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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, [CH₄] 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 (ΔO_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 [CH₄] 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 ΔO_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 μl H_3PO_4 (85 %) to ensure that all DIC in the water sample would
204 be converted to CO_2 and filled with 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 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 CH_4 and 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 CH_4 and TCD for 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 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 CO_2)
220 have been presented in Rinta et al. (2015). As described in their publication, 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 μm 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 NH_4Cl 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 μg . 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 (1σ) 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 (1σ) 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 ρ 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, [CH₄], and indicators of stratification (N_s and
305 ΔO₂). Nutrient concentrations appear to be higher in the shallower lakes, and higher
306 [CH₄] 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 δ¹³C values of Cladocera ranged from -45.6 to -26.8 ‰ and were
314 positively related to the δ¹³C values of SOM in the deep water zone (ρ = 0.73, p =
315 0.002; Table 1; Fig. 3) and nearly significantly correlated to δ¹³C values of Bryozoa
316 (ρ = 0.70, p = 0.051). In contrast, a significant relationship with SOM δ¹³C for the
317 near-shore zone was not observed for bryozoan δ¹³C values that ranged from -40.7 to
318 -24.2 ‰. Cladocera δ¹³C values were significantly negatively correlated with deep
319 water [CH₄] (ρ = -0.60, p = 0.026; Fig. 4; Table 1), whereas the relationship with
320 stratification stability (N_s) was almost statistically significant (ρ = -0.52, p = 0.054).
321 Similarly, the δ¹³C 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), ΔO₂ (ρ
323 = -0.62, p < 0.001), and N_s (ρ = -0.44, p = 0.033).

324 Mean Bryozoa δ¹³C values, in contrast, were not significantly correlated with
325 stratification indicators and [CH₄] (Table 1), but only with the lake:catchment ratio (ρ
326 = -0.67, p = 0.014). The δ¹⁵N values of Bryozoa, ranging from -1.2 to 10.4 ‰, were
327 significantly correlated with δ¹⁵N of SOM in the near-shore zone (ρ = 0.76, p = 0.008,
328 Fig. 5). A correlation between δ¹⁵N values of Cladocera and δ¹⁵N values of deep
329 water SOM was not observed, but δ¹⁵N 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 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 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 CH_4 concentrations (Table 1). This suggests that either

365 ¹³C-depleted CH₄-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 δ¹³C values correlated with deep-water CH₄ concentrations and
368 reinforced the apparent relationship between CH₄ concentrations and ehippia δ¹³C
369 values. Methane concentrations in the lakes included in this study overlap with typical
370 [CH₄] values reported for other small lakes in the literature. [CH₄] 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 [CH₄] 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 δ¹³C values of POM or SOM collected from
377 the hypolimnion of lakes with high deep water CH₄ concentrations can be strongly
378 depleted in ¹³C, and have higher methanogen activity (West et al. 2012), supporting
379 that CH₄-derived carbon is also included in and can affect the δ¹³C values of organic
380 matter floating and sedimenting in lakes. Further evidence for the importance of CH₄
381 in driving δ¹³C values of Cladocera ehippia and SOM, comes from the significant
382 negative correlation between these δ¹³C values and stratification stability (N_s) and
383 with differences between surface and bottom water oxygen concentrations (ΔO₂)
384 (Table 1). In strongly stratified lakes with reduced deep water oxygen availability, it
385 can be expected that more CH₄ 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 δ¹³C values in *Daphnia* and those in SOM. The δ¹³C 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 ¹³C and lower δ¹³C 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 δ¹³C 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 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 CH_4 -derived carbon in 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}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}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‰ in early spring and -29.8‰ 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‰) 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‰) 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 ± 4.6
533 ‰). The same was found for *Ceriodaphnia* ehippia in flotsam (-42.9 ± 4.8 ‰) and
534 those in surface sediments (-42.9 ‰). *Plumatella* statoblasts, however, were more
535 ^{13}C -depleted in flotsam (-36.3 ± 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 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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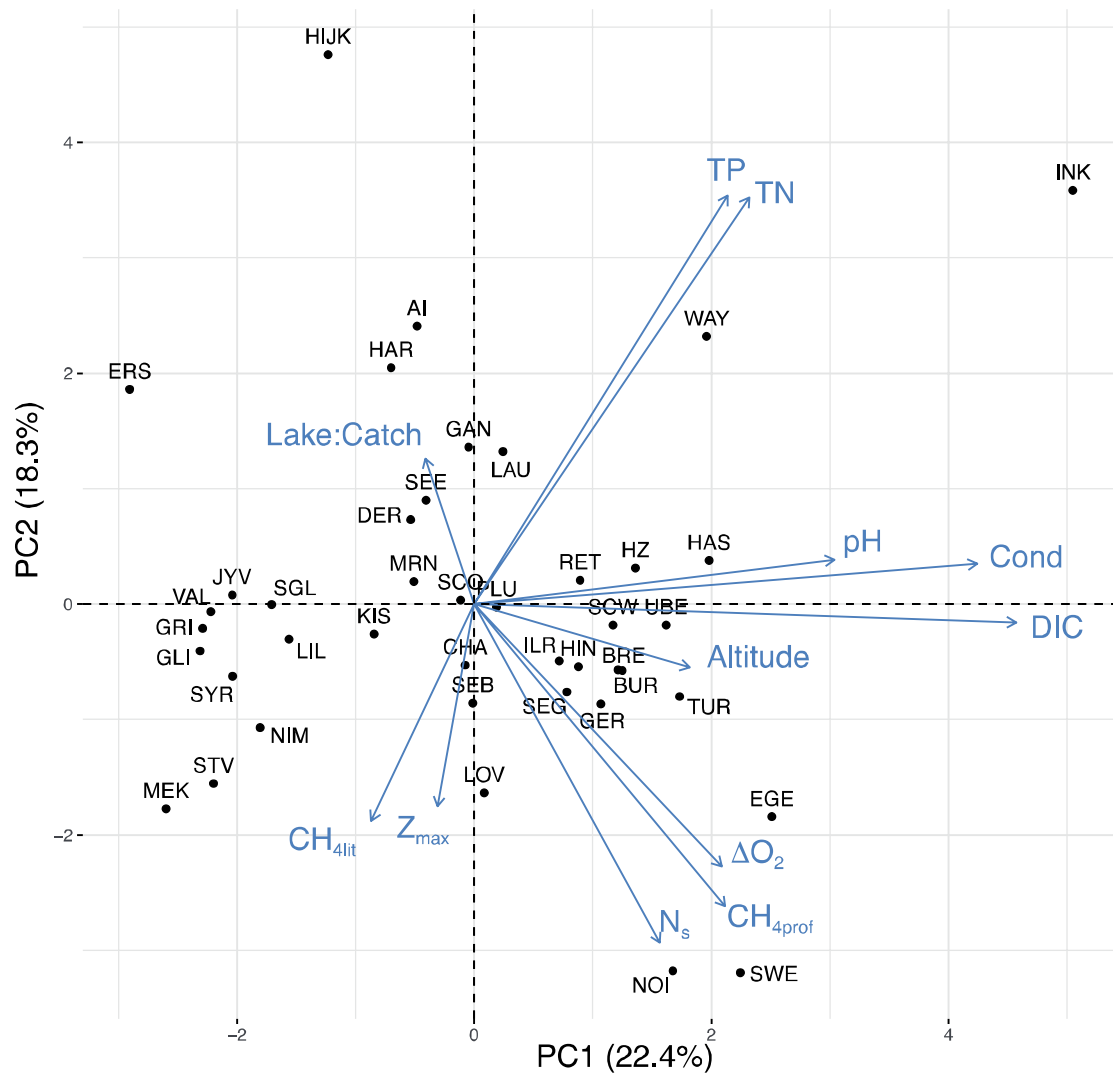
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810 **Figures**

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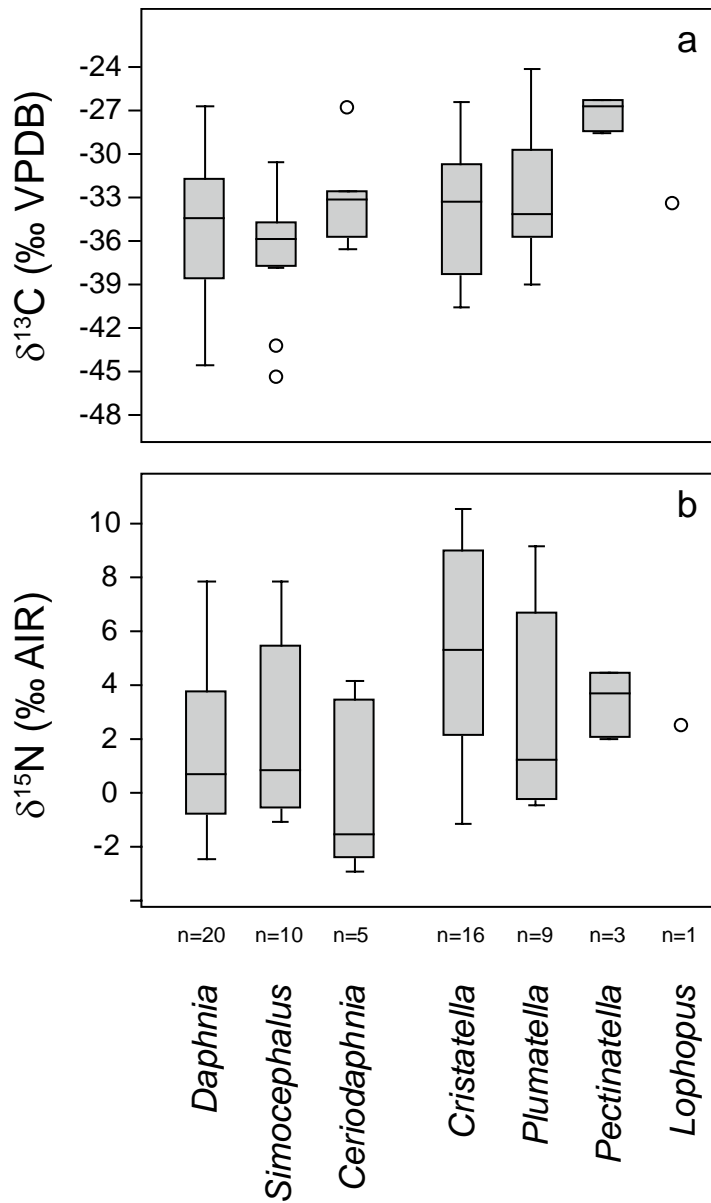
814 **Fig. 1** Principal Component Analysis of physical lake characteristics and water

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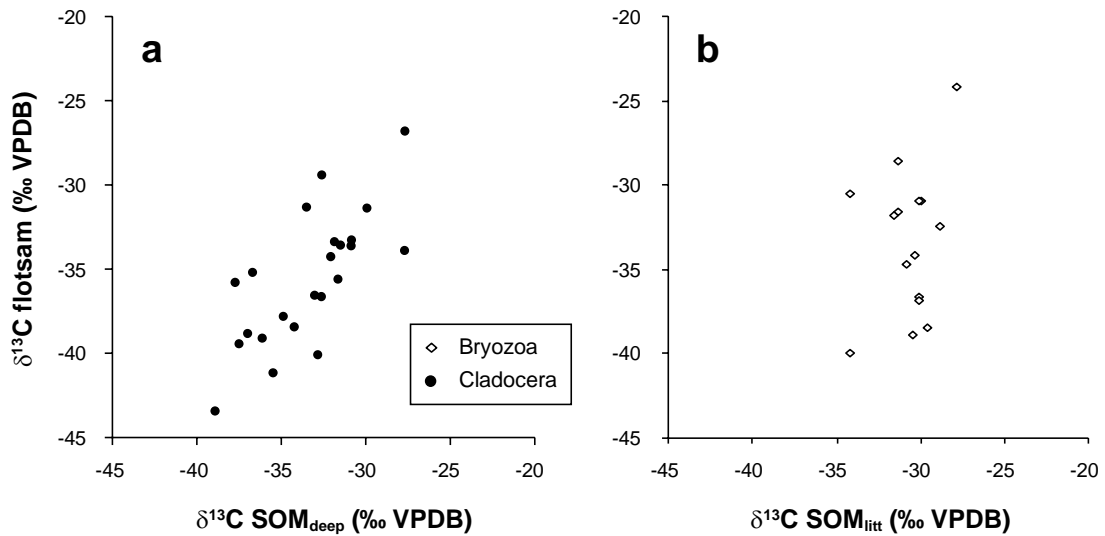
821 **Fig. 2** Boxplot with $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for the different taxa collected in

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

823 analysed for the respective taxon.

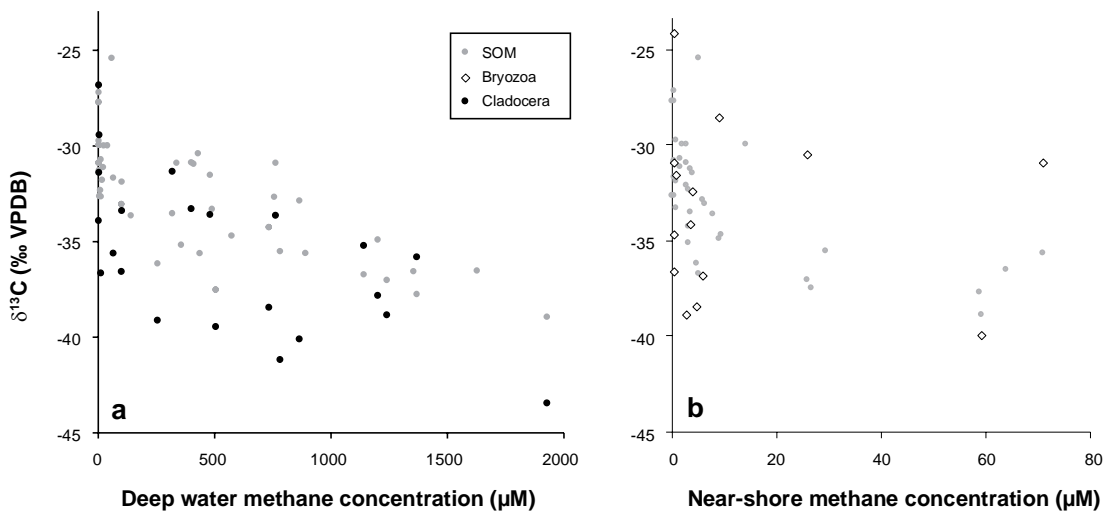
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Fig. 3 Average $\delta^{13}\text{C}$ values of Cladocera ehippia plotted against $\delta^{13}\text{C}$ of SOM in the deep water zone (SOM_{deep}) (a) and average $\delta^{13}\text{C}$ values Bryozoa statoblasts plotted against $\delta^{13}\text{C}$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



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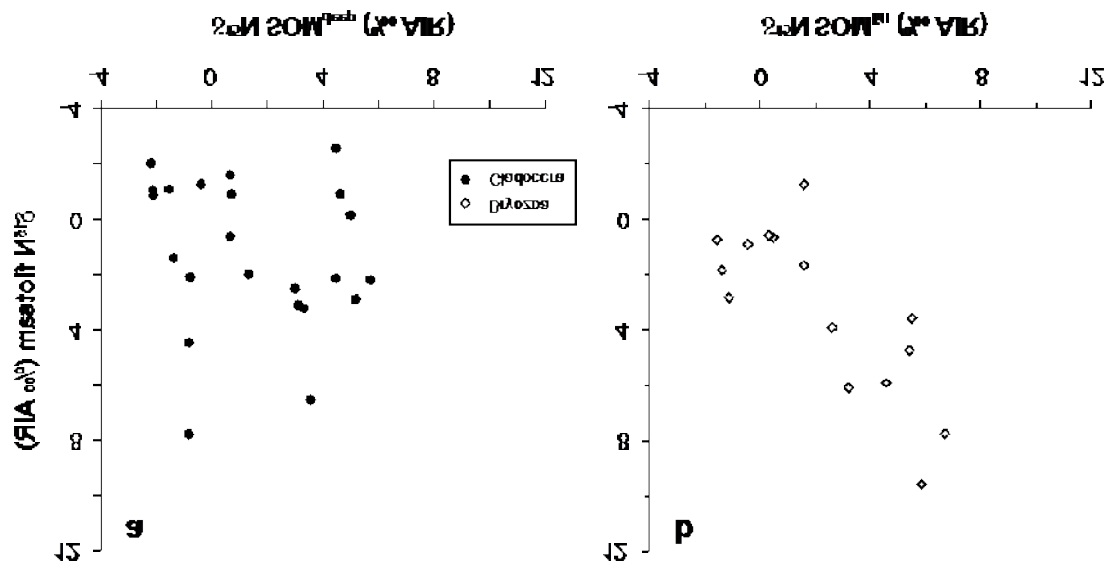
Fig. 4 Average $\delta^{13}\text{C}$ values of Cladocera and deep water SOM plotted against deep water methane concentrations 10 cm above the sediments (a), and average $\delta^{13}\text{C}$ of Bryozoa and near-shore SOM plotted against near-shore methane concentrations measured 10 cm above the sediments.

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

850 shore SOM (SOM_{litt}) (b).

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