



HAL
open science

Lipid-correction models for $\delta^{13}\text{C}$ values across small pelagic fishes (Clupeiformes) from the Atlantic Ocean

Fany Sardenne, Thomas Raynon, Jean Marie Munaron, Carl van der Lingen, O. Sadio, Khady Diop, Pablo Brosset, Christophe Lebigre, Philippe Soudant, Marie Vagner, et al.

► To cite this version:

Fany Sardenne, Thomas Raynon, Jean Marie Munaron, Carl van der Lingen, O. Sadio, et al.. Lipid-correction models for $\delta^{13}\text{C}$ values across small pelagic fishes (Clupeiformes) from the Atlantic Ocean. *Marine Environmental Research*, 2023, 192, pp.106213. 10.1016/j.marenvres.2023.106213 . hal-04305861

HAL Id: hal-04305861

<https://hal.science/hal-04305861v1>

Submitted on 29 Aug 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Lipid-correction models for $\delta^{13}\text{C}$ values across small pelagic fishes (Clupeiformes) from the Atlantic Ocean

Fany Sardenne¹, Thomas Raynon¹, Jean-Marie Munaron¹, Carl D. van der Lingen², Oumar Sadio³, Khady Diop³, Pablo Brosset⁴, Christophe Lebigre⁴, Philippe Soudant¹, Marie Vagner¹, Laure Pecquerie¹

¹ IRD, Univ Brest, CNRS, Ifremer, LEMAR, F-29280 Plouzané, France

² Department of Forestry, Fisheries and the Environment (DFFE), Cape Town, South Africa

³ IRD, Univ Brest, CNRS, Ifremer, LEMAR, Dakar, Senegal

⁴ UMR DECOD, Ifremer, INRAE, L'Institut Agro, Plouzané, France

Highlights

- Changes in $\delta^{13}\text{C}$ values following lipid removal depend on bulk C:N ratio and species
- Corrected $\delta^{13}\text{C}$ values from linear and mass balance models were similar
- Linear lipid correction model can be applied at the Clupeiforme order level
- Clupeiforme order has an average lipid-free C:N ratio of 3.1

Abstract

The interpretation of $\delta^{13}\text{C}$ values in trophic ecology requires standardization of the lipid content of organisms estimated through their C:N ratio. To avoid time-consuming lipid extractions, the use of mathematical corrections has been developed for many years, and the conclusions generally point in the direction of species-specific adjustment of the models. This study aimed at defining the maximum taxonomic level required to obtain the best corrected $\delta^{13}\text{C}$ values in small pelagic fish of the order Clupeiformes. $\delta^{13}\text{C}$ values of six species were analyzed bulk and lipid-free, and were used to fit and validate linear and mass-balance models at different taxonomic levels. Despite a species effect combined with the C:N ratio effect, the corrected $\delta^{13}\text{C}$ values produced by a global model for the Clupeiformes were as good as or better when compared to lipid-free samples than those produced by species-specific models, paving the way for possible generalization to other species in this order. At the order level, the linear model outperformed the mass-balance model.

Keywords: carbon isotope, Clupeiformes, C:N ratio, lipid removal, trophic ecology

1. Introduction

Stable isotopes of carbon and nitrogen have been used for decades in trophic ecology. In the marine environment they allow to discriminate the feeding habitats through carbon isotopic composition ($\delta^{13}\text{C}$ values ; France, 1995) and the trophic level of organisms through nitrogen isotopic composition ($\delta^{15}\text{N}$ values ; Minagawa and Wada, 1984). This notably allow clarifying habitat uses, feeding migrations and lengths of trophic chains of an ecosystem (Fry, 2002; McMahon et al., 2015; Trueman et al., 2012).

In order to optimize the trophic interpretation of isotopic compositions, it is often necessary to standardize $\delta^{13}\text{C}$ values especially with the lipid content of organisms. Lipids have up to 20 ‰ lower $\delta^{13}\text{C}$ values than other tissue components (notably proteins ; DeNiro and Epstein, 1977) and the amount of lipids in tissues can vary with many non-trophic factors (e.g. reproduction, season), hence the $\delta^{13}\text{C}$ values of organisms can vary with their lipid content regardless of their trophic ecology. The need to move away from the effect of lipids on $\delta^{13}\text{C}$ values is left to the discretion of scientists, as it depends on the research question (Arostegui et al., 2019). Nevertheless, this effect is generally undesirable to reconstruct food webs and different methods have been developed to standardize $\delta^{13}\text{C}$ values for lipid contents. These methods are either (1) chemical, *i.e.* extracting lipids using different solvents - often optimized to avoid changes in $\delta^{15}\text{N}$ values and thus to be able to make a single analysis provide the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values simultaneously (e.g. Bodin et al., 2009; Chouvelon et al., 2014), or (2) mathematical, *i.e. a posteriori* correcting of the bulk $\delta^{13}\text{C}$ values based on the (estimated) lipid content: the higher the lipid content, the greater the correction of $\delta^{13}\text{C}$ values. The second method has been more widely used as it is faster and avoids time consuming laboratory work (Fry, 2002; Hoffman and Sutton, 2010; Logan et al., 2008; McConnaughey and McRoy, 1979; Post et al., 2007). To estimate lipid content and the need for correction, the elemental C:N ratio of the bulk tissue is generally used, with most studies relying on the recommendations of Post et al. (2007) who suggested that correction due to high lipid levels is required when C:N ratios are > 3.5 . Once the need for correction has been established, mathematical corrections are applied and the resulting corrected $\delta^{13}\text{C}$ values are tested in a first step against chemically treated samples, *i.e.* lipid-free $\delta^{13}\text{C}$ values (e.g. Clark et al., 2019; Hoffman and Sutton, 2010; Sardenne et al., 2015). Several mathematical correction methods have been proposed, generally based on linear (Mintenbeck et al., 2008; Post et al., 2007), non-linear (Kiljunen et al., 2006; McConnaughey and McRoy, 1979) or mass-balance models (Fry, 2002; Logan et al., 2008; Smyntek et al., 2007; Sweeting et al., 2006). Mathematical correction methods encompassing many organisms

have also been proposed (Hoffman and Sutton, 2010; McConnaughey and McRoy, 1979; Post et al., 2007) but it is often recommended to adjust model parameters to the studied species due to high inter-specific difference in both lipid content and bulk $\delta^{13}\text{C}$ values (Cloyed et al., 2020). Phylogenetic and ecological proximities between species sometimes allow the use of the same parameters but this requires an evaluation of a subsample of the dataset (Cloyed et al., 2020; Hoffman and Sutton, 2010; Sardenne et al., 2015; Smyntek et al., 2007).

Small pelagic fishes (order Clupeiformes) are important ecological, economical and nutritional resources, accounting for about 20% of global fish catch (FAO, 2020). They feed on phyto- and zooplankton with some disparities in trophic dynamics between coexisting species (Peck et al., 2021), which are particularly documented for anchovy and sardine (van der Lingen et al., 2009; Garrido and van der Lingen, 2014; Saraux et al., 2019). Their lipid content is also highly variable between species and seasons for some species (Bertrand et al., 2022; Lloret-Lloret et al., 2022; Njinkoué et al., 2002; Pethybridge et al., 2014), with an accumulation of lipids in the muscle before reproduction particularly marked in sardines (Albo-Puigserver et al., 2020; Garrido et al., 2008).

The objective of this study is to test the hypothesis that the taxonomic proximity of species sharing similar habitat allows the application of a single set of parameters for lipid-correction models of $\delta^{13}\text{C}$ values, and to identify the maximum taxonomic level required to produce appropriate corrections. To undertake this study, six species of small pelagic fishes (Clupeiformes order) from three distant regions of the eastern Atlantic were considered, including two genera of sardine (*Sardina* and *Sardinops*), two species of *Sardinella*, two populations of anchovy *Engraulis encrasicolus*, and one species of round herring (*Etrumeus*). The isotopic compositions of each species were analyzed in bulk and lipid-free samples of muscle and used to test for the influence of taxonomic levels on the fit of linear and mass-balance models for the lipid correction of bulk $\delta^{13}\text{C}$ values.

2. Material & methods

2.1. Fish and tissue collection

A total of 149 individuals from six small pelagic fish species with slightly different trophic levels (*Engraulis encrasicolus*, *Etrumeus whiteheadi*, *Sardina pilchardus*, *Sardinella aurita*, *Sardinella maderensis* and *Sardinops sagax*) were collected from three world regions during scientific surveys (**Table 1**). These three regions are distributed over a latitudinal gradient along the eastern Atlantic and belong to three distinct large marine ecosystems: the Bay of Biscay,

France from the Celtic-Biscay shelf ecosystem (EVHOE survey of Ifremer in October 2020, EVHOE, 2020), the harbor of Thiarroye-sur-mer, Senegal from the Canary current ecosystem (survey of the OMEGA project in January 2022, with fish collected by artisanal fisherman), and the south coast of South Africa from the Agulhas current ecosystem (DFFE Pelagic biomass and recruit surveys in November 2020 and May 2021, respectively). Fish were dead by the time of sampling. They were sorted and total length (in cm), weight (in g) and sex (indeterminate when the gonads were underdeveloped, male or female otherwise) were estimated when possible (**Table 1**). A piece of white dorsal muscle was collected from each individual shortly after sampling and subsequently stored frozen at -80°C . Muscle samples were then freeze-dried for 72 h and ground to a homogeneous powder with a ball mill.

Table 1. Sampling design with the number of samples analyzed for each species and sampling regions (n total = 149), with N the number of samples detailed by gender in brackets (Male/Female/Indeterminate), as well as length and weight (mean \pm standard deviation).

Species (FAO code)	Trophic level*	Countries (FAO code)	N total (M/F/IND)	Total length (cm)	Total weight (g)
<i>Engraulis encrasicolus</i> (ANE)	3.1 \pm 0.4	France (FR)	20 (0/3/17)	9.0 \pm 2.0	4.9 \pm 4.2
		South Africa (ZA)	18 (7/9/2)	11.7 \pm 2.5	10.6 \pm 5.1
<i>Etrumeus whiteheadi</i> (WRR)	3.4 \pm 0.5	South Africa (ZA)	22 (2/1/19)	13.6 \pm 2.8	18.2 \pm 7.2
<i>Sardina pilchardus</i> (PIL)	3.1 \pm 0.1	France (FR)	22 (9/9/4)	16.6 \pm 3.5	38.8 \pm 18.9
<i>Sardinella aurita</i> (SAA)	3.4 \pm 0.5	Senegal (SN)	20 (12/8/0)	29.7 \pm 1.3	224.5 \pm 19.7
<i>Sardinella maderensis</i> (SAE)	3.2 \pm 0.4	Senegal (SN)	29 (22/7/0)	27.8 \pm 1.0	190.8 \pm 23.0
<i>Sardinops sagax</i> (CHP)	2.8 \pm 0.1	South Africa (ZA)	18 (5/4/9)	13.6 \pm 3.0	19.5 \pm 9.9

* estimation from FishBase, based on diet items

2.2. Lipid extraction

A subsample of ca. 70 mg of dry powder from each powder sample was lipid-extracted with 3 rinses of 2 mL of solvent mixture (CHCl_3 :MeOH, 2:1, v:v) directly added into glass vials and sonicated for 10 min according to a modified method of Folch et al. (1957) (Sardenne et al., 2019). After each rinse, vials were left to settle for 24 h for powder decantation and the lipid-containing solvent was removed using Pasteur pipettes. After the last rinse, the lipid-free powders were left to dry in a fume hood for 72 h.

2.3. Carbon and nitrogen stable isotope analysis

Bulk and lipid-free powders were weighed into tin capsules (8x5 mm, Microanalysis) and analyzed by continuous flow on a Flash EA2000 elemental analyzer coupled to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher scientific) at the Pôle Spectrométrie Océan, University of Brest, France. Calibrations were based on reference materials (IAEA-600, IAEA-CH-6, IAEA-N1 and IAEA-N2). Results were reported in the δ unit notation and expressed as parts per thousand (‰) relative to the international standards (atmospheric N₂ for nitrogen and Vienna- Pee Dee Belemnite for carbon). Analytical precision based on replicate measurements (after every 6 samples) of a Thermo-Acetanilide standard was < 0.15 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. C:N ratios were determined from % element weight.

2.4. Data analysis

Type III ANOVAs were used to determine if the difference between bulk and lipid-free $\delta^{13}\text{C}$ values ($\Delta\delta^{13}\text{C}$, log transformed) depends on bulk C:N ratio and different taxonomic levels, *i.e.* if it depends on bulk C:N and the population origin (*E. encrasicolus* only), on bulk C:N and the species (six species), and on bulk C:N and the genus (*Sardinella* spp. only). For each analysis the homogeneity and normality of residuals were tested with Levene and Shapiro tests, respectively. Proportions of explained variance were reported as ω^2 .

Once the highest taxonomic level required to establish the best correction model was determined (*i.e.* species, see results section 3.1.), parameters of linear model and mass-balance models were fitted to predict corrected $\delta^{13}\text{C}$ values. They were also fitted to the whole dataset for comparison (Clupeiformes models). The linear (equation 1) and mass-balance (equation 2) models followed the general structure proposed by Post et al. (2007) and Fry (2002), respectively:

$$(1) \delta^{13}\text{C}_{corrected} = \delta^{13}\text{C}_{bulk} + a \times C:N_{bulk} + b, \text{ with } a \text{ and } b \text{ adjusted to the dataset.}$$

$$(2) \delta^{13}\text{C}_{corrected} = \delta^{13}\text{C}_{bulk} + D - \left(\frac{D \times C:N_{pure\ proteins}}{C:N_{bulk}} \right), \text{ with } D \text{ (the protein-lipid discrimination factor) adjusted to the dataset and } C:N \text{ of pure proteins set to the average value of lipid-free } C:N.$$

A cross-validation procedure was used to assess the predictive performance of these models and quantify the stability of their parameters (*i.e.* whether a, b and D were highly dependent on the dataset used for model fitting or not). To this end, models were trained with a random subset of the dataset (*i.e.* 2/3 of the dataset) and validated based on the predictive quality of the unused data (validation dataset). This process of cross-validation on random subsets was repeated 100 times. Prediction quality is the best when the difference between lipid-free and corrected $\delta^{13}\text{C}$ values is the smallest possible and insignificant. This was tested for each of the 100 validation steps using a Wilcoxon test for paired samples.

All statistical analyses and graphics were performed with R, with packages *effectsize* (Ben-Shachar et al., 2020), *ggplot2* (Wickham, 2016) and *stats* (R Core Team, 2020).

3. Results

3.1. Changes in C:N ratio and $\delta^{13}\text{C}$ after chemical lipid extraction

Bulk C:N ratio values were highly variable among groups, with an average C:N of 3.6 ± 0.9 , and with 91% of samples above 3.5 for PIL and 41% for WRR but only few individuals for the other groups (**Fig. 1A** ; **Table S1**). After lipid removal, the average C:N was 3.1 ± 0.1 for all groups (**Fig. 1A**). Bulk $\delta^{13}\text{C}$ values were also variable among groups, with an average of -17.8 ± 1.8 ‰ which slightly increased after lipid removal to -17.0 ± 1.4 ‰ (**Fig. 1B**).

The $\Delta\delta^{13}\text{C}$ (*i.e.* the difference between bulk and lipid-free $\delta^{13}\text{C}$ values) was also highly variable among groups. At the population level (ANE only), bulk C:N explained 70% of the $\Delta\delta^{13}\text{C}$ variability (ANOVA, $df = 1$, $F = 90.0$, $p < 0.001$) while the population origin (France FR vs South Africa ZA) explained 7% of this variability ($df=1$, $F=4.0$, $p = 0.06$) with no effect of the interaction term ($df = 1$, $F = 3.1$, $p = 0.09$).

At the species level (six species), the interaction between bulk C:N and species explained 39% of the $\Delta\delta^{13}\text{C}$ variability and was significant, indicating that the effect of bulk C:N on $\Delta\delta^{13}\text{C}$ variability was specie-dependent (ANOVA, $df = 5$, $F = 23.7$, $p < 0.001$). The main effects of this model suggested that bulk C:N explained 18% of the $\Delta\delta^{13}\text{C}$ variability ($df = 1$, $F = 34.4$, $p < 0.001$) and the species explained 43% of this variability ($df = 5$, $F = 23.7$, $p < 0.001$).

At the genus level (*Sardinella* only, SAA and SAE species), the interaction between bulk C:N and species explained 11% of the $\Delta\delta^{13}\text{C}$ variability and was significant, indicating that the effect of bulk C:N on $\Delta\delta^{13}\text{C}$ variability was stronger in SAA than in SAE (ANOVA, $df = 1$, $F = 7.3$, $p < 0.01$). The main effects of this model suggested that bulk C:N explained 0% of the $\Delta\delta^{13}\text{C}$

variability ($df = 1$, $F = 0.5$, $p = 0.467$) and the species explained 14% of this variability ($df = 1$, $F = 8.8$, $p < 0.01$).

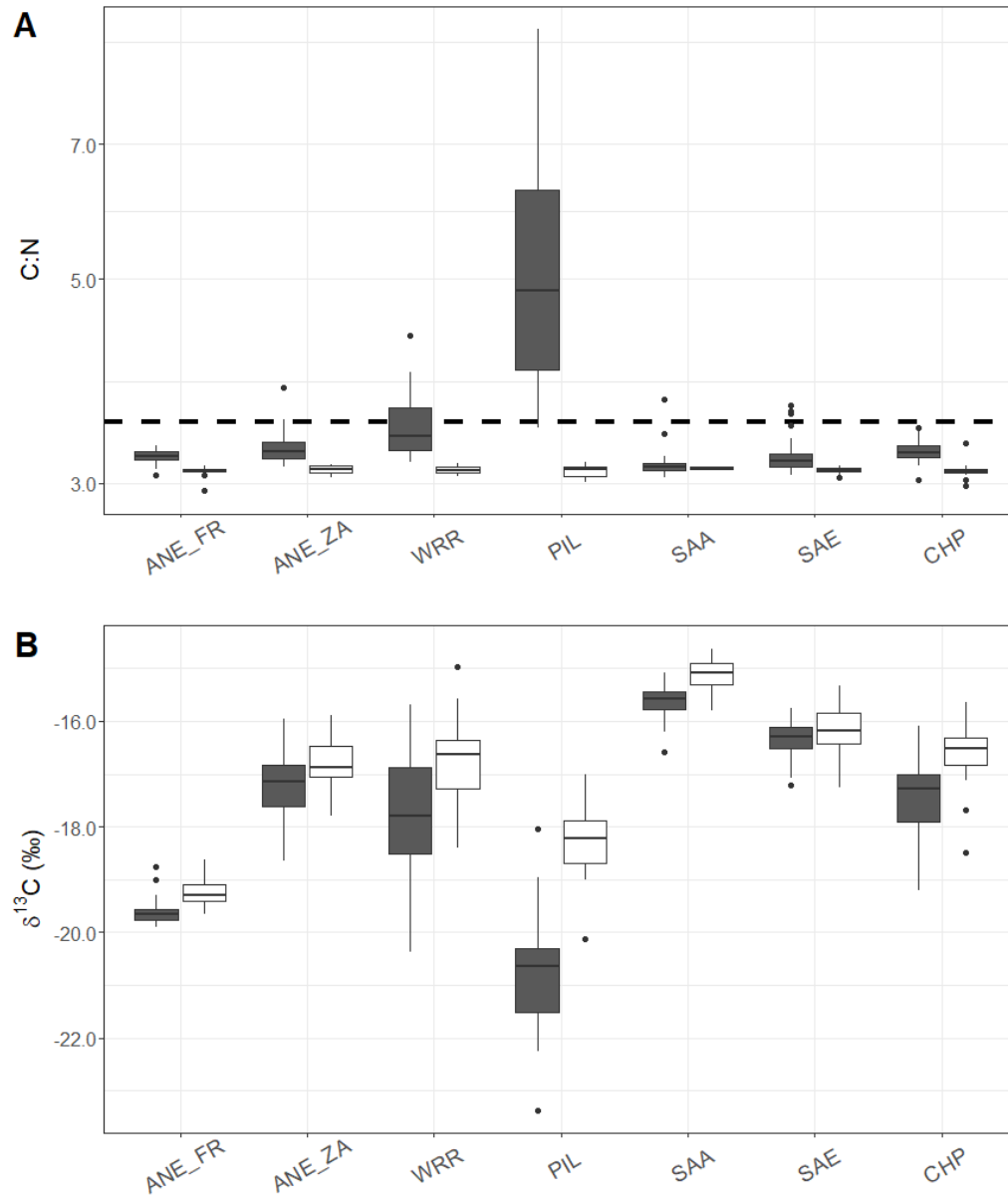


Fig.1. Distributions of (A) C:N ratio and (B) $\delta^{13}\text{C}$ values for all groups of small pelagic fish considered (see table 1 for species codes) before (bulk samples, grey color) and after chemical lipid extraction (lipid-free samples, white color). The horizontal line in panel A is the limit generally used to decide to standardize $\delta^{13}\text{C}$ values for lipids (C:N above 3.5).

3.2. Lipid correction models for $\delta^{13}\text{C}$ values

Since $\Delta\delta^{13}\text{C}$ (*i.e.* the difference between bulk and lipid-free $\delta^{13}\text{C}$ values) varied between species of the same genus (SAA and SAE) but not between populations of the same species (ANE, see section 3.1.), the most relevant taxonomic level for fitting $\delta^{13}\text{C}$ correction models is the species. Estimated parameters of species-specific models were particularly variable (**Table 2**) and the most difficult adjustment was for SAA, regardless of model type (with coefficients of variation of parameters a, b, and D of 125 %, 161 % and 57 %, respectively). This is reflected in the prediction errors for corrected $\delta^{13}\text{C}$ values for this species: the corrected $\delta^{13}\text{C}$ values were significantly different from those lipid-free in 29% and 69% of the random subsets for the linear and mass-balance models, respectively (Paired Wilcoxon tests, $p < 0.05$ with maximum errors of 1.69 ‰ and 3.66 ‰, respectively; **Fig. 2D**). For the other five species, the prediction errors were smaller and corrected and lipid-free $\delta^{13}\text{C}$ values were significantly different in 7–4% of cases and 2–8% of cases for the linear and mass-balance models, respectively (**Fig. 2A, B, C, E, F**).

The predictions of the linear model for the Clupeiformes order (all species) were as good as those from the species-specific models, with only 13% of predictions significantly different from the lipid-free values (Paired Wilcoxon tests, $p < 0.05$; mean error of 0.01 ± 0.07 ‰ and a maximum error of 1.89 ‰). In contrast, the mass-balance model for all the studied clupeiformes underestimated the corrected $\delta^{13}\text{C}$ values in 80% of cases, but with less variability (Paired Wilcoxon tests, $p < 0.05$; mean error -0.14 ± 0.05 ‰ and maximum error of 1.61‰) (**Fig. 2G**).

Table 2. Adjusted parameters for linear and mass-balance models (see section 2.4. for the equations) for six species of small pelagic fish and for all species together (Clupeiforme order), estimated during a cross validation procedure repeated 100 times. Values are mean \pm standard deviation.

Species (FAO code)	Linear model		Mass-balance model
	a	b	D
<i>Engraulis encrasicolus</i> (ANE)	1.898 \pm 1.283	-5.777 \pm 0.912	8.044 \pm 0.347
<i>Etrumeus whiteheadi</i> (WRR)	2.050 \pm 0.137	-6.226 \pm 0.513	8.763 \pm 0.218
<i>Sardina pilchardus</i> (PIL)	0.681 \pm 0.023	-1.114 \pm 0.122	6.651 \pm 0.128
<i>Sardinella aurita</i> (SAA)	0.741 \pm 0.927	-1.791 \pm 2.882	6.942 \pm 3.978
<i>Sardinella maderensis</i> (SAE)	1.477 \pm 0.089	-4.539 \pm 0.292	5.648 \pm 0.229
<i>Sardinops sagax</i> (CHP)	0.627 \pm 0.376	-1.264 \pm 1.229	13.073 \pm 1.342
Clupeiformes order	0.894 \pm 0.046	-2.377 \pm 0.158	6.970 \pm 0.123

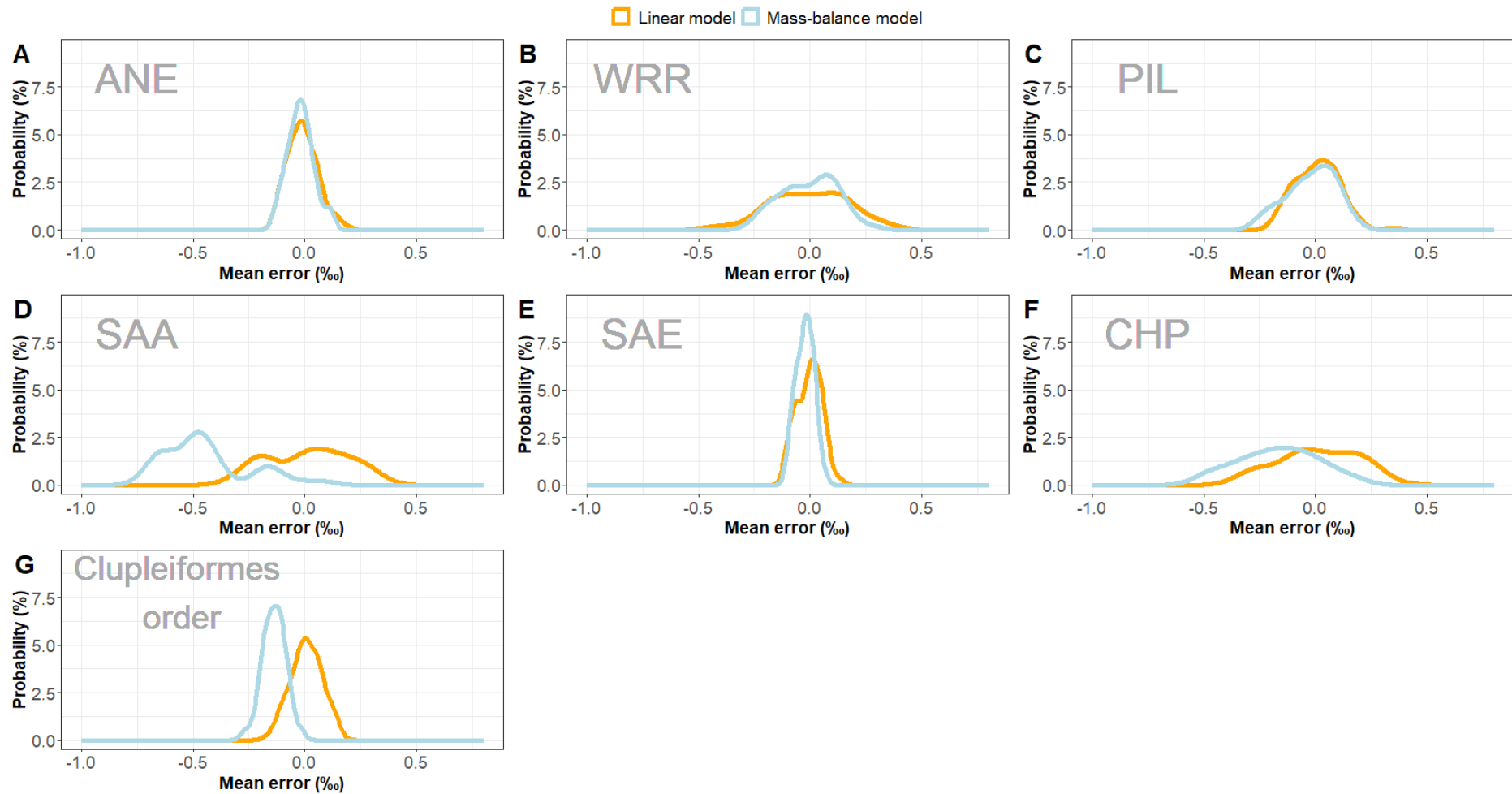


Fig. 2. Distributions of the errors for corrected $\delta^{13}\text{C}$ values for each species (A to F) and the Clupleiformes order (G), *i.e.* the difference between the lipid-free and corrected $\delta^{13}\text{C}$ values from the lipid-correction models (linear and mass-balance models), obtained during a cross validation procedure repeated 100 times (see table 1 for species codes). Colors displayed model types (orange = linear model and blue = mass-balance model).

4. Discussion

In order to correct the bulk $\delta^{13}\text{C}$ values for the non-trophic influence of lipid quantity, linear correction and mass balance models were fitted at different taxonomic levels for small pelagic fish (population, species, genus, order) using lipid-free $\delta^{13}\text{C}$ values as reference. Our data indicate that there was no need for population-specific adjustments but that the quality of predictions varied with species. The fit of models with all individuals greatly benefited from the large number of samples available, which means that the quality of prediction at the order level was *in fine* as good as that at the species level. In this case, the linear model was better than the mass-balance model, as shown by the lower mean error in $\delta^{13}\text{C}$ prediction.

4.1. Fitting a linear $\delta^{13}\text{C}$ correction model for the Clupeiformes: the best compromise
 The main limitation to obtaining quality corrected $\delta^{13}\text{C}$ data is most probably the number of samples used for parameter fitting, whatever the type of model. For example, the linear model for the Clupeiformes order managed to compensate for the species-specific effects thanks to this large number of samples, and produced corrected $\delta^{13}\text{C}$ values of at least equivalent quality to those from species-specific models. Conversely, the poorest fits were obtained with both types of model for SAA, possibly due to the low number of samples available ($n=20$, *i.e.* 13 individuals used to fit the parameters and seven to validate them in the cross-validation procedure used in our study). However, this low number does not entirely explain these poor predictions, since for some species a similar number of individuals resulted in better fits (e.g. PIL, CHP and WRR). As SAA is migratory, it is also possible that these poor fits result from the interaction between lipid content (C:N) and the recent change in $\delta^{13}\text{C}$ isoscapes of some individuals. Thus, for the four species with a number of individuals ≤ 22 (WRR, PIL, SAA, CHP), the use of the linear model for the Clupeiformes order provided better predictions than the mass balance model and species-specific linear models: these species can then benefit from the data available for the other two species (*i.e.* ANE and SAE). Furthermore, it is interesting to note that the parameters of this model ($a = 0.89$ and $b = -2.38$) are particularly close to those obtained by Svensson et al. (2014) on the muscle of 27 fish species, including Clupeidae ($a = 0.82$ and $b = -2.21$). As many Clupeiformes also have bulk C:N of muscle equivalent to those obtained here (*e.g.* average values of 3.5 for *Ethmalosa fimbriata* (Faye et al., 2011), 4.3 for *Etrumeus wongratanai* (Vorsatz et al., 2019), 3.6 for *Clupea harengus* and 3.3 for *Sprattus sprattus* (Svensson et al., 2014)), these parameters may be common to Clupeiformes other than those considered here.

It should nevertheless be noted that the maximum prediction error of the linear model for the Clupeiformes order can reach 1.89 ‰ which is ecologically significant, especially for small pelagic fishes (e.g. Brosset et al., 2016). This maximum error is nevertheless close to those obtained when fitting linear or mass-balance models in other fish species: 1.5 ‰ for deep-sea fishes (Hoffman & Sutton 2010), 0.5-1.6 ‰ for four tissues of tropical tunas (Sardenne et al 2015), and 0.3-1.6 ‰ for brook trout muscle (Skinner et al., 2016).

For species-specific model fitting, the mass-balance model produced data with less error than that of linear models, except for the SAA species (underestimation of corrected $\delta^{13}\text{C}$ values by an average of 0.5 ‰). This quality deteriorates sharply when scaling up to the Clupeiformes order, with an average underestimation of corrected $\delta^{13}\text{C}$ values of -0.14 ‰ in 80% of cases.

4.2. A C:N threshold for lipid correction?

Lipid-free C:N was 3.1 ± 0.1 for the six studied species, which is close to the value reported for Atlantic herring *Clupea harengus* (Logan and Lutcavage, 2008). This could indicate a property of the Clupeiformes order, possibly related to the amino acid composition of their proteins. Amino acid composition seems however to vary between species and seasons: for instance, the main amino acids of sardine and anchovy in % weight are aspartic acid ($\text{C}_4\text{H}_7\text{NO}_4$) and glutamic acid ($\text{C}_5\text{H}_9\text{NO}_4$), or histidine ($\text{C}_6\text{H}_9\text{N}_3\text{O}_2$) and arginine ($\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$) (Öğretmen, 2022; Šimat et al., 2020), with C:N ratio of 4, 5, 2 and 1.5, respectively.

Nevertheless, this lipid-free C:N value has a notable implication in the case of correction equations: the application of a correction equation must be applied to the whole dataset, and not only to individuals whose bulk C:N is above the generally used limit of 3.5. Indeed, the correction equations will correct the bulk $\delta^{13}\text{C}$ to a $\delta^{13}\text{C}$ value for a “theoretical” lipid-free sample, *i.e.* a sample with a C:N of 3.1 (and not 3.5). As a consequence, correction models are highly dependent on the lipid removal method used to produce the reference lipid-free $\delta^{13}\text{C}$ values. The use of solvents other than the Folch mixture for lipid removal would have led to different C:N value (Logan and Lutcavage, 2008). For instance, the Folch mixture is among the strongest lipid extraction (it extracts both apolar and polar lipids, including lipoproteins), and the use of apolar solvents such as cyclohexane or dichloromethane leave some polar lipids in the tissue and would therefore provide slightly a higher lipid-free C:N ratio (Bodin et al., 2009). Correction models based on such lipid-free samples will therefore correct the $\delta^{13}\text{C}$ values more moderately than correction models adjusted on lipid-free samples obtained from the Folch mixture.

Conclusion

To correct the bulk $\delta^{13}\text{C}$ values for a species in the Clupeiformes order for which no lipid-free data are available, the following equation can be used, although a species-specific adjustment based on at least 30 individuals would be preferable: $\delta^{13}\text{C}_{corrected} = \delta^{13}\text{C}_{bulk} + 0.894 \times C:N_{bulk} - 2.377$. If a lipid correction is applied, then this equation must be applied to the entire dataset (including individuals with bulk C:N < 3.5), the equation producing a correction proportional to the bulk C:N.

Acknowledgements

We would like to thank all those who collected samples during the campaigns at the three study sites. Thanks also to the anonymous reviewer for his/her comments.

Funding

This work is a contribution to the OMEGA project, supported by ISblue project, Interdisciplinary graduate school for the blue planet (ANR-17-EURE-0015) and co-funded by a grant from the French government under the program "Investissements d'Avenir" embedded in France 2030.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

FS – Conceptualization, Methodology, Formal analysis, Writing - Original Draft

TR – Investigation, Data Curation

JMM – Validation, Investigation, Resources

CvdL – Investigation, Writing - Review & Editing

OS – Investigation

KD – Investigation

PB – Investigation, Writing - Review & Editing

CL – Investigation, Writing - Review & Editing

PS – Investigation, Writing - Review & Editing

MV – Funding acquisition, Writing - Review & Editing

LP – Investigation, Funding acquisition

References

- Albo-Puigserver, M., Sánchez, S., Coll, M., Bernal, M., Sáez-Liante, R., Navarro, J., Palomera, I., 2020. Year-round energy dynamics of sardine and anchovy in the north-western Mediterranean Sea. *Marine Environmental Research* 159, 105021. <https://doi.org/10.1016/j.marenvres.2020.105021>
- Arostegui, M.C., Schindler, D.E., Holtgrieve, G.W., 2019. Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. *Oecologia* 191, 745–755. <https://doi.org/10.1007/s00442-019-04525-7>
- Barange, M., Coetzee, J., Takasuka, A., Hill, K., Gutierrez, M., Oozeki, Y., van der Lingen, C., Agostini, V., 2009. Habitat expansion and contraction in anchovy and sardine populations. *Progress in Oceanography* 83, 251–260.
- Ben-Shachar, M., Lüdtke, D., Makowski, D., 2020. effectsize: Estimation of Effect Size Indices and Standardized Parameters. *JOSS* 5, 2815. <https://doi.org/10.21105/joss.02815>
- Bertrand, M., Brosset, P., Soudant, P., Lebigre, C., 2022. Spatial and ontogenetic variations in sardine feeding conditions in the Bay of Biscay through fatty acid composition. *Marine Environmental Research* 173, 105514. <https://doi.org/10.1016/J.MARENVRES.2021.105514>
- Bodin, N., Budzinski, H., Le Ménach, K., Tapie, N., 2009. ASE extraction method for simultaneous carbon and nitrogen stable isotope analysis in soft tissues of aquatic organisms. *Analytica Chimica Acta* 643, 54–60. <https://doi.org/10.1016/j.aca.2009.03.048>
- Brosset, P., Bourg, B.L., Costalago, D., Bănar, D., Beveren, E.V., Bourdeix, J.-H., Fromentin, J.-M., Ménard, F., Saraux, C., 2016. Linking small pelagic dietary shifts with ecosystem changes in the Gulf of Lions. *Marine Ecology Progress Series* 554, 157–171. <https://doi.org/10.3354/meps11796>
- Chouvelon, T., Caurant, F., Cherel, Y., Simon-Bouhet, B., Spitz, J., Bustamante, P., 2014. Species- and size-related patterns in stable isotopes and mercury concentrations in fish help refine marine ecosystem indicators and provide evidence for distinct management units for hake in the Northeast Atlantic. *ICES Journal of Marine Science: Journal du Conseil* 199. <https://doi.org/10.1093/icesjms/fst199>
- Clark, C.T., Horstmann, L., Misarti, N., 2019. Lipid normalization and stable isotope discrimination in Pacific walrus tissues. *Sci Rep* 9, 5843. <https://doi.org/10.1038/s41598-019-42095-z>
- Cloyd, C.S., DaCosta, K.P., Hodanbosi, M.R., Carmichael, R.H., 2020. The effects of lipid extraction on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and use of lipid-correction models across tissues, taxa and trophic groups. *Methods in Ecology and Evolution* 11, 751–762. <https://doi.org/10.1111/2041-210X.13386>
- DeNiro, M., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197, 261–263. <https://doi.org/10.1126/science.327543>
- FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. FAO, Rome. <https://doi.org/10.4060/ca9229en>
- Faye, D., Tito de Morais, L., Raffray, J., Sadio, O., Thiaw, O.T., Le Loc'h, F., 2011. Structure and seasonal variability of fish food webs in an estuarine tropical marine protected area (Senegal): Evidence from stable isotope analysis. *Estuarine, Coastal and Shelf Science* 92, 607–617. <https://doi.org/10.1016/j.ecss.2011.02.017>

- Folch, J., Lees, M., Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497–509.
- France, R.L., 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine Ecology Progress Series* 124, 307–312. <https://doi.org/10.3354/meps124307>
- Fry, B., 2002. Stable isotopic indicators of habitat use by Mississippi River fish. *Journal of the North American Benthological Society* 21, 676–685.
- Garrido, S., Rosa, R., Ben-Hamadou, R., Cunha, M.E., Chícharo, M.A., Van Der Lingen, C.D., 2008. Spatio-temporal variability in fatty acid trophic biomarkers in stomach contents and muscle of Iberian sardine (*Sardina pilchardus*) and its relationship with spawning. *Marine Biology* 2008 154:6 154, 1053–1065. <https://doi.org/10.1007/S00227-008-0999-7>
- 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>
- Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., Jones, R.I., 2006. A revised model for lipid-normalizing $\delta^{13}\text{C}$ values from aquatic organisms, with implications for isotope mixing models: Revised lipid-normalization model for C isotope analysis. *Journal of Applied Ecology* 43, 1213–1222. <https://doi.org/10.1111/j.1365-2664.2006.01224.x>
- Laffargue, P., Garren, F., Duhamel, E., 2020. EVHOE 2020 cruise, RV Thalassa. <https://doi.org/10.17600/18000661>
- Lloret-Lloret, E., Albo-Puigserver, M., Giménez, J., Navarro, J., Pennino, M.G., Steenbeek, J., Bellido, J.M., Coll, M., 2022. Small pelagic fish fitness relates to local environmental conditions and trophic variables. *Progress in Oceanography* 202, 102745. <https://doi.org/10.1016/j.pocean.2022.102745>
- 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.
- McConnaughey, T., McRoy, C.P., 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* 53, 257–262.
- McMahon, K.W., Thorrold, S.R., Elsdon, T.S., McCarthy, M.D., 2015. Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. *Limnology and Oceanography* 60. <https://doi.org/10.1002/lno.10081>
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48, 1135–1140. [https://doi.org/10.1016/0016-7037\(84\)90204-7](https://doi.org/10.1016/0016-7037(84)90204-7)
- Mintenbeck, K., Brey, T., Jacob, U., Knust, R., Struck, U., 2008. How to account for the lipid effect on carbon stable-isotope ratio ($\delta^{13}\text{C}$): sample treatment effects and model bias. *Journal of Fish Biology* 72, 815–830. <https://doi.org/10.1111/j.1095-8649.2007.01754.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 - B Biochemistry and Molecular Biology 131, 395–402. [https://doi.org/10.1016/S1096-4959\(01\)00506-1](https://doi.org/10.1016/S1096-4959(01)00506-1)
- Öğretmen, Ö.Y., 2022. The effect of migration on fatty acid, amino acid, and proximate compositions of the Black Sea anchovy (*Engraulis encrasicolus*, Linne 1758) from Turkey, Georgia, and Abkhazia. *Journal of Food Composition and Analysis* 105, 104197. <https://doi.org/10.1016/j.jfca.2021.104197>
- Peck, M.A., Alheit, J., Bertrand, A., Catalán, I.A., Garrido, S., Moyano, M., Rykaczewski, R.R., Takasuka, A., van der Lingen, C.D., 2021. Small pelagic fish in the new millennium: A bottom-up view of global research effort. *Progress in Oceanography* 191, 102494. <https://doi.org/10.1016/J.POCEAN.2020.102494>
- Pethybridge, H., Bodin, N., Arsenaault-Pernet, E.J., Bourdeix, J.H., Brisset, B., Bigot, J.L., Roos, D., Peter, M., 2014. Temporal and inter-specific variations in forage fish feeding conditions in the NW Mediterranean: lipid content and fatty acid compositional changes. *Marine Ecology Progress Series* 512, 39–54.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, 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>
- R Core Team, 2020. R: A Language and Environment for Statistical Computing.
- Saraux, C., Van Beveren, E., Brosset, P., Queiros, Q., Bourdeix, J.-H., Dutto, G., Gasset, E., Jac, C., Bonhommeau, S., Fromentin, J.-M., 2019. Small pelagic fish dynamics: A review of mechanisms in the Gulf of Lions. *Deep Sea Research Part II: Topical Studies in Oceanography, Drivers of dynamics of small pelagic fish resources: environmental control of long-term changes* 159, 52–61. <https://doi.org/10.1016/j.dsr2.2018.02.010>
- Sardenne, F., Bodin, N., Metral, L., Crottier, A., Le Grand, F., Bideau, A., Brisset, B., Bourjea, J., Saraux, C., Bonhommeau, S., 2019. Effects of extraction method and storage of dry tissue on marine lipids and fatty acids. *Analytica Chimica Acta* 1051, 82–93.
- Sardenne, F., Ménard, F., Degroote, M., Fouché, E., Guillou, G., Lebreton, B., Hollanda, S.J., 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>
- Šimat, V., Hamed, I., Petričević, S., Bogdanović, T., 2020. Seasonal changes in free amino acid and fatty acid compositions of sardines, *Sardina pilchardus* (Walbaum, 1792): implications for nutrition. *Foods* 9, 867. <https://doi.org/10.3390/foods9070867>
- Skinner, M.M., Martin, A.A., Moore, B.C., 2016. Is lipid correction necessary in the stable isotope analysis of fish tissues? *Rapid Communications in Mass Spectrometry* 30, 881–889. <https://doi.org/10.1002/rcm.7480>
- Smyntek, P.M., Teece, M.A., Schulz, K.L., Thackeray, S.J., 2007. A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnology and Oceanography* 52, 2135–2146.
- Svensson, E., Freitas, V., Schouten, S., Middelburg, J.J., van der Veer, H.W., Sinninghe Damsté, J.S., 2014. Comparison of the stable carbon and nitrogen isotopic values of gill and white muscle tissue of fish. *Journal of Experimental Marine Biology and Ecology* 457, 173–179. <https://doi.org/10.1016/j.jembe.2014.04.014>
- Sweeting, C.J., Polunin, N.V.C., 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>

- Trueman, C.N., MacKenzie, K.M., Palmer, M.R., 2012. Identifying migrations in marine fishes through stable-isotope analysis. *Journal of Fish Biology* 81, 826–847. <https://doi.org/10.1111/j.1095-8649.2012.03361.x>
- Vorsatz, L.D., van der Lingen, C.D., Gibbons, M.J., 2019. Observations on the biology and seasonal variation in feeding of the east coast round herring *Etrumeus wongratanai* (Clupeiformes), off Scottburgh, KwaZulu-Natal, South Africa. *Journal of Fish Biology* 94, 498–511. <https://doi.org/10.1111/jfb.13914>
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

SUPPLEMENTARY MATERIAL

Lipid-correction models for $\delta^{13}\text{C}$ values across small pelagic fishes (Clupeiformes) from the Atlantic Ocean

Fany Sardenne¹, Thomas Raynon¹, Jean-Marie Munaron¹, Carl D. van der Lingen², Oumar Sadio³, Khady Diop³, Pablo Brosset⁴, Christophe Lebigre⁴, Philippe Soudant¹, Marie Vagner¹, Laure Pecquerie¹

¹ IRD, Univ Brest, CNRS, Ifremer, LEMAR, F-29280 Plouzané, France

² Department of Forestry, Fisheries and the Environment (DFFE), Cape Town, South Africa

³ IRD, Univ Brest, CNRS, Ifremer, LEMAR, Dakar, Senegal

⁴ UMR DECOD, Ifremer, INRAE, L'Institut Agro, Plouzané, France

Table S1. Bulk isotopic compositions and elemental carbon to nitrogen ratio of the six Clupeiformes species studied here (see Material & Methods for details on sampling design).

Species (FAO code)	Countries (FAO code)	Bulk $\delta^{13}\text{C}$ (‰)	Bulk $\delta^{15}\text{N}$ (‰)	Bulk C:N
<i>Engraulis encrasicolus</i> (ANE)	France (FR)	-19.6 ± 0.3	8.8 ± 0.7	3.2 ± 0.1
	South Africa (ZA)	-17.2 ± 0.6	11.7 ± 1.0	3.3 ± 0.2
<i>Etrumeus whiteheadi</i> (WRR)	South Africa (ZA)	-17.7 ± 1.2	12.4 ± 1.0	3.5 ± 0.3
<i>Sardina pilchardus</i> (PIL)	France (FR)	-20.8 ± 1.2	10.8 ± 0.9	5.2 ± 1.6
<i>Sardinella aurita</i> (SAA)	Senegal (SN)	-15.7 ± 0.4	10.8 ± 0.6	3.2 ± 0.1
<i>Sardinella maderensis</i> (SAE)	Senegal (SN)	-16.4 ± 0.4	10.9 ± 0.5	3.2 ± 0.2
<i>Sardinops sagax</i> (CHP)	South Africa (ZA)	-17.4 ± 0.7	10.9 ± 0.3	3.3 ± 0.1