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1  **Flotsam samples can help explain the $\delta^{13}$C and $\delta^{15}$N values of invertebrate**

2  **resting stages in lake sediment**

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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 $\delta^{13}$C and $\delta^{15}$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 $\delta^{13}$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 $\delta^{15}$N values of bryozoan

33  statoblasts and SOM were correlated, suggesting that both reflect the $\delta^{15}$N values of

34  phytoplankton. Our results provide information on how environmental variables in

35  lakes can influence the $\delta^{13}C$ and $\delta^{15}N$ 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; Ephippia; 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 ephippium 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  Ephippia 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 $\delta^{13}C$ and

53  $\delta^{15}N$ values, respectively), which reflects the $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ values in *Daphnia*

68 and some other aquatic invertebrates if this carbon source provides a significant

69 comtribution 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 $\delta^{13}C$ 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 $\delta^{13}C$ values are thought to predominantly represent $\delta^{13}C$

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 $\delta^{13}C$

115 and $\delta^{15}N$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ and $\delta^{15}N$ values are therefore expected to reflect the overall $\delta^{13}C$

127 and $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ values of sedimentary

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

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

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

*2. Materials and methods*

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

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, [$CH_4$] 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 [$CH_4$] 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 $\delta^{13}C$ 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 $\delta^{13}C$ and $\delta^{15}N$ (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 $\delta^{13}C$ 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 $\delta^{13}C$ and $\delta^{15}N$ values of *Daphnia*

245   ephippia (Schilder et al. 2015a) or on the $\delta^{13}C$ 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 ($\delta^{13}C$ and $\delta^{15}N$)

261   relative to Vienna Pee Dee Belemnite (VPDB) and AIR, respectively. Analytical

262   precision (1σ) for bulk $\delta^{13}C$ and $\delta^{15}N$ were 0.1 and 0.3‰, respectively.

263        SOM samples for $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ values and 0.33‰ for $\delta^{15}N$ 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  $\delta^{13}C$ and $\delta^{15}N$ 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 [$CH_4$] 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 [$CH_4$] in bottom waters made this site very different from the deep water [$CH_4$]

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 $\delta^{13}C$ values or $\delta^{15}N$ 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, $[CH_4]$, and indicators of stratification ($N_s$ and

305     $\Delta O_2$). Nutrient concentrations appear to be higher in the shallower lakes, and higher

306     $[CH_4]$ 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 $\delta^{13}C$ values of Cladocera ranged from -45.6 to -26.8 ‰ and were

314     positively related to the $\delta^{13}C$ values of SOM in the deep water zone ($\rho = 0.73$, p =

315     0.002; Table 1; Fig. 3) and nearly significantly correlated to $\delta^{13}C$ values of Bryozoa

316     ($\rho = 0.70$, p = 0.051). In contrast, a significant relationship with SOM $\delta^{13}C$ for the

317     near-shore zone was not observed for bryozoan $\delta^{13}C$ values that ranged from -40.7 to

318     -24.2 ‰. Cladocera $\delta^{13}C$ values were significantly negatively correlated with deep

319     water $[CH_4]$ ($\rho = -0.60$, p = 0.026; Fig. 4; Table 1), whereas the relationship with

320     stratification stability ($N_s$) was almost statistically significant ($\rho = -0.52$, p = 0.054).

321     Similarly, the $\delta^{13}C$ values of deep water SOM ranged from -38.9 to -25.4 ‰ and were

322     correlated to deep water methane concentrations ($\rho = -0.76$, p < 0.001; Fig. 4), $\Delta O_2$ ($\rho$

323     = -0.62, p < 0.001), and $N_s$ ($\rho = -0.44$, p = 0.033).

324         Mean Bryozoa $\delta^{13}C$ values, in contrast, were not significantly correlated with

325     stratification indicators and $[CH_4]$ (Table 1), but only with the lake:catchment ratio ($\rho$

326     = -0.67, p = 0.014). The $\delta^{15}N$ values of Bryozoa, ranging from -1.2 to 10.4 ‰, were

327     significantly correlated with $\delta^{15}N$ of SOM in the near-shore zone ($\rho = 0.76$, p = 0.008,

328     Fig. 5). A correlation between $\delta^{15}N$ values of Cladocera and $\delta^{15}N$ values of deep

329     water SOM was not observed, but $\delta^{15}N$ values of deep water SOM were correlated to

330     TN ($\rho = 0.54$, p = 0.004) and TP ($\rho = 0.44$, p = 0.033).

331

332 *4. Discussion*

333

334 *4.1 δ¹³C values of resting stages and SOM related to CH₄ concentrations*

335

336 This study shows a similar pattern as reported in studies assessing $\delta^{13}C$ 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 $\delta^{13}C$ values in flotsam and in-lake methane concentrations (Fig. 4). The $\delta^{13}C$

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 $\delta^{13}C$ 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 Uebeschisee, 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 $\delta^{13}C$ values of cladoceran

350 resting stages in lake sediments have revealed that exceptionally low $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 CH₄-derived carbon originating from

362 MOB, as earlier studies described for *Daphnia* and *Ceriodaphnia*.

363        In our dataset $\delta^{13}C$ values of Cladocera ephippia are correlated with both $\delta^{13}C$

364 values of SOM and deep water CH₄ concentrations (Table 1). This suggests that either

365   $^{13}$C-depleted CH$_4$-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$^{13}$C values correlated with deep-water CH$_4$ concentrations and

368   reinforced the apparent relationship between CH$_4$ concentrations and ephippia δ$^{13}$C

369   values. Methane concentrations in the lakes included in this study overlap with typical

370   [CH$_4$] values reported for other small lakes in the literature. [CH$_4$] 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$_4$] 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$^{13}$C values of POM or SOM collected from

377   the hypolimnion of lakes with high deep water CH$_4$ concentrations can be strongly

378   depleted in $^{13}$C, and have higher methanogen activity (West et al. 2012), supporting

379   that CH$_4$-derived carbon is also included in and can affect the δ$^{13}$C values of organic

380   matter floating and sedimenting in lakes. Further evidence for the importance of CH$_4$

381   in driving δ$^{13}$C values of Cladocera ephippia and SOM, comes from the significant

382   negative correlation between these δ$^{13}$C values and stratification stability (N$_s$) and

383   with differences between surface and bottom water oxygen concentrations (ΔO$_2$)

384   (Table 1). In strongly stratified lakes with reduced deep water oxygen availability, it

385   can be expected that more CH$_4$ 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$^{13}$C values in *Daphnia* and those in SOM. The δ$^{13}$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 $^{13}$C and lower δ$^{13}$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$^{13}$C values. If

398    productivity-related variation in phytoplankton $\delta^{13}C$ values is an important factor in

399    our data set, we would expect to find higher $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values of flotsam (Table 1), making it

404    unlikely that the observed $\delta^{13}C$ values of flotsam are driven predominantly by

405    productivity.

406         In our dataset, however, the effect of productivity on DIC concentrations and

407    $\delta^{13}C$ 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    $\delta^{13}C$ 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 $\delta^{13}C$ values of SOM as discussed above. Indirect evidence

413    suggests that variations in $\delta^{13}C$ values of phytoplankton cannot be the main driver of

414    variations in ephippia $\delta^{13}C$ values: If phytoplankton $\delta^{13}C$ values would be the main

415    driver for $\delta^{13}C$ values of SOM and cladoceran ephippia we would expect to see a

416    similarly strong relationship between $\delta^{13}C$ 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 $\delta^{13}C$ values is noticeably weaker than observed

421    between cladoceran ephippia and SOM (Fig 3; Table 1). Furthermore, the extremely

422    negative $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{15}N$ values of invertebrates and SOM*

430

431 Bryozoan statoblast $\delta^{15}$N values were positively correlated with $\delta^{15}$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 $\delta^{15}$N

434 values could be expected to represent the $\delta^{15}$N values of phytoplankton, which in turn

435 reflects $\delta^{15}$N values of the dissolved inorganic nitrogen (DIN) pool available to

436 planktonic algae. SOM $\delta^{15}$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, $\delta^{15}$N

441 values of bryzoan statoblasts were not significantly correlated with TN.

442        The $\delta^{15}$N values of Cladocera were not found to correlate to the $\delta^{15}$N values of

443 SOM, and neither were they significantly correlated with $\delta^{15}$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 μm (Geller and Müller 1981; Jürgens 1994; Taipale et al. 2007). Bacterial biomass

448 can have relatively low $\delta^{15}$N values, which has been shown subsequently to affect the

449 $\delta^{15}$N values of *Daphnia* (Taipale et al. 2012). It is possible that trends in the $\delta^{15}$N

450 values of Cladocera related to $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$N values in SOM and benthic chironomids, whereas

457 *Daphnia* $\delta^{15}$N values were continuously high throughout the record, even before $\delta^{15}$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 $\delta^{15}$N values of cladoceran carapaces

463 preserved in lake sediment records, and these suggest that variations in $\delta^{15}$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 $\delta^{13}C$ of

476  Cladocera ephippia and $[CH_4]$ and between $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}$C and $\delta^{15}$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          Ephippia 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 ephippia production varies between species (Cáceres 1998;

507    Cáceres and Tessier 2004a, b) but most of the investigated species have ephippia

508    production peaks around April-June and September-November. A detailed study in

509    Gerzensee (Switzerland) indicated that living *Daphnia* $\delta^{13}$C values varied seasonally

510    between -44.2 ‰ in early spring and -29.8 ‰ in autumn, tracking the $\delta^{13}$C values of

511    POM (Morlock et al. 2017). In contrast, the $\delta^{13}$C values of *Daphnia* ephippia 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 ephippia were produced during one or both of these time intervals and that

515    ephippia from these production peaks remained afloat or where re-suspended for at

516    least six months. In contrast, studies on *Ceriodaphnia* ephippia and *Plumatella*

517    statoblasts from Gerzensee (Morlock et al. 2017) but also on *Daphnia* ephippia from

518    Lake De Waay (Schilder et al. 2017), showed pronounced seasonal variations in

519    ephippia $\delta^{13}$C values. This suggests that ephippia 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* ephippia from Lake De Waay had extremely variable $\delta^{13}$C values in

525    flotsam samples (-41.7 ± 4.8‰), which was also found for *Daphnia* themselves (-36.6

526    ± 6.8 ‰), but less so for ephippia in surface sediments (-39.2 ± 0.5 ‰) (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 $\delta^{13}$C value of Daphnia ephippia in surface

530    sediments (-39.8‰) was nearly identical to the average $\delta^{13}$C value of flotsam

531    collected throughout the year from the lake surface (-39.5 ± 0.9 ‰) and to the average

532    $\delta^{13}$C value of living *Daphnia* collected during the 2-year study period (-39.4 ± 4.6

533    ‰). The same was found for *Ceriodaphnia* ephippia 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 $\delta^{13}$C values are more

540    complex than for ephippia.

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 $\delta^{13}$C values in

546    flotsam and $CH_4$ or TP concentrations. It could be expected that $\delta^{13}$C values of resting

547    stages in flotsam for some of the lakes in the present study will be representative for

548    ephippia production peaks in spring and/or autumn (at least for *Daphnia* ephippia),

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 ephippia 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 $\delta^{13}$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 $\delta^{13}$C values of Cladocera ephippia 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 $\delta^{13}C$ values of Cladocera and SOM were significantly correlated with

568    each other, but this was not found for Bryozoa, possibly because $\delta^{13}C$ values of

569    Cladocera and SOM were both driven by methane-derived carbon. In addition, the

570    $\delta^{15}N$ 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 $\delta^{15}N$ values in

574    phytoplankton and SOM.

575          In contrast to our initial expectations, we found no clear relationship between

576    nutrient concentrations and $\delta^{13}C$ values of resting stages. Such a relationship would be

577    expected if nutrients and growth rates were a main driver of $\delta^{13}C$ values of

578    phytoplankton (and ultimately the $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ and $\delta^{15}N$ 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    $\delta^{13}C$ and $\delta^{15}N$ 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

**References**

Bastviken, D., Ejlertsson, J., Sundh, I., Tranvik, L., 2003. Methane as a source of carbon and energy for lake pelagic food webs. Ecology 84, 969-981.

Bastviken, D., Cole, J.J., Pace, M.L., de Bogert, M.C.V., 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and $CH_4$ emissions. J. Geophys. Res.-Biogeo. 113, G02024.

Bastviken, D., Santoro, A.L., Marotta, H., Pinho, L.Q., Calheiros, D.F., Crill, P., Enrich-Prast, A., 2010. Methane Emissions from Pantanal, South America, during the Low Water Season: Toward More Comprehensive Sampling. Environ. Sci. Technol. 44, 5450-5455.

Battarbee, R.W., Anderson, N.J., Bennion, H., Simpson, G.L., 2012. Combining limnological and palaeolimnological data to disentangle the effects of nutrient pollution and climate change on lake ecosystems: problems and potential. Freshwat. Biol. 57, 2091-2106.

Belle, S., Millet, L., Lami, A., Verneaux, V., Musazzi, S., Hossann, C., Magny, M., 2016a. Increase in benthic trophic reliance on methane in 14 French lakes during the Anthropocene. Freshwat. Biol. 61, 1105-1118.

Belle, S., Millet, L., Verneaux, V., Lami, A., David, E., Murgia, L., Parent, C., Musazzi, S., Gauthier, E., Bichet, V., 2016b. 20th century human pressures drive reductions in deepwater oxygen leading to losses of benthic methane-based food webs. Quaternary Sci. Rev. 137, 209-220.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological) 57, 289-300.

Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences 93, 10844-10847.

633 Cáceres, C.E., 1998. Interspecific variation in the abundance, production, and
634      emergence of *Daphnia* diapausing eggs. Ecology 79, 1699-1710.

635 Cáceres, C.E., Tessier, A.J., 2004a. Incidence of Diapause Varies among Populations
636      of *Daphnia pulicaria*. Oecologia 141: 425-431.

637 Cáceres CE, Tessier AJ (2004b) To sink or swim: Variable diapause strategies among
638      Daphnia species Limnol. Oceanogr. 49, 133-1340.

639 Deines, P., Fink, P., 2011. The potential of methanotrophic bacteria to compensate for
640      food quantity or food quality limitations in *Daphnia*. Aquat. Microb. Ecol. 65,
641      197-206.

642 Elvert, M., Pohlman, J.W., Becker, K.W., Gaglioti, B., Hinrichs, K.-U., Wooller,
643      M.J., 2016. Methane turnover and environmental change from Holocene lipid
644      biomarker records in a thermokarst lake in Arctic Alaska. Holocene 26, 1766-
645      1777.

646 Flössner, D., 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas.
647      Backhuys Publishers, Leiden.

648 Fogel, M.L., Cifuentes, L.A., 1993. Isotopic fractionation during primary production,
649      in: Engel, M.H., Macko, S.A. (Eds.), Org. Geochem. Plenum, New York, pp.
650      73-98.

651 France, R.L., 1995. Carbon-13 enrichment in benthic compared to planktonic algae:
652      foodweb implications. Mar. Ecol. Prog. Ser. 124, 307-312.

653 Geller, W., Müller, H., 1981. The filtration apparatus of Cladocera: Filter mesh-sizes
654      and their implications on food selectivity. Oecologia 49, 316-321.

655 Grey, J., 2016. The Incredible Lightness of Being Methane-Fuelled: Stable Isotopes
656      Reveal Alternative Energy Pathways in Aquatic Ecosystems and Beyond.
657      Frontiers in Ecology and Evolution 4, doi: 10.3389/fevo.2016.00008.

658 Griffiths, K., Michelutti, N., Blais, J., Kimpe, L., Smol, J., 2010. Comparing nitrogen
659      isotopic signals between bulk sediments and invertebrate remains in High
660      Arctic seabird-influenced ponds. J. Paleolimnol. 44, 405–412.

661 Heiri, O., Schilder, J., van Hardenbroek, M., 2012. Stable isotopic analysis of fossil
662      chironomids as an approach to environmental reconstruction: State of
663      development and future challenges. Proceedings of the 18th International
664      Symposium on Chironomidae, Trondheim 4-6. August 2011, Fauna Norvegica
665      31, 7-18.

666    Hershey, A.E., Northington, R.M., Hart-Smith, J., Bostick, M., Whalen, S.C., 2015.
667        Methane efflux and oxidation, and use of methane-derived carbon by larval
668        Chironomini, in arctic lake sediments. Limnol. Oceanogr. 60, 276-285.

669    Holgerson, M.A., Raymond, P.A., 2016. Large contribution to inland water $CO_2$ and
670        $CH_4$ emissions from very small ponds. Nature Geoscience 9, 222.

671    Hollander, D.J., Smith, M.A., 2001. Microbially mediated carbon cycling as a control
672        on the $\delta^{13}C$ of sedimentary carbon in eutrophic Lake Mendota (USA): new
673        models for interpreting isotopic excursions in the sedimentary record.
674        Geochim. Cosmochim. Acta 65, 4321-4337.

675    Jürgens, K., 1994. Impact of Daphnia on planktonic microbial food webs - A
676        review  Marine Microbial Food Webs 8, 295-324.

677    Kaminski, M., 1984. Food composition of three bryozoan species (Bryozoa,
678        Phylactolaemata) in a mesotrophic lake. Polish Archive of Hydrobiology 31,
679        45-53.

680    Kamjunke, N., Boing, W., Voigt, H., 1997. Bacterial and primary production under
681        hypertrophic conditions. Aquat. Microb. Ecol. 13, 29-35.

682    Kirilova, E., Cremer, H., Heiri, O., Lotter, A., 2010. Eutrophication of moderately
683        deep Dutch lakes during the past century: flaws in the expectations of water
684        management? Hydrobiologia 637, 157-171.

685    Kleiven, O.T., Larsson, P., Hobæk, A., 1992. Sexual Reproduction in *Daphnia magna*
686        requires three stimuli. Oikos 65, 197-206.

687    Lampert, W., 2006. Daphnia: model herbivore, predator and prey. Polish J. Ecol. 54,
688        607-620.

689    Lampert, W., 2011. *Daphnia*: development of a model organism in ecology and
690        evolution. International Ecology Institute, Oldendorf/Luhe.

691    Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J.A., 2002. Preservation
692        of organic matter and alteration of its carbon and nitrogen isotope composition
693        during simulated and in situ early sedimentary diagenesis. Geochim.
694        Cosmochim. Acta 66, 3573-3584.

695    Lehmann, M.F., Bernasconi, S.M., McKenzie, J.A., Barbieri, A., Simona, M.,
696        Veronesi, M., 2004. Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and
697        dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical
698        cycling in a eutrophic lake. Limnol. Oceanogr. 49, 415-429.

699    McAuliffe, C.D., 1971. GC determination of solutes by multiple phase equilibration.

Chem Technol 1, 46–51.

Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry--an overview of indicators of organic matter sources and diagenesis in lake sediments. Org. Geochem. 20, 867-900.

Morlock, M.A., Schilder, J., van Hardenbroek, M., Szidat, S., Wooller, M.J., Heiri, O., 2017. Seasonality of cladoceran and bryozoan resting stage $\delta^{13}C$ values and implications for their use as palaeolimnological indicators of lacustrine carbon cycle dynamics. J. Paleolimnol. 57, 141-156.

Perga, M.E., Gerdeaux, D., 2006. Seasonal variability in the $\delta^{13}C$ and $\delta^{15}N$ values of the zooplankton taxa in two alpine lakes. Acta Oecol. 30, 69-77.

Perga, M.E., Desmet, M., Enters, D., Reyss, J.-L., 2010. A century of bottom-up and top-down driven changes on a lake planktonic food web: A paleoecological and paleoisotopic study of Lake Annecy, France. Limnol. Oceanogr. 55, 803-816.

Perga, M.E., 2011. Taphonomic and early diagenetic effects on the C and N stable isotope composition of cladoceran remains: implications for paleoecological studies. J. Paleolimnol. 46, 203-213.

R core team, 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rantala, M.V., Luoto, T.P., Weckström, J., Perga, M.-E., Rautio, M., Nevalainen, L., 2015. Climate controls on the Holocene development of a subarctic lake in northern Fennoscandia. Quaternary Sci. Rev. 126, 175-185.

Rinta, P., Bastviken, D., van Hardenbroek, M., Kankaala, P., Leuenberger, M., Schilder, J., Stötter, T., Heiri, O., 2015. An inter-regional assessment of concentrations and $\delta^{13}C$ values of methane and dissolved inorganic carbon in small European lakes. Aquat. Sci. 77, 667-680.

Rinta, P., van Hardenbroek, M., Jones, R.I., Kankaala, P., Rey, F., Szidat, S., Wooller, M.J., Heiri, O., 2016. Land Use Affects Carbon Sources to the Pelagic Food Web in a Small Boreal Lake. PLOS ONE 11, e0159900.

Rinta, P., Bastviken, D., Schilder, J., van Hardenbroek, M., Stötter, T., Heiri, O., 2017. Higher late summer methane emission from central than northern European lakes. J. Limnol. 76, 52-67.

Schilder, J., Tellenbach, C., Möst, M., Spaak, P., van Hardenbroek, M., Wooller, M.J., Heiri, O., 2015a. The stable isotopic composition of *Daphnia* ephippia

734         reflects changes in $\delta^{13}$C and $\delta^{18}$O values of food and water. Biogeosciences
735         12, 3819-3830.

736 Schilder, J., Bastviken, D., van Hardenbroek, M., Leuenberger, M., Rinta, P., Stötter,
737         T., Heiri, O., 2015b. The stable carbon isotopic composition of Daphnia
738         ephippia in small, temperate lakes reflects in-lake methane availability.
739         Limnol. Oceanogr. 60, 1064-1075.

740 Schilder, J., van Hardenbroek, M., Bodelier, P., Kirilova, E.P., Leuenberger, M.,
741         Lotter, A.F., Heiri, O., 2017. Trophic state changes can affect the importance
742         of methane-derived carbon in aquatic food webs. Proc. Roy. Soc. Lond. Ser. B
743         Biol. Sci. 284, 20170278.

744 Ślusarczyk, M., Pietrzak, B., 2008. To sink or float: the fate of dormant offspring is
745         determined by maternal behaviour in Daphnia. Freshwat. Biol. 53, 569-576.

746 Spigel, R.H., Imberger, J., 1987. Mixing processes relevant to phytoplankton
747         dynamics in lakes. N. Z. J. Mar. Freshw. Res. 21, 361–377.

748 Stevenson, F.J., 1982. Extraction, fractionation, and general chemical composition of
749         soil organic matter. In: Stevenson, F.J., ed., Humus Chemistry. Genesis,
750         Composition, Reactions. John Wiley and Sons, New York, pp. 26-54.

751 Stumm, W., Morgan, J.J., 1996. Aquatic chemistry: chemical equilibria and rates in
752         natural waters, 3rd edn. Wiley, New York.

753 Taipale, S., Kankaala, P., Hämäläinen, H., Jones, R.I., 2009. Seasonal shifts in the
754         diet of lake zooplankton revealed by phospholipid fatty acid analysis.
755         Freshwat. Biol. 54, 90-104.

756 Taipale, S., Kankaala, P., Jones, R., 2007. Contributions of different organic carbon
757         sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: results
758         from mesocosm DI13C-additions. Ecosystems 10, 757-772.

759 Taipale, S.J., Brett, M.T., Pulkkinen, K., Kainz, M.J., 2012. The influence of bacteria-
760         dominated diets on Daphnia magna somatic growth, reproduction, and lipid
761         composition. FEMS Microbiol. Ecol. 82, 50-62.

762 Teranes, J.L., Bernasconi, S.M., 2005. Factors controlling δ13C values of sedimentary
763         carbon in hypertrophic Baldeggersee, Switzerland, and implications for
764         interpreting isotope excursions in lake sedimentary records. Limnol.
765         Oceanogr. 50, 914-922.

766     Turney, C.S.M., 1999. Lacustrine Bulk Organic $\delta^{13}$C in the British Isles during the

767     Last Glacial-Holocene Transition (14-9 ka $^{14}$C BP). Arct. Antarct. Alp. Res.

768     31, 71-81.

769     Vanderkerkhove, J., Declerck, S., Vanhove, M., Brendonck, L., Jeppesen, E.,

770     Porcuna, J.M., De Meester, L., 2004. Use of ephippial morphology to assess

771     richness of anomopods: potentials and pitfalls. J. Limnol. 63, 75-84.

772     van Hardenbroek, M., Lotter, A.F., Bastviken, D., Andersen, T.J., Heiri, O., 2014.

773     Taxon-specific $\delta^{13}$C analysis of chitinous invertebrate remains in sediments

774     from Strandsjön, Sweden. J. Paleolimnol. 52, 95-105.

775     van Hardenbroek, M., Heiri, O., Parmentier, F.J.W., Bastviken, D., Ilyashuk, B.P.,

776     Wiklund, J.A., Hall, R.I., Lotter, A.F., 2013. Evidence for past variations in

777     methane availability in a Siberian thermokarst lake based on $\delta^{13}$C of chitinous

778     invertebrate remains. Quaternary Sci. Rev. 66, 74-84.

779     van Hardenbroek, M., Leuenberger, M., Hartikainen, H., Okamura, B., Heiri, O.,

780     2016. Bryozoan stable carbon and hydrogen isotopes: relationships between

781     the isotopic composition of zooids, statoblasts and lake water. Hydrobiologia

782     765, 209-223.

783     van Riel, M.C., Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F., Vaate, A.b.,

784     2006. Trophic relationships in the Rhine food web during invasion and after

785     establishment of the Ponto-Caspian invader Dikerogammarus villosus, in:

786     Leuven, R.S.E.W., Ragas, A.M.J., Smits, A.J.M., Velde, G. (Eds.), Living

787     Rivers: Trends and Challenges in Science and Management. Springer

788     Netherlands, pp. 39-58.

789     Verbruggen, F., Heiri, O., Reichart, G.J., Lotter, A.F., 2010. Chironomid $\delta^{18}$O as a

790     proxy for past lake water $\delta^{18}$O: a Lateglacial record from Rotsee

791     (Switzerland). Quaternary Sci. Rev. 29, 2271-2279.

792     Vuorio, K., Meili, M., Sarvala, J., 2006. Taxon-specific variation in the stable isotopic

793     signatures ($\delta^{13}$C and $\delta^{15}$N) of lake phytoplankton. Freshwat. Biol. 51, 807-822.

794     Wang, Y., Francis, D., O'Brien, D., Wooller, M., 2008. A protocol for preparing

795     subfossil chironomid head capsules (Diptera: Chironomidae) for stable isotope

796     analysis in paleoclimate reconstruction and considerations of contamination

797     sources. J. Paleolimnol. 40, 771-781.

798    West, W.E., Coloso, J.J., Jones, S.E., 2012. Effects of algal and terrestrial carbon on

799          methane production rates and methanogen community structure in a temperate

800          lake sediment. Freshwat. Biol. 57, 949-955.

801    Wood, T.S., Okamura, B., 2005. A new key to freshwater bryozoans of Britain,

802          Ireland and continental Europe, with notes on their ecology. Freshwater
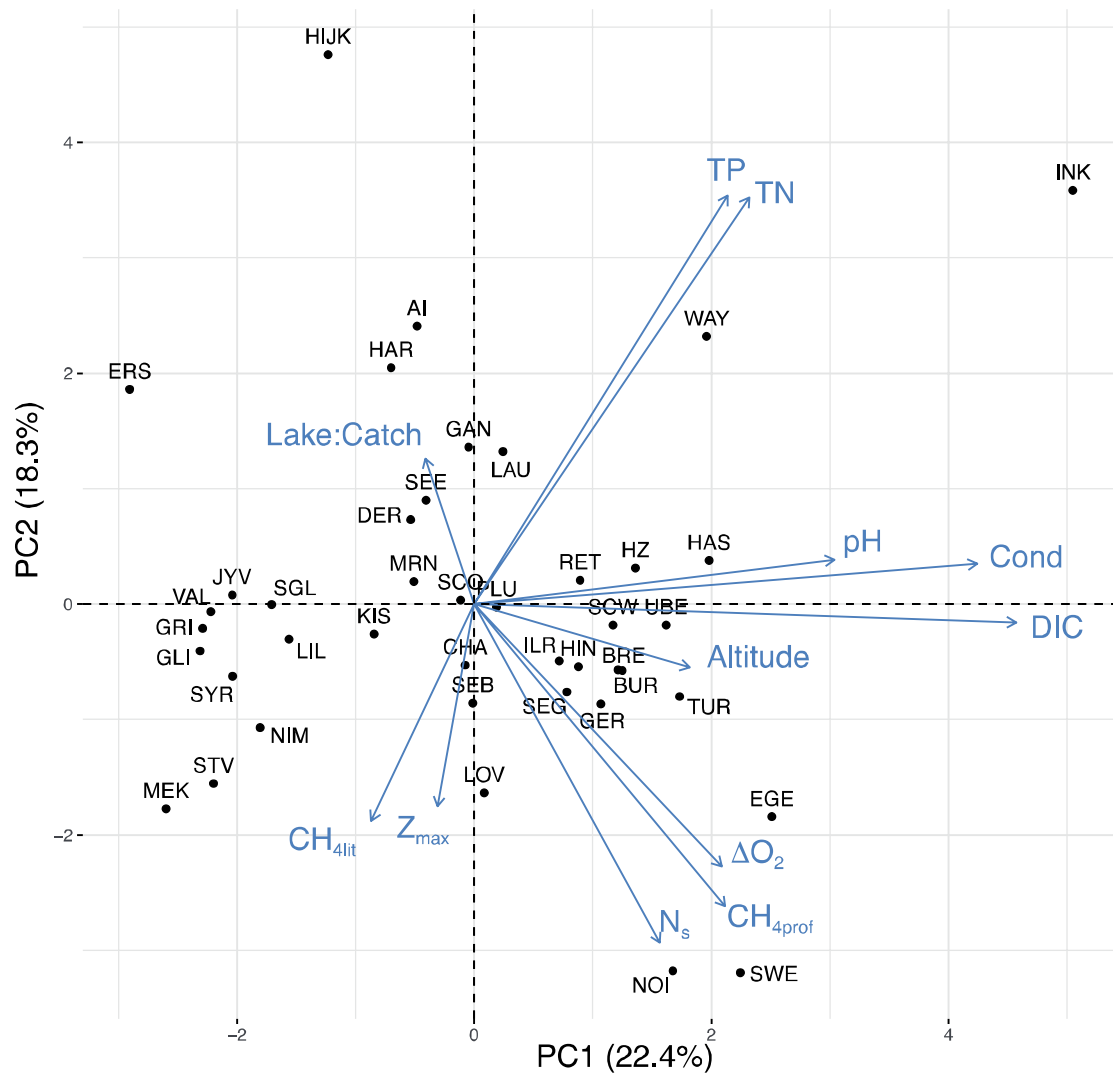
803          Biological Association, London.

804    Wooller, M., Pohlman, J., Gaglioti, B., Langdon, P., Jones, M., Walter Anthony, K.,

805          Becker, K., Hinrichs, K.-U., Elvert, M., 2012. Reconstruction of past methane

806          availability in an Arctic Alaska wetland indicates climate influenced methane

807          release during the past ~12,000 years. J. Paleolimnol. 48, 27-42.

808

809

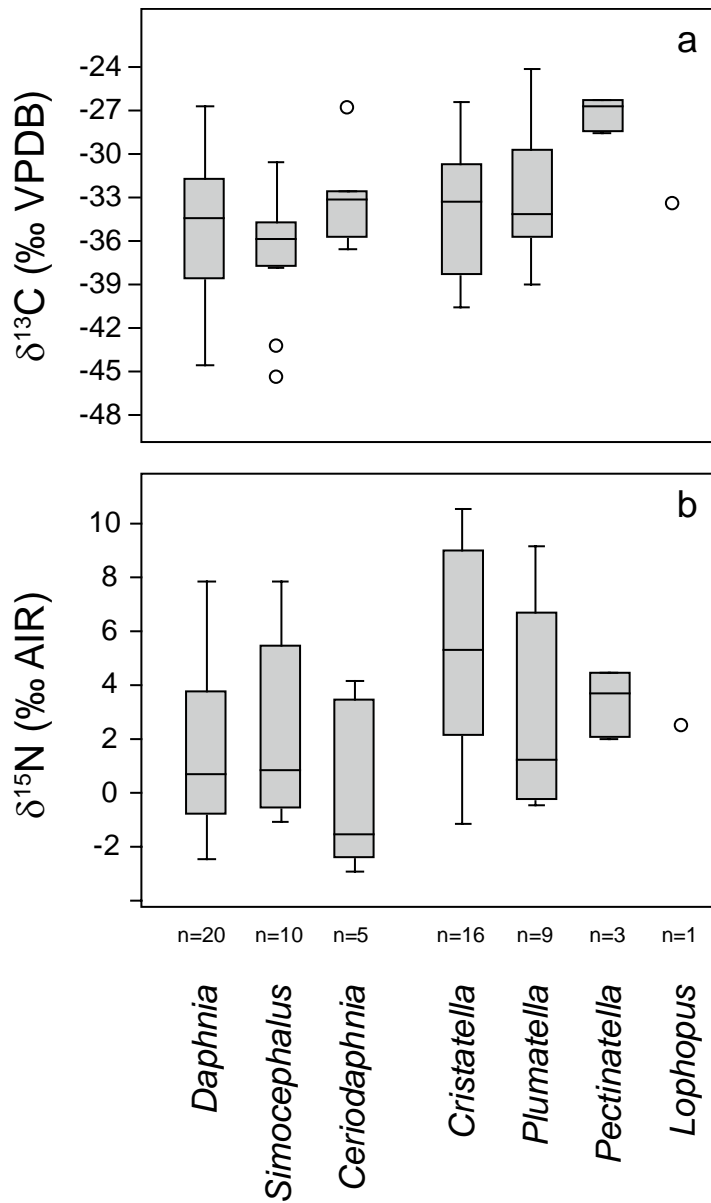810 **Figures**

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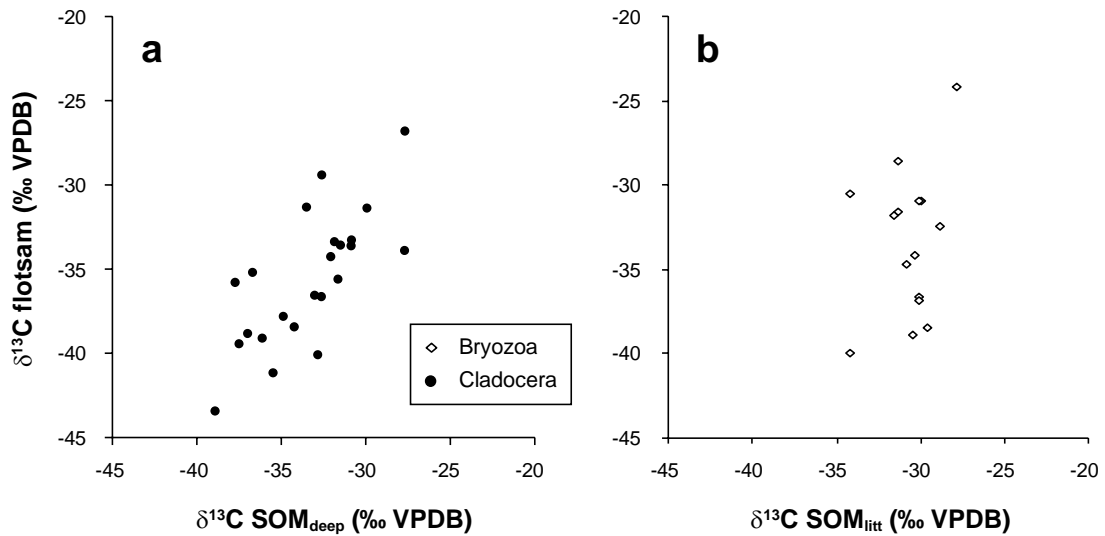**Fig. 2** Boxplot with $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values for the different taxa collected in flotsam samples; n indicates the number of lakes for which stable isotopes were analysed for the respective taxon.

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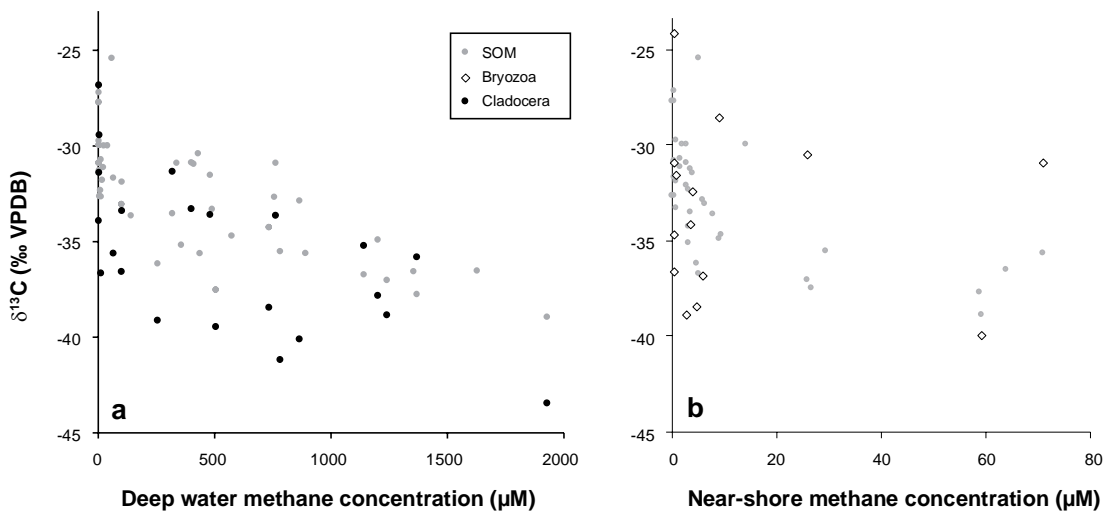
**Fig. 3** Average $\delta^{13}C$ values of Cladocera ephippia plotted against $\delta^{13}C$ of SOM in the

deep water zone (SOM$_{deep}$) (a) and average $\delta^{13}C$ values Bryozoa statoblasts plotted

against $\delta^{13}C$ of SOM in the near-shore zone (SOM$_{litt}$) (b).
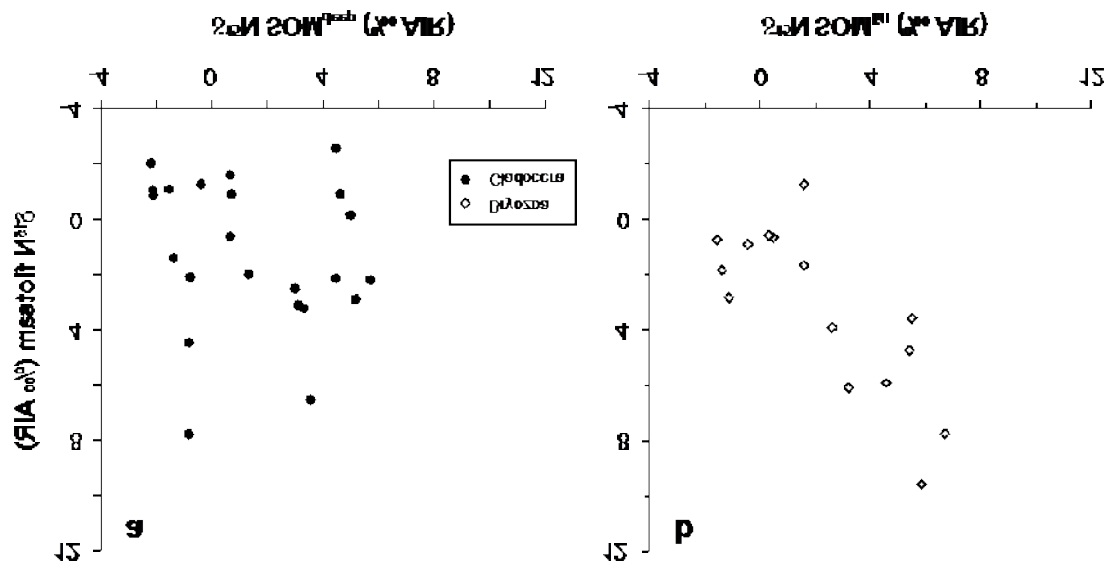
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**Fig. 4** Average $\delta^{13}C$ values of Cladocera and deep water SOM plotted against deep

water methane concentrations 10 cm above the sediments (a), and average $\delta^{13}C$ of

Bryozoa and near-shore SOM plotted against near-shore methane concentrations

measured 10 cm above the sediments.

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

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