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Flotsam samples can help explain the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of invertebrate resting stages in lake sediment

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Abstract

The stable isotopic composition of chitinous remains of Cladocera (water fleas) and freshwater Bryozoa (moss animals) preserved in lake sediment records can provide supporting insights into past environmental and ecosystem changes in lakes. Here we explore whether analyses of these remains isolated from lake flotsam can provide information on the driving variables affecting the isotopic composition of these remains. We collected flotsam in 53 lakes and found enough material in 33 lakes to measure the stable carbon and nitrogen isotope ratios (expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively) of resting stages. These values were compared with lake characteristics, water chemistry measurements, and the isotopic composition of sedimentary organic matter (SOM) in the lakes. Mean $\delta^{13}\text{C}$ values of cladoceran ephippia and SOM were correlated and both were also negatively correlated with deep water methane concentrations and indicators of lake stratification. This supports the findings of previous studies that methane-derived carbon can provide a significant proportion of carbon entering planktonic food webs. Mean $\delta^{15}\text{N}$ values of bryozoan statoblasts and SOM were correlated, suggesting that both reflect the $\delta^{15}\text{N}$ values of

phytoplankton. Our results provide information on how environmental variables in lakes can influence the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in resting stages, but flotsam samples can also potentially be used to assess seasonal stable isotope variability of resting stages. Both types of information are important to improve palaeoenvironmental interpretations of stable isotope records based on these remains in lake sediments.

Keywords: Stable isotopes; Invertebrates; Ehippia; Statoblasts; Lakes; Flotsam; Sediment; Methane

1. Introduction

Resting stages are produced by several aquatic invertebrate groups and they can remain dormant until more suitable environmental conditions return. Resting stages of water fleas (Cladocera) and moss animals (Bryozoa) are protected by robust chitinous external structures (the ehippium in the case of Cladocera and the statoblast valves in the case of freshwater bryozoans). These organisms are usually abundant in lakes (Lampert 2006, 2011; Ślusarczyk & Pietrzak 2008; Wood and Okamura 2005). Ehippia and statoblasts can be found well-preserved in lake sediments and can be analysed for their stable carbon and nitrogen isotope ratios (expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively), which reflects the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of their parent organisms on average within 0-1‰ (Perga 2011; Schilder et al. 2015a; van Hardenbroek et al. 2016). Together with analyses on other chitinous remains, such as head capsules and fossilising mouthparts of aquatic insects, stable isotope measurements on these resting stages can be used to study the impact of land use and nutrient input on productivity and carbon cycling in lake ecosystems (van Hardenbroek et al. 2014; Rinta et al. 2016). Analysing the stable isotopic composition of invertebrates and their remains in sediment records can also provide valuable insights in the long-term carbon balance of lakes in response to climate change and eutrophication (Wooller et al. 2012; Hershey et al. 2015; Belle et al. 2016a, b; Elvert et al. 2016; Schilder et al. 2017).

For example, *Daphnia* is a filter-feeding cladoceran zooplankter that can feed on algae in lakes but also on methane-oxidizing bacteria (MOB) (Kamjunke et al 1996; Taipale et al. 2007) or other organisms (e.g., ciliates) feeding on MOB. This

67 reliance on methane-derived carbon can lead to relatively low $\delta^{13}\text{C}$ values in *Daphnia*
68 and some other aquatic invertebrates if this carbon source provides a significant
69 contribution to their diet (Bastviken et al. 2003; Grey 2016). Earlier studies
70 examining the stable isotope composition of *Daphnia* ephippia in lake surface
71 sediments have found strong and significant relationships between $\delta^{13}\text{C}$ values of
72 *Daphnia* ephippia and methane availability in lakes (van Hardenbroek et al. 2013;
73 Schilder et al. 2015b; Morlock et al. 2017).

74 Few studies have investigated the stable isotope composition of Bryozoa and
75 their statoblasts (Turney 1999; Van Riel et al. 2006; van Hardenbroek et al. 2014,
76 2016; Rinta et al. 2016; Morlock et al. 2017). Bryozoan colonies are attached to plants
77 and other substrates, predominantly in the near-shore zone of lakes, where they filter
78 particulate organic matter (POM) and feed on this material and associated
79 microorganisms. Their $\delta^{13}\text{C}$ values are thought to predominantly represent $\delta^{13}\text{C}$
80 values of photosynthetic primary producers (Van Riel et al. 2006; van Hardenbroek et
81 al. 2014, 2016). However, no studies are available that have examined the stable
82 isotope ratios of bryozoan statoblasts produced in multiple lakes to compare with
83 environmental variables expected to influence these values. The relationship between
84 the stable isotopic composition of bryozoan remains and potentially driving variables
85 such as nutrient and methane concentrations or catchment geology (e.g., Schilder et
86 al. 2015b) is therefore poorly constrained and this complicates the interpretation of
87 stable isotope composition measured on these remains in lake sediment records.

88 Bryozoan statoblasts and cladoceran ephippia are not only found in lake
89 sediments but also abundantly in flotsam drifting on the surface of lakes or
90 accumulating on the lake shore. Flotsam can easily be collected from the (leeward)
91 shore of a lake and samples from a large number of sites can therefore be collected
92 within a single field day. In contrast to sediment samples, which typically encompass
93 material accumulating over several years (typically 2-10 years per cm, Kirilova et al.
94 2010; Battarbee et al 2012), flotsam on the lake surface or accumulating on the shore
95 can be expected to represent resting stages produced over a shorter period. Stable
96 isotope analyses of floating resting stages may therefore provide more current
97 information on the state of lake ecosystems and their cladoceran and bryozoan
98 communities than analyses of lake sediments and can help to bridge the gap between
99 isotopic analyses on modern, live organisms and their fossilising remains in lake
100 sediments. In addition, resting stages can be isolated from flotsam samples rapidly

under low power microscopes, whereas sorting of these remains from lake sediment samples requires considerably more time and training (e.g., Wang et al. 2008). For these reasons, stable isotope analyses of ephippia and statoblasts from lake flotsam may provide, e.g., a rapid screening tool to provide a first-order assessment of variations of ephippia or statoblast isotopic compositions at a large number of lakes, allow the identification of sites with bryozoan or cladoceran populations with unusual isotopic composition for future down-core studies, or help to assess how rapidly the isotopic composition of these remains responds to environmental change between seasons or between years. However, no systematic multi-lake studies are presently available which demonstrate the potential of isotopic analyses on resting stages in lake flotsam for providing supporting information for interpreting down-core isotope records based on statoblasts and ephippia.

In our study, we collected flotsam samples in late summer from the lake surface of 53 lakes in central, northwestern and northern Europe and analysed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bryozoan and cladoceran resting stages for the 33 lakes providing sufficient material for stable isotope analysis. The study is intended to provide a first assessment of the extent to which such isotopic analyses can contribute to the interpretation of down-core isotopic measurements of these remains in lake sediment records and to examine how resting stages differ in their isotopic composition in different lake environments. Most (42) of these lakes form part of more extensive, ongoing studies intended to assess the relationship between methane concentrations and environmental variables and how in-lake methane concentrations are related to the $\delta^{13}\text{C}$ values of invertebrate remains in lake surface sediments (Rinta et al. 2015, 2016b; Schilder et al. 2015b).

We investigated cladoceran and bryozoan taxa that are filter feeders, living on seston. Their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are therefore expected to reflect the overall $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of POM suspended in water column or of particular components of this material (e.g. associated algae, microorganisms). Detailed assessments of POM isotopic values in the study lakes were not available and beyond the scope of our survey, since this would have necessitated multiple sampling campaigns at the study lakes to assess long-time (i.e. multi-week) averages of the isotopic composition of POM available to bryozoans and cladocerans prior to the production of resting stages. Instead we compare the observed values with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sedimentary

organic matter (SOM) in the top 2 cm of sediment, as this was expected to provide a time-averaged approximation of POM sedimented at the study lakes, although sedimentary organic matter may still be subject to considerable alteration after deposition (Meyers and Ishiwatari 1993; Lehmann et al. 2002).

We also compare flotsam stable isotope ratios with methane concentrations measured 10 cm above the sediment-water interface and measures of stratification stability of the lakes, since earlier studies have shown that $\delta^{13}\text{C}$ values of the ephippia of *Daphnia* in small lakes are related with methane emissions (van Hardenbroek et al. 2013) and methane concentrations (Schilder et al. 2015b). Unusually low $\delta^{13}\text{C}$ values typical for organisms feeding on methane-derived carbon have also been reported in a few cases for bryozoan colonies (van Hardenbroek et al. 2016). Finally, resting stage $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are compared with surface water dissolved inorganic carbon (DIC), total phosphorus (TP) and total nitrogen concentrations (TN). Phosphorus is typically the limiting plant nutrient in lakes and can affect algal growth rates, exerting a strong indirect influence on algal $\delta^{13}\text{C}$ values (Fogel and Cifuentes 1993). Algal $\delta^{13}\text{C}$ values are also strongly influenced by concentrations and $\delta^{13}\text{C}$ values of dissolved inorganic carbon (DIC) in lakes (Fogel and Cifuentes 1993), which are linked to catchment geology (presence of carbonate bedrock) and pH.

If the stable isotope analysis of invertebrate resting stages in lake flotsam provides a promising avenue to assess potential drivers for the isotopic composition of bryozoans and planktonic cladocerans in the study lakes we would expect: (1) positive relationships with the stable carbon and nitrogen isotopic composition of SOM, since these should be related to the isotopic composition of POM, the main food source of bryozoans and planktonic cladocerans; (2) negative relationships between $\delta^{13}\text{C}$ values of *Daphnia* ephippia and methane concentrations as have been reported for earlier studies based ephippia preserved in lake sediments; and/or (3) positive relationships between TP and resting stage $\delta^{13}\text{C}$ values if nutrient availability is an important determinant of algal $\delta^{13}\text{C}$ values.

2. Materials and methods

2.1 Field sites and sampling of flotsam, water and sediment.

Flotsam was collected with a 1 mm mesh hand net from the shore of 17 lakes in Finland, Germany, the Netherlands, and Switzerland between 5 August and 22 September 2011, 9 lakes in Sweden between 22 and 31 October 2011, and 16 Swiss lakes between in 8 August and 4 October 2012. Lake characteristics included in our study are altitude, maximum water depth, and ratio between lake area and catchment area. In addition, we measured surface water TP and TN concentrations in the lake centre, [CH₄] 10 cm above the sediment in the deepest part and in the near-shore zone, difference between dissolved oxygen concentrations in surface and bottom water (ΔO_2), the stratification stability, and the $\delta^{13}C$ and $\delta^{15}N$ values of SOM from the deepest part of the lake and the near-shore zone. Most physio-chemical measurements are described in Rinta et al. (2015, 2017) and therefore just briefly summarized here. The variables were measured and surface sediment collections were made at the time of flotsam collection, with the exception of the 9 Swedish lakes for which physio-chemical parameters and surface sediments were collected a year earlier, in September 2010. Additional flotsam samples were collected from 11 Swiss lakes in September 2011 and 2012. For these 11 sites limnological, water chemistry, and sediment samples were not collected during fieldwork. Therefore, environmental data for these sites are only partly available from published reports (see Supplementary Table 1).

The investigated Cladocera genera were mainly planktonic (Flössner 2000) and can be found in the deep water zone of lakes, whereas the bryozoan colonies were sessile and only found in the near-shore zone (Wood and Okamura 2005). For this reason, we compared the $\delta^{13}C$ and $\delta^{15}N$ values of Cladocera with [CH₄] and the $\delta^{13}C$ and $\delta^{15}N$ values of SOM from deep water samples. For Bryozoa these comparisons were made with near-shore samples. All other water chemistry was measured on surface water samples from the centre of the lakes.

Oxygen and temperature profiles were measured at the sites using a multi-probe (WTW, Oxi 1970i, Germany) and used to calculate ΔO_2 values. The strength of water column stratification was estimated based on the Brunt-Väisälä stability frequency (N_s) (s^{-1}) (Spigel and Imberger 1987), which is based on a density gradient calculated from measured temperature profiles. Surface water samples were obtained in a 5 litre water sampler 0.5 m below the lake surface. Water samples were taken for TP and TN measurements in the laboratory (see Rinta et al. 2015 for details).

For the analysis of concentrations and $\delta^{13}\text{C}$ values of DIC, 60 ml of water was immediately collected from the water sampler using a plastic syringe (Becton–Dickinson, USA) and injected into a 118 ml glass vial. The vials were prepared in the laboratory with 200 μl H_3PO_4 (85 %) to ensure that all DIC in the water sample would be converted to CO_2 and filled with N_2 gas and capped with a 10 mm thick butyl rubber septum (Apodan, Denmark) following Bastviken et al. (2008). Water samples for CH_4 concentrations 10 cm above the sediments were obtained using a gravity corer (UWITEC, Austria) and sampling 60 ml of water 10 cm above the undisturbed sediment-water interface into the same 118 ml prepared glass vials for storage. From the obtained sediment cores, the top 2 cm of sediment was sampled in plastic bags for measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (described in section 2.2). Sediment samples were frozen and freeze-dried upon arrival in the laboratory.

CH_4 and CO_2 concentrations in the headspace of the vials were quantified by gas chromatography using a flame ionization detector with a methanizer (Agilent 6890 N, PlotQ capillary column, with FID for CH_4 and TCD for CO_2 for the samples from Finnish lakes and GC-FID, Shimadzu GC-8, PoropackN column for the others, see Rinta et al. 2015 for details). The concentration of CH_4 and DIC in the lake water was calculated using the headspace equilibration method (McAuliffe 1971) according to Henry's law describing gas–water partitioning (Stumm and Morgan 1996; see methods in Bastviken et al. 2010). Measurements of $\delta^{13}\text{C}$ values of DIC (as CO_2) have been presented in Rinta et al. (2015). As described in their publication, CO_2 was cryogenically separated from the sample gas mixture by means of liquid nitrogen, then volatilized and via a helium carrier gas stream transferred to the isotope ratio mass-spectrometer (ThermoFinnigan MAT Delta Plus XL, Germany) at the Division of Climate and Environmental Physics at the Physics Institute of University of Bern (Switzerland) with an accuracy better than 0.3‰.

Further details about collection dates and measurements of flotsam, lake characteristics, water chemistry, and surface sediments are presented in Supplementary Table 1. After collection, flotsam samples were transferred to plastic bags, frozen and kept dark until processing in the laboratory. Samples were sieved over nested sieves with 2 mm, 0.5 mm, and 100 μm mesh sizes. Cladoceran ephippia of the genera *Ceriodaphnia*, *Daphnia*, and *Simocephalus* and all bryozoan statoblasts (including *Cristatella mucedo*, *Lophopus crystallinus*, *Pectinatella magnifica*, and

Plumatella) were manually picked with forceps under a stereo microscope (4-40x magnification) and stored separately for each taxon in demineralized water until further processing for stable isotope analysis. Identification of resting stages followed Vanderkerkhove et al. (2004) for ehippia and Wood and Okamura (2005) for statoblasts.

2.2 Stable isotope analysis

For the flotsam samples, easily degradable organic material was removed by manually taking out the soft tissue from ehippia and statoblasts and treating the remaining chitinous material with 10% KOH for 2h at room temperature. Previous studies have shown that this treatment has no detectable effect on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Daphnia* ehippia (Schilder et al. 2015a) or on the $\delta^{13}\text{C}$ values of chitinous head capsules of chironomid larvae (Heiri et al. 2012). Furthermore, this treatment also removes adsorbed fulvic and humic acids from organic particles (Stevenson 1982). Samples were then treated with 2M NH_4Cl solution buffered with NaOH to dissolve residual carbonates at a pH of 7.5 (Verbruggen et al. 2010), rinsed in de-ionized water and directly picked into tin capsules for stable carbon and nitrogen isotope analysis. The target weight for each sample was 30 μg . Samples were air dried, crimped into tin capsules, shipped to the isotope laboratory and stored in a desiccator until analysis. A Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo Delta Plus XP isotope ratio mass spectrometer (IRMS) at the Alaska Stable Isotope Facility (University of Alaska Fairbanks) was used for stable carbon and nitrogen isotope and elemental (%C and %N) analyses. All analytical precisions are expressed as one standard deviation from the mean based on the results from multiple analyses of a laboratory peptone standard with known relation to international reference materials conducted during the run of samples. Stable carbon and nitrogen isotope compositions of samples are expressed in standard delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relative to Vienna Pee Dee Belemnite (VPDB) and AIR, respectively. Analytical precision (1σ) for bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were 0.1 and 0.3‰, respectively.

SOM samples for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis were exposed to 2.5% HCl for 15 minutes to remove carbonates, rinsed three times with deionized water, centrifuged 4 min. at 3000 rpm to remove excess water, and freeze-dried. Elemental C:N ratios, as

well as stable carbon and nitrogen isotopes of SOM were analyzed on a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 IRMS at the UC Davis Stable Isotope Facility. Replicate measurements on four reference materials (nylon, bovine liver, USGS41 glutamic acid, and peach leaves) of known relation to international standards indicated that the analytical error (1σ) was better than 0.14‰ for $\delta^{13}\text{C}$ values and 0.33‰ for $\delta^{15}\text{N}$ values.

2.3 Statistical analysis

To summarise lake characteristics and water chemistry data, a Principal Component Analysis (PCA) was performed. Further statistical analyses were based on average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all Cladocera taxa (i.e. *Ceriodaphnia*, *Daphnia*, and *Simocephalus*) and of all Bryozoa (*C. mucedo*, *L. crystallinus*, *P. magnifica*, and *Plumatella*) available for each lake. These averages were used because the planktonic Cladocera genera of which ephippia were collected have similar feeding behaviour (Flössner 2000) and the same applies to the investigated Bryozoa genera (Kaminski 1984).

The $[\text{CH}_4]$ in bottom waters in Lovojärvi, the only meromictic lake in the data set, was not included in numerical analyses; the lack of seasonal mixing and resulting high $[\text{CH}_4]$ in bottom waters made this site very different from the deep water $[\text{CH}_4]$ at other lakes.

Not all environmental variables were normally distributed and therefore non-parametric correlation tests were used. All statistical analyses were performed using R software (R core team, 2013). Spearman's ρ and statistical significance were calculated between $\delta^{13}\text{C}$ values or $\delta^{15}\text{N}$ values of resting stages in lake flotsam and the environmental variables including the stable isotopic composition of SOM. All reported p-values are adjusted p-values using the Benjamini and Hochberg (1995) correction for multiple testing with the R package 'psych'.

3. Results

Lake characteristics and detailed water chemistry data, which is available for 42 of the 53 study lakes, have been summarised in a PCA biplot (Fig. 1). The first two PCA

axes explained 22.4% and 18.3%, of the variance in the environmental data, respectively, and indicated that [DIC], conductivity and pH are important variables in the data set that plot along the first PCA axis. For our campaign, lakes with high [DIC], conductivity and pH are generally located in areas with carbonate bedrock, which is discussed in more detail by Rinta et al. (2015). The second PCA axis represents a combination of nutrients, [CH₄], and indicators of stratification (N_s and ΔO₂). Nutrient concentrations appear to be higher in the shallower lakes, and higher [CH₄] are found in deeper, more strongly stratified lakes. A more detailed discussion of the methane cycling and limnological profiles in the study lakes is provided elsewhere (Rinta et al. 2015, 2017).

In the flotsam of the 52 investigated lakes enough material for stable carbon and nitrogen isotope analysis was found for *Ceriodaphnia* in 5 lakes, *Daphnia* in 20 lakes, for *Simocephalus* in 10 lakes, for *Cristatella* in 16 lakes, or *Plumatella* in 9 lakes, for *Pectinatella* in 3 lakes, and for *Lophopus* in 1 lake (Fig. 2).

Mean δ¹³C values of Cladocera ranged from -45.6 to -26.8 ‰ and were positively related to the δ¹³C values of SOM in the deep water zone (ρ = 0.73, p = 0.002; Table 1; Fig. 3) and nearly significantly correlated to δ¹³C values of Bryozoa (ρ = 0.70, p = 0.051). In contrast, a significant relationship with SOM δ¹³C for the near-shore zone was not observed for bryozoan δ¹³C values that ranged from -40.7 to -24.2 ‰. Cladocera δ¹³C values were significantly negatively correlated with deep water [CH₄] (ρ = -0.60, p = 0.026; Fig. 4; Table 1), whereas the relationship with stratification stability (N_s) was almost statistically significant (ρ = -0.52, p = 0.054). Similarly, the δ¹³C values of deep water SOM ranged from -38.9 to -25.4 ‰ and were correlated to deep water methane concentrations (ρ = -0.76, p < 0.001; Fig. 4), ΔO₂ (ρ = -0.62, p < 0.001), and N_s (ρ = -0.44, p = 0.033).

Mean Bryozoa δ¹³C values, in contrast, were not significantly correlated with stratification indicators and [CH₄] (Table 1), but only with the lake:catchment ratio (ρ = -0.67, p = 0.014). The δ¹⁵N values of Bryozoa, ranging from -1.2 to 10.4 ‰, were significantly correlated with δ¹⁵N of SOM in the near-shore zone (ρ = 0.76, p = 0.008, Fig. 5). A correlation between δ¹⁵N values of Cladocera and δ¹⁵N values of deep water SOM was not observed, but δ¹⁵N values of deep water SOM were correlated to TN (ρ = 0.54, p = 0.004) and TP (ρ = 0.44, p = 0.033).

4. Discussion

4.1 $\delta^{13}\text{C}$ values of resting stages and SOM related to CH_4 concentrations

This study shows a similar pattern as reported in studies assessing $\delta^{13}\text{C}$ values of cladoceran ephippia in the surface sediments of small lakes (van Hardenbroek et al. 2013; Schilder et al. 2015b); Our results also show a negative correlation between ephippia $\delta^{13}\text{C}$ values in flotsam and in-lake methane concentrations (Fig. 4). The $\delta^{13}\text{C}$ values at some of the studied sites are clearly lower for the ephippia of some cladoceran groups (e.g. *Daphnia*, *Simocephalus*, Fig. 2) than expected for algal organic matter, which typically ranges between -36 and -20 ‰ (France 1995; Vuorio et al. 2006). For example, *Simocephalus* ephippia had $\delta^{13}\text{C}$ values as low as -43.4 and -45.6 ‰ in Schwendisee and Lobsigensee, respectively, and *Daphnia* ephippia had values as low as -43.4 and -44.6 ‰ in Mekkojärvi and Uebeschisee, respectively.

Laboratory and field studies of *Daphnia* have shown that this organism group is able to ingest and assimilate methane-derived carbon from MOB if these bacteria are an abundant resource in the open water column (Taipale et al. 2007, 2009, 2011; Deines and Fink 2011). Furthermore, surveys examining $\delta^{13}\text{C}$ values of cladoceran resting stages in lake sediments have revealed that exceptionally low $\delta^{13}\text{C}$ values of the ephippia of *Daphnia*, and other filter feeding cladocerans such as *Ceriodaphnia*, occur regularly in lakes with high methane concentrations. Schilder et al. (2015b) reported $\delta^{13}\text{C}$ values as low as -52.8 ‰ for *Daphnia* ephippia in surface sediments of lake Mekkojärvi (Finland), and Morlock et al. (2017) values as low as -43.3 ‰ for *Ceriodaphnia* ephippia in sediments of lake Gerzensee (Switzerland), confirming that methane-derived carbon is incorporated by these cladocerans. Exceptionally negative $\delta^{13}\text{C}$ values as low as -50.1 ‰ were also reported for the ephippia of *Ceriodaphnia* isolated from flotsam on lake Gerzensee during winter (Morlock et al. 2017). The $\delta^{13}\text{C}$ values observed for ephippia of *Simocephalus* in our dataset are also low (-45.6 ‰ in lake Lobsigensee and -43.4 ‰ in lake Schwendisee), and indicate that this group of filter-feeding cladocerans can also ingest CH_4 -derived carbon originating from MOB, as earlier studies described for *Daphnia* and *Ceriodaphnia*.

In our dataset $\delta^{13}\text{C}$ values of Cladocera ephippia are correlated with both $\delta^{13}\text{C}$ values of SOM and deep water CH_4 concentrations (Table 1). This suggests that either

¹³C-depleted CH₄-derived carbon contributed to both Cladocera biomass and SOM, or that other carbon sources available to filtering cladocerans in our lakes (e.g. algal material in POM) had $\delta^{13}\text{C}$ values correlated with deep-water CH₄ concentrations and reinforced the apparent relationship between CH₄ concentrations and ephippia $\delta^{13}\text{C}$ values. Methane concentrations in the lakes included in this study overlap with typical [CH₄] values reported for other small lakes in the literature. [CH₄] in surface waters in our study lakes range between 0.1 and 8.8 μM (the mean $\pm$ standard deviation is $1.8 \pm 2.0 \mu\text{M}$ (Rinta et al. 2017)). This fits well within the range of surface water [CH₄] of small lakes and ponds found in a recent literature review (range: 0.01 – 59 μM , mean 3.4 ± 7.7) by Holgerson and Raymond (2016).

Some studies (Hollander and Smith 2001; Lehmann et al. 2004; Teranes and Bernasconi 2005) have demonstrated that $\delta^{13}\text{C}$ values of POM or SOM collected from the hypolimnion of lakes with high deep water CH₄ concentrations can be strongly depleted in ¹³C, and have higher methanogen activity (West et al. 2012), supporting that CH₄-derived carbon is also included in and can affect the $\delta^{13}\text{C}$ values of organic matter floating and sedimenting in lakes. Further evidence for the importance of CH₄ in driving $\delta^{13}\text{C}$ values of Cladocera ephippia and SOM, comes from the significant negative correlation between these $\delta^{13}\text{C}$ values and stratification stability (N_s) and with differences between surface and bottom water oxygen concentrations (ΔO_2) (Table 1). In strongly stratified lakes with reduced deep water oxygen availability, it can be expected that more CH₄ is produced and that more MOB biomass will be available in the water column and surface sediments compared with other lakes. However, direct evidence for higher MOB biomass in stratified lakes would be desirable and could be obtained, e.g., via analysis of lipids, environmental DNA, or compound-specific stable isotope analysis to support this hypothesis.

Other processes could also explain, in part, the observed correlation between $\delta^{13}\text{C}$ values in *Daphnia* and those in SOM. The $\delta^{13}\text{C}$ values of phytoplankton can be strongly influenced by concentrations of dissolved organic carbon (DIC) in the lake water and by algal productivity. In lakes where DIC is not limiting (e.g., in unproductive and in high-DIC lakes), phytoplankton can more easily discriminate against ¹³C and lower $\delta^{13}\text{C}$ values in phytoplankton may be expected (Fogel and Cifuentes 1993). In situations where DIC is limiting (e.g., in low-DIC or in very productive lakes), phytoplankton is typically characterised by higher $\delta^{13}\text{C}$ values. If

productivity-related variation in phytoplankton $\delta^{13}\text{C}$ values is an important factor in our data set, we would expect to find higher $\delta^{13}\text{C}$ values in organisms feeding on phytoplankton (i.e. Cladocera and Bryozoa) and in SOM of lakes with higher nutrient concentrations. Although the highest $\delta^{13}\text{C}$ values in flotsam (-30 to -24 ‰) were found in the lakes with high TP and TN concentrations, we did not find a significant correlation between TP or TN and $\delta^{13}\text{C}$ values of flotsam (Table 1), making it unlikely that the observed $\delta^{13}\text{C}$ values of flotsam are driven predominantly by productivity.

In our dataset, however, the effect of productivity on DIC concentrations and $\delta^{13}\text{C}$ values of phytoplankton cannot be studied in isolation, because the more productive lakes in our data are naturally high in DIC concentrations and have higher $\delta^{13}\text{C}$ values of DIC, caused by underlying bedrock type (Schilder et al. 2015b; Rinta et al. 2017). As a result, the productive lakes in our data set generally have high DIC concentrations as well as high deep water methane concentrations, and both of these could lead to lower $\delta^{13}\text{C}$ values of SOM as discussed above. Indirect evidence suggests that variations in $\delta^{13}\text{C}$ values of phytoplankton cannot be the main driver of variations in ephippia $\delta^{13}\text{C}$ values: If phytoplankton $\delta^{13}\text{C}$ values would be the main driver for $\delta^{13}\text{C}$ values of SOM and cladoceran ephippia we would expect to see a similarly strong relationship between $\delta^{13}\text{C}$ values of statoblasts and of SOM as observed between cladoceran ephippia and SOM. This is because planktonic algae and POM are also the main food source for bryozoan colonies in near-shore zone of lakes (Wood and Okamura 2005; Kaminski 1984). However, the relationship between bryozoan statoblasts and SOM $\delta^{13}\text{C}$ values is noticeably weaker than observed between cladoceran ephippia and SOM (Fig 3; Table 1). Furthermore, the extremely negative $\delta^{13}\text{C}$ values below -36‰ for some cladoceran ephippia samples cannot be explained by the uptake of algal organic matter (typically > -36 ‰). Regardless of the direct cause, the apparent correlation between cladoceran ephippia $\delta^{13}\text{C}$ values and CH_4 concentrations agrees with earlier studies indicating that the stable isotopic composition of filter feeding planktonic cladocerans, and particularly *Daphnia*, is strongly affected by the assimilation of CH_4 -derived carbon in CH_4 -rich lakes.

4.2 $\delta^{15}\text{N}$ values of invertebrates and SOM

Bryozoan statoblast $\delta^{15}\text{N}$ values were positively correlated with $\delta^{15}\text{N}$ values of SOM. This was to be expected, since Bryozoa feed on seston in the near-shore zone of lakes, mostly dead and dying phytoplankton cells (Kaminski 1984). Therefore, their $\delta^{15}\text{N}$ values could be expected to represent the $\delta^{15}\text{N}$ values of phytoplankton, which in turn reflects $\delta^{15}\text{N}$ values of the dissolved inorganic nitrogen (DIN) pool available to planktonic algae. SOM $\delta^{15}\text{N}$ was in turn positively correlated with TN measured in the lake water, suggesting that nitrogen tended to be isotopically enriched in ^{15}N in lakes receiving high external nitrogen loads. This is also expected, since lakes with high TN concentrations often receive more anthropogenic N sources (sewage and manure), which are enriched in ^{15}N (Cabana and Rasmussen 1996). However, $\delta^{15}\text{N}$ values of bryozoan statoblasts were not significantly correlated with TN.

The $\delta^{15}\text{N}$ values of Cladocera were not found to correlate to the $\delta^{15}\text{N}$ values of SOM, and neither were they significantly correlated with $\delta^{15}\text{N}$ values of Bryozoa ($\rho = 0.60$, $p = 0.12$). This might be related to the feeding ecology of Cladocera, which is more varied than that of Bryozoa: Cladocera can migrate vertically in the water column (Lampert 2011) and filter living algae and bacteria in the size range of 0.5 to 30 μm (Geller and Müller 1981; Jürgens 1994; Taipale et al. 2007). Bacterial biomass can have relatively low $\delta^{15}\text{N}$ values, which has been shown subsequently to affect the $\delta^{15}\text{N}$ values of *Daphnia* (Taipale et al. 2012). It is possible that trends in the $\delta^{15}\text{N}$ values of Cladocera related to $\delta^{15}\text{N}$ of phytoplankton are partially obscured by assimilation of bacterial biomass, but data are not presently available to rigorously test this hypothesis.

Only one study to date has compared the $\delta^{15}\text{N}$ values of *Daphnia ephippia* and SOM in a sediment record from a shallow pond in the Canadian High Arctic (Griffiths et al. 2010). This study showed how marine-derived nutrients from sea-bird colonies can lead to increasing $\delta^{15}\text{N}$ values in SOM and benthic chironomids, whereas *Daphnia* $\delta^{15}\text{N}$ values were continuously high throughout the record, even before $\delta^{15}\text{N}$ values of chironomid remains and SOM increased. The results indicate that the filter-feeding zooplankton had access to different food sources (living phytoplankton and bacterial biomass in POM) compared with the food sources of benthic invertebrates (epiphytic algae and decomposing SOM) in this very shallow lake. Studies by Perga et al. (2010) and Rantala et al. (2015) measured $\delta^{15}\text{N}$ values of cladoceran carapaces preserved in lake sediment records, and these suggest that variations in $\delta^{15}\text{N}$ values

are linked to changes in diet and trophic position. Results from these studies and the dataset presented here highlight that invertebrate groups with specific habitats and feeding preferences (e.g., bryozoans feeding on POM in the near-shore zone, cladocerans feeding on bacteria and small algae in the water column, or chironomids feeding on epiphytic algae) will assimilate different nitrogen (and carbon) sources, which can lead to significantly different stable isotopic compositions.

4.3 Timing and location of resting stage production and relation to parent organisms

Our study confirms that the stable carbon and nitrogen isotope composition of ephippia and statoblasts in lake flotsam relate to in-lake processes (at least during stratification in late summer) as is evidenced by the relationships between $\delta^{13}\text{C}$ of Cladocera ephippia and $[\text{CH}_4]$ and between $\delta^{15}\text{N}$ values of Bryozoa and SOM. The results therefore suggest that resting stages can provide information about different carbon and nitrogen sources in the water column (phytoplankton and MOB). However, several limitations of our pioneering study restrict the extent to which we can assess the utility of flotsam $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis for interpreting down core isotope analyses based on these remains.

Firstly, flotsam was sampled floating along the downwind shore on the lake surface, providing an amalgamated sample of resting stages. The exact location where analysed resting stages were formed is not known, however. There will be variability in stable isotope ratios of resting stages related to the diet and habitat of parent specimens, but a more detailed spatial study of flotsam is required to quantify this variability. As a result, correlations found between the stable isotope values of resting stages and environmental parameters measured mostly in the centre of the lake may not be as strong as they may have been if these would have been measured in exactly the same location. This applies more strongly to bryzoan statoblasts as the microhabitat of the colonies in the near-shore zone might have an important influence on the available food (and ultimately on the stable isotope composition of the statoblast, van Hardenbroek et al. (2016)). In comparison, the environment in which *Daphnia* live and feed is relatively well-represented by the variables measured in the lake centre, since *Daphnia* is found abundantly in the central, open water zone of lakes (Flössner 2000, Lampert 2011).

Secondly, samples were collected in late summer, and it is unclear how

seasonal variations in stable isotope values of flotsam resting stages influence our results. Several studies have indicated seasonal variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of aquatic invertebrates, usually tracking variations in the isotopic composition of phytoplankton (e.g., Perga and Gerdeaux 2006; Morlock et al. 2017), but this does not necessarily translate to similar changes in the isotopic composition of their resting stages.

Ephippia formation is stimulated by food availability, day length and population density (Kleiven et al. 1992; Lampert 2011). Field observations have revealed that the timing of ephippia production varies between species (Cáceres 1998; Cáceres and Tessier 2004a, b) but most of the investigated species have ephippia production peaks around April-June and September-November. A detailed study in Gerzensee (Switzerland) indicated that living *Daphnia* $\delta^{13}\text{C}$ values varied seasonally between -44.2 ‰ in early spring and -29.8 ‰ in autumn, tracking the $\delta^{13}\text{C}$ values of POM (Morlock et al. 2017). In contrast, the $\delta^{13}\text{C}$ values of *Daphnia* ephippia in flotsam were nearly constant (-41.7 to -38.8 ‰) and in the range of values that the living *Daphnia* had during turnover in spring and autumn. This indicates that in Gerzensee ephippia were produced during one or both of these time intervals and that ephippia from these production peaks remained afloat or were re-suspended for at least six months. In contrast, studies on *Ceriodaphnia* ephippia and *Plumatella* statoblasts from Gerzensee (Morlock et al. 2017) but also on *Daphnia* ephippia from Lake De Waay (Schilder et al. 2017), showed pronounced seasonal variations in ephippia $\delta^{13}\text{C}$ values. This suggests that ephippia and statoblasts were continuously produced during the seasonal cycle.

When using resting stages in palaeolimnological studies, it is important to realise that resting stages accumulating in lake sediments provide an integrated average of stable isotope composition in resting stages produced over longer time periods. *Daphnia* ephippia from Lake De Waay had extremely variable $\delta^{13}\text{C}$ values in flotsam samples ($-41.7 \pm 4.8\text{‰}$), which was also found for *Daphnia* themselves ($-36.6 \pm 6.8\text{‰}$), but less so for ephippia in surface sediments ($-39.2 \pm 0.5\text{‰}$) (Schilder et al. 2017). Schilder et al. (2017) only analysed a limited number of samples collected four times during the annual cycle. A more detailed study by Morlock et al. (2017) in Lake Gerzensee found that the time-averaged $\delta^{13}\text{C}$ value of *Daphnia* ephippia in surface sediments (-39.8‰) was nearly identical to the average $\delta^{13}\text{C}$ value of flotsam collected throughout the year from the lake surface ($-39.5 \pm 0.9\text{‰}$) and to the average

$\delta^{13}\text{C}$ value of living *Daphnia* collected during the 2-year study period (-39.4 ± 4.6 ‰). The same was found for *Ceriodaphnia ehippia* in flotsam (-42.9 ± 4.8 ‰) and those in surface sediments (-42.9 ‰). *Plumatella* statoblasts, however, were more ^{13}C -depleted in flotsam (-36.3 ± 1.7 ‰) than in the surface sediments (-32.7 ‰). It is possible that this difference was caused by the low number statoblasts measurements in summer flotsam samples, or because statoblasts from the top 7 cm of the core had to be pooled to get enough material for stable isotope analysis. However, it is also possible that the impact of taphonomic processes on statoblast $\delta^{13}\text{C}$ values are more complex than for ehippia.

The examples from Gerzensee and Lake De Waay demonstrate that the timing of resting stage production, and the isotopic composition of food sources available for the examined invertebrate groups just before resting stage production may have influenced our dataset and may be responsible for some of the scatter between stable isotopic composition of resting stages and that of SOM, or between $\delta^{13}\text{C}$ values in flotsam and CH_4 or TP concentrations. It could be expected that $\delta^{13}\text{C}$ values of resting stages in flotsam for some of the lakes in the present study will be representative for ehippia production peaks in spring and/or autumn (at least for *Daphnia ehippia*), whereas at other sites they will represent resting stages produced in the days to weeks before sampling in late summer. More detailed research is needed on individual species of Cladocera and Bryozoa to constrain during which period of the annual cycle they produce the majority of resting stages.

5. Conclusions

We have shown that cladoceran ehippia and bryozoan statoblasts are abundant in the flotsam of lakes and can be used for stable isotope studies. Our data demonstrate that studies based on resting stages in lake flotsam can detect large between-lake variations in stable isotope values that are driven by in-lake processes. A strong negative relationship between $\delta^{13}\text{C}$ values of Cladocera resting stages and methane concentrations was observed, as we expected based on previous studies. In addition, we found that $\delta^{13}\text{C}$ values of Cladocera ehippia are related to the strength of water column stratification, consistent with requirements for high rates of methanogenesis in lake basins.

Our initial expectations that positive correlations would be observed between the stable carbon and nitrogen isotope ratios of flotsam and SOM were partially confirmed. The $\delta^{13}\text{C}$ values of Cladocera and SOM were significantly correlated with each other, but this was not found for Bryozoa, possibly because $\delta^{13}\text{C}$ values of Cladocera and SOM were both driven by methane-derived carbon. In addition, the $\delta^{15}\text{N}$ values of Bryozoa and SOM were correlated with each other, but as similar relationship was not observed for Cladocera. It is likely that the feeding mode and habitat of Cladocera as pelagic zooplankton leads to a larger proportion of microbial biomass in the cladoceran diet, which can obscure a relationship with $\delta^{15}\text{N}$ values in phytoplankton and SOM.

In contrast to our initial expectations, we found no clear relationship between nutrient concentrations and $\delta^{13}\text{C}$ values of resting stages. Such a relationship would be expected if nutrients and growth rates were a main driver of $\delta^{13}\text{C}$ values of phytoplankton (and ultimately the $\delta^{13}\text{C}$ values of the invertebrates feeding on phytoplankton). In our lakes, however, a combination of (1) methane-derived carbon and (2) high DIC concentrations related to calcareous bedrock were apparently affecting $\delta^{13}\text{C}$ values of ephippia and statoblasts more strongly.

Ephippia and statoblasts in flotsam can be produced in specific habitats and throughout the seasonal cycle and the variability of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be either related to specific peaks in resting stage production during spring and early autumn, or else represent the stable isotope composition of the parent organism for a restricted interval before sampling of lake flotsam. Therefore, studying the stable isotope composition of resting stages in flotsam may provide key information about which environmental variables are related to the stable isotope composition of resting stages on shorter time scales than possible from studies based on surface sediment samples. This may provide crucial supplementary information for the interpretation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of ephippia and statoblast in sediment records. Future studies should continue to explore the seasonal and spatial variability in the isotopic composition of resting stages, particularly in relation to the isotopic composition of algae and microbial biomass in lake water. This would provide a better understanding in respect to the time interval represented by resting stages (i.e. a distinct production peak versus continuous production during the growing season) and the assimilated food sources during that time interval.

599

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Figures

Fig. 1 Principal Component Analysis of physical lake characteristics and water chemistry of 42 of the lakes for which detailed surveys were undertaken. Lake ID codes are explained in Supplementary Table 1.

Fig. 2 Boxplot with $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for the different taxa collected in flotsam samples; n indicates the number of lakes for which stable isotopes were analysed for the respective taxon.

Fig. 3 Average $\delta^{13}\text{C}$ values of Cladocera ephippia plotted against $\delta^{13}\text{C}$ of SOM in the deep water zone (SOM_{deep}) (a) and average $\delta^{13}\text{C}$ values Bryozoa statoblasts plotted against $\delta^{13}\text{C}$ of SOM in the near-shore zone (SOM_{litt}) (b).

Fig. 4 Average $\delta^{13}\text{C}$ values of Cladocera and deep water SOM plotted against deep water methane concentrations 10 cm above the sediments (a), and average $\delta^{13}\text{C}$ of Bryozoa and near-shore SOM plotted against near-shore methane concentrations measured 10 cm above the sediments.

Fig. 5 Average $\delta^{15}\text{N}$ values of Cladocera plotted against $\delta^{15}\text{N}$ of deep water SOM (SOM_{deep}) in (a) and average $\delta^{15}\text{N}$ values of Bryozoa plotted against $\delta^{15}\text{N}$ of near-shore SOM (SOM_{litt}) (b).

Table 1: Spearman's rank correlation coefficient (ρ) for correlations between stable carbon and nitrogen isotope values of cladoceran ephippia and bryozoan statoblasts and environmental variables: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM; Altitude, Maximum water depth ($Z_{\max}$); Lake:Catchment ratio; Total Phosphorus (TP); Total Nitrogen (TN); pH; Conductivity; Stratification stability (N_s); Difference in oxygen concentrations between surface and bottom water (ΔO_2); Methane concentrations 10 cm above the sediment ($[\text{CH}_4]$); Surface water dissolved inorganic carbon concentrations ($[\text{DIC}]$) and DIC $\delta^{13}\text{C}$ values. All presented p-values are adjusted p-values using the Benjamini and Hochberg (1995) correction for multiple testing with the R package 'psych'. Significant correlations after correction are shown in bold font.

	$\delta^{13}\text{C}$ Cladocera		$\delta^{13}\text{C}$ Bryozoa		$\delta^{15}\text{N}$ Cladocera		$\delta^{15}\text{N}$ Bryozoa		$\delta^{13}\text{C}$ SOM		$\delta^{15}\text{N}$ SOM	
$\delta^{13}\text{C}$ SOM	0.73	**	0.11	†	-0.03		-0.02	†	0.53	** §	-0.18	§
$\delta^{15}\text{N}$ SOM	0.43		0.13	†	0.28		0.71	* †	-0.19	§	0.82	** §
Altitude	-0.36		-0.07		0.10		0.11		-0.23		-0.59	**
$Z_{\max}$	-0.30		0.01		0.04		0.46		-0.18		0.12	
Lake:Catchment	0.12		0.67	*	-0.08		-0.04		-0.20		-0.09	
TP	0.36		0.26		0.39		0.47		0.08		0.44	*
TN	0.47		0.61		0.08		0.39		-0.04		0.54	**
pH	-0.07		0.37		0.32		0.46		-0.20		-0.31	
Conductivity	-0.24		0.08		0.36		0.57		-0.48	*	0.32	
N_s	-0.52		-0.29		0.47		0.19		-0.44	*	0.18	
ΔO_2	-0.50		0.01		0.06		-0.20		-0.62	**	0.16	
$[\text{CH}_4]$	-0.60	*	0.02	†	-0.03		-0.04	†	-0.76	**	0.06	
$[\text{DIC}]$	-0.26		0.08		0.32		0.51		-0.58	**	0.19	
$\delta^{13}\text{C}$ DIC	0.03		0.57		-0.06		0.54		-0.38		0.26	

* $p < 0.05$

** $p < 0.01$

† using SOM samples or $[\text{CH}_4]$ measurements from the near-shore sediments of the lakes

§ comparing SOM collected in near-shore and deep water sediments of lakes