Zooplankton, lipids and stable isotopes: Importance of seasonal, latitudinal, and taxonomic differences

Article *in* Canadian Journal of Fisheries and Aquatic Sciences · November 2010

DOI: 10.1139/F10-091

CITATIONS
57

READS
796

2 authors:

Jari Syväranta
University of Eastern Finland
55 PUBLICATIONS **1,045** CITATIONS

SEE PROFILE

Milla Rautio
University of Québec in Chicoutimi
88 PUBLICATIONS **3,478** CITATIONS

SEE PROFILE

Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences

Jari Syväranta and Milla Rautio

Abstract: We found considerable seasonal, latitudinal, and taxonomic differences in zooplankton lipid content and concurrent $\delta^{13}C$ values of zooplankton. We collected cladoceran as well as cyclopoid and calanoid copepod zooplankton from boreal and subarctic lakes throughout a year, allowing us to study zooplankton likely subjected to different isotopic fractionation processes and with highly variable lipid contents. Considerable seasonal variation was observed in the difference between bulk and lipid-extracted zooplankton $\delta^{13}C$ values, indicating that seasonally changing lipid content introduced notable variation in zooplankton $\delta^{13}C$ values. The difference between bulk and lipid-extracted material was most amplified in lipid-rich subarctic zooplankton in winter, $\delta^{13}C$ difference being >5 units. Significant differences were also observed among zooplankton taxa, with copepods showing a greater lipid impact on $\delta^{13}C$ than cladocerans. Published lipid correction models failed to produce satisfying fits to our data, and considerable variation was left even after recalibrating the model parameters. This was likely due to taxonomic differences in lipid effects on $\delta^{13}C$ values. We therefore produced separate mass balance-based lipid correction models for cladocerans and also cyclopoid and calanoid copepods. We conclude that arithmetic lipid correction models perform well with zooplankton samples, but taxonomic differences need to be considered.

Résumé : Nous avons observé des différences saisonnières, latitudinales et taxonomiques considérables dans le contenu lipidique et les valeurs associées de $\delta^{13}C$ chez divers taxons zooplanctoniques. Nous avons récolté des cladocères et des copépodes cyclopoïdes et calanoïdes dans des lacs boréaux et subarctiques au cours d'une année complète de façon à étudier le zooplancton sous divers processus de fractionnement isotopique et une forte variation de contenus lipidiques individuels. Des variations saisonnières considérables ont été notées dans les valeurs de $\delta^{13}C$ entre des extraits d'organismes entiers et leurs lipides seuls. Ceci montre que les variations saisonnières du contenu lipidique du zooplancton peuvent influencer les valeurs de $\delta^{13}C$ du zooplancton. D'ailleurs, cette différence de $\delta^{13}C$ entre les organismes entiers et les lipides extraits pouvait atteindre plus de 5 unités pour le zooplancton riche en lipides des milieux subarctiques en hiver. Des différences significatives ont aussi été observées entre taxons zooplanctonique, les lipides des copépodes affectant davantage les valeurs de $\delta^{13}C$ que ceux des cladocères. Les modèles de correction pour les lipides provenant de la littérature ne montrent pas un ajustement satisfaisant avec nos données, même avec une re-calibration de leurs paramètres, ceci étant probablement dû aux différences d'impact des valeurs lipidiques sur le $\delta^{13}C$ entre les groupes taxonomiques. C'est pourquoi, nous avons mis au point des modèles correctifs pour les lipides basés sur le bilan massique séparément pour les cladocères et les copépodes cyclopoïdes et calanoïdes. Nous concluons que les modèles correctifs arithmétiques du contenu en lipides produisent de bons résultats avec des échantillons zooplanctoniques, mais les différences entre les taxons doivent toutefois être considérées.

Introduction

Stable isotope analysis (SIA) is now routinely used in studies of food webs and ecosystem structure. Stable carbon isotopes in particular are commonly used to quantify food sources and energy flow in aquatic ecosystems, since carbon stable isotopes are known to fractionate little between each trophic transfer (DeNiro and Epstein 1978; Peterson and Fry 1987). Stable nitrogen isotopes fractionate more and are typically used to infer trophic positions of consumers in food webs (Minagawa and Wada 1984; Peterson and Fry 1987). While the technical aspects of stable isotope analysis have become easier and more affordable in recent years because of instrumental developments and increased number of commercial SIA laboratories, more concerns are being expressed on how to deal with the inherent variability of stable isotope ratios in sample materials and their many potential sources of error.

Received 07 December 2009. Accepted 28 July 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 1 October 2010. J21552

J. Syväranta.[1,2] Department of Biological and Environmental Science, University of Jyväskylä, Finland; EcoLab, UMR 5245 (CNRS-UPS-INPT), Université Paul Sabatier, Toulouse, France.
M. Rautio.[3] Department of Biological and Environmental Science, University of Jyväskylä, Finland.

[1]Corresponding author (e-mail: jari.syvaranta@jyu.fi).
[2]Present address: Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, D-12561 Berlin, Germany.
[3]Present address: Département des sciences fondamentales & Centre for Northern Studies (CEN), Université du Québec à Chicoutimi, Chicoutimi, QC G7H 2B1, Canada.

One such potential source of error in SIA is the variable lipid content in sample tissues. Lipids are known to be depleted in the heavier carbon isotope (^{13}C) leading to lower $\delta^{13}C$ values analyzed from tissues with greater lipid content (DeNiro and Epstein 1977; McConnaughey and McRoy 1979; Kling et al. 1992), which can mask the carbon signature of the food source and introduce bias into mixing model calculations or baseline estimates. Especially, temporally variable lipid content can introduce considerable bias into interpretations of $\delta^{13}C$ data because increases in tissue lipid content could result in lower $\delta^{13}C$ values, although the source of carbon (or diet) could remain the same. Lipid content in zooplankton is of particular importance, since some species (such as many copepods) can accumulate considerable lipid storages during the growing season to cope with less productive periods (Kuosa and Gyllenberg 1989; Lee et al. 2006; Kattner et al. 2007), especially in Arctic or subarctic environments, thus potentially reflecting greater lipid-induced variation in their $\delta^{13}C$ values (Grey 2006). Although the lipids in zooplankton are partly incorporated directly from their diet and may therefore be considered as part of their actual diet, another part of lipids is synthesized in situ by zooplankton (Graeve et al. 2005; Kattner et al. 2007) altering the $\delta^{13}C$ values of their body constituents. In addition, since zooplankton accumulate greater lipid reserves than their algal prey, bulk zooplankton $\delta^{13}C$ values may be considerably ^{13}C depleted compared with their diet (Smyntek et al. 2007).

Also, lipids in zooplankton that are accumulated during more productive periods may have a very different $\delta^{13}C$ value than the dietary lipid that the zooplankton is acquiring from diet later in the year. Identifying such temporal variation in zooplankton stable isotope values is of crucial importance to many ecological studies of aquatic ecosystems utilizing SIA, such as those assessing seasonally variable carbon sources to zooplankton (Grey et al. 2001; Karlsson and Säwström 2009). Zooplankton is also an important dietary source to many consumers at higher trophic levels in food webs and is frequently used to establish isotopic baseline levels in the pelagic habitat of aquatic systems (Matthews and Mazumder 2007). Seasonal variation in zooplankton $\delta^{15}N$ values has recently received increasing attention (e.g., Matthews and Mazumder 2007; Syväranta et al. 2008), but less such attention has been given to seasonal variation in $\delta^{13}C$ values (but see Matthews and Mazumder 2005). Although some papers reported no appreciable temporal differences between untreated and lipid-extracted zooplankton $\delta^{13}C$ values (Syväranta et al. 2006; Ventura and Catalan 2008), seasonal increases in lipid storage for overwintering and reproduction purposes could introduce substantial changes on zooplankton $\delta^{13}C$ values (Matthews and Mazumder 2005; Grey 2006), especially in copepods (Smyntek et al. 2007) and in higher latitude regions.

One potential way of accounting for the effects of increasing lipid content on $\delta^{13}C$ is simply to extract the lipids prior to measuring stable isotopes. However, controversial impacts of lipid extraction on nitrogen stable isotope values of sample materials have already been reported (e.g., Sotiropoulos et al. 2004; Ingram et al. 2007; Logan and Lutcavage 2008). If chemical lipid extraction is used for zooplankton, any notable impacts of chemical lipid extraction on sample $\delta^{15}N$ values would then result in doubled analysis costs and extra labor due to preparation of subsamples for lipid extraction.

Greater lipid content in sample materials is typically associated with higher C:N ratios, which can potentially be used to mathematically correct $\delta^{13}C$ values for lipid content (McConnaughey and McRoy 1979), thereby avoiding the need for chemical lipid extraction, such as in chloroform–methanol solution (Bligh and Dyer 1959). Not surprisingly, different models and model revisions to correct the $\delta^{13}C$ values of tissues with variable lipid content have recently received increasing attention in the literature (e.g., Kiljunen et al. 2006; Smyntek et al. 2007; Logan et al. 2008). However, most published papers have concentrated more on the lipid effects on $\delta^{13}C$ values of fish tissues, while invertebrates and zooplankton in particular have received notably less attention. Smyntek et al. (2007) proposed a revised mass balance model (Fry et al. 2003) for lipid correction of zooplankton samples, but this model remains virtually untested. We can expect that models designed for lipid corrections in fish muscle tissues may not perform well on invertebrate samples, which are typically analyzed whole, because of differences in tissue compositions (e.g., chitin in many invertebrates). We might also expect some differences in body compositions between different invertebrate and zooplankton taxa, which could ultimately mean that one model, even if particularly designed for zooplankton lipid correction, may not always perform well if the studied taxa is very different from the one used for model calibration.

Here we present data from seasonal collections of zooplankton samples covering a wide latitudinal gradient from boreal lake systems (central Finland) to subarctic lakes (northern Finland). Our aims were (*i*) to evaluate the impact of seasonally varying lipid content on freshwater zooplankton $\delta^{13}C$ values, (*ii*) evaluate the influence of increasing geographical latitude on zooplankton lipid impacts on $\delta^{13}C$ values, and (*iii*) test the validity of existing mathematical lipid correction models on different zooplankton taxa using a data set that covers one of the largest reported ranges of zooplankton lipid content (20%–62%) and C:N ratios (3.8–19.3) studied for $\delta^{13}C$ values.

Materials and methods

Zooplankton samples were collected in 2005–2007 from four lakes in central boreal Finland near the city of Jyväskylä (62°15′N, 25°46′E, ca. 95 m above sea level) and in 2007–2008 from Lake Saanajärvi in northern Finland near the Kilpisjärvi village (69°05′N, 20°52′E, ca. 670 m above sea level). Samples were collected during open water season (May–November) in lakes in the boreal sampling area by sampling two lakes one to two times a month and two other lakes only in spring and autumn. Mean monthly values for each lake are used in this paper. In the subarctic sampling area, samples were taken four times in winter from under ice (November–May) and four times in open water season (June–October). Zooplankton was sampled by several vertical hauls with zooplankton nets (50–250 μm), and replicate samples were collected during each sampling period from different sites. Samples were then brought to the laboratory

and left overnight in clean tap water or to GF/F filtered lake water to allow gut evacuation. The next day these samples were identified and sorted. Samples from the boreal sampling area were sorted into cladocerans and cyclopoid and calanoid copepods, calanoid copepods being mostly *Eudiaptomus graciloides*. Samples from northern Finland were sorted similarly, but almost all samples of cladocera were *Daphnia umbra*, cyclopoids were all *Cyclops abyssorum*, while calanoids were all *E. graciloides*. After sorting, the samples were stored frozen (–20 °C) in Eppendorf vials and subsequently freeze-dried. These dried zooplankton samples were divided in two to create an untreated sample for SIA and a subsample for lipid-extracted SIA sample. Chemical lipid extraction was done using a 2:1 chloroform–methanol (*v*/*v*) solution (Bligh and Dyer 1959) and 24 h extraction period. The resultant lipid-free material was then dried in an oven at 60 °C and analyzed for comparison with untreated samples. Lipid percentage of dry mass (lipid-%) was determined only for zooplankton samples collected from northern Finland by weighing the dried sample material before and after lipid extraction and calculating the lipid content as a weight difference.

All stable isotope analyses were done at the Institute for Environmental Research, University of Jyväskylä, using a FlashEA 1112 elemental analyzer coupled to a Thermo Finnigan DELTA^{plus} Advantage mass spectrometer (Thermo Electron Corporation, Waltham, Massachusetts, USA). Pike muscle tissue was used as a laboratory working standard, and replicate standards were run repeatedly in every analysis to ensure accurate analysis and to allow any required correction for linearity and drift. Stable isotope ratios are expressed as parts per thousand (‰) delta values ($^{13}C/^{12}C$ or $^{15}N/^{14}N$, i.e., $\delta^{13}C$‰ and $\delta^{15}N$‰), referring to the international standards for carbon (PeeDee Belemnite) and nitrogen (atmospheric nitrogen) (Peterson and Fry 1987). Internal precision for standards was always better than 0.2‰ for both C and N in each run.

Influence of increasing lipid content on zooplankton $\delta^{13}C$ values ($\Delta\delta^{13}C$) was calculated by subtracting values analyzed from lipid-extracted material ($\delta^{13}C_{le}$) from that of bulk material ($\delta^{13}C_{bulk}$). The $\Delta\delta^{13}C$ values were then compared among separate zooplankton taxa (cladocerans and cyclopoid and calanoid copepods) and between boreal and subarctic sampling areas using analysis of variance (ANOVA) followed by Tukey's multiple comparisons. A Box–Cox transformation was applied when needed to achieve homogeneity of variances (Box and Cox 1964). A multiple linear regression method was used to test for the significance of surface (0–2 m) water temperature, taxa, and sampling area (boreal–subarctic) on $\Delta\delta^{13}C$. Simple *F* tests were applied to test for potential increases in $\delta^{13}C$ or $\delta^{15}N$ variances before and after chemical lipid extraction, and impacts of this procedure on $\delta^{15}N$ values of zooplankton was tested using paired *t* tests between lipid-extracted and untreated samples.

To test published mathematical lipid correction models (Table 1) to our data, we first selected the McConnaughey and McRoy (1979) model, which is widely used for lipid normalization of fish tissues and was recently revised by Kiljunen et al. (2006). The most often used lipid correction models for $\delta^{13}C$ values are either based on lipid normalization model (McConnaughey and McRoy 1979; Kiljunen et al. 2006) or mass balance model (Fry et al. 2003; Sweeting et al. 2006; Smyntek et al. 2007). The difference is that the lipid normalization model by McConnaughey and McRoy (1979) first uses C:N ratio of bulk sample material to calculate a lipid factor (*L*)

$$(1) \qquad L = \frac{93}{1 + [0.246 \times (\mathrm{C{:}N_{bulk}}) - 0.775]^{-1}}$$

which is then used to calculate $\delta^{13}C$ of lipid-extracted tissue ($\delta^{13}C_{le}$)

$$(2) \qquad \delta^{13}C_{le} = \delta^{13}C_{bulk} + D \times \left(I + \frac{3.9}{1 + 287/L}\right)$$

where *D* is the difference in $\delta^{13}C$ between proteins and lipids and *I* is a constant value. The model revision by Kiljunen et al. (2006) estimated a value of 7.018 for *D* and 0.048 for *I* instead of the original 6 and –0.207, respectively.

The second model is simpler and based on mass balance correction (Fry et al. 2003) and was recently recalibrated more specifically for freshwater zooplankton lipid correction by Smyntek et al. (2007). The mass balance model uses both bulk and lipid-extracted C:N ratios with *D* to calculate $\delta^{13}C_{le}$

$$(3) \qquad \delta^{13}C_{le} = \delta^{13}C_{bulk} + D \times \left(\frac{\mathrm{C{:}N_{bulk}} - \mathrm{C{:}N_{le}}}{\mathrm{C{:}N_{bulk}}}\right)$$

where $C{:}N_{le}$ and $C{:}N_{bulk}$ are the C:N ratios of the lipid-extracted and untreated sample materials. Appropriate $C{:}N_{le}$ has to be assessed either from the literature or by chemically extracting lipids from a small subsample and analyzing the lipid free C:N value. Smyntek et al. (2007) estimated a *D* value 6.3 and $C{:}N_{le}$ value 4.2 appropriate for freshwater zooplankton.

The third model is derived from Logan et al. (2008), who also compared different correction methods with chemical lipid extraction on a variety of sample materials. The model assumes a log-transformed relationship between $\Delta\delta^{13}C$ and C:N ratios:

$$(4) \qquad \delta^{13}C_{le} = \delta^{13}C_{bulk} + \beta_0 + \beta_1 \ln(\mathrm{C{:}N_{bulk}})$$

where values for variables β_0 and β_1 were taken from parameter estimates for all invertebrates given in Logan et al. (2008). All previous three methods (eqs. 1–4) assume a nonlinear relationship between the lipid content (i.e., C:N ratios) and the $\Delta\delta^{13}C$. However, Post et al. (2007) found a linear relationship between the C:N ratios and $\Delta\delta^{13}C$ in aquatic organisms. According to Post et al. (2007), the impact of lipids on $\delta^{13}C$ values can be corrected by

$$(5) \qquad \delta^{13}C_{le} = \delta^{13}C_{bulk} - 3.32 + 0.99 \times \mathrm{C{:}N_{bulk}}$$

We tested these models on our zooplankton data and followed published recommendations (e.g., Logan et al. 2008) of calibrating the existing models to the present data. We chose to recalibrate the parameters used in the mass balance model by calculating the appropriate $C{:}N_{le}$ value from our lipid-extracted zooplankton samples and then iterating a new value of *D* using Marquardt–Levenberg algorithm, which seeks the values of the parameters that minimize the sum of the squared differences between the values of the ob-

Table 1. Summary of lipid correction models compared in this study with details on taxa and C:N range used to develop and calibrate the models.

Reference	Studied taxa	Studied C:N range	Model*	R^2
Kiljunen et al. 2006	14 fish species from marine, freshwater, and brackish water habitats	2.9–63	$\delta^{13}C_{le} = \delta^{13}C_{bulk} + 7.018 \times \left(\frac{3.90}{1+287/L}\right)$	0.65
Logan et al. 2008†	10 species of marine and freshwater invertebrates	3.7–10.7	$\delta^{13}C_{le} = \delta^{13}C_{bulk} - 2.06 + 1.91 \times \ln(C:N_{bulk})$	0.03
Smyntek et al. 2007	Freshwater zooplankton (cladocera, cyclopoid and calanoid copepods)	4.7–17.8	$\delta^{13}C_{le} = \delta^{13}C_{bulk} + 6.3 \times \left(\frac{C:N_{bulk}-4.2}{C:N_{bulk}}\right)$	0.54
Post et al. 2007	16 aquatic animals, including reptiles, fish, and invertebrates	3.0–6.9	$\delta^{13}C_{le} = \delta^{13}C_{bulk} - 3.32 + 0.99 \times C:N_{bulk}$	0.00
This study	Freshwater zooplankton (cladocera, cyclopoid and calanoid copepods)	3.8–19.3	$\delta^{13}C_{le} = \delta^{13}C_{bulk} + 7.95 \times \left(\frac{C:N_{bulk}-3.8}{C:N_{bulk}}\right)$	0.86

Note: R^2 values indicate the model fits to the present data (Fig. 4).
*In the model by Kiljunen et al. 2006, the variable L is defined in eq. 1.
†C:N values and model parameters taken from invertebrate data.

served and predicted values. Each model was evaluated against the recalibrated model using the Akaike information criterion, AIC (Akaike 1974), with a second-order correction for small sample sizes, AIC_c (Burnham and Anderson 1998)

$$(6) \qquad AIC_c = 2k - 2\ln(L) + \frac{2k(k+1)}{n-k-1}$$

where k is the number of model parameters, and L is the likelihood value for the estimated model. Models were evaluated by calculating AIC_c values for each model based on the fits to our chemically lipid-extracted zooplankton data, with lower AIC_c values indicating better model performance.

We were also able to estimate the values of D directly from zooplankton samples collected from northern Finland, since these were accurately weighted before and after lipid extraction and lipid-% was calculated. The value of D was estimated from

$$(7) \qquad \delta^{13}C_{bulk} = f_{le} \times \delta^{13}C_{le} + f_{lipid} \times \delta^{13}C_{lipid}$$

which rearranges to

$$(8) \qquad \delta^{13}C_{lipid} = \frac{\delta^{13}C_{bulk} - f_{le} \times \delta^{13}C_{le}}{f_{lipid}}$$

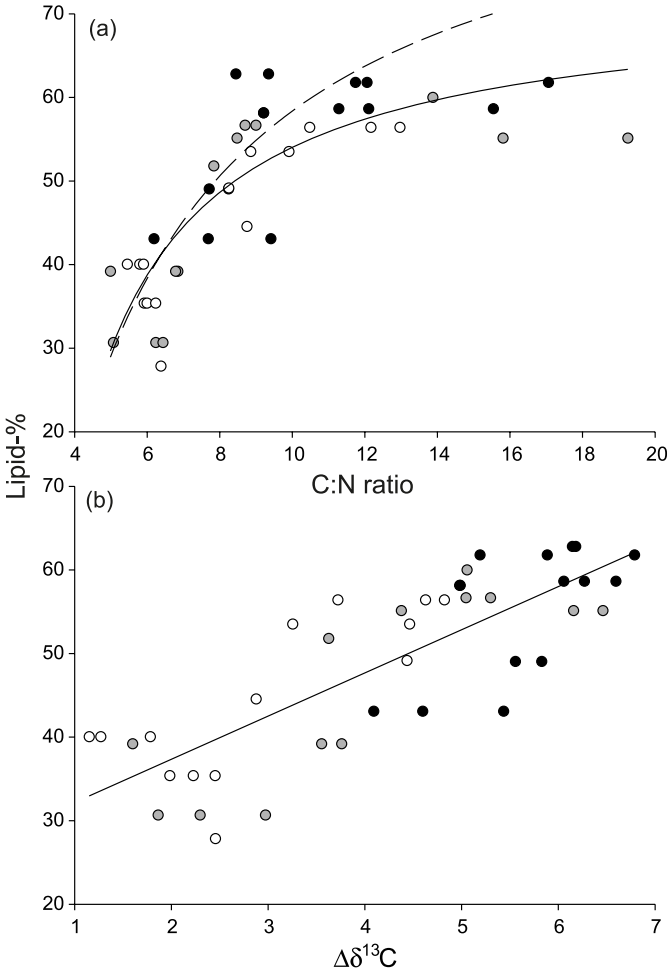
where f_{lipid} is the zooplankton lipid fraction (lipid-%) and f_{le} is $1 - f_{lipid}$. Values of D for northern Finland zooplankton were then simply extracted from $\delta^{13}C_{lipid}$ and $\delta^{13}C_{le}$. All statistical analyses and modeling were done using SYSTAT SigmaPlot for Windows v. 10.0 (Systat Software Inc., Point Richmond, California, USA) and SPSS for Windows v. 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Fifty-two zooplankton samples of cladocerans (21) and cyclopoid (20) and calanoid copepods (11) from the boreal sampling area and 51 samples (18, 15, and 18, respectively) from the subarctic sampling area were analyzed for this study. Collecting samples from such different habitats and at different times of year increased the observed range in C:N ratios in our zooplankton samples from 3.8 to as high as 19.3. Lipid-% in zooplankton samples collected from northern Finland varied from ~20% in May to over 60% in early winter, cladocerans typically having the lowest and calanoid copepods (*E. graciloides*) having the highest lipid content. The lipid-% had a clear inverse relationship with C:N ratios of zooplankton and fitting eq. 1 to the observed data resulted in an $R^2 = 0.50$ model fit (Fig. 1*a*). Iterating new parameters to the model using our observed data improved the fit to $R^2 = 0.70$ and resulted in a model to estimate lipid fraction as $72.2/[1 + (0.453 \times C:N - 1.562)^{-1}]$. Lipid-% was also linearly related to the difference between lipid-extracted and untreated $\delta^{13}C$ values ($\Delta\delta^{13}C$) of zooplankton ($F_{[1,41]} = 74.9$, $p < 0.001$, Fig. 1*b*).

The $\Delta\delta^{13}C$ values showed significant differences among taxa in both boreal ($F_{[2,39]} = 3.3$, $p = 0.049$) and subarctic sampling areas ($F_{[2,50]} = 11.7$, $p < 0.001$) but were significantly higher in samples collected from subarctic Finland ($F_{[1,91]} = 134.4$, $p < 0.001$). However, only the means of cladocerans and calanoid copepods differed significantly ($p = 0.046$ in southern and $p < 0.001$ in northern areas) in annual $\Delta\delta^{13}C$ values. Zooplankton samples from both sampling areas showed considerable temporal variation in their $\Delta\delta^{13}C$ values (Fig. 2). The $\Delta\delta^{13}C$ value for cladocerans from boreal sampling area ranged from 0.7‰ to 1.4‰ in May–November, while the mean (± standard error, SE) was 1.1‰ ± 0.1‰. Similarly, cyclopoid and calanoid copepods $\Delta\delta^{13}C$ ranged between 0.9‰–1.8‰ and 0.7‰–3.5‰, with mean values of 1.5‰ ± 0.1‰ and 1.9‰ ± 0.3‰, respectively. The variation was more pronounced in samples from subarctic sampling area, where $\Delta\delta^{13}C$ values of cladocerans ranged between 1.8‰ and 4.4‰ (mean 3.4‰ ± 0.3‰) and those of cyclopoid and calanoid copepods between 2.4‰–5.7‰ (mean 4.1‰ ± 0.4‰) and 2.8‰–6.3‰ (mean 5.2‰ ± 0.3‰), respectively. A linear regression indicated that water temperature was an important factor determining the $\Delta\delta^{13}C$ of zooplankton ($F_{[1,39]} = 47.4$, $p < 0.001$, $R^2 = 0.56$; Fig. 3). Adding taxa and sampling site as other explaining factors increased the model performance in explaining zooplankton $\Delta\delta^{13}C$ ($F_{[2,39]} = 37.0$, $p < 0.05$, $R^2 = 0.67$; and $F_{[3,39]} = 47.9$, $p < 0.005$, $R^2 = 0.80$, respectively). All explaining variables (water temperature, taxa, and sampling site) in the final model were highly significant (all $p < 0.005$).

Fig. 1. (*a*) Zooplankton lipid content (lipid-%) in samples from the subarctic sampling area plotted against their C:N ratios. Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods. The dashed line indicates lipid content estimated using the McConnaughey and McRoy (1979) equation to calculate the lipid factor (*L*) shown in eq. 1 ($R^2 = 0.50$). The solid line indicates the eq. 1 fit after iterating new model parameters ($R^2 = 0.70$). (*b*) A linear relationship ($y = 5.2x + 27.02$, $R^2 = 0.65$ is shown between the difference of bulk and lipid-extracted $\delta^{13}C$ values ($\Delta\delta^{13}C$) and lipid content.



Although the mean (± SE) difference between untreated and lipid-extracted $\delta^{15}N$ values was only 0.15 ± 0.07, and therefore biologically negligible, it was statistically significant (paired *t* test; $t_{90} = -2.1$, $p = 0.04$). However, the values were tightly correlated with little scatter along a very considerable range of $\delta^{15}N$ values, and there was no correlation between the C:N ratios and the difference in untreated and lipid-extracted $\delta^{15}N$ values, indicating that any small effect on $\delta^{15}N$ values is likely meaningless. Lipid extraction had no effect on the variance of $\delta^{15}N$ values ($p > 0.05$) but significantly increased the $\delta^{13}C$ variance ($p = 0.024$).

Testing the lipid correction models from Kiljunen et al. (2006), Post et al. (2007), Smyntek et al. (2007), and Logan et al. (2008) to our zooplankton data revealed very different predictions of $\delta^{13}C_{le}$ from bulk sample C:N ratios. None of the tested models satisfactorily fitted the present data with the original model parameters (Fig. 4, Table 1). Particularly

Fig. 2. Temporal variation in $\Delta\delta^{13}C$ values of different zooplankton taxa in subarctic sampling areas (*a*) and boreal sampling area (*b*). Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods. Mean values (± standard error, SE) are given for replicated samples.

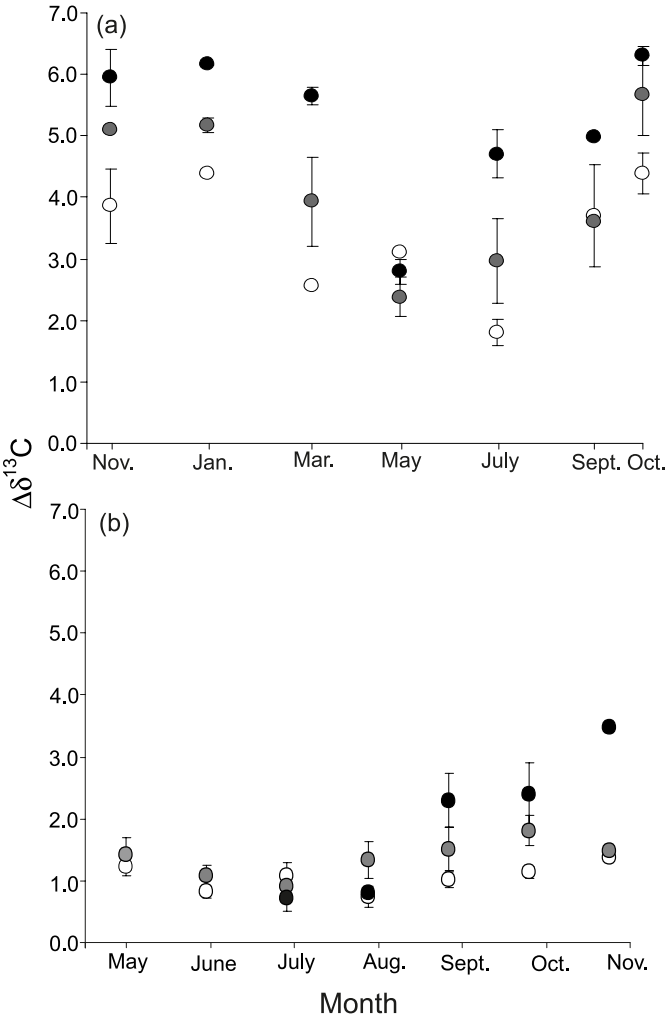
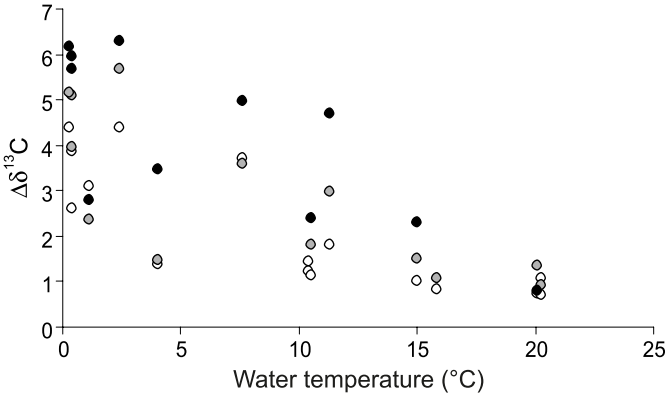


Fig. 3. Relationship between surface (0–2 m) water temperature at the time of sampling and the difference in $\delta^{13}C$ values of bulk and lipid-extracted zooplankton material ($\Delta\delta^{13}C$). Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods.



the linear model by Post et al. (2007) and the log-transformed model by Logan et al. (2008) did not fit to the observed zooplankton data. The models by Kiljunen et al. (2006) and Smyntek et al. (2007) produced predicted $\delta^{13}C_{le}$ values with generally similar trends but both slightly underestimated the $\Delta\delta^{13}C$. After recalibrating the mass balance model parameters (D and $C:N_{le}$) according to our data, the model fit improved from $R^2 = 0.54$ by Smyntek et al. (2007) to $R^2 = 0.86$. The calibrated parameters (± SE) of the best fit for the whole data were $D = 7.9 \pm 0.2$ and $C:N_{le} = 3.8 \pm 0.1$. Some individual samples were excluded when calculating the $C:N_{le}$ because of incomplete lipid removal ($C:N_{le} > 5$). As expected, the AIC_c values were lowest, indicating the best fit, for our recalibrated mass balance model, followed by Kiljunen, Smyntek, Logan, and Post models. The two parameters in the Logan et al. (2008) model can also be easily recalibrated using the observed data, seriously improving the model fit ($R^2 = 0.84$ with $\beta_0 = 5.06 \pm 0.4$ and $\beta_1 = 4.30 \pm 0.2$), but the calibrated mass balance model would still have the best performance. Our lipid-% data also allowed a recalibration of the parameters to calculate the lipid factor (L, eq. 1, Fig. 1*a*) for the lipid normalization model. However, this did not notably improve the overall performance of the lipid normalization (by only 9%). But scatter remained even in the mass balance model between the predicted and observed values even after calibrating the model parameters. This scatter was considerably reduced when separate models were applied for individual taxa (Fig. 5). After iterating separate D values for cladocerans ($D = 6.4 \pm 0.2$) and cyclopoid ($D = 7.9 \pm 0.2$) and calanoid ($D = 9.1 \pm 0.2$) copepods, the model fits to individual taxa were $R^2 = 0.84$, $R^2 = 0.94$, and $R^2 = 0.93$, respectively.

Calculating the D values based on $\delta^{13}C_{bulk}$, $\delta^{13}C_{le}$, and lipid-% of zooplankton collected from subarctic Finland resulted in mean D values (± SE) of 6.4 ± 0.5, 8.2 ± 0.5, and 10.3 ± 0.4 for cladocerans, cyclopoids, and calanoids, respectively. The values for cladocerans and cyclopoid copepods matched closely the D values iterated using the mass balance model for the same subarctic data set (6.4 ± 0.3 and 8.0 ± 0.2, respectively), but was somewhat higher than the iterated value for the calanoid copepods (9.2 ± 0.2) from subarctic Finland. The D values for calanoid copepods ranged from 8.3 to 13.2 but did not reflect any clear pattern, whereas lower D values were calculated from cladocerans and cyclopid copepods, with lower C:N ratios collected during the summer period.

Fig. 4. Observed relationship between the difference in $\delta^{13}C$ values of bulk and lipid-extracted zooplankton material ($\Delta\delta^{13}C$) and the bulk zooplankton C:N ratio. Solid lines represent the expected relationship based on the lipid correction models tested for the present data; the dashed line indicates the expected relationship based on our recalibrated mass balance correction model.

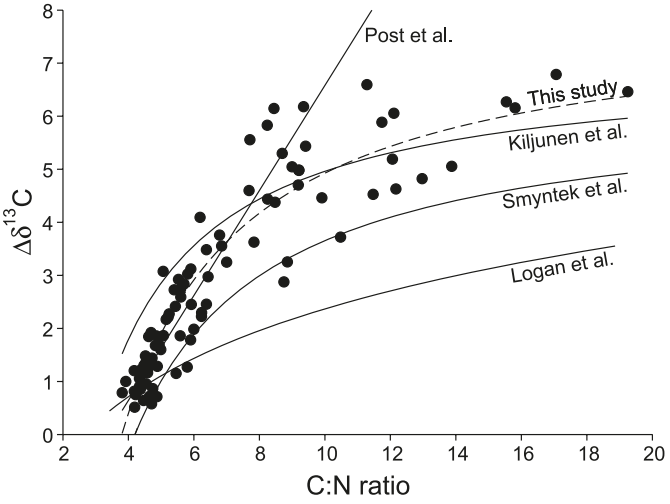
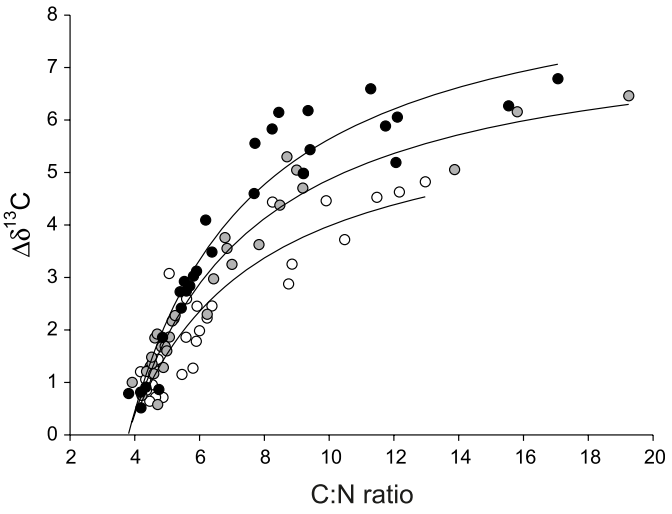


Fig. 5. Mass balance lipid correction models calibrated for separate zooplankton taxa (bottom line, open symbols: cladocerans $R^2 = 0.84$; middle line, grey symbols: cyclopoid copepods $R^2 = 0.94$; top line, black symbols: calanoid copepods $R^2 = 0.93$).



Discussion

We analyzed three zooplankton taxa (cladocerans and cyclopoid and calanoid copepods) exposed to very different environmental conditions from boreal and subarctic Finland. Sampling from these distinct areas resulted in highly variable body compositions (lipid content) and considerably high C:N ratios (up to 19.3) in our zooplankton samples. Increased lipid content in zooplankton collected from subarctic Finland was clearly reflected as both increased C:N ratios and greater difference between bulk zooplankton and lipid-extracted $\delta^{13}C$ values ($\Delta\delta^{13}C$), allowing a thorough testing of the existing lipid correction models. The relationship between C:N and lipid content was clearly not linear but rather asymptotic, similar to what has been typically reported between C:N ratios and $\Delta\delta^{13}C$ values in literature (e.g., Kiljunen et al. 2006; Smyntek et al. 2007; Logan et al. 2008). Therefore the linear lipid correction model proposed by Post et al. (2007) does not seem appropriate for aquatic samples with higher lipid content and elevated C:N ratios and should not be used for samples with a greater range in C:N ratios than the relatively small range ($C:N < 7$) studied by Post et al. (2007). Within the appropriate range of C:N ratios, the model by Post et al. (2007) does seem to perform equally well to the calibrated mass balance model.

The lipid-% in our samples reached its upper limit (50%–60%) when the C:N ratios were approaching 10. These gravimetrically calculated lipid-% values may be a slight

overestimation because of other constituents such as salts and solids, which are also soluble with chloroform–methanol, and some of them may have been unintentionally removed with the lipid phase. Nevertheless, even if the lipid-% in reality were slightly smaller, the relationship between C:N and lipid-% would still be the same. However, while the lipid-% remained more stagnant after C:N ratios of 10, the C:N ratios continued increasing almost up to 20. Such increase in C:N ratios, but not in lipid-%, might be due to increases in other carbon body parts in zooplankton carapace, which could increase the total carbon concentrations of samples but not total nitrogen or lipid content. But such increases should be evident as larger body sizes of these zooplankton individuals, which did not seem to be the case in our samples. It is perhaps more likely that lipid content around 60% is close to a physiological maximum for many zooplankton species, but the C:N ratios can still be increasing because of assimilation of carbon from diet. The highest C:N ratios in our data were analyzed from samples collected from northern sampling sites in early winter, which would be just after the zooplankton had accumulated energy storages for the winter.

The zooplankton samples analyzed here showed that the $\Delta\delta^{13}C$ varied considerably over time and among taxa. Samples from lower latitude showed notably less variation than those from northern latitude, but the $\Delta\delta^{13}C$ values clearly increased toward autumn and winter in both regions, especially in calanoid copepods with a $\Delta\delta^{13}C$ value up to 3.5‰ in November. This seasonal shift was also reflected as a clear relationship between surface water temperatures and zooplankton $\Delta\delta^{13}C$ values. Water temperature alone explained 56% of the variation in $\Delta\delta^{13}C$ values, but adding zooplankton taxa and sampling area (boreal–subarctic) increased the model performance up to 80%. Water temperature, together with taxa and sampling area, affect the lipid content of the zooplankton, as lipid contents are highest in copepods of the higher latitude region in autumn just before winter and early winter when the water temperatures are low. Samples from the lakes in the lower latitude region were taken only during the ice-free period (typically from May to November), and the increasing $\Delta\delta^{13}C$ values likely reflected increasing lipid storages in zooplankton for the winter period. The ice-free period is much shorter in our subarctic sampling area, typically from late June to late October, but the short open water period was still reflected as notable decrease (over 3‰ in calanoid copepods) in $\Delta\delta^{13}C$ values. The $\Delta\delta^{13}C$ values in zooplankton from the subarctic sampling area were much higher than in our boreal sampling area throughout the year, and in summer the values only briefly dropped to similar levels to those of zooplankton in the south. This greater difference in $\delta^{13}C$ values between bulk and lipid free zooplankton body tissues observed in northern latitudes may not solely be due to greater lipid content of these zooplankters, but the cold climate may also affect the isotope fractionation in lipid synthesis differently because of increased enzymatic fractionation (DeNiro and Epstein 1977; Smyntek et al. 2007). There was also a consistent difference in $\Delta\delta^{13}C$ values among different taxa in both sampling regions, with higher values in copepods than in cladocerans, which can explain the often observed difference in $\delta^{13}C$ values between these taxa (Syväranta et al. 2006; Rautio and Vincent 2007; Smyntek et al. 2007). The highly variable $\Delta\delta^{13}C$ values in both sampled regions indicate that studies using zooplankton $\delta^{13}C$ values to assess baseline levels or especially carbon sources to zooplankton should carefully consider the potential influence of varying lipid content in zooplankton tissues.

Lipid extraction had a significant impact of the $\delta^{15}N$ values of zooplankton in our study. This is contrary to the findings of Ingram et al. (2007). However, although statistically significant, the mean difference of 0.15‰ between lipid-extracted and untreated zooplankton $\delta^{15}N$ values is biologically insignificant, since the typical natural variation among samples is by far greater than 0.15‰ and even the instrument precision may not always reach this accuracy. Since the variation in $\delta^{15}N$ values did not increase after lipid extraction, the lipid-extracted and untreated values were highly correlated along the wide range of $\delta^{15}N$ values, and there was no correlation between C:N value and $\Delta\delta^{15}N$, we can confidently agree with Ingram et al. (2007) that lipid extraction does not significantly alter the zooplankton $\delta^{15}N$ values.

The lipid correction models tested here produced rather unsatisfactory estimates of $\Delta\delta^{13}C$ when using the original model parameters. The AIC_c values were much higher for these models when compared with our calibrated mass balance model. Comparing the individual AIC_c values of each model to the lowest AIC_c value from our mass balance model revealed differences in AIC_c values > 10, basically indicating very little support for these models (Burnham and Anderson 1998). Particularly, the linear model by Post et al. (2007) did not fit to samples with higher lipid content and C:N ratios and therefore should not be used to correct such samples (C:N ratios > 7). Also, the Logan et al. (2008) model with its parameters derived from a variety of invertebrate samples did not fit to the zooplankton data tested here. The invertebrates used to calibrate model parameters were mostly macroinvertebrates in which the higher C:N ratios are more dependent on other body materials (like chitin) than lipids. However, calibrating the two parameters in Logan et al. (2008) model with the present data resulted in improved fit and second-best AIC_c values (the difference to our mass balance model being still 7.7). The models by Kiljunen et al. (2006) and Smyntek et al. (2007) in general provided similar trends and fits to our zooplankton data, but neither model satisfactorily fitted the data when published parameters were used. This was not surprising for the model by Kiljunen et al. (2006), which was calibrated for lipid normalization of fish muscle tissues. However, the lack of fit of the model by Smyntek et al. (2007) specifically calibrated for zooplankton samples was more surprising and is likely due to our samples from subarctic Finland having very high C:N and $\Delta\delta^{13}C$ values.

In addition, our zooplankton samples spanned a very considerable range of C:N ratios (from just 3.8 to 19.3), enabling us to efficiently test different models but to also detect clear among-taxa differences in C:N–$\Delta\delta^{13}C$ relationships, resulting in relatively poor fit for a single model even after recalibration. We chose to use a single $C:N_{le}$ parameter value (3.8) in all our taxa-specific mass balance models, which was calculated from the whole lipid free zooplankton data and fit perfectly for cyclopoid and calanoid copepod data. However, a slightly higher value might have been

more appropriate for cladocerans, potentially the 4.2 proposed by Smyntek et al. (2007) for all zooplankton. But the data presented here clearly indicate that a single lipid correction model may not perform satisfactorily if the data consist of several taxa with unequal C:N–$\Delta\delta^{13}C$ relationships. Therefore, separate models with taxa-specific parameter *D* values (and potentially also $C:N_{le}$ values) are clearly needed when analyzing zooplankton samples from such contrasting environments and especially with very high lipid contents and C:N ratios. This conclusion agrees with Logan et al. (2008), who compared chemical and arithmetic lipid correction and presented parameter values for diverse taxa of aquatic fishes and invertebrates. However, when using models with parameters such as *D*, it is worth remembering the underlying assumptions for such parameters. For example, *D* is the difference in $\delta^{13}C$ values between lipids and both proteins and carbohydrates. Contrary to more homogenous tissues like muscle tissue, many aquatic invertebrates including zooplankton are typically analyzed as whole and may contain variable amounts of other materials such as chitin and glycogen (Kiljunen et al. 2006). In other words, the $\delta^{13}C$ of lipid-extracted zooplankton is not the same as $\delta^{13}C$ of zooplankton proteins, as is in the muscle tissue.

The *D* values calculated for samples collected from subarctic Finland were in good agreement with the modeled values for cladocerans and cyclopoid copepods, while slightly higher for calanoid copepods. In general, these values were higher than those presented by Smyntek et al. (2007), who estimated the *D* for different taxa from temperate lakes by directly analyzing the $\delta^{13}C$ of fatty acids. This difference is likely explained by differences in lipid fractionation and synthesis in the colder climate of our higher latitude lake (DeNiro and Epstein 1977). However, our method of calculating the $\delta^{13}C_{lipid}$ from lipid-% is less accurate and may introduce some bias to the final estimates of *D*. Nevertheless, these estimates agreed to our iterated *D* values and showed also some within-taxa variation for cladocerans and cyclopoid copepods. This variation was linked with lower C:N ratios and seasonally to summer period, when the lipid reserves were lowest. Changes in $\delta^{13}C$ of algae and algal lipids that are in part directly incorporated into zooplankton lipids could at this time be quickly reflected as the $\delta^{13}C$ difference between zooplankton lipids and proteins.

The results presented in this paper clearly illustrate how increasing lipid content impacts the $\delta^{13}C$ values analyzed from freshwater zooplankton. However, as concluded in previous studies (e.g., Post et al. 2007), the need for lipid correction only becomes important when the lipid content of samples is variable and (or) high, as indicated by higher (>3.5) and variable C:N ratios. Moreover, all studied zooplankton taxa showed considerable temporal variation in the extent of lipid impact on $\delta^{13}C$ values and in general greater impacts were observed in samples from the subarctic sampling area with colder water temperatures. Equally important is the conclusion that $\delta^{13}C$ of different zooplankton taxa responds differently to increases in lipid content and C:N ratios. This highlights the importance of separating bulk zooplankton samples into different taxa before SIA to minimize observed variation (Matthews and Mazumder 2003; Syväranta et al. 2006). Our results also highlight the importance of calibrating existing models to the present data and using taxa-specific parameters when possible. If appropriate parameters are not available in the literature, we stress the previously published recommendations (Smyntek et al. 2007; Logan et al. 2008) of taking a subsample for chemical lipid extraction to calculate $C:N_{le}$ and estimate *D*. Preferentially, samples representing the greatest range in $C:N_{bulk}$ should be selected to assure an accurate estimate of parameter *D*. The models can be rather sensitive to different values of *D* (Matthews and Mazumder 2005; Logan et al. 2008; this study), and care has to be taken when deciding the final model parameters.

Acknowledgements

Pekka Majuri, Katie Aoki, Sari Oksanen, Heather Mariash, and Laura Forsström gave valuable assistance in the field and laboratory. Tuula Sinisalo helped with operating the SIA instrument in Jyväskylä, and many constructive discussions with Mikko Kiljunen are greatly appreciated. Helpful comments from Martin Kainz, Marc Trudel, and two anonymous reviewers are also acknowledged. This work was funded by grants from the Maj and Tor Nessling Foundation, Finnish Cultural Foundation, and Kone Foundation to JS and from the Academy of Finland (grant 119205) to MR.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. **19**(6): 716–723. doi:10.1109/TAC.1974.1100705.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**(8): 911–917. PMID:13671378.

Box, G.E.P., and Cox, D.R. 1964. An analysis of transformations. J. R. Stat. Soc. B, **26**: 211–234.

Burnham, K.P., and Anderson, D.R. 1998. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.

DeNiro, M.J., and Epstein, S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science (Washington, D.C.), **197**(4300): 261–263. doi:10.1126/science.327543. PMID:327543.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**(5): 495–506. doi:10.1016/0016-7037(78)90199-0.

Fry, B., Baltz, D.M., Benfield, M.C., Fleeger, J.W., Gace, A., Haas, H.L., and Quiñones-Rivera, Z.J. 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries, **26**(1): 82–97. doi:10.1007/BF02691696.

Graeve, M., Albers, C., and Kattner, G. 2005. Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a ^{13}C labeled diatom. J. Exp. Mar. Biol. Ecol. **317**(1): 109–125. doi:10.1016/j.jembe.2004.11.016.

Grey, J. 2006. The use of stable isotope analyses in freshwater ecology: current awareness. Pol. J. Ecol. **54**: 563–584.

Grey, J., Jones, R.I., and Sleep, D. 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnol. Oceanogr. **46**(3): 505–513. doi:10.4319/lo.2001.46.3.0505.

Ingram, T., Matthews, B., Harrod, C., Stephens, T., Grey, J., and Markel, R. 2007. Lipid extraction has little effect on the $\delta^{15}N$ of aquatic consumers. Limnol. Oceanogr. Methods, **5**: 338–343.

Karlsson, J., and Säwström, C. 2009. Benthic algae support zooplankton growth during winter in a clear-water lake. Oikos, **118**: 539–544.

Kattner, G., Hagen, W., Lee, R.F., Campbell, R., Deibel, D., Falk-Petersen, S., Graeve, M., Hansen, B.W., Hirche, H.J., Jónasdóttir, S.H., Madsen, M.L., Mayzaud, P., Müller-Navarra, D., Nichols, P.D., Paffenhöfer, G.-A., Pond, D., Saito, H., Stübing, D., and Virtue, P. 2007. Perspectives on marine zooplankton lipids. Can. J. Fish. Aquat. Sci. **64**(11): 1628–1639. doi:10.1139/F07-122.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., and Jones, R.I. 2006. A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. J. Appl. Ecol. **43**(6): 1213–1222. doi:10.1111/j.1365-2664.2006.01224.x.

Kling, G.W., Fry, B., and O'Brien, W.J. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology, **73**(2): 561–566. doi:10.2307/1940762.

Kuosa, H., and Gyllenberg, G. 1989. Lipid content and utilization of lipids in planktonic copepods in Lake Pääjärvi, southern Finland. Hydrobiologia, **171**(3): 215–222. doi:10.1007/BF00008144.

Lee, R.F., Hagen, W., and Kattner, G. 2006. Lipid storage in marine zooplankton. Mar. Ecol. Prog. Ser. **307**: 273–306. doi:10.3354/meps307273.

Logan, J.M., and 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 Commun. Mass Spectrom. **22**(7): 1081–1086. doi:10.1002/rcm.3471. PMID:18327856.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. J. Anim. Ecol. **77**(4): 838–846. doi:10.1111/j.1365-2656.2008.01394.x. PMID:18489570.

Matthews, B., and Mazumder, A. 2003. Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. Limnol. Oceanogr. **48**(5): 1977–1987. doi:10.4319/lo.2003.48.5.1977.

Matthews, B., and Mazumder, A. 2005. Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}C$. Freshw. Biol. **50**(3): 502–515. doi:10.1111/j.1365-2427.2005.01336.x.

Matthews, B., and Mazumder, A. 2007. Distinguishing trophic variation from seasonal and size-based isotopic ($\delta^{15}N$) variation of zooplankton. Can. J. Fish. Aquat. Sci. **64**(1): 74–83. doi:10.1139/F06-168.

McConnaughey, T., and McRoy, C.P. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar. Biol. (Berlin), **53**(3): 257–262. doi:10.1007/BF00952434.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta, **48**(5): 1135–1140. doi:10.1016/0016-7037(84)90204-7.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**(1): 293–320. doi:10.1146/annurev.es.18.110187.001453.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montaña, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia (Berlin), **152**(1): 179–189. doi:10.1007/s00442-006-0630-x.

Rautio, M., and Vincent, W.F. 2007. Isotopic analysis of the sources of organic carbon for zooplankton in shallow subarctic and arctic waters. Ecography, **30**: 77–87.

Smyntek, P.M., Teece, M.A., Schultz, K.L., and Thackeray, S.J. 2007. A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. Limnol. Oceanogr. **52**: 2135–2146.

Sotiropoulos, M.A., Tonn, W.M., and Wassenaar, L.I. 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. Ecol. Freshw. Fish, **13**(3): 155–160. doi:10.1111/j.1600-0633.2004.00056.x.

Sweeting, C.J., Polunin, N.V.C., and Jennings, S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Commun. Mass Spectrom. **20**(4): 595–601. doi:10.1002/rcm.2347. PMID:16429479.

Syväranta, J., Hämäläinen, H., and Jones, R.I. 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshw. Biol. **51**(6): 1090–1102. doi:10.1111/j.1365-2427.2006.01557.x.

Syväranta, J., Tiirola, M., and Jones, R.I. 2008. Seasonality in lake pelagic $\delta^{15}N$ values: patterns, possible explanations, and implications for food web studies. Fund. Appl. Limnol. **172**(3): 255–262. doi:10.1127/1863-9135/2008/0172-0255.

Ventura, M., and Catalan, J. 2008. Incorporating life histories and diet quality in stable isotope interpretations of crustacean zooplankton. Freshw. Biol. **53**(7): 1453–1469. doi:10.1111/j.1365-2427.2008.01976.x.