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Variability in δ¹³C values between individual *Daphnia* ephippia: Implications for palaeo-studies

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ABSTRACT

The stable carbon isotope ratio (δ^{13}C value) of *Daphnia* spp. resting egg shells (ephippia) provides information on past changes in *Daphnia* diet. Measurements are typically performed on samples of $\geq$20 ephippia, which obscures the range of values associated with individual ephippia. Using a recently developed laser ablation-based technique, we perform multiple δ^{13}C analyses on individual ephippia, which show a high degree of reproducibility (standard deviations 0.1–0.5‰). We further measured δ^{13}C values of 13 ephippia from surface sediments of three Swiss lakes. In the well-oxygenated lake with low methane concentrations, δ^{13}C values are close to values typical for algae (−31.4‰) and the range in values is relatively small (5.8‰). This variability is likely driven by seasonal (or inter-annual) variability in algae δ^{13}C values. In two seasonally anoxic lakes with higher methane concentrations, average values were lower (−41.4 and −43.9‰, respectively) and the ranges much larger (10.7 and 20.0‰). We attribute this variability to seasonal variation in incorporation of methane-derived carbon. In one lake we identify two statistically distinct isotopic populations, which may reflect separate production peaks. The potentially large within-sample variability should be considered when interpreting small-amplitude, short-lived isotope excursions based on samples consisting of few ephippia. We show that measurements on single ephippia can be performed using laser ablation, which allows for refined assessments of past *Daphnia* diet and carbon cycling in lake food webs. Furthermore, our study provides a basis for similar measurements on other chitinous remains (e.g. from chironomids, bryozoans).

1. Introduction

The use of the stable isotopic composition of organic remains in lake sediments has been emerging as a means to reconstruct changes in past ecological and climatological conditions in and around lakes (Leng and Henderson, 2013). Because they are often abundantly found in sediments and are resistant to degradation (Verbruggen et al., 2010), the chitinous remains of aquatic invertebrates have a strong potential as a proxy for such reconstructions (Heiri et al., 2012). For example, the stable carbon isotopic composition (expressed as δ^{13}C values) of the shells (ephippia) of the resting eggs of water fleas (*Daphnia* spp.) has been shown to reflect that of the maternal *Daphnia* and its diet (Schilder et al., 2015b). This allows for the use of δ^{13}C values of ephippia to investigate the δ^{13}C value of *Daphnia* diet in the past (see e.g. Morlock et al., 2016; Rinta et al., 2016; Schilder et al., 2017). Chitinous remains of other organisms, most notably chironomid head capsules, bryozoan statoblasts and *Ceriodaphnia* ephippia have also been used to investigate past changes in the stable carbon isotopic composition of aquatic invertebrates in lake food webs, often in combination with *Daphnia* ephippia (Belle et al., 2014; Frossard et al., 2013; Morlock et al., 2016; Rinta et al., 2016; van Hardenbroek et al., 2013; Wooller et al., 2012).

Daphnia feed on suspended organic particles, predominantly algae, in the water column of lakes (Lampert, 2011). In addition, *Daphnia* may ingest methanotrophic bacteria (MOB) or other microorganisms feeding on MOB. This leads to δ^{13}C values in *Daphnia* that are much lower (Kankaala et al., 2006) than typical for freshwater algae (−35 to −25‰ (Vuorio et al., 2006)), because the δ^{13}C

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values of biogenic methane in lake ecosystems (−85 to −50‰ (Jedrysek, 2005; Rinta et al., 2015; Whiticar et al., 1986)) and MOB metabolizing this methane (Templeton et al., 2006) are exceptionally low. Schilder et al. (2015a) found that $\delta^{13}C$ values of ephippia from surface sediments were lower in small temperate lakes with high methane concentrations in the water column than in those with low methane concentrations in the water column. This suggests that the amount of methane-derived carbon in these ephippia (and consequently their $\delta^{13}C$ values) are systematically related to in-lake methane concentrations. The $\delta^{13}C$ values of ephippia deposited in lake sediments may thus provide an indication of past availability of methane in lakes. Given the importance of lakes (and other freshwater bodies) in the global carbon cycle as producers and emitters of methane (Bastviken et al., 2011; Battin et al., 2009), this method can provide valuable insight into the past response of lakes to environmental and climatic change in terms of methane productivity and carbon cycling in general (e.g. Perga et al., 2016; van Hardenbroek et al., 2014, 2013; Wooller et al., 2012). This information, in turn, can be used to better predict future responses of the lacustrine carbon cycle, and particularly of lacustrine methane production and emission, to changes in the environment and in the climate.

Seasonal fluctuations in $\delta^{13}C$ values of *Daphnia* exceed 10‰ in some lakes (Morlock et al., 2016; Perga and Gerdeaux, 2006; Smyntek et al., 2012) and are partly driven by changes in the $\delta^{13}C$ value of phytoplankton. For example, the seasonal succession of dominant algae species with different carbon fractionation rates can drive seasonal shifts in algal $\delta^{13}C$ values (e.g. Hollander and McKenzie, 1991) and therefore the stable carbon isotopic composition of food available to *Daphnia*. Additionally, changes in CO_2 concentrations (due to e.g. uptake of CO_2 by algae, CO_2 produced as a product of decomposition of organic matter or changes in pH that shift the equilibrium between the various dissolved carbonate species) can also influence both the baseline $\delta^{13}C$ value of CO_2 and algal carbon fractionation rates (Laws et al., 1995; Smyntek et al., 2012), forming another source of seasonal variability in *Daphnia* $\delta^{13}C$ values. As a consequence, higher $\delta^{13}C$ values of algae are typically observed during (late) spring and summer and lower values during fall and winter (Hollander and McKenzie, 1991; Morlock et al., 2016; Schilder et al., 2017; Smyntek et al., 2012). However, incorporation of MOB by *Daphnia* also likely occurs mostly in fall when algal abundance declines and hypolimnetic methane is exposed to oxygen upon lake mixing (Morlock et al., 2016; Taipale et al., 2007). This can promote large seasonal fluctuations in *Daphnia* $\delta^{13}C$ values in dimictic lakes with high methane production, leading to *Dapnia* $\delta^{13}C$ values much lower than reported for algae (−40‰ and lower, see e.g. Kankaala et al. (2010) and Schilder et al. (2017)). In addition, there is a seasonality in the production of ephippia themselves: Ephippia may be produced throughout the year, but there typically are distinct production peaks in spring and in fall (Cáceres, 1998; Cáceres and Tessier, 2004).

The extent to which these sources of (potential) seasonal variability affect down-core variations in $\delta^{13}C$ values of *Daphnia* ephippia is poorly constrained. In part, this is because $\delta^{13}C$ analysis of single (sub)fossil ephippia shells has to date been impossible. $\delta^{13}C$ measurements on a large number of shells from a sample (hereafter called bulk measurements) represent a weighted average of $\delta^{13}C$ values of individual ephippia which may originate from populations of very uniform or highly variable $\delta^{13}C$ values. Measurements on individual ephippia would provide insights into this within-sample variation. Furthermore, they could provide information on whether ephippia in a sample represent one or more isotopically distinct ephippia production peaks or *Daphnia* populations (e.g. spring and autumn production peaks of the same or different *Daphnia* species), and how food sources different from algae (e.g. MOB) contributed to the diet of *Daphnia* producing these ephippia.

The amount of *Daphnia* ephippia needed for down-core ephippia $\delta^{13}C$ analysis constrains the resolution of presently available records, often resulting in records with a relatively low level of taxonomic and temporal resolution (e.g. Morlock et al., 2016; Schilder et al., 2017). The number of ephippia needed is in turn strongly constrained by the size (mass) of the ephippia found in a sediment record and the lower limit of sample mass that can be measured with a given analytical setup. For standard total combustion isotope ratio mass spectrometry (TC/IRMS), 20 or more individual ephippia are typically measured (Morlock et al., 2016; Rinta et al., 2016; Schilder et al., 2017). The $\delta^{13}C$ analysis on invertebrate remains is at times performed on samples as small as 20 μg (see e.g. Belle et al., 2017) and given the potentially large weight of *Daphnia* ephippia (0.5–5 μg per ephippium, with very large ephippia even exceeding 5 μg; Van Hardenbroek et al., 2018) measurements could be performed on samples containing only 5 to 10 individual ephippia (or even less). However, if the variability in $\delta^{13}C$ values is high in a sediment sample, measurements on bulk samples containing a low number of ephippia may also lead to high apparent variability in down-core records, which can complicate their interpretation. Here we apply a novel analytical setup capable of analysing $\delta^{13}C$ values of individual ephippia which we use to investigate within-bulk sample variability.

Recent advances in analytical procedures (e.g. approaches based on laser-ablation or Spooling Wire Microcombustion) now allow the measurement of the isotopic composition of samples considerably smaller than those conventionally measured in isotope studies of lacustrine invertebrate remains (see e.g. Eek et al., 2007; Moran et al., 2011; Pearson et al., 2016), potentially allowing single or even multiple measurements on individual microfossils (e.g. Nelson et al., 2007; van Roij et al., 2017; Zhao et al., 2017). We use a laser-ablation based setup for measuring the $\delta^{13}C$ values of individual ephippia. The setup has been developed for $\delta^{13}C$ analysis of small organic particles, such as organic microfossils, and has been shown to produce high accuracy and precision data for an international standard, as well as for single grains of pollen and dinoflagellate cysts (Sluijs et al., 2018; van Roij et al., 2017). We analyse individual *Daphnia* ephippia from surface sediments from three Central European lakes that vary in their geographical and elevational setting (subalpine versus lowland), occurrence of anoxia in deeper water layers, and the extent to which methane enters the open water column. We aim to explore the potential of this approach to investigate the variability behind $\delta^{13}C$ values based on bulk *Daphnia* ephippia samples such as presented by Schilder et al. (2015a). For each lake we present multiple $\delta^{13}C$ measurements on the same ephippium to constrain the reproducibility of the approach and assess whether individual ephippia were homogeneous in their stable carbon isotopic composition. Furthermore, for each lake we analysed another 13 individual ephippia from the same surface sediment sample once to investigate the variability in *Daphnia* ephippia $\delta^{13}C$ values within a fossil ephippia assemblage. We expected to find larger variability in ephippia $\delta^{13}C$ values in the stratified lakes with high methane concentrations than in the well-mixed lake with low methane concentrations, since it can be expected that in the lakes with high methane concentrations *Daphnia* diet may be supplemented by strongly ^{13}C-depleted, methane-derived carbon during part of the seasonal cycle (e.g. Morlock et al., 2016; Rinta et al., 2016; Schilder et al., 2017; Taipale et al., 2007).