

NOTE

Stable isotope food web studies: a case for standardized sample treatment

Ute Jacob*, Katja Mintenbeck, Thomas Brey, Rainer Knust, Kerstin Beyer

Alfred Wegener Institute for Polar and Marine Research, PO Box 120161, 27515 Bremerhaven, Germany

ABSTRACT: Enrichment of the stable isotopes ^{13}C and ^{15}N across trophic levels is a commonly used tool in studies on organic matter flow and food webs. However, there is still no accepted standard for pre-analysis sample preparation. Thus, potential methodological bias in single studies may hamper comparability and scalability of data from different sources. Sample CaCO_3 content introduces a positive bias in $\delta^{13}\text{C}$ measurements and a negative bias in $\delta^{15}\text{N}$ measurements. The acidification of samples to remove inorganic carbonate significantly reduces both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. As a standard procedure we recommend (1) acidifying samples with as little hydrochloric acid (HCl) as possible using the drop-by-drop technique, and (2) restraining from rinsing after HCl application.

KEY WORDS: Isotope signatures · Carbonates · Sample acidification

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INTRODUCTION

Metabolic processing of ingested organic matter causes isotopic fractionation of ^{13}C : ^{12}C and ^{15}N : ^{14}N stable isotope pairs. Therefore, the stable isotope ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are proxies of the trophic distance of an organism from the origin of the corresponding food chain. Although the underlying physiological, biochemical and biophysical processes are not yet fully understood (Ponsard & Averbuch 1999), enrichment of the stable isotopes ^{13}C and ^{15}N across trophic levels is a commonly used tool in studies on organic matter flow and food webs in aquatic and terrestrial ecosystems. On average, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ increase by 0.5 to 1 and 2.8 to 3.4‰, respectively, from one trophic level to the next (e.g. Minagawa & Wada 1984, Peterson & Fry 1987, Michener & Schell 1994). Early trophic stable isotope studies relied on $\delta^{13}\text{C}$ only (Fry 1984, Fry & Sherr 1984). The finding that $\delta^{13}\text{C}$ correlates with tissue fat content, whereas $\delta^{15}\text{N}$ does not, led to the parallel application of both isotope ratios in more recent studies (e.g. Fry 1988, Rau et al. 1991, 1992). Although the magnitude of variation in $\delta^{13}\text{C}$ fractionation is the

major source of error in quantitative stable isotope models (Vander Zanden & Rasmussen 2001), $\delta^{13}\text{C}$ is still used as it serves as a valuable carbon source tracer (e.g. Lesage et al. 2001). The major methodological question regarding the simultaneous measurement of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is whether the removal of inorganic carbonate (CaCO_3 , for instance in red algae, cnidarians, bryozoans, mollusks, echinoderms) from the sample will bias tissue stable isotope ratios (Fry 1988, Cloern et al. 2002). Carbon incorporated in tissue and in carbonate are of different origin and hence differ in $\delta^{13}\text{C}$; therefore, carbonate must be removed by acidification prior to mass spectrometry (Fry 1988, Rau et al. 1991, Cloern et al. 2002). It remains unclear, however, whether tissue isotopic ratios are affected by acidification or not; there is contradictory evidence regarding $\delta^{15}\text{N}$, whereas no significant impact on $\delta^{13}\text{C}$ has been reported (Bunn et al. 1995, Bosley & Wainright 1999, Pinnegar & Polunin 1999). Our study, based on 193 samples from 29 species (11 major taxa), analyzes (1) how sample CaCO_3 content affects stable isotope ratios, and (2) whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are affected by sample acidification.

*Email: ujacob@awi-bremerhaven.de

MATERIALS AND METHODS

A total of 92 invertebrate samples (18 species among crustaceans, bryozoans, brachiopods, cephalopods, sponges, cnidarians, echiurids, polychetes, pantopods and echinoderms) and 101 fish samples (11 species) were collected for stable isotope analysis from fauna sampled along the Scotia Arc and the Antarctic Peninsula. Small organisms were analyzed whole, whereas in macro- and megafaunal specimens only body wall pieces or muscle tissue samples were used. All samples were kept frozen at -30°C until further analysis. Samples were lyophilised for 24 h in a Finn-Aqua Lyovac GT2E and then ground to a fine powder. Each sample was split in half: one part was acidified to remove CaCO_3 in accordance with Fry (1988) and Cloern et al. (2002) by adding 1 mol l^{-1} hydrochloric acid (HCl) drop-by-drop until no more CO_2 was released, re-dried at 60°C without rinsing to minimize loss of DOM (dissolved organic matter) and ground again; the other half did not receive any further treatment. Stable isotope analysis was conducted with an isotope-ratio mass spectrometer (Thermo/Finnigan Delta plus, GeoBioCenter). Experimental precision (based on standard deviation of replicates of a peptone standard) was $\leq 0.15\text{‰}$ for carbon and nitrogen.

A paired Student's *t*-test was used to evaluate whether stable isotope ratios differed between 'crude' and acidified samples. Sample CaCO_3 content had not been determined; hence, we used a CaCO_3 proxy to analyze the effect of CaCO_3 on stable isotope ratios:

$$\text{carbonate proxy} = \frac{[\text{C:N}]_{\text{crude}}}{[\text{C:N}]_{\text{acid}}} - 1 \quad (1)$$

where $[\text{C:N}]_{\text{crude}}$ is the C:N ratio of a non-acidified sample and $[\text{C:N}]_{\text{acid}}$ is the C:N ratio of an acidified sample. This proxy should be linearly related to sample CaCO_3 content, provided that tissue C:N is independent of sample CaCO_3 .

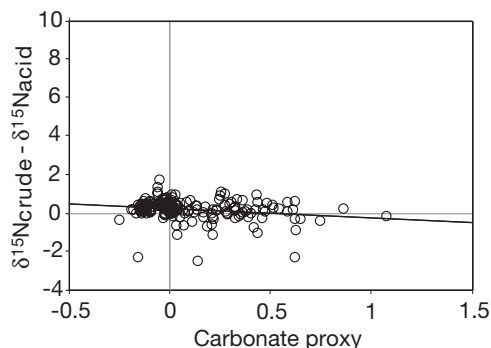


Fig. 1. Effect of sample CaCO_3 content on the difference in $\delta^{15}\text{N}$ between acidified and non-acidified samples. Sample CaCO_3 is approximated by the carbonate proxy $[\text{C:N}]_{\text{crude}}/[\text{C:N}]_{\text{acid}}$. Significant linear relation: $y = 0.248 - 0.486 \times x$, $r^2 = 0.047$, $p = 0.0033$, $N = 193$

RESULTS AND DISCUSSION

Surprisingly, a number of samples showed negative carbonate proxy values (Figs. 1 & 2). Analysis of presumably CaCO_3 free samples (fish and polychaete tissue) showed that, on average, acidification decreased tissue N content ($N_{\text{acid}} = -0.15N_{\text{crude}}$) proportionally more than tissue C content ($C_{\text{acid}} = -0.11C_{\text{crude}}$), thus causing higher C/N ratios in acidified samples. There is no need to correct the carbonate proxy value for this effect, as we assume it to be independent of sample CaCO_3 content.

$\delta^{15}\text{N}_{\text{crude}} - \delta^{15}\text{N}_{\text{acid}}$ was negatively related to sample CaCO_3 content (linear regression: $\text{df} = 192$, $p = 0.0033$, Fig. 1), whereas tissue $\delta^{13}\text{C}_{\text{crude}} - \delta^{13}\text{C}_{\text{acid}}$ was positively related to sample CaCO_3 content (linear regression: $\text{df} = 192$, $p < 0.0001$, Fig. 2). In samples containing no CaCO_3 (defined by carbonate proxy ≤ 0.03), acid treatment showed a significant negative effect on both $\delta^{15}\text{N}$ (paired *t*-test: mean difference = 0.320‰ , $\text{SE} = 0.037$, $\text{df} = 115$, $p = 0.0001$) and $\delta^{13}\text{C}$ (paired *t*-test: mean difference = 0.117‰ , $\text{SE} = 0.044$, $\text{df} = 115$, $p = 0.0081$).

The ongoing discussion of whether samples should be treated with HCl, how this treatment should be carried out, and what effect the treatment may have on isotope ratios as well as the contradictory findings (Bunn et al. 1995, Bosley & Wainright 1999, Pinnegar & Polunin 1999, McCutchan et al. 2003) shows that there is an urgent need for sample treatment standardization.

Regarding $\delta^{13}\text{C}$, our results indicate the expected positive relation between the acidification effect on $\delta^{13}\text{C}$ and sample CaCO_3 content (Fig. 2). The bias caused by CaCO_3 was in the range of the distance between subsequent trophic levels (0.5 to 1.0‰) at carbonate proxy values as low as 0.08 (Fig. 2). Regarding $\delta^{15}\text{N}$, this is the first study that demonstrates a significant negative, albeit weak, effect of sample CaCO_3 on tissue $\delta^{15}\text{N}$ (Fig. 1), the mechanism of which, however, remains unclear.

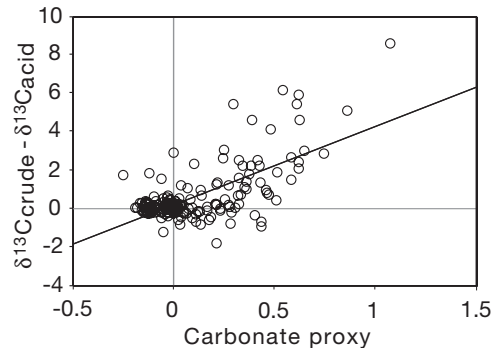


Fig. 2. Effect of sample CaCO_3 content on the difference in $\delta^{13}\text{C}$ between acidified and non-acidified samples. Sample CaCO_3 is approximated by the carbonate proxy $[\text{C:N}]_{\text{crude}}/[\text{C:N}]_{\text{acid}}$. Significant linear relation: $y = 0.181 + 4.08 \times x$, $r^2 = 0.449$, $p = 0.0001$, $N = 193$

The analysis of samples containing no CaCO_3 showed that acidification caused a significant (average = 0.117‰) decrease in $\delta^{13}\text{C}$. This deviation is (1) about 16% of the average shift of 0.5 to 1‰ from one trophic level to the next, (2) within the $\delta^{13}\text{C}$ variability observed in one trophic compartment (e.g. Rau et al. 1991, Vander Zanden & Rasmussen 2001, Cloern et al. 2002), and (3) in the range of measurement precision ($\leq 0.15\%$). $\delta^{15}\text{N}$ was also negatively affected (average = 0.320‰), but to a much lesser extent regarding the average shift of 2.8 to 3.4‰ from one trophic level to the next as well as $\delta^{15}\text{N}$ variability within one trophic compartment (e.g. Adams & Sterner 2000, Dunton 2001, Iken et al. 2001, Nyssen et al. 2002). The shift was, however, distinctly above measurement precision ($\leq 0.15\%$). Our findings support Bosley & Wainright's (1999) view that the way and extent to which $\delta^{15}\text{N}$ is affected by acidification depends on the methods applied, mainly on the rinsing of the acidified sample with distilled water. Our drop-by-drop acidification technique without subsequent rinsing appeared to produce, on average, little bias in $\delta^{15}\text{N}$ and low inter-sample variability in bias (Figs. 1 & 2). The 3 outliers in Fig. 1 were most likely to be caused by mistakes during sample processing.

CONCLUSION

Tissue samples must be acidified in order to make stable isotope data comparable across taxa with varying CaCO_3 content. Data from non-acidified but carbonate-free samples can be made comparable to data from acidified samples by the corresponding correction factors for $\delta^{13}\text{C}$ (-0.117‰) and $\delta^{15}\text{N}$ (-0.320‰). It remains to be seen, however, whether the factors found here are also valid for non-Antarctic ecosystems.

For future food web studies using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ simultaneously, we recommend the following procedure to standardize sample treatment: (1) Acidify samples prior to stable isotope analysis by careful application of as little HCl as possible. Apply 1 mol l⁻¹ HCl drop-by-drop until no further CO_2 development is visible.

(2) Dry acidified samples directly, do not rinse with water after acidification.

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