39\text{‰}$; C:N_{protein}=3.76) follows the curvilinear form of our data, though it slightly underestimates $\Delta\delta^{13}\text{C}$ for samples with C:N_{bulk} > 8 in comparison to the best-fit exponential equation.

The mass balance correction produced a similar result, whether C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ were estimated from all individuals (model 1) or were species-specific (model 2; Fig. 4). Both methods produced a correction with a small mean residual error and bias relative to ecological variation ($< 0.5\text{‰}$), indicating the mass balance correction accurately predicted $\delta^{13}\text{C}_{\text{protein}}$ with an acceptable precision (Table 4). The mean residual error was slightly lower for the species-based model compared to the individual-based model and the RSS was lower, indicating the species-based correction provided a better fit to the data. The difference in the maximum residual error, however, was $< 1\text{‰}$ among the two models and the mean bias in both models was $< 0.1\text{‰}$, similar to an IRMS error. The mass balance correction produced similar results to models 1 and 2 when using published values (model 3). The mean residual error was slightly higher than models 1 and 2, the maximum residual error lower than either, the RSS higher, and the mean bias slightly higher, about $+0.3\text{‰}$. For all three models, the maximum residual error was $> 1.5\text{‰}$, which is potentially ecologically significant.

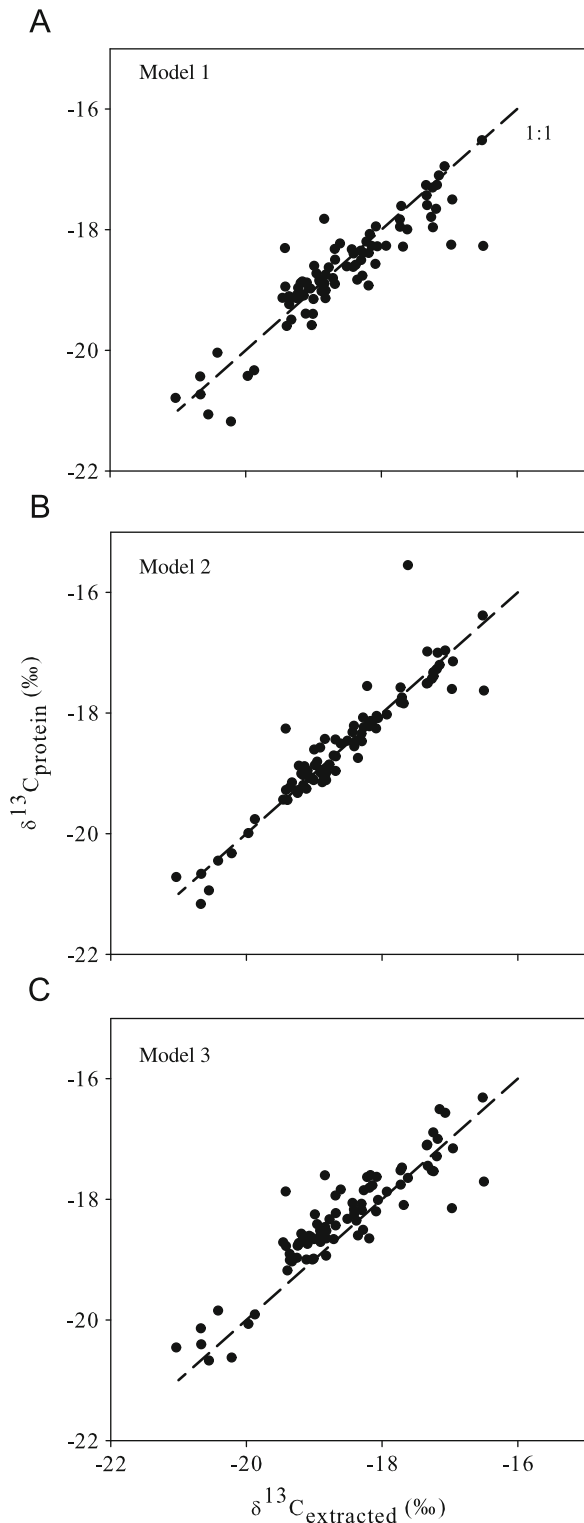


Fig. 4. Comparison among the mass balance correction models using three different values for C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$. Model 1 uses the average values among all fish sampled (A), model 2 species-specific values (B), and model 3 published values (C). The dashed line shows the 1:1 relationship if the $\delta^{13}\text{C}_{\text{protein}}$ predicted by the mass balance model (Eq. (5)) were equal to that measured after lipid extraction ($\delta^{13}\text{C}_{\text{extracted}}$).

4. Discussion

On an average, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish muscle tissue were enriched by lipid extraction and the mean C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$

Table 4

Comparison of the mean, 95% confidence intervals and range of the residual errors, the residual sum of squares (RSS), and mean bias between the lipid-extracted $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{extracted}}$) and the lipid-corrected $\delta^{13}\text{C}$ predicted by the mass balance correction ($\delta^{13}\text{C}_{\text{protein}}$; Eq. (5)) using different values for C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$. Model 1 uses the average values among all fish sampled (C:N_{protein}=3.76, $\Delta\delta^{13}\text{C}_{\text{lipid}}$ = -6.11‰), including outliers. Model 2 uses species-specific values (Table 2). We excluded two species, *Argyroleucus hemigymnus* and *Vinciguerria poweria*, because only one sample from each species was analyzed. Model 3 uses published values (C:N_{protein}=3.7, $\Delta\delta^{13}\text{C}_{\text{lipid}}$ = -7‰).

	Model 1	Model 2	Model 3
Mean residual error (SD)	0.29 (0.30)	0.20 (0.29)	0.37 (0.28)
95% CI of residuals	0.22–0.36	0.13–0.27	0.31–0.44
Range of residuals	0.00–1.77	0.00–2.07	0.03–1.55
RSS	14.77	10.33	18.70
Mean bias	-0.09	0.02	0.33
n	86	84	86

were similar to published values. Using estimates of C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$, the C:N-based mass balance correction generally provided an accurate and unbiased correction to the $\delta^{13}\text{C}_{\text{bulk}}$ data. The mass balance correction produced similar results under any of the approaches to parameter estimation, suggesting the correction method is not highly sensitive to taxonomic resolution or small variations in C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$, although the species-by-species approach produced the least error, best fit, and smallest overall bias in the model prediction. We discuss here a comparison between this correction and other published methods, potential error in the data, and the implications of our study for the application of the mass balance correction.

4.1. Method comparison

We compared our results to a recently published mass balance correction and two versions of the McConnaughey and McRoy (1979) $\delta^{13}\text{C}$ correction because the correction methods are widely used and take different mathematical forms. The mass balance model (Eq. (5)) predicts a curvilinear change in $\Delta\delta^{13}\text{C}$ in relation to C:N_{bulk}. Normalization in the form of a curvilinear relationship as described originally by McConnaughey and McRoy (1979) may closely approximate a mass-balance correction (Logan et al. 2008).

We compared corrections proposed by Kiljunen et al. (2006) and Logan et al. (2008) to our own (Eq. (6)). Both Kiljunen et al. (2006) and Logan et al. (2008) used similar lipid extraction methods to this study. Kiljunen et al. (2006) estimated parameters (mean \pm 95% confidence intervals) for the model proposed by McConnaughey and McRoy (1979) using marine and freshwater fishes with C:N_{bulk} of 3.4–10.9 (molar ratios; Kiljunen et al.'s (2006) model, as presented, uses mass ratios): D (our $\Delta\delta^{13}\text{C}_{\text{lipid}}$) = $7.018 \pm 0.263\text{‰}$ and I (a constant) = 0.048 ± 0.013 . When pooling fish species, Logan et al. (2008) also recommends using a modified version of the McConnaughey and McRoy (1979) model; they provide a simplified three-parameter form of the model. Logan et al. (2008) further recommends a mass balance model for a species- and tissue-specific approach (e.g., *Benthosema glaciale* muscle), for which they estimate parameter means \pm standard error based on five freshwater and marine fish species: $\Delta\delta^{13}\text{C}_{\text{lipid}}$ = $6.699 \pm 0.119\text{‰}$ and C:N_{protein} = 3.614 ± 0.021 (C:N values converted from the originally reported mass to molar ratios). For all four models, we determined the 95% confidence interval using a Monte-Carlo simulation (1000 trials) based on the reported parameters' errors.

When the four corrections are directly compared, the differences among models are relatively small, $< 1\text{‰}$ for any given C:N_{bulk} value (Table 5). The 95% confidence interval of the correction proposed by Logan et al. (2008, Eq. (1a)) based on the

Table 5
Correction of $\delta^{13}\text{C}$ ($\Delta\delta^{13}\text{C}$ [‰]; 95% confidence intervals in parentheses) of fish muscle tissue for its lipid content as a function of the molar C:N of the muscle tissue (C:N_{bulk}) based on four different models: corrections based on McConnaughey and McRoy (1979) proposed by Kiljunen et al. (2006) and Logan et al. (2008; Eq. (1a)) and mass-balance corrections proposed by Logan et al. (2008; Eq. (2)) and from the results of this study (MAR-ECO; Eq. (6)).

C:N _{bulk}	$\Delta\delta^{13}\text{C}$ Kiljunen et al. (2006)	$\Delta\delta^{13}\text{C}$ Logan et al. (2008), Eq. (1a)	$\Delta\delta^{13}\text{C}$ Logan et al. (2008), Eq. (2)	$\Delta\delta^{13}\text{C}$ MAR-ECO
4	0.89 (0.79–1.00)	0.66 (–0.60–1.91)	0.65 (0.57–0.72)	0.38 (0.35–0.42)
6	2.97 (2.82–3.12)	2.64 (1.39–3.89)	2.66 (2.56–2.77)	2.38 (2.29–2.48)
8	4.00 (3.82–4.18)	3.27 (2.48–4.96)	3.67 (3.54–3.81)	3.38 (3.25–3.52)
12	5.02 (4.81–5.23)	4.87 (3.67–6.07)	4.68 (4.51–4.85)	4.38 (4.21–4.56)
15	5.42 (5.20–5.65)	5.35 (4.17–6.52)	5.08 (4.90–5.27)	4.78 (4.59–4.97)

McConnaughey and McRoy (1979) model encompasses all other models. The 95% confidence intervals of the Kiljunen et al. (2006) and Logan et al. (2008, Eq. (2)) mass balance corrections overlap for tissue with C:N > 8, indicating that the two models, though different in form, largely propose a similar $\delta^{13}\text{C}$ correction. The difference in the models for tissue with C:N_{bulk} < 8 is less than 0.5‰ and will not be ecologically significant in most applications. The 95% confidence interval associated with our correction for deep-sea fishes overlaps the mass balance correction proposed by Logan et al. (2008, Eq. (2)) for tissue with C:N_{bulk} > 12. For tissue with C:N_{bulk} < 12, the difference was ca. 0.3–0.4‰ (Table 5), similar to the greatest difference found among the various mass balance models we considered (Table 4). The correction derived by Kiljunen et al. (2006) overestimates the $\Delta\delta^{13}\text{C}$ for deep-sea fishes at all C:N values, though the difference is < 1‰ $\delta^{13}\text{C}$.

4.2. Potential error

In this study, we attempted to measure potentially subtle differences in stable isotope ratios; thus, an IRMS analytical error (Jardine and Cunjak, 2005) and inter-instrument variation (Mill et al., 2008) are important to consider. Both of the facilities that analyzed samples use methods that reduce an analytical error: internal standards are analyzed periodically among the samples, values for calibration material both previous and prior to the analysis are reported to assess accuracy and variation over time (Table 1), and samples of similar mass were analyzed (ca. 1000 µg). We do not believe that inter-instrument variation affected our results. Both IRMS facilities reported measures for standards that are within the range of an analytical error compared to the expected values (Table 1). Also, both facilities used similar calibration materials.

Two concerns arise from the data with respect to the general use of the mass balance approach for correcting $\delta^{13}\text{C}$ of untreated samples. First, the slope of the regression between C:N_{bulk} and C:N_{extracted}, although not significant, was slightly positive, possibly indicating a loss in extraction efficiency as the lipid content increases (Fig. 1). If so, it raises concerns about correcting $\delta^{13}\text{C}$ for lipid content for fish with very high lipid content (C:N_{bulk} > 7). Assuming a linear relationship between C:N_{bulk} and % lipids, fish with a C:N_{bulk} of 7–10 would have a lipid content ranging from 23% to 42%, respectively (Post et al., 2007). Only 12 specimens had C:N_{bulk} > 7, and so the individual-based averages used in the modeling application were only slightly affected. At the species level, there could be a risk of over-estimating C:N_{protein}. *Hoplostethus atlanticus*, *Arctozenus rissoi*, *Sigmops bathyphilum*, and *Lampadena speculigera* had an average C:N_{bulk} > 7 (Table 2). There was, however, no evidence that C:N_{extracted} was systematically higher for these fishes. Only two of the C:N_{extracted} values for these four fishes were greater than the overall mean (Table 2), and these by a small amount (< 0.1).

Second, there was high variability in the estimates of $\Delta\delta^{13}\text{C}_{\text{lipid}}$ for samples with low lipid content, C:N_{bulk} < 5 (Fig. 2). The cause is not known, but the mass balance approach used to estimate

$\Delta\delta^{13}\text{C}_{\text{lipid}}$ is likely responsible (Eqs. (3) and (4)). In the first step, Eq. (3), $\Delta\text{C:N}$ is the denominator in the second term ($(\delta^{13}\text{C}_{\text{protein}} \times \text{C:N}_{\text{protein}})/\Delta\text{C:N}$); thus, $\Delta\delta^{13}\text{C}_{\text{lipid}}$ is sensitive to $\Delta\text{C:N}$ of < 1.0 (i.e., ca. C:N_{bulk} < 5). In this study, the overall effect of the few samples with high variability is small because most fish had C:N_{bulk} > 5. We recommend caution when estimating $\Delta\delta^{13}\text{C}_{\text{lipid}}$ based on a few samples with C:N_{bulk} < 5. However, because these samples have low lipid content, the necessary correction would be < 1.0‰ (Fig. 3). For most ecological applications, using published values to generate the small correction is likely sufficient.

4.3. Correction application

When using a multispecies data set, developing species-specific C:N_{protein} and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ by analyzing a large number of individuals from each species is apparently not critical. Although the sampling design does not allow us to determine if there were significant differences among species, the three different modeling approaches obtained ecologically similar results—in all cases, the model mean residual error and bias were small (< 1‰). The results suggest that, for deep-sea fishes, using the multispecies data set to determine an average (\pm SD) C:N_{protein} (3.76 \pm 0.07) and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ (–6.39 \pm 1.21‰; i.e., outliers removed) for general application to correct $\delta^{13}\text{C}$ values without lipid extraction is sufficient:

$$\delta^{13}\text{C}_{\text{protein}} = \delta^{13}\text{C}_{\text{bulk}} + (-6.39\text{‰} \times (3.76 - \text{C:N}_{\text{bulk}})) / \text{C:N}_{\text{bulk}} \quad (6)$$

We caution, however, that using a mean from a multispecies data set may reduce the precision and increase the bias of the correction with respect to individual species.

The potential effect of using a general correction on the data interpretation will depend on the isotopic difference among diet sources of interest. A correction bias of 0.4‰ or residual error > 1‰ could be important if the isotopic difference among diet sources is small (e.g., 2‰) or if only a few samples are available. In these instances, we recommend developing species-specific corrections. We note that in some isolated cases, the deviation between $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{extracted}}$ was > 1‰, even when parameters were species-specific (model 2). Moreover there was considerable variation in the estimate of $\Delta\delta^{13}\text{C}_{\text{lipid}}$ on a species-by-species basis compared to C:N_{extracted} (Table 2). By species, the mean standard deviation for $\Delta\delta^{13}\text{C}_{\text{lipid}}$ was 1.2‰ (generally based on three individuals), implying that at least seven individuals should be analyzed to obtain a species-specific $\Delta\delta^{13}\text{C}_{\text{lipid}}$ estimate with a standard error \leq 0.5‰.

We conclude that a generalized lipid correction can be applied to multispecies data sets in most applications without compromising the ecological interpretation of the data set. We did not find large differences among our mass balance correction and alternative, published corrections based on fishes from different habitats (i.e., freshwater and coastal marine versus deep-sea fishes). The small differences between these corrections are likely the result of experimental error and random selection influencing

model outcomes, though plausibly could relate to physiological differences among fish species. Thus, we concur with Logan et al. (2008) that the form of the model correction is not critical; however, we emphasize that if an alternative correction method is used, its form should be curvilinear with respect to C:N, rather than linear. Also, error in the correction should be taken into account when applying a published correction that was based on a multispecies data set (e.g., Table 5). In this respect, an important consideration is that species are not distributed randomly across the correction with respect to $C:N_{\text{bulk}}$, but rather are distributed in clusters because individuals within a species tend to have a similar $C:N_{\text{bulk}}$. A species can therefore influence the shape of these curves, and it is notable that both Kiljunen et al. (2006) and Logan et al. (2008) conclude that a species-by-species approach minimizes potential error—a similar finding to our own. If very precise corrections are required for a species, species-specific corrections should be determined.

The similarity in our estimates of $C:N_{\text{protein}}$ (ca. 3.76) and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ (-6.39‰) for fish muscle tissue to those found in other studies using fish muscle (Schlechtriem et al. 2003; Kiljunen et al. 2006; Sweeting et al. 2006), as well as shrimp muscle (Fry et al. 2003; $C:N_{\text{protein}}=3.7$, $\Delta\delta^{13}\text{C}_{\text{lipid}}=-5$ to -8‰), and crustacean zooplankton (Smyntek et al. 2007; $C:N_{\text{protein}}=4.2$, $\Delta\delta^{13}\text{C}_{\text{lipid}}=-6.3\text{‰}$; Kiljunen et al. 2006; $C:N_{\text{protein}}=4.07$) supports the hypothesis that $C:N_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ may be similar among aquatic organisms. The number of species that have been analyzed to date, however, is not extensive. At this time, differences among taxa at this broad level (i.e., Order) appear to be sufficiently different to warrant a separate treatment. Given the uncertainty of whether the differences among corrections reflect experimental error or physiological differences, we recommend that the correction developed in this study, rather than one derived from freshwater and other marine fishes, be applied to deep-sea fishes. Further, we advise caution for investigators analyzing fishes with high lipid content ($C:N > 8$, lipid $> 35\%$). In this study, only a few species with high lipid content were captured. Estimates of $C:N_{\text{extracted}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ from more fishes would reduce uncertainty and further development of a generalized correction that might encompass a broad array of fishes from a variety of aquatic habitats.

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