



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

Maarten van Hardenbroek ^{a, b, *}, Päivi Rinta ^b, Matthew J. Wooller ^{c, d}, Jos Schilder ^{b, e}, Tabea Stötter ^b, Oliver Heiri ^b

^a School of Geography Politics and Sociology, Newcastle University, Newcastle-upon-Tyne, NE1 7RU, UK

^b Oeschger Centre for Climate Change Research & Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013, Bern, Switzerland

^c Alaska Stable Isotope Facility, Water and Environmental Research Center, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

^d College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

^e Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, 40014 Jyväskylä, Finland

ARTICLE INFO

Article history:

Received 5 October 2017

Received in revised form

7 February 2018

Accepted 6 April 2018

Available online 25 April 2018

Keywords:

Stable isotopes

Invertebrates

Ephippia

Statoblasts

Lakes

Flotsam

Sediment

Methane

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.

© 2018 Elsevier Ltd. All rights reserved.

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 ephippium 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 and Pietrzak, 2008; Wood and Okamura, 2005).

Ephippia 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 fossilizing 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

* Corresponding author. School of Geography Politics and Sociology, Newcastle University, Newcastle-upon-Tyne, NE1 7RU, UK.

E-mail address: maarten.vanhardenbroek@ncl.ac.uk (M. van Hardenbroek).

et al., 2016a; b; Elvert et al., 2016; Schilder et al., 2017).

For example, *Daphnia* is a filter-feeding cladoceran zooplankton that can feed on algae in lakes but also on methane-oxidizing bacteria (MOB) (Kamjunke et al., 1997; Taipale et al., 2007) or other organisms (e.g., ciliates) feeding on MOB. This reliance on methane-derived carbon can lead to relatively low $\delta^{13}\text{C}$ values in *Daphnia* and some other aquatic invertebrates if this carbon source provides a significant contribution to their diet (Bastviken et al., 2003; Grey, 2016). Earlier studies examining the stable isotope composition of *Daphnia* ephippia in lake surface sediments have found strong and significant relationships between $\delta^{13}\text{C}$ values of *Daphnia* ephippia and methane availability in lakes (van Hardenbroek et al., 2013; Schilder et al., 2015b; Morlock et al. 2017).

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

Bryozoan statoblasts and cladoceran ephippia are not only found in lake sediments but also abundantly in flotsam drifting on the surface of lakes or accumulating on the lake shore. Flotsam can easily be collected from the (leeward) shore of a lake and samples from a large number of sites can therefore be collected within a single field day. In contrast to sediment samples, which typically encompass material accumulating over several years (typically 2–10 years per cm, Kirilova et al., 2010; Battarbee et al., 2012), flotsam on the lake surface or accumulating on the shore can be expected to represent resting stages produced over a shorter period. Stable isotope analyses of floating resting stages may therefore provide more current information on the state of lake ecosystems and their cladoceran and bryozoan communities than analyses of lake sediments and can help to bridge the gap between isotopic analyses on modern, live organisms and their fossilising remains in lake 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., 2016; 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 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, $[CH_4]$ 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 $[CH_4]$ 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 L 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 $\delta^{13}C$ 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 μ l 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 $\delta^{13}C$ and $\delta^{15}N$ (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 $\delta^{13}C$ 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 μ m 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 (4–40 \times 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 ephippia 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 ephippia and statoblasts and treating the remaining chitinous material with 10% KOH for 2 h at room temperature. Previous studies have shown that this treatment has no detectable effect on $\delta^{13}C$ and $\delta^{15}N$ values of *Daphnia* ephippia (Schilder et al., 2015a) or on the $\delta^{13}C$ 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 μ g. 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 ($\delta^{13}C$ and $\delta^{15}N$) relative to Vienna Pee Dee Belemnite (VPDB) and AIR, respectively. Analytical precision (1σ) for bulk $\delta^{13}C$ and $\delta^{15}N$ were 0.1 and 0.3‰, respectively.

SOM samples for $\delta^{13}C$ and $\delta^{15}N$ analysis were exposed to 2.5% HCl for 15 min 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 analysed 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 Lovöjärvi, 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.

All statistical analyses were performed using R software (R core team, 2013). 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 summarized 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, $[\text{CH}_4]$, and indicators of stratification (N_s and ΔO_2). Nutrient concentrations appear to be higher in the shallower lakes, and higher $[\text{CH}_4]$ 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 $\delta^{13}\text{C}$ values of Cladocera ranged from -45.6 to -26.8 ‰ and were positively related to the $\delta^{13}\text{C}$ values of SOM in the deep water zone ($\rho = 0.73$, $p = 0.002$; Table 1; Fig. 3) and nearly significantly correlated to $\delta^{13}\text{C}$ values of Bryozoa ($\rho = 0.70$, $p = 0.051$). In contrast, a significant relationship with SOM $\delta^{13}\text{C}$ for the near-shore zone was not observed for bryozoan $\delta^{13}\text{C}$ values that ranged from -40.7 to -24.2 ‰. Cladocera $\delta^{13}\text{C}$ values were significantly negatively correlated with deep water $[\text{CH}_4]$ ($\rho = -0.60$, $p = 0.026$; Fig. 4; Table 1), whereas the relationship with stratification stability

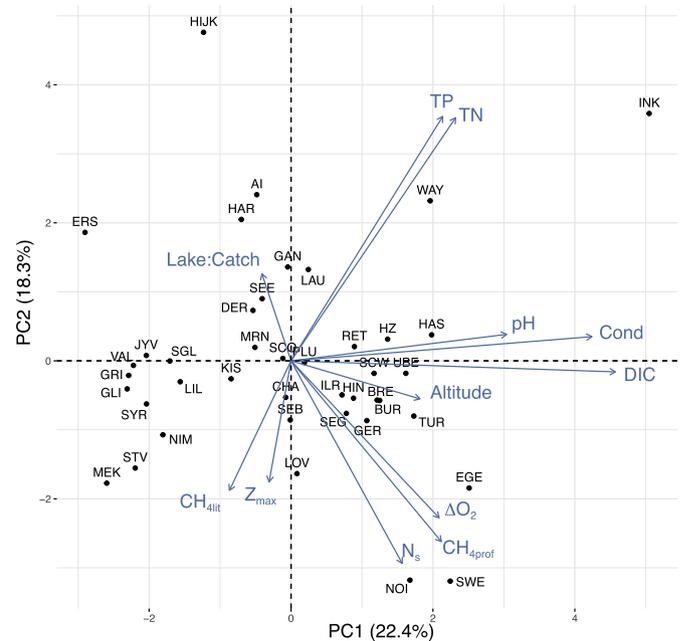


Fig. 1. Principal Component Analysis of physical lake characteristics and water chemistry of 42 of the lakes for which detailed surveys were undertaken. Lake ID codes are explained in Supplementary Table 1.

(N_s) was almost statistically significant ($\rho = -0.52$, $p = 0.054$). Similarly, the $\delta^{13}\text{C}$ values of deep water SOM ranged from -38.9 to -25.4 ‰ and were correlated to deep water methane concentrations ($\rho = -0.76$, $p < 0.001$; Fig. 4), ΔO_2 ($\rho = -0.62$, $p < 0.001$), and N_s ($\rho = -0.44$, $p = 0.033$).

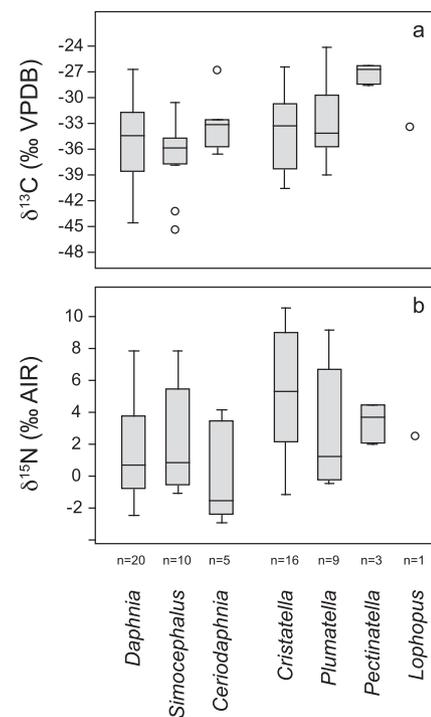


Fig. 2. Boxplot with $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for the different taxa collected in flotsam samples; n indicates the number of lakes for which stable isotopes were analysed for the respective taxon.

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 ([DIC]) and DIC $\delta^{13}\text{C}$ values. All presented p-values are adjusted p-values calculated 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 ^a	-0.03	-0.02 ^a	0.53 ** ^b	-0.18 ^b
$\delta^{15}\text{N}$ SOM	0.43	0.13 ^a	0.28	0.71 * ^a	-0.19 ^b	0.82 ** ^b
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 ^a	-0.03	-0.04 ^a	-0.76 **	0.06
[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

* $p < 0.05$; ** $p < 0.01$.

^a Using SOM samples or $[\text{CH}_4]$ measurements from the near-shore sediments of the lakes.

^b Comparing SOM collected in near-shore and deep water sediments of lakes.

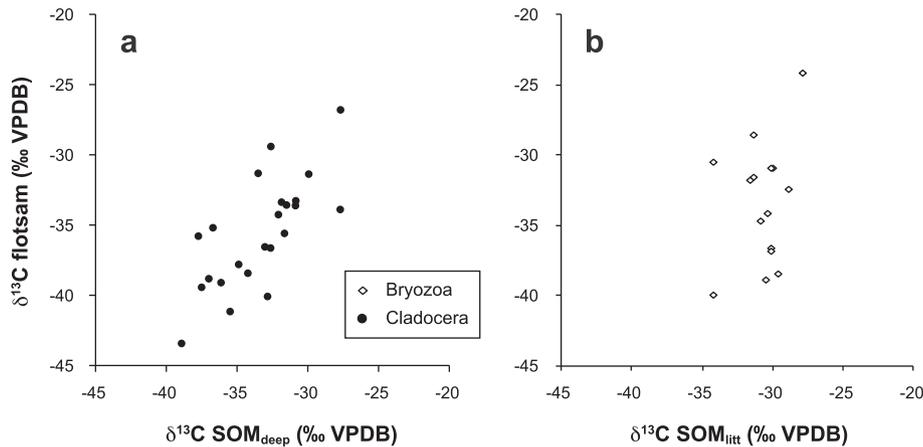


Fig. 3. Average $\delta^{13}\text{C}$ values of Cladocera ephippia plotted against $\delta^{13}\text{C}$ of SOM in the deep water zone (SOM_{deep}) (a) and average $\delta^{13}\text{C}$ values Bryozoa statoblasts plotted against $\delta^{13}\text{C}$ of SOM in the near-shore zone (SOM_{litt}) (b).

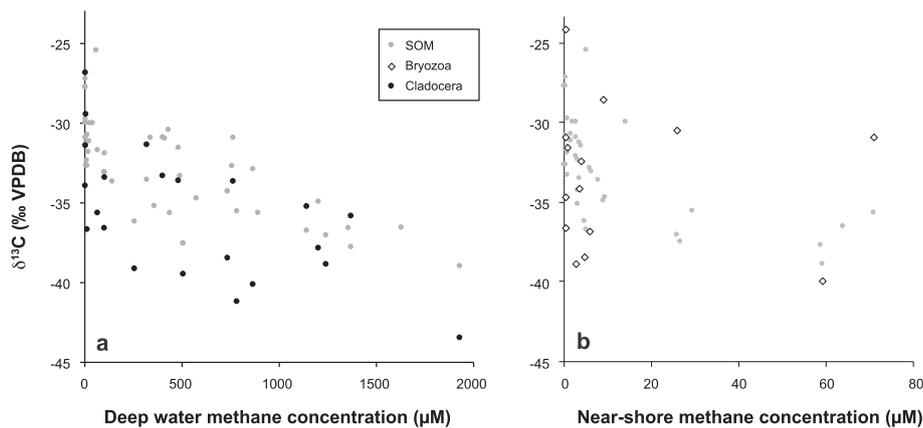


Fig. 4. Average $\delta^{13}\text{C}$ values of Cladocera and deep water SOM plotted against deep water methane concentrations 10 cm above the sediments (a), and average $\delta^{13}\text{C}$ of Bryozoa and near-shore SOM plotted against near-shore methane concentrations measured 10 cm above the sediments.

Mean Bryozoa $\delta^{13}\text{C}$ values, in contrast, were not significantly correlated with stratification indicators and $[\text{CH}_4]$ (Table 1), but only with the lake:catchment ratio ($\rho = -0.67$, $p = 0.014$). The $\delta^{15}\text{N}$

values of Bryozoa, ranging from -1.2 – 10.4‰ , were significantly correlated with $\delta^{15}\text{N}$ of SOM in the near-shore zone ($\rho = 0.76$, $p = 0.008$, Fig. 5). A correlation between $\delta^{15}\text{N}$ values of Cladocera

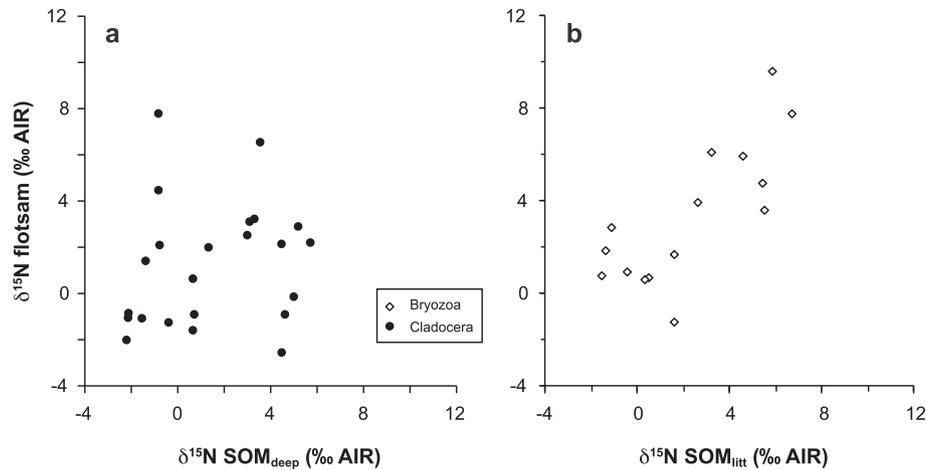


Fig. 5. Average $\delta^{15}\text{N}$ values of Cladocera plotted against $\delta^{15}\text{N}$ of deep water SOM (SOM_{deep}) in (a) and average $\delta^{15}\text{N}$ values of Bryozoa plotted against $\delta^{15}\text{N}$ of near-shore SOM (SOM_{litt}) (b).

and $\delta^{15}\text{N}$ values of deep water SOM was not observed, but $\delta^{15}\text{N}$ values of deep water SOM were correlated to TN ($\rho = 0.54$, $p = 0.004$) and TP ($\rho = 0.44$, $p = 0.033$).

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, 2012; 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 ^{13}C -depleted CH_4 -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 CH_4 concentrations and reinforced the apparent relationship between CH_4 concentrations and ephippia $\delta^{13}\text{C}$ values. Methane concentrations in the lakes included in this study overlap with typical $[\text{CH}_4]$ values reported for other small lakes in the literature. $[\text{CH}_4]$ in surface waters in our study lakes range between 0.1 and 8.8 μM (the mean \pm standard deviation is 1.8 ± 2.0 μM (Rinta et al., 2017)). This fits well within the range of surface water $[\text{CH}_4]$ 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 CH_4 concentrations can be strongly depleted in ^{13}C , and have higher methanogen activity (West et al., 2012), supporting that CH_4 -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 CH_4 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 CH_4 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 ^{13}C 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 in 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 $> -36\text{‰}$). 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 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 $0.5\text{--}30\ \mu\text{m}$ (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 bryozoan 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.

Ehippia 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 ehippia production varies between species (Cáceres, 1998; Cáceres and Tessier, 2004a; b) but most of the investigated species have ehippia 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* ehippia 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 ehippia were produced during one or both of these time intervals and that ehippia from these production peaks remained afloat or were re-suspended for at least six months. In contrast, studies on *Ceriodaphnia* ehippia and *Plumatella* statoblasts from Gerzensee (Morlock et al. 2017) but also on *Daphnia* ehippia from Lake De Waay (Schilder et al., 2017), showed pronounced seasonal variations in ehippia $\delta^{13}\text{C}$ values. This suggests that ehippia 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* ehippia from 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 ehippia 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 Gerzensee found that the time-averaged $\delta^{13}\text{C}$ value of *Daphnia* ehippia 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 ($-39.4 \pm 4.6\text{‰}$). The same was found for *Ceriodaphnia* ehippia in flotsam ($-42.9 \pm 4.8\text{‰}$) and those in surface sediments (-42.9‰). *Plumatella* statoblasts, however, were more ^{13}C -depleted in flotsam ($-36.3 \pm 1.7\text{‰}$) than in the surface sediments (-32.7‰). It is possible that this difference was caused by the low number of 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 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 a 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 ehippia and statoblasts more strongly.

Ehippia 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 ehippia and statoblasts 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.

Acknowledgements

This study was supported by the European Research Council (ERC) Starting Grant project RECONMET 239858. We thank Tim Howe for his work on the stable isotope measurements. We also thank two anonymous reviewers for their constructive feedback.

Appendix A. Supplementary data

Supplementary data related to this article can be found at

<https://doi.org/10.1016/j.quascirev.2018.04.008>.

References

- Bastviken, D., Ejlertsson, J., Sundh, I., Tranvik, L., 2003. Methane as a source of carbon and energy for lake pelagic food webs. *Ecology* 84, 969–981.
- Bastviken, D., Cole, J.J., Pace, M.L., de Bogert, M.C.V., 2008. Fates of methane from different lake habitats: connecting whole-lake budgets and CH₄ emissions. *J. Geophys. Res.-Biogeo.* 113, G02024.
- Bastviken, D., Santoro, A.L., Marotta, H., Pinho, L.Q., Calheiros, D.F., Crill, P., Enrich-Prast, A., 2010. Methane emissions from Pantanal, South America, during the low water season: toward more comprehensive sampling. *Environ. Sci. Technol.* 44, 5450–5455.
- Battarbee, R.W., Anderson, N.J., Bennion, H., Simpson, G.L., 2012. Combining limnological and palaeolimnological data to disentangle the effects of nutrient pollution and climate change on lake ecosystems: problems and potential. *Freshw. Biol.* 57, 2091–2106.
- Belle, S., Millet, L., Lami, A., Verneaux, V., Musazzi, S., Hossann, C., Magny, M., 2016a. Increase in benthic trophic reliance on methane in 14 French lakes during the Anthropocene. *Freshw. Biol.* 61, 1105–1118.
- Belle, S., Millet, L., Verneaux, V., Lami, A., David, E., Murgia, L., Parent, C., Musazzi, S., Gauthier, E., Bichet, V., 2016b. 20th century human pressures drive reductions in deepwater oxygen leading to losses of benthic methane-based food webs. *Quat. Sci. Rev.* 137, 209–220.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B* 57, 289–300.
- Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. Unit. States Am.* 93, 10844–10847.
- Cáceres, C.E., 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. *Ecology* 79, 1699–1710.
- Cáceres, C.E., Tessier, A.J., 2004a. Incidence of diapause varies among populations of *Daphnia pulicaria*. *Oecologia* 141, 425–431.
- Cáceres, C.E., Tessier, A.J., 2004b. To sink or swim: variable diapause strategies among *Daphnia* species. *Limnol. Oceanogr.* 49, 133–1340.
- Deines, P., Fink, P., 2011. The potential of methanotrophic bacteria to compensate for food quantity or food quality limitations in *Daphnia*. *Aquat. Microb. Ecol.* 65, 197–206.
- Elvert, M., Pohlman, J.W., Becker, K.W., Gaglioti, B., Hinrichs, K.-U., Wooller, M.J., 2016. Methane turnover and environmental change from Holocene lipid biomarker records in a thermokarst lake in Arctic Alaska. *Holocene* 26, 1766–1777.
- Flössner, D., 2000. Die Haplozoa und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publishers, Leiden.
- Fogel, M.L., Cifuentes, L.A., 1993. Isotopic fractionation during primary production. In: Engel, M.H., Macko, S.A. (Eds.), *Org. Geochem. Plenum*, New York, pp. 73–98.
- France, R.L., 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Mar. Ecol. Prog. Ser.* 124, 307–312.
- Geller, W., Müller, H., 1981. The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. *Oecologia* 49, 316–321.
- Grey, J., 2016. The incredible lightness of being methane-fuelled: stable isotopes reveal alternative energy pathways in aquatic ecosystems and beyond. *Front. Ecol. Evol.* 4 <https://doi.org/10.3389/fevo.2016.00008>.
- Griffiths, K., Michelutti, N., Blais, J., Kimpe, L., Smol, J., 2010. Comparing nitrogen isotopic signals between bulk sediments and invertebrate remains in High Arctic seabird-influenced ponds. *J. Paleolimnol.* 44, 405–412.
- Heiri, O., Schilder, J., van Hardenbroek, M., 2012. Stable isotopic analysis of fossil chironomids as an approach to environmental reconstruction: State of development and future challenges. In: *Proceedings of the 18th International Symposium on Chironomidae*, Trondheim 4–6, August 2011, 31. *Fauna Norvegica*, pp. 7–18.
- Hershey, A.E., Northington, R.M., Hart-Smith, J., Bostick, M., Whalen, S.C., 2015. Methane efflux and oxidation, and use of methane-derived carbon by larval Chironominae, in arctic lake sediments. *Limnol. Oceanogr.* 60, 276–285.
- Holgerson, M.A., Raymond, P.A., 2016. Large contribution to inland water CO₂ and CH₄ emissions from very small ponds. *Nat. Geosci.* 9, 222.
- Hollander, D.J., Smith, M.A., 2001. Microbially mediated carbon cycling as a control on the $\delta^{13}\text{C}$ of sedimentary carbon in eutrophic Lake Mendota (USA): new models for interpreting isotopic excursions in the sedimentary record. *Geochem. Cosmochim. Acta* 65, 4321–4337.
- Jürgens, K., 1994. Impact of *Daphnia* on planktonic microbial food webs - a review marine microbial food webs, 8, 295–324.
- Kaminski, M., 1984. Food composition of three bryozoan species (Bryozoa, Phylactolaemata) in a mesotrophic lake. *Pol. Arch. Hydrobiol.* 31, 45–53.
- Kamjunke, N., Boing, W., Voigt, H., 1997. Bacterial and primary production under hypertrophic conditions. *Aquat. Microb. Ecol.* 13, 29–35.
- Kirilova, E., Cremer, H., Heiri, O., Lotter, A., 2010. Eutrophication of moderately deep Dutch lakes during the past century: flaws in the expectations of water management? *Hydrobiologia* 637, 157–171.
- Kleiven, O.T., Larsson, P., Hobæk, A., 1992. Sexual Reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65, 197–206.
- Lampert, W., 2006. *Daphnia*: model herbivore, predator and prey. *Pol. J. Ecol.* 54, 607–620.
- Lampert, W., 2011. *Daphnia*: Development of a Model Organism in Ecology and Evolution. International Ecology Institute, Oldendorf/Luhe.
- Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J.A., 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. *Geochem. Cosmochim. Acta* 66, 3573–3584.
- Lehmann, M.F., Bernasconi, S.M., McKenzie, J.A., Barbieri, A., Simona, M., Veronesi, M., 2004. Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. *Limnol. Oceanogr.* 49, 415–429.
- McAuliffe, C.D., 1971. GC determination of solutes by multiple phase equilibration. *Chem. Technol.* 1, 46–51.
- Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* 20, 867–900.
- Morlock, M.A., Schilder, J., van Hardenbroek, M., Szidat, S., Wooller, M.J., Heiri, O., 2017. Seasonality of cladoceran and bryozoan resting stage $\delta^{13}\text{C}$ values and implications for their use as palaeolimnological indicators of lacustrine carbon cycle dynamics. *J. Paleolimnol.* 57, 141–156.
- Perga, M.E., Gerdeaux, D., 2006. Seasonal variability in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the zooplankton taxa in two alpine lakes. *Acta Oecol.* 30, 69–77.
- Perga, M.E., Desmet, M., Enters, D., Reys, J.-L., 2010. A century of bottom-up and top-down driven changes on a lake planktonic food web: a paleoecological and paleoisotopic study of Lake Annecy, France. *Limnol. Oceanogr.* 55, 803–816.
- Perga, M.E., 2011. Taphonomic and early diagenetic effects on the C and N stable isotope composition of cladoceran remains: implications for paleoecological studies. *J. Paleolimnol.* 46, 203–213.
- R core team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rantala, M.V., Luoto, T.P., Weckström, J., Perga, M.-E., Rautio, M., Nevalainen, L., 2015. Climate controls on the Holocene development of a subarctic lake in northern Fennoscandia. *Quat. Sci. Rev.* 126, 175–185.
- Rinta, P., Bastviken, D., van Hardenbroek, M., Kankaala, P., Leuenberger, M., Schilder, J., Stötter, T., Heiri, O., 2015. An inter-regional assessment of concentrations and $\delta^{13}\text{C}$ values of methane and dissolved inorganic carbon in small European lakes. *Aquat. Sci.* 77, 667–680.
- Rinta, P., van Hardenbroek, M., Jones, R.I., Kankaala, P., Rey, F., Szidat, S., Wooller, M.J., Heiri, O., 2016. Land use affects carbon sources to the pelagic food web in a small boreal lake. *PLoS One* 11 e0159900.
- Rinta, P., Bastviken, D., Schilder, J., van Hardenbroek, M., Stötter, T., Heiri, O., 2017. Higher late summer methane emission from central than northern European lakes. *J. Limnol.* 76, 52–67.
- Schilder, J., Tellenbach, C., Möst, M., Spaak, P., van Hardenbroek, M., Wooller, M.J., Heiri, O., 2015a. The stable isotopic composition of *Daphnia* ephippia reflects changes in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of food and water. *Biogeosciences* 12, 3819–3830.
- Schilder, J., Bastviken, D., van Hardenbroek, M., Leuenberger, M., Rinta, P., Stötter, T., Heiri, O., 2015b. The stable carbon isotopic composition of *Daphnia* ephippia in small, temperate lakes reflects in-lake methane availability. *Limnol. Oceanogr.* 60, 1064–1075.
- Schilder, J., van Hardenbroek, M., Bodelier, P., Kirilova, E.P., Leuenberger, M., Lotter, A.F., Heiri, O., 2017. Trophic state changes can affect the importance of methane-derived carbon in aquatic food webs. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 284, 20170278.
- Ślusarczyk, M., Pietrzak, B., 2008. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia*. *Freshw. Biol.* 53, 569–576.
- Spigel, R.H., Imberger, J., 1987. Mixing processes relevant to phytoplankton dynamics in lakes. *N. Z. J. Mar. Freshw. Res.* 21, 361–377.
- Stevenson, F.J., 1982. Extraction, fractionation, and general chemical composition of soil organic matter. In: Stevenson, F.J. (Ed.), *Humus Chemistry. Genesis, Composition, Reactions*. John Wiley and Sons, New York, pp. 26–54.
- Stumm, W., Morgan, J.J., 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, third ed. Wiley, New York.
- Taipale, S., Kankaala, P., Hämäläinen, H., Jones, R.I., 2009. Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. *Freshw. Biol.* 54, 90–104.
- Taipale, S., Kankaala, P., Jones, R., 2007. Contributions of different organic carbon sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: results from mesocosm DI13C-additions. *Ecosystems* 10, 757–772.
- Taipale, S.J., Brett, M.T., Pulkkinen, K., Kainz, M.J., 2012. The influence of bacteria-dominated diets on *Daphnia magna* somatic growth, reproduction, and lipid composition. *FEMS Microbiol. Ecol.* 82, 50–62.
- Teranes, J.L., Bernasconi, S.M., 2005. Factors controlling $\delta^{13}\text{C}$ values of sedimentary carbon in hypertrophic Baldeggersee, Switzerland, and implications for interpreting isotope excursions in lake sedimentary records. *Limnol. Oceanogr.* 50, 914–922.
- Turney, C.S.M., 1999. Lacustrine bulk organic $\delta^{13}\text{C}$ in the British isles during the last glacial-holocene transition (14–9 ka ^{14}C BP). *Arctic Antarct. Alpine Res.* 31, 71–81.
- Vanderkerkhove, J., Declerck, S., Vanhove, M., Brendonck, L., Jeppesen, E., Porcuna, J.M., De Meester, L., 2004. Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls. *J. Limnol.* 63, 75–84.
- van Hardenbroek, M., Lotter, A.F., Bastviken, D., Andersen, T.J., Heiri, O., 2014. Taxon-specific $\delta^{13}\text{C}$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden. *J. Paleolimnol.* 52, 95–105.
- van Hardenbroek, M., Heiri, O., Parmentier, F.J.W., Bastviken, D., Ilyashuk, B.P., Wiklund, J.A., Hall, R.I., Lotter, A.F., 2013. Evidence for past variations in

- methane availability in a Siberian thermokarst lake based on $\delta^{13}\text{C}$ of chitinous invertebrate remains. *Quat. Sci. Rev.* 66, 74–84.
- van Hardenbroek, M., Leuenberger, M., Hartikainen, H., Okamura, B., Heiri, O., 2016. Bryozoan stable carbon and hydrogen isotopes: relationships between the isotopic composition of zooids, statoblasts and lake water. *Hydrobiologia* 765, 209–223.
- van Riel, M.C., Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F., Vaate, A.B., 2006. Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. In: Leuven, R.S.E.W., Ragas, A.M.J., Smits, A.J.M., Velde, G. (Eds.), *Living Rivers: Trends and Challenges in Science and Management*. Springer, Netherlands, pp. 39–58.
- Verbruggen, F., Heiri, O., Reichart, G.J., Lotter, A.F., 2010. Chironomid $\delta^{18}\text{O}$ as a proxy for past lake water $\delta^{18}\text{O}$: a lateglacial record from Rotsee (Switzerland). *Quat. Sci. Rev.* 29, 2271–2279.
- Vuorio, K., Meili, M., Sarvala, J., 2006. Taxon-specific variation in the stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of lake phytoplankton. *Freshwat. Biol.* 51, 807–822.
- Wang, Y., Francis, D., O'Brien, D., Wooller, M., 2008. A protocol for preparing sub-fossil chironomid head capsules (Diptera: Chironomidae) for stable isotope analysis in paleoclimate reconstruction and considerations of contamination sources. *J. Paleolimnol.* 40, 771–781.
- West, W.E., Coloso, J.J., Jones, S.E., 2012. Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. *Freshw. Biol.* 57, 949–955.
- Wood, T.S., Okamura, B., 2005. *A New Key to Freshwater Bryozoans of Britain, Ireland and Continental Europe, with Notes on Their Ecology*. Freshwater Biological Association, London.
- Wooller, M., Pohlman, J., Gaglioti, B., Langdon, P., Jones, M., Walter Anthony, K., Becker, K., Hinrichs, K.-U., Elvert, M., 2012. Reconstruction of past methane availability in an Arctic Alaska wetland indicates climate influenced methane release during the past ~12,000 years. *J. Paleolimnol.* 48, 27–42.