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

3

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16

17 **Abstract**

18

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

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

39

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

42

### 43 *1. Introduction*

44

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

64 For example, *Daphnia* is a filter-feeding cladoceran zooplankter that can feed  
65 on algae in lakes but also on methane-oxidizing bacteria (MOB) (Kamjunke et al  
66 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

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

113 In our study, we collected flotsam samples in late summer from the lake  
114 surface of 53 lakes in central, northwestern and northern Europe and analysed  $\delta^{13}\text{C}$   
115 and  $\delta^{15}\text{N}$  values of bryozoan and cladoceran resting stages for the 33 lakes providing  
116 sufficient material for stable isotope analysis. The study is intended to provide a first  
117 assessment of the extent to which such isotopic analyses can contribute to the  
118 interpretation of down-core isotopic measurements of these remains in lake sediment  
119 records and to examine how resting stages differ in their isotopic composition in  
120 different lake environments. Most (42) of these lakes form part of more extensive,  
121 ongoing studies intended to assess the relationship between methane concentrations  
122 and environmental variables and how in-lake methane concentrations are related to  
123 the  $\delta^{13}\text{C}$  values of invertebrate remains in lake surface sediments (Rinta et al. 2015,  
124 2016b; Schilder et al. 2015b).

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

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

138 We also compare flotsam stable isotope ratios with methane concentrations  
139 measured 10 cm above the sediment-water interface and measures of stratification  
140 stability of the lakes, since earlier studies have shown that  $\delta^{13}\text{C}$  values of the ephippia  
141 of *Daphnia* in small lakes are related with methane emissions (van Hardenbroek et al.  
142 2013) and methane concentrations (Schilder et al. 2015b). Unusually low  $\delta^{13}\text{C}$  values  
143 typical for organisms feeding on methane-derived carbon have also been reported in a  
144 few cases for bryozoan colonies (van Hardenbroek et al. 2016). Finally, resting stage  
145  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are compared with surface water dissolved inorganic carbon  
146 (DIC), total phosphorus (TP) and total nitrogen concentrations (TN). Phosphorus is  
147 typically the limiting plant nutrient in lakes and can affect algal growth rates, exerting  
148 a strong indirect influence on algal  $\delta^{13}\text{C}$  values (Fogel and Cifuentes 1993). Algal  
149  $\delta^{13}\text{C}$  values are also strongly influenced by concentrations and  $\delta^{13}\text{C}$  values of  
150 dissolved inorganic carbon (DIC) in lakes (Fogel and Cifuentes 1993), which are  
151 linked to catchment geology (presence of carbonate bedrock) and pH.

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

162

## 163 2. Materials and methods

164

### 165 2.1 Field sites and sampling of flotsam, water and sediment.

166

167 Flotsam was collected with a 1 mm mesh hand net from the shore of 17 lakes in  
168 Finland, Germany, the Netherlands, and Switzerland between 5 August and 22  
169 September 2011, 9 lakes in Sweden between 22 and 31 October 2011, and 16 Swiss  
170 lakes between in 8 August and 4 October 2012. Lake characteristics included in our  
171 study are altitude, maximum water depth, and ratio between lake area and catchment  
172 area. In addition, we measured surface water TP and TN concentrations in the lake  
173 centre, [CH<sub>4</sub>] 10 cm above the sediment in the deepest part and in the near-shore  
174 zone, difference between dissolved oxygen concentrations in surface and bottom  
175 water ( $\Delta O_2$ ), the stratification stability, and the  $\delta^{13}C$  and  $\delta^{15}N$  values of SOM from the  
176 deepest part of the lake and the near-shore zone. Most physio-chemical measurements  
177 are described in Rinta et al. (2015, 2017) and therefore just briefly summarized here.  
178 The variables were measured and surface sediment collections were made at the time  
179 of flotsam collection, with the exception of the 9 Swedish lakes for which physio-  
180 chemical parameters and surface sediments were collected a year earlier, in  
181 September 2010. Additional flotsam samples were collected from 11 Swiss lakes in  
182 September 2011 and 2012. For these 11 sites limnological, water chemistry, and  
183 sediment samples were not collected during fieldwork. Therefore, environmental data  
184 for these sites are only partly available from published reports (see Supplementary  
185 Table 1).

186 The investigated Cladocera genera were mainly planktonic (Flössner 2000)  
187 and can be found in the deep water zone of lakes, whereas the bryozoan colonies were  
188 sessile and only found in the near-shore zone (Wood and Okamura 2005). For this  
189 reason, we compared the  $\delta^{13}C$  and  $\delta^{15}N$  values of Cladocera with [CH<sub>4</sub>] and the  $\delta^{13}C$   
190 and  $\delta^{15}N$  values of SOM from deep water samples. For Bryozoa these comparisons  
191 were made with near-shore samples. All other water chemistry was measured on  
192 surface water samples from the centre of the lakes.

193 Oxygen and temperature profiles were measured at the sites using a multi-  
194 probe (WTW, Oxi 1970i, Germany) and used to calculate  $\Delta O_2$  values. The strength of  
195 water column stratification was estimated based on the Brunt-Väisälä stability  
196 frequency ( $N_s$ ) ( $s^{-1}$ ) (Spigel and Imberger 1987), which is based on a density gradient  
197 calculated from measured temperature profiles. Surface water samples were obtained  
198 in a 5 litre water sampler 0.5 m below the lake surface. Water samples were taken for  
199 TP and TN measurements in the laboratory (see Rinta et al. 2015 for details).

200 For the analysis of concentrations and  $\delta^{13}\text{C}$  values of DIC, 60 ml of water was  
201 immediately collected from the water sampler using a plastic syringe (Becton–  
202 Dickinson, USA) and injected into a 118 ml glass vial. The vials were prepared in the  
203 laboratory with 200  $\mu\text{l}$   $\text{H}_3\text{PO}_4$  (85 %) to ensure that all DIC in the water sample would  
204 be converted to  $\text{CO}_2$  and filled with  $\text{N}_2$  gas and capped with a 10 mm thick butyl  
205 rubber septum (Apodan, Denmark) following Bastviken et al. (2008). Water samples  
206 for  $\text{CH}_4$  concentrations 10 cm above the sediments were obtained using a gravity  
207 corer (UWITEC, Austria) and sampling 60 ml of water 10 cm above the undisturbed  
208 sediment-water interface into the same 118 ml prepared glass vials for storage. From  
209 the obtained sediment cores, the top 2 cm of sediment was sampled in plastic bags for  
210 measurements of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (described in section 2.2). Sediment samples were  
211 frozen and freeze-dried upon arrival in the laboratory.

212  $\text{CH}_4$  and  $\text{CO}_2$  concentrations in the headspace of the vials were quantified by  
213 gas chromatography using a flame ionization detector with a methanizer (Agilent  
214 6890 N, PlotQ capillary column, with FID for  $\text{CH}_4$  and TCD for  $\text{CO}_2$  for the samples  
215 from Finnish lakes and GC-FID, Shimadzu GC-8, PoropackN column for the others,  
216 see Rinta et al. 2015 for details). The concentration of  $\text{CH}_4$  and DIC in the lake water  
217 was calculated using the headspace equilibration method (McAuliffe 1971) according  
218 to Henry's law describing gas–water partitioning (Stumm and Morgan 1996; see  
219 methods in Bastviken et al. 2010). Measurements of  $\delta^{13}\text{C}$  values of DIC (as  $\text{CO}_2$ )  
220 have been presented in Rinta et al. (2015). As described in their publication,  $\text{CO}_2$  was  
221 cryogenically separated from the sample gas mixture by means of liquid nitrogen,  
222 then volatilized and via a helium carrier gas stream transferred to the isotope ratio  
223 mass-spectrometer (ThermoFinnigan MAT Delta Plus XL, Germany) at the Division  
224 of Climate and Environmental Physics at the Physics Institute of University of Bern  
225 (Switzerland) with an accuracy better than 0.3‰.

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



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

238

## 239 2.2 Stable isotope analysis

240

241 For the flotsam samples, easily degradable organic material was removed by manually  
242 taking out the soft tissue from ephippia and statoblasts and treating the remaining  
243 chitinous material with 10% KOH for 2h at room temperature. Previous studies have  
244 shown that this treatment has no detectable effect on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *Daphnia*  
245 ephippia (Schilder et al. 2015a) or on the  $\delta^{13}\text{C}$  values of chitinous head capsules of  
246 chironomid larvae (Heiri et al. 2012). Furthermore, this treatment also removes  
247 adsorbed fulvic and humic acids from organic particles (Stevenson 1982). Samples  
248 were then treated with 2M  $\text{NH}_4\text{Cl}$  solution buffered with NaOH to dissolve residual  
249 carbonates at a pH of 7.5 (Verbruggen et al. 2010), rinsed in de-ionized water and  
250 directly picked into tin capsules for stable carbon and nitrogen isotope analysis. The  
251 target weight for each sample was 30  $\mu\text{g}$ . Samples were air dried, crimped into tin  
252 capsules, shipped to the isotope laboratory and stored in a desiccator until analysis. A  
253 Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo  
254 Delta Plus XP isotope ratio mass spectrometer (IRMS) at the Alaska Stable Isotope  
255 Facility (University of Alaska Fairbanks) was used for stable carbon and nitrogen  
256 isotope and elemental (%C and %N) analyses. All analytical precisions are expressed  
257 as one standard deviation from the mean based on the results from multiple analyses  
258 of a laboratory peptone standard with known relation to international reference  
259 materials conducted during the run of samples. Stable carbon and nitrogen isotope  
260 compositions of samples are expressed in standard delta notation ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ )  
261 relative to Vienna Pee Dee Belemnite (VPDB) and AIR, respectively. Analytical  
262 precision ( $1\sigma$ ) for bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were 0.1 and 0.3‰, respectively.

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

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

272

### 273 2.3 Statistical analysis

274

275 To summarise lake characteristics and water chemistry data, a Principal Component  
276 Analysis (PCA) was performed. Further statistical analyses were based on average  
277  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all Cladocera taxa (i.e. *Ceriodaphnia*, *Daphnia*, and  
278 *Simocephalus*) and of all Bryozoa (*C. mucedo*, *L. crystallinus*, *P. magnifica*, and  
279 *Plumatella*) available for each lake. These averages were used because the planktonic  
280 Cladocera genera of which ephippia were collected have similar feeding behaviour  
281 (Flössner 2000) and the same applies to the investigated Bryozoa genera (Kaminski  
282 1984).

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

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

294

### 295 3. Results

296

297 Lake characteristics and detailed water chemistry data, which is available for 42 of the  
298 53 study lakes, have been summarised in a PCA biplot (Fig. 1). The first two PCA

299 axes explained 22.4% and 18.3%, of the variance in the environmental data,  
300 respectively, and indicated that [DIC], conductivity and pH are important variables in  
301 the data set that plot along the first PCA axis. For our campaign, lakes with high  
302 [DIC], conductivity and pH are generally located in areas with carbonate bedrock,  
303 which is discussed in more detail by Rinta et al. (2015). The second PCA axis  
304 represents a combination of nutrients, [CH<sub>4</sub>], and indicators of stratification (N<sub>s</sub> and  
305 ΔO<sub>2</sub>). Nutrient concentrations appear to be higher in the shallower lakes, and higher  
306 [CH<sub>4</sub>] are found in deeper, more strongly stratified lakes. A more detailed discussion  
307 of the methane cycling and limnological profiles in the study lakes is provided  
308 elsewhere (Rinta et al. 2015, 2017).

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

313 Mean δ<sup>13</sup>C values of Cladocera ranged from -45.6 to -26.8 ‰ and were  
314 positively related to the δ<sup>13</sup>C values of SOM in the deep water zone (ρ = 0.73, p =  
315 0.002; Table 1; Fig. 3) and nearly significantly correlated to δ<sup>13</sup>C values of Bryozoa  
316 (ρ = 0.70, p = 0.051). In contrast, a significant relationship with SOM δ<sup>13</sup>C for the  
317 near-shore zone was not observed for bryozoan δ<sup>13</sup>C values that ranged from -40.7 to  
318 -24.2 ‰. Cladocera δ<sup>13</sup>C values were significantly negatively correlated with deep  
319 water [CH<sub>4</sub>] (ρ = -0.60, p = 0.026; Fig. 4; Table 1), whereas the relationship with  
320 stratification stability (N<sub>s</sub>) was almost statistically significant (ρ = -0.52, p = 0.054).  
321 Similarly, the δ<sup>13</sup>C values of deep water SOM ranged from -38.9 to -25.4 ‰ and were  
322 correlated to deep water methane concentrations (ρ = -0.76, p < 0.001; Fig. 4), ΔO<sub>2</sub> (ρ  
323 = -0.62, p < 0.001), and N<sub>s</sub> (ρ = -0.44, p = 0.033).

324 Mean Bryozoa δ<sup>13</sup>C values, in contrast, were not significantly correlated with  
325 stratification indicators and [CH<sub>4</sub>] (Table 1), but only with the lake:catchment ratio (ρ  
326 = -0.67, p = 0.014). The δ<sup>15</sup>N values of Bryozoa, ranging from -1.2 to 10.4 ‰, were  
327 significantly correlated with δ<sup>15</sup>N of SOM in the near-shore zone (ρ = 0.76, p = 0.008,  
328 Fig. 5). A correlation between δ<sup>15</sup>N values of Cladocera and δ<sup>15</sup>N values of deep  
329 water SOM was not observed, but δ<sup>15</sup>N values of deep water SOM were correlated to  
330 TN (ρ = 0.54, p = 0.004) and TP (ρ = 0.44, p = 0.033).

331

332 4. Discussion

333

334 4.1  $\delta^{13}\text{C}$  values of resting stages and SOM related to  $\text{CH}_4$  concentrations

335

336 This study shows a similar pattern as reported in studies assessing  $\delta^{13}\text{C}$  values of  
337 cladoceran ephippia in the surface sediments of small lakes (van Hardenbroek et al.  
338 2013; Schilder et al. 2015b); Our results also show a negative correlation between  
339 ephippia  $\delta^{13}\text{C}$  values in flotsam and in-lake methane concentrations (Fig. 4). The  $\delta^{13}\text{C}$   
340 values at some of the studied sites are clearly lower for the ephippia of some  
341 cladoceran groups (e.g. *Daphnia*, *Simocephalus*, Fig. 2) than expected for algal  
342 organic matter, which typically ranges between -36 and -20 ‰ (France 1995; Vuorio  
343 et al. 2006). For example, *Simocephalus* ephippia had  $\delta^{13}\text{C}$  values as low as -43.4 and  
344 -45.6 ‰ in Schwendisee and Lobsigensee, respectively, and *Daphnia* ephippia had  
345 values as low as -43.4 and -44.6 ‰ in Mekkojärvi and Uebesisee, respectively.

346 Laboratory and field studies of *Daphnia* have shown that this organism group  
347 is able to ingest and assimilate methane-derived carbon from MOB if these bacteria  
348 are an abundant resource in the open water column (Taipale et al. 2007, 2009, 2011;  
349 Deines and Fink 2011). Furthermore, surveys examining  $\delta^{13}\text{C}$  values of cladoceran  
350 resting stages in lake sediments have revealed that exceptionally low  $\delta^{13}\text{C}$  values of  
351 the ephippia of *Daphnia*, and other filter feeding cladocerans such as *Ceriodaphnia*,  
352 occur regularly in lakes with high methane concentrations. Schilder et al. (2015b)  
353 reported  $\delta^{13}\text{C}$  values as low as -52.8 ‰ for *Daphnia* ephippia in surface sediments of  
354 lake Mekkojärvi (Finland), and Morlock et al. (2017) values as low as -43.3 ‰ for  
355 *Ceriodaphnia* ephippia in sediments of lake Gerzensee (Switzerland), confirming that  
356 methane-derived carbon is incorporated by these cladocerans. Exceptionally negative  
357  $\delta^{13}\text{C}$  values as low as -50.1 ‰ were also reported for the ephippia of *Ceriodaphnia*  
358 isolated from flotsam on lake Gerzensee during winter (Morlock et al. 2017). The  
359  $\delta^{13}\text{C}$  values observed for ephippia of *Simocephalus* in our dataset are also low (-45.6  
360 ‰ in lake Lobsigensee and -43.4 ‰ in lake Schwendisee), and indicate that this group  
361 of filter-feeding cladocerans can also ingest  $\text{CH}_4$ -derived carbon originating from  
362 MOB, as earlier studies described for *Daphnia* and *Ceriodaphnia*.

363 In our dataset  $\delta^{13}\text{C}$  values of Cladocera ephippia are correlated with both  $\delta^{13}\text{C}$   
364 values of SOM and deep water  $\text{CH}_4$  concentrations (Table 1). This suggests that either

365 <sup>13</sup>C-depleted CH<sub>4</sub>-derived carbon contributed to both Cladocera biomass and SOM, or  
366 that other carbon sources available to filtering cladocerans in our lakes (e.g. algal  
367 material in POM) had δ<sup>13</sup>C values correlated with deep-water CH<sub>4</sub> concentrations and  
368 reinforced the apparent relationship between CH<sub>4</sub> concentrations and ephippia δ<sup>13</sup>C  
369 values. Methane concentrations in the lakes included in this study overlap with typical  
370 [CH<sub>4</sub>] values reported for other small lakes in the literature. [CH<sub>4</sub>] in surface waters in  
371 our study lakes range between 0.1 and 8.8 μM (the mean ± standard deviation is 1.8  
372 ± 2.0 μM (Rinta et al. 2017)). This fits well within the range of surface water [CH<sub>4</sub>] of  
373 small lakes and ponds found in a recent literature review (range: 0.01 – 59 μM, mean  
374 3.4 ± 7.7) by Holgerson and Raymond (2016).

375         Some studies (Hollander and Smith 2001; Lehmann et al. 2004; Teranes and  
376 Bernasconi 2005) have demonstrated that δ<sup>13</sup>C values of POM or SOM collected from  
377 the hypolimnion of lakes with high deep water CH<sub>4</sub> concentrations can be strongly  
378 depleted in <sup>13</sup>C, and have higher methanogen activity (West et al. 2012), supporting  
379 that CH<sub>4</sub>-derived carbon is also included in and can affect the δ<sup>13</sup>C values of organic  
380 matter floating and sedimenting in lakes. Further evidence for the importance of CH<sub>4</sub>  
381 in driving δ<sup>13</sup>C values of Cladocera ephippia and SOM, comes from the significant  
382 negative correlation between these δ<sup>13</sup>C values and stratification stability (N<sub>s</sub>) and  
383 with differences between surface and bottom water oxygen concentrations (ΔO<sub>2</sub>)  
384 (Table 1). In strongly stratified lakes with reduced deep water oxygen availability, it  
385 can be expected that more CH<sub>4</sub> is produced and that more MOB biomass will be  
386 available in the water column and surface sediments compared with other lakes.  
387 However, direct evidence for higher MOB biomass in stratified lakes would be  
388 desirable and could be obtained, e.g., via analysis of lipids, environmental DNA, or  
389 compound-specific stable isotope analysis to support this hypothesis.

390         Other processes could also explain, in part, the observed correlation between  
391 δ<sup>13</sup>C values in *Daphnia* and those in SOM. The δ<sup>13</sup>C values of phytoplankton can be  
392 strongly influenced by concentrations of dissolved organic carbon (DIC) in the lake  
393 water and by algal productivity. In lakes where DIC is not limiting (e.g., in  
394 unproductive and in high-DIC lakes), phytoplankton can more easily discriminate  
395 against <sup>13</sup>C and lower δ<sup>13</sup>C values in phytoplankton may be expected (Fogel and  
396 Cifuentes 1993). In situations where DIC is limiting (e.g., in low-DIC or in very  
397 productive lakes), phytoplankton is typically characterised by higher δ<sup>13</sup>C values. If

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

406 In our dataset, however, the effect of productivity on DIC concentrations and  
407  $\delta^{13}\text{C}$  values of phytoplankton cannot be studied in isolation, because the more  
408 productive lakes in our data are naturally high in DIC concentrations and have higher  
409  $\delta^{13}\text{C}$  values of DIC, caused by underlying bedrock type (Schilder et al. 2015b; Rinta  
410 et al. 2017). As a result, the productive lakes in our data set generally have high DIC  
411 concentrations as well as high deep water methane concentrations, and both of these  
412 could lead to lower  $\delta^{13}\text{C}$  values of SOM as discussed above. Indirect evidence  
413 suggests that variations in  $\delta^{13}\text{C}$  values of phytoplankton cannot be the main driver of  
414 variations in ephippia  $\delta^{13}\text{C}$  values: If phytoplankton  $\delta^{13}\text{C}$  values would be the main  
415 driver for  $\delta^{13}\text{C}$  values of SOM and cladoceran ephippia we would expect to see a  
416 similarly strong relationship between  $\delta^{13}\text{C}$  values of statoblasts and of SOM as  
417 observed between cladoceran ephippia and SOM. This is because planktonic algae  
418 and POM are also the main food source for bryozoan colonies in near-shore zone of  
419 lakes (Wood and Okamura 2005; Kaminski 1984). However, the relationship between  
420 bryozoan statoblasts and SOM  $\delta^{13}\text{C}$  values is noticeably weaker than observed  
421 between cladoceran ephippia and SOM (Fig 3; Table 1). Furthermore, the extremely  
422 negative  $\delta^{13}\text{C}$  values below -36‰ for some cladoceran ephippia samples cannot be  
423 explained by the uptake of algal organic matter (typically > -36 ‰). Regardless of the  
424 direct cause, the apparent correlation between cladoceran ephippia  $\delta^{13}\text{C}$  values and  
425  $\text{CH}_4$  concentrations agrees with earlier studies indicating that the stable isotopic  
426 composition of filter feeding planktonic cladocerans, and particularly *Daphnia*, is  
427 strongly affected by the assimilation of  $\text{CH}_4$ -derived carbon in  $\text{CH}_4$ -rich lakes.

428

429 *4.2  $\delta^{15}\text{N}$  values of invertebrates and SOM*

430

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

442 The  $\delta^{15}\text{N}$  values of Cladocera were not found to correlate to the  $\delta^{15}\text{N}$  values of  
443 SOM, and neither were they significantly correlated with  $\delta^{15}\text{N}$  values of Bryozoa ( $\rho =$   
444  $0.60$ ,  $p = 0.12$ ). This might be related to the feeding ecology of Cladocera, which is  
445 more varied than that of Bryozoa: Cladocera can migrate vertically in the water  
446 column (Lampert 2011) and filter living algae and bacteria in the size range of  $0.5$  to  
447  $30\ \mu\text{m}$  (Geller and Müller 1981; Jürgens 1994; Taipale et al. 2007). Bacterial biomass  
448 can have relatively low  $\delta^{15}\text{N}$  values, which has been shown subsequently to affect the  
449  $\delta^{15}\text{N}$  values of *Daphnia* (Taipale et al. 2012). It is possible that trends in the  $\delta^{15}\text{N}$   
450 values of Cladocera related to  $\delta^{15}\text{N}$  of phytoplankton are partially obscured by  
451 assimilation of bacterial biomass, but data are not presently available to rigorously  
452 test this hypothesis.

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

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

470

#### 471 4.3 Timing and location of resting stage production and relation to parent organisms

472

473 Our study confirms that the stable carbon and nitrogen isotope composition of  
474 ephippia and statoblasts in lake flotsam relate to in-lake processes (at least during  
475 stratification in late summer) as is evidenced by the relationships between  $\delta^{13}\text{C}$  of  
476 Cladocera ephippia and  $[\text{CH}_4]$  and between  $\delta^{15}\text{N}$  values of Bryozoa and SOM. The  
477 results therefore suggest that resting stages can provide information about different  
478 carbon and nitrogen sources in the water column (phytoplankton and MOB).

479 However, several limitations of our pioneering study restrict the extent to which we  
480 can assess the utility of flotsam  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis for interpreting down core  
481 isotope analyses based on these remains.

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

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



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

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

521 When using resting stages in palaeolimnological studies, it is important to  
522 realise that resting stages accumulating in lake sediments provide an integrated  
523 average of stable isotope composition in resting stages produced over longer time  
524 periods. *Daphnia* ehippia from Lake De Waay had extremely variable  $\delta^{13}\text{C}$  values in  
525 flotsam samples ( $-41.7 \pm 4.8\text{‰}$ ), which was also found for *Daphnia* themselves ( $-36.6$   
526  $\pm 6.8\text{‰}$ ), but less so for ehippia in surface sediments ( $-39.2 \pm 0.5\text{‰}$ ) (Schilder et al.  
527 2017). Schilder et al. (2017) only analysed a limited number of samples collected four  
528 times during the annual cycle. A more detailed study by Morlock et al. (2017) in Lake  
529 Gerzensee found that the time-averaged  $\delta^{13}\text{C}$  value of *Daphnia* ehippia in surface  
530 sediments ( $-39.8\text{‰}$ ) was nearly identical to the average  $\delta^{13}\text{C}$  value of flotsam  
531 collected throughout the year from the lake surface ( $-39.5 \pm 0.9\text{‰}$ ) and to the average

532  $\delta^{13}\text{C}$  value of living *Daphnia* collected during the 2-year study period ( $-39.4 \pm 4.6$   
533 ‰). The same was found for *Ceriodaphnia* ehippia in flotsam ( $-42.9 \pm 4.8$  ‰) and  
534 those in surface sediments ( $-42.9$  ‰). *Plumatella* statoblasts, however, were more  
535  $^{13}\text{C}$ -depleted in flotsam ( $-36.3 \pm 1.7$  ‰) than in the surface sediments ( $-32.7$  ‰). It is  
536 possible that this difference was caused by the low number statoblasts measurements  
537 in summer flotsam samples, or because statoblasts from the top 7 cm of the core had  
538 to be pooled to get enough material for stable isotope analysis. However, it is also  
539 possible that the impact of taphonomic processes on statoblast  $\delta^{13}\text{C}$  values are more  
540 complex than for ehippia.

541         The examples from Gerzensee and Lake De Waay demonstrate that the timing  
542 of resting stage production, and the isotopic composition of food sources available for  
543 the examined invertebrate groups just before resting stage production may have  
544 influenced our dataset and may be responsible for some of the scatter between stable  
545 isotopic composition of resting stages and that of SOM, or between  $\delta^{13}\text{C}$  values in  
546 flotsam and  $\text{CH}_4$  or TP concentrations. It could be expected that  $\delta^{13}\text{C}$  values of resting  
547 stages in flotsam for some of the lakes in the present study will be representative for  
548 ehippia production peaks in spring and/or autumn (at least for *Daphnia* ehippia),  
549 whereas at other sites they will represent resting stages produced in the days to weeks  
550 before sampling in late summer. More detailed research is needed on individual  
551 species of Cladocera and Bryozoa to constrain during which period of the annual  
552 cycle they produce the majority of resting stages.

553

## 554 5. Conclusions

555

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

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

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

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

598

599

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601

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606

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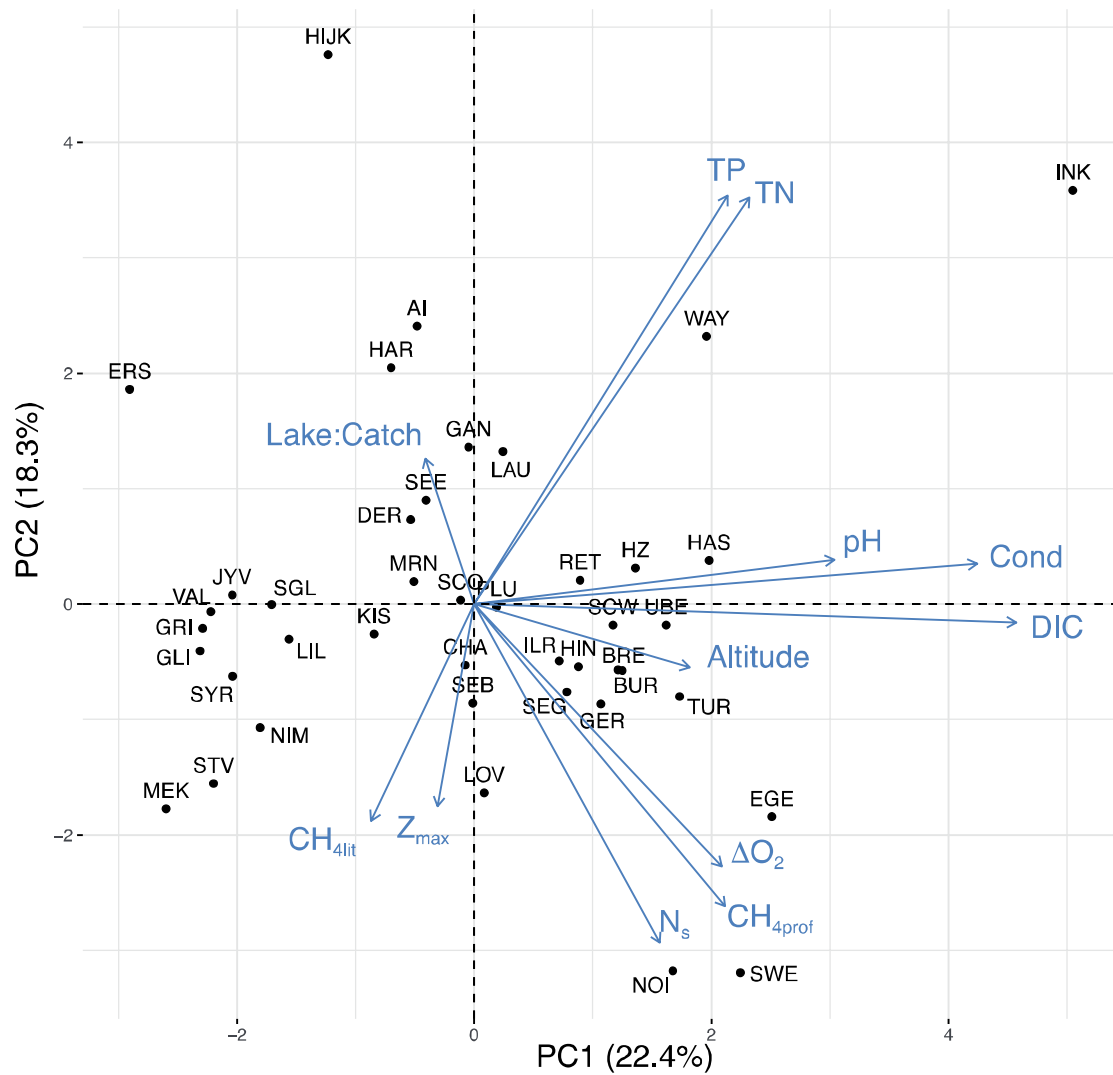
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810 **Figures**

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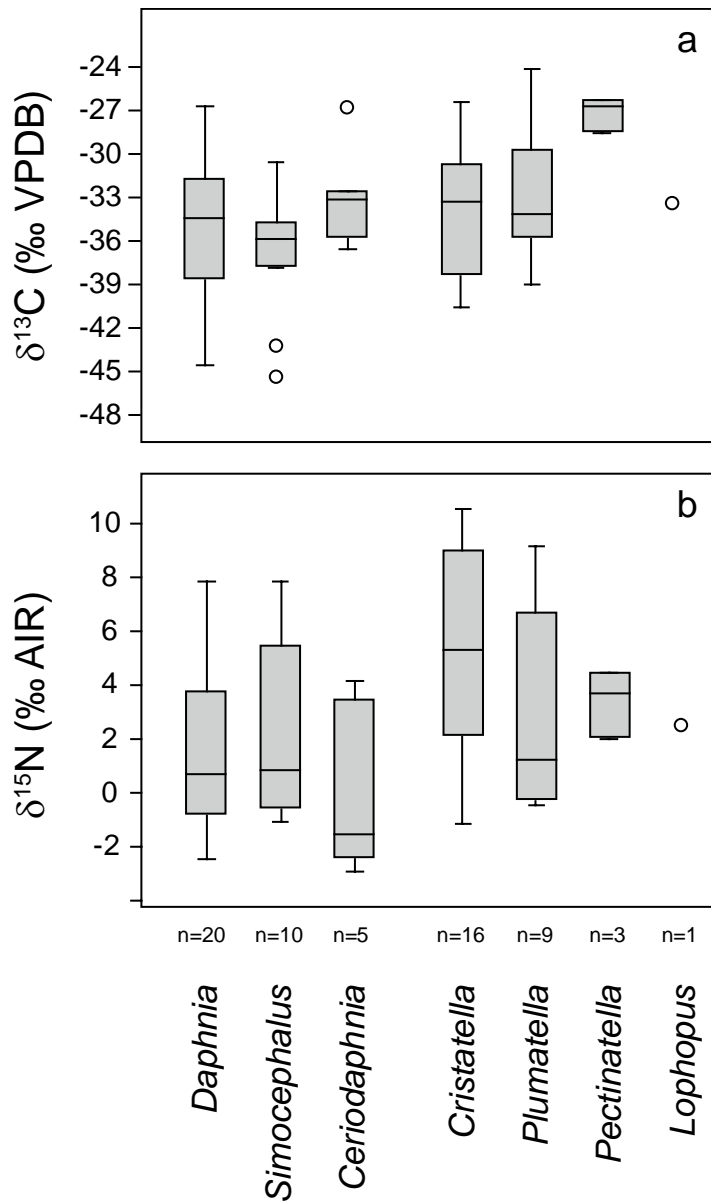
814 **Fig. 1** Principal Component Analysis of physical lake characteristics and water

815 chemistry of 42 of the lakes for which detailed surveys were undertaken. Lake ID

816 codes are explained in Supplementary Table 1.

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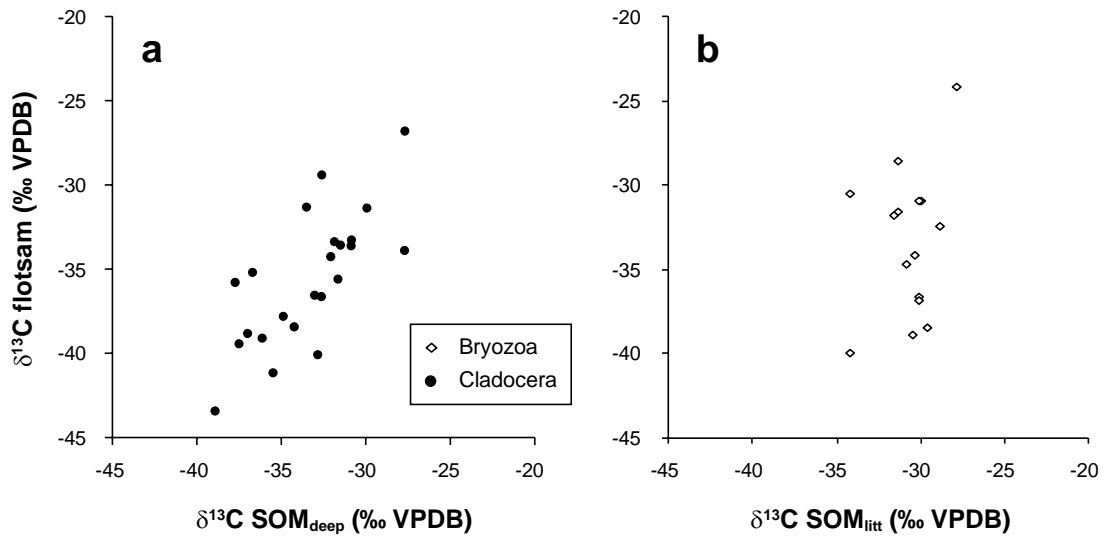
821 **Fig. 2** Boxplot with  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b) values for the different taxa collected in

822 flotsam samples; n indicates the number of lakes for which stable isotopes were

823 analysed for the respective taxon.

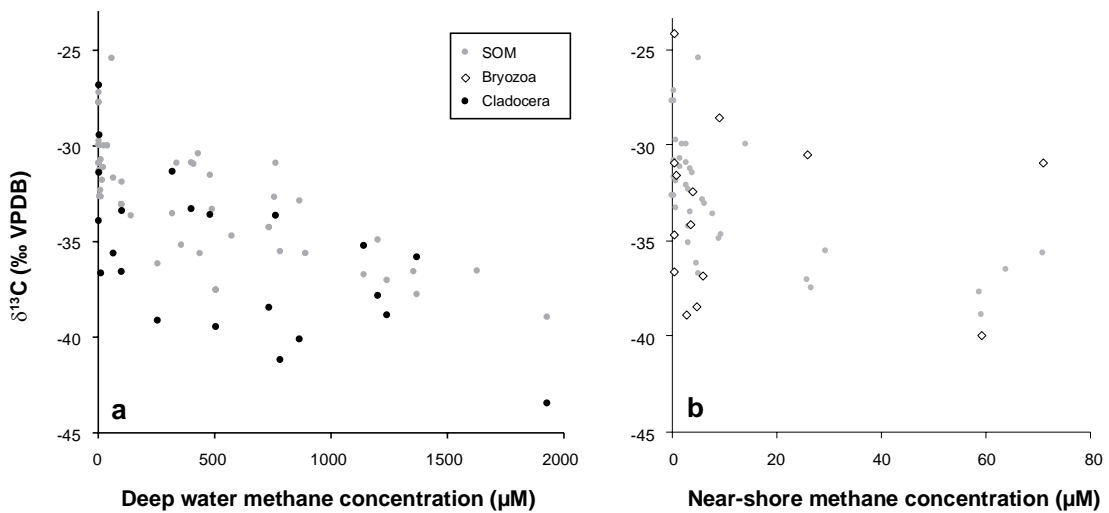
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**Fig. 3** Average  $\delta^{13}\text{C}$  values of Cladocera ephippia plotted against  $\delta^{13}\text{C}$  of SOM in the deep water zone ( $\text{SOM}_{\text{deep}}$ ) (a) and average  $\delta^{13}\text{C}$  values Bryozoa statoblasts plotted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



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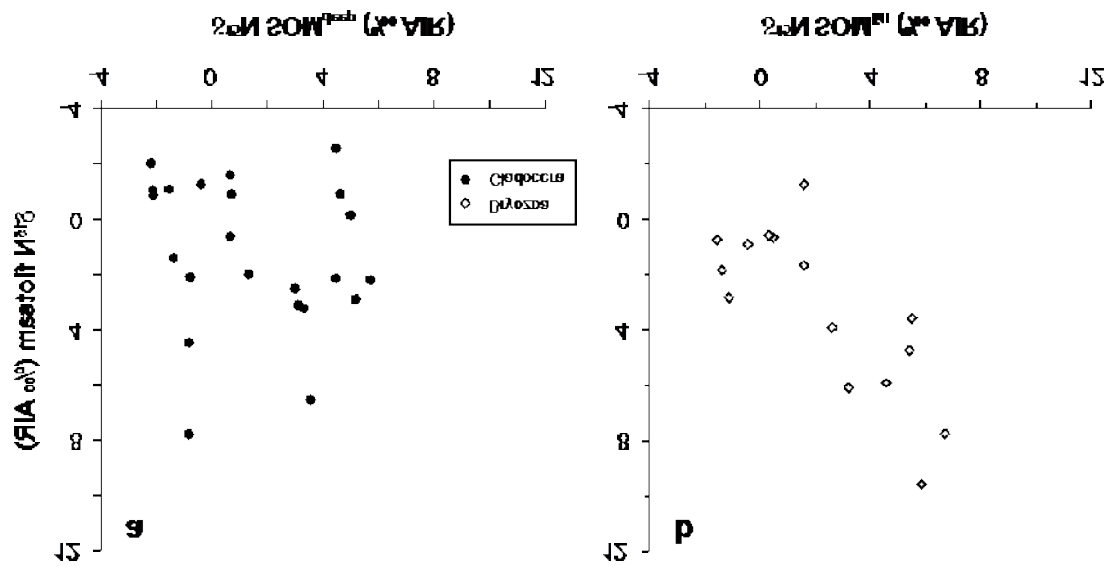
**Fig. 4** Average  $\delta^{13}\text{C}$  values of Cladocera and deep water SOM plotted against deep water methane concentrations 10 cm above the sediments (a), and average  $\delta^{13}\text{C}$  of Bryozoa and near-shore SOM plotted against near-shore methane concentrations measured 10 cm above the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

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848 **Fig. 5** Average  $\delta^{15}\text{N}$  values of Cladocera plotted against  $\delta^{15}\text{N}$  of deep water SOM

849 ( $\text{SOM}_{\text{deep}}$ ) in (a) and average  $\delta^{15}\text{N}$  values of Bryozoa plotted against  $\delta^{15}\text{N}$  of near-

850 shore SOM ( $\text{SOM}_{\text{litt}}$ ) (b).

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