1 **Seasonality of cladoceran and bryozoan resting stage δ^{13}C values**

2 **and implications for their use as palaeolimnological indicators of**

3 **lacustrine carbon cycle dynamics**

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17 **Key words** Methane, *Daphnia*, Stable carbon isotopes, Invertebrates, Bryozoa, Cladocera

18

19   **Abstract**

20

21   The stable carbon isotope composition, expressed as $\delta^{13}C$ values, of chitinous resting

22   stages of planktivorous invertebrates can provide information on past changes in carbon

23   cycling in lakes. For example, the $\delta^{13}C$ values of cladoceran ephippia and bryozoan

24   statoblasts have been used to estimate the past contribution of methane-derived carbon to

25   lake food webs and variations in the $\delta^{13}C$ value of planktonic algae. Limited information,

26   however, is available concerning seasonal variations in $\delta^{13}C$ values of these organisms and

27   their resting stages. We measured the seasonal variation in $\delta^{13}C$ values of *Daphnia*

28   (Branchiopoda: Cladocera: Daphniidae) and their floating ephippia over a 2-year period in

29   small, dimictic Lake Gerzensee, Switzerland. Floating ephippia of *Ceriodaphnia*

30   (Branchiopoda: Cladocera: Daphniidae) and statoblasts of *Plumatella* (Phylactolaemata:

31   Plumatellida: Plumatellidae) were analysed during parts of this period. Furthermore, $\delta^{13}C$

32   values of remains from all three organism groups were analysed in a 62-cm-long sediment

33   core. Throughout the year, *Daphnia* $\delta^{13}C$ values tracked the $\delta^{13}C$ values of particulate

34   organic matter (POM), but were more negative than POM, indicating that *Daphnia* also

35   utilize a relatively ^{13}C-depleted carbon source. *Daphnia* ephippia $\delta^{13}C$ values did not show

36   any pronounced seasonal variation, suggesting that they are produced batch-wise in autumn

37   and/or spring and float for several months. In contrast, $\delta^{13}C$ values of *Ceriodaphnia* ephippia

38   and *Plumatella* statoblasts followed variations in $\delta^{13}C_{POM}$ values, *Ceriodaphnia* values being

39   the most negative of the resting stages. Average cladoceran ephippia $\delta^{13}C$ values in the

40   flotsam agreed well with ephippia values from Gerzensee surface sediments. In contrast,

41   average *Plumatella* statoblast $\delta^{13}C$ values from the flotsam were 4 ‰ more negative than in

42   the surface sediments. In the sediment core, $\delta^{13}C$ values of the two cladocerans remained

43   low (mean -39.0 ‰ and -41.9 ‰) throughout the record. In contrast, *Plumatella* had distinctly

44   less negative $\delta^{13}C$ values (mean -32.0 ‰). Our results indicate that in Gerzensee, *Daphnia*

45   and *Ceriodaphnia* strongly relied on a ^{13}C-depleted food source throughout the past 150

46   years, most likely methane-oxidising bacteria, whereas this food source was not a major

47   contribution to the diet of bryozoans.

Introduction

Chitinous remains of aquatic invertebrates are readily preserved in lake sediments and their stable carbon isotope compositions, expressed as $\delta^{13}C$ values, have been used as a proxy to study past changes in the availability and importance of different carbon pathways in lakes (Frossard et al. 2014; Rinta et al. 2016; van Hardenbroek et al. 2010; Wooller et al. 2012). Two such pathways are the fixation of dissolved CO_2 by algae and the recycling of carbon from methane (CH_4) by methane-oxidising bacteria (MOB). Up to now, palaeolimnological studies that analysed the $\delta^{13}C$ values of chitinous invertebrate remains have focused mainly on benthic invertebrates, most prominently chironomid larvae (Insecta: Diptera: Chironomidae), whose chitinous head capsules are regularly found in sediments (Belle et al. 2014; Heiri et al. 2012; van Hardenbroek et al. 2010). Additionally, exoskeleton fragments of planktonic invertebrates such as *Bosmina* (Branchiopoda: Cladocera: Bosminidae) have been analysed for their $\delta^{13}C$ values (Perga 2009, 2011). It has recently been suggested that similar information may be gained from $\delta^{13}C$ analysis of resting stages of planktivorous invertebrates (Schilder et al. 2015a, 2015b; van Hardenbroek et al. 2013, 2014; Wooller et al. 2012). Examples of these resting stages are ephippia, produced by *Daphnia* (Branchiopoda: Cladocera: Daphniidae) and other planktonic cladocerans, and statoblasts, produced by bryozoans that form sessile colonies on hard substrates in the shallow parts of lakes (Wood and Okamura 2005). Ephippia and statoblasts are abundant in lake sediments (Francis 2001; Korhola and Rautio 2001), providing a potential archive for palaeoecological reconstructions.

In modern lake food web studies, the analysis of the $\delta^{13}C$ value of different food components is a widely used technique to differentiate between carbon sources (Bunn and Boon 1993). The main organic carbon sources for filter-feeding zooplankton are algae, detritus, and heterotrophic bacteria (Edmondson 1957; Lampert 2011). Using characteristic isotopic signatures of these different sources, carbon flow can be traced through a lake's

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75   food web (Fry 2006; Taipale et al. 2007). Fundamental for such studies is that the carbon

76   isotopic composition of an organism closely reflects the isotopic signature of its diet (DeNiro

77   and Epstein 1978; Peterson and Fry 1987). For *Daphnia* and their ephippia, Perga (2011)

78   and Schilder et al. (2015b) showed that ephippia $\delta^{13}C$ values closely reflect the $\delta^{13}C$ values

79   of the living *Daphnia* at the time of ephippia production. Van Hardenbroek et al. (2016)

80   recently demonstrated that the $\delta^{13}C$ values of bryozoan statoblasts are related to the $\delta^{13}C$

81   values of the colonies that produced them. More work is required, however, to show that

82    $\delta^{13}C$ values of *Daphnia* ephippia and bryozoan statoblasts reflect the diet of the parent

83   organisms during or shortly before resting stage formation.

84   Understanding the extent to which lakes change their capacity to sequester or

85   release carbon in response to global warming and eutrophication is a key issue for

86   palaeoecologists today (Seddon et al. 2014). New insights into lake carbon cycling can be

87   gained from analysing $\delta^{13}C$ values of aquatic invertebrate remains. Recently, it was

88   suggested that carbon from CH_4 may be an important additional transfer pathway of carbon

89   in lake food webs, indicated by remarkably low $\delta^{13}C$ values of aquatic invertebrates when

90   they incorporate CH_4-derived, ^{13}C-depleted carbon (Bastviken et al. 2003; Bunn and Boon

91   1993; Grey et al. 2016). The $\delta^{13}C$ values of biogenic CH_4 in small European lakes range

92   between -86 and -61 ‰ (Rinta et al. 2015), which is markedly depleted in ^{13}C relative to

93   algae with $\delta^{13}C$ values that typically fall in the range from -35 to -25 ‰ (Jones et al. 1999;

94   Peterson and Fry 1987). The large difference in $\delta^{13}C$ values thus allows for differentiation

95   between photosynthetically produced organic matter and MOB as potential carbon sources

96   for primary consumers in lakes. Significant relationships have been observed between $\delta^{13}C$

97   of *Daphnia* ephippia in surface sediments and diffusive CH_4 flux (Van Hardenbroek et al.

98   2013) and within-lake CH_4 concentrations (Schilder et al. 2015a). Cladoceran remains may

99   thus record changes in past diffusive CH_4 flux in lakes, though they do not provide direct

100   information on other forms of CH_4 fluxes such as ebullition or plant-mediated transport.

101   Palaeoecological studies using $\delta^{13}C$ values of chitinous remains have largely

102   overlooked the use of bryozoan statoblasts, which have been suggested as a resource for

103 reconstructing δ¹³C values of primary production (Turney 1999; van Hardenbroek et al. 2014,

104 2016). As filter feeders, Bryozoa rely on algae, particulate organic matter (POM) and

105 associated microorganisms (e.g. bacteria) as their main food source (Kaminsky 1984).

106 Bryozoa are mainly bound to their substrate and do not have access to food sources from

107 deeper water layers and are therefore not, or only to a small degree, influenced by CH_4-

108 derived carbon (van Hardenbroek et al. 2016). By combining δ¹³C measurements of mobile

109 filter feeders like *Daphnia* with sessile filter feeders like *Plumatella* (Phylactolaemata:

110 Plumatellida: Plumatellidae), it is possible to separate changes in the importance of CH_4-

111 derived carbon from changes in algal δ¹³C values, or more generally, changes in lake

112 productivity (van Hardenbroek et al. 2014; Rinta et al. 2016). At present, however, no

113 information is available about seasonal changes in δ¹³C values of cladoceran and bryozoan

114 resting stages or about the extent to which this seasonality influences the δ¹³C values of

115 statoblast and ephippia remains in lake sediments. Furthermore, it is unknown whether the

116 δ¹³C values of deposited organism remains reflect integrated δ¹³C values for statoblasts and

117 ephippia that float on the lake surface, or whether the sedimentary assemblages are

118 characterized by systematically higher or lower values. Such differences could appear as a

119 consequence of degradation, transport processes, or production of resting stages in cryptic

120 habitats that do not contribute to floating resting stages at the lake surface (e.g. from deeper

121 littoral, lower epilimnetic or profundal habitats). These uncertainties currently hinder the

122 development of δ¹³C analysis of invertebrate resting stages as a proxy for reconstructing

123 past variations in carbon cycling in lakes.

124   The main aim of this study was to assess the influence of seasonality on δ¹³C values

125 of zooplankton remains and evaluate their potential use as palaeoecological indicators for

126 lacustrine carbon cycle dynamics. This study provides a first assessment of seasonal

127 variations in δ¹³C values of cladoceran and bryozoan resting stages in a small temperate

128 European lake, with respect to physical, chemical and biological variables. In particular, the

129 focus was on assessing seasonal variations in transfer pathways of carbon in the lake, and

130 their influence on the δ¹³C values of organisms in the water column, as well as their

131   chitinous resting stages (i.e. planktonic Cladocera and their ephippia, bryozoan statoblasts).

132   In a field campaign, $\delta^{13}C$ values of *Daphnia* and their ephippia were analysed over a two-

133   year period, and $\delta^{13}C$ values of floating resting stages of *Ceriodaphnia* (Branchiopoda:

134   Cladocera: Daphniidae) and *Plumatella* were analysed when present during this period. In a

135   second step, we investigated the implications of seasonal changes for interpretation of $\delta^{13}C$

136   values in fossil invertebrate remains. To that end, ephippia of the cladocerans *Daphnia* and

137   *Ceriodaphnia*, and statoblasts of the bryozoan *Plumatella* were analysed in the lake surface

138   sediment and in a short sediment core covering roughly the past 150 years. Our study was

139   conducted on Lake Gerzensee (7°33'E, 46°50'N, 606 m a.s.l.), a small temperate lake in the

140   foreland of the Swiss Alps, about 20 km south of Bern (Fig. 1). The lake is characterised by

141   exceptionally high lake water CH_4 concentrations (Rinta et al. 2015; Schilder et al. 2016).

142   Lake Gerzensee has a surface area of 0.27 km^2, a total water volume of 0.16 km^3, and a

143   maximum depth of 10 m. The catchment area (2.6 km^2) consists of 80 % agricultural land,

144   5 % wooded land, and 15 % urban areas (Lotter et al. 2000). The mean annual temperature

145   is 8.8 °C (Bern Zollikofen 1981-2010, Bundesamt für Meteorologie und Klimatologie

146   MeteoSchweiz 2014). Today, Lake Gerzensee is eutrophic, with summer anoxia in the

147   hypolimnion (Zeh et al. 2004).

148

149   **Materials and methods**

150

151   Lake Gerzensee was sampled on 15 one-to-three-day visits between October 2012 and July

152   2014, throughout all seasons (Electronic Supplementary Material [ESM] Table S1). In the

153   first period, between October 2012 and September 2013, measurements were taken every

154   other month (6 visits). Based on the first year of data, the fieldwork protocol was adjusted to

155   gain more detailed information about deeper-water POM and chlorophyll *a* concentrations

156   (ESM Table S1). In the second year, intervals between field visits were reduced to two

157   weeks from September 2013 to December 2013, to cover the destratification period (4 visits),

158   and sampling continued with 2-4 week intervals after the lake ice thawed in February 2014,

159   until July 2014 (5 visits). Data for March and July 2014 were collected along with other

160   fieldwork activities, using a shortened protocol (ESM Table S1).

161

162   Sample collection

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164   Vertical profiles of temperature and dissolved oxygen concentration ($[O_2]_{aq}$) were measured

165   for every metre in the water column at the lake centre (location C1, Fig. 1), using a multi-

166   sensor probe (WTW CellOx$^©$ 325 oxi1970i, Germany). *Daphnia* individuals were collected

167   from the oxic part of the water column at the lake centre (location C1) in multiple vertical

168   hauls, with a 40-µm mesh plankton net. Flotsam was collected from the lake surface with a

169   hand net (mesh size ~120 µm). Water samples were collected at C1 in 0.7 m and 8 m water

170   depth (top epilimnion and bottom hypolimnion, respectively), using a 5-L water sampler

171   (UWITEC, Austria). For these water samples, pH was measured (Waterproof pHTestr 20,

172   Oakton, USA), and 60 ml of water was injected with a syringe into a 118-ml glass vial

173   through a 10-mm-thick butyl rubber stopper (Apodan, Denmark) to determine the abundance

174   and $δ^{13}C$ value of the dissolved inorganic carbon (DIC). The vials were prepared beforehand

175   with 200 µl of H_3PO_4 (85 %), closed, and repeatedly vacuumed and flushed with N_2 to

176   ensure that no CO_2 remained in the vials (Rinta et al. 2015; Schilder et al. 2015a). Samples

177   for the $δ^{13}C$ analysis of POM were collected from the lake water at C1 (0.7 m water depth)

178   by passing the water through a 250-µm sieve before manually pushing water through a glass

179   fibre filter (Whatman GF/C 25 mm, pore size 1.2 µm) with a syringe (water volume recorded

180   in the field). A second sample of POM was obtained in a similar fashion, immediately put into

181   90 % undenatured ethanol, and covered with aluminium foil for chlorophyll *a* analysis.

182   Immediately after returning from fieldwork, POM filters designated for chlorophyll *a* analysis

183   were heated to 70 °C in 90 % undenatured ethanol for 10 min, put in an ultrasound bath for

184   5 min, and stored in a refrigerator for at least 48 hours. The samples were then filtered

185   through a membrane filter to remove suspended particles. Chlorophyll *a* measurements

186   were based on absorbance at characteristic wavelengths (Schwoerbel 1994). Samples were

187   injected into glass cuvettes (1 cm light path) and absorbance at wavelengths (λ) 665 nm and

188   750 nm was measured with a spectrophotometer (Jenway, UK). Chlorophyll *a* content in the

189   lake water was calculated following EAWAG (1995).

190

191   Isotope analysis

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193   *δ^{13}C analysis of DIC and POM*

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195   The concentration of CH_4 and CO_2 in the headspace of the DIC samples was measured by

196   gas chromatography with a flame ionisation detector and methanizer (GC-FID; Shimadzu

197   GC8, PoropackN column, see Rinta et al. 2015 for details). Lake water DIC concentrations

198   and dissolved CH_4 concentrations ($[CH_4]_{aq}$) were back-calculated from measured headspace

199   CO_2 and CH_4 concentrations. Dissolved CO_2 concentrations ($[CO_2]_{aq}$) (as the sum of

200   dissolved CO_2 and H_2CO_3) were calculated following Stumm and Morgan (1996), accounting

201   for lake water pH, temperature, and DIC concentrations.

202   Filters containing lake water POM were freeze-dried and a maximum amount of filter

203   material was separated from the seston. The seston was then transferred into ultra-clean tin

204   cups. For δ^{13}C analysis of DIC, 15-20 ml of gas from the headspace of the vial was allowed

205   to escape into a 60-ml syringe. The gas was then injected into a pre-vacuumed 12-ml glass

206   vial such that slight overpressure was applied. The procedure was repeated for δ$^{13}CH_4$

207   measurements. Isotope samples of CO_2, CH_4, and POM were analysed at the Stable

208   Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL Cube

209   or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 isotope ratio mass

210   spectrometer (IRMS). Analytical uncertainties for gas sample δ^{13}C values were < 0.1 ‰ (one

211   standard deviation) for CO_2 for two to three replicate measurements of three laboratory

212   standards (δ^{13}C = -40.73 ‰, -10.39 ‰, and -3.59 ‰) and < 0.1 ‰ (one standard deviation)

213   for CH_4 for replicate measurements of a laboratory standard (δ^{13}C = -36.7 ‰, n = 6). Results

214   are reported in conventional δ-notation relative to the international standard Vienna PeeDee

215   Belemnite (V-PDB).

216

217   *δ¹³C analysis of zooplankton*

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219   Living *Daphnia* were kept in approximately 1 L of unfiltered lake water for 1-2 days. They

220   were then separated from other organisms under a dissecting microscope (magnification

221   20x–50x), and were frozen in demineralised water. Samples were freeze-dried and weighed

222   into ultra clean tin cups (Lüdi Swiss AG, Switzerland). Because *Daphnia* ephippia cannot be

223   identified to species level under the microscope, the species of living *Daphnia* was not

224   determined for this analysis. Flotsam was sieved at 100 μm and examined under a

225   dissecting microscope. Ephippia of the planktonic cladocerans *Daphnia* and *Ceriodaphnia*,

226   and statoblasts of the bryozoan *Plumatella* were the only invertebrate resting stages found

227   regularly and abundantly enough for δ^{13}C analysis. These resting stages were identified

228   according to Vandekerkhove et al. (2004) (Cladocera) and Wood and Okamura (2005)

229   (Bryozoa), exposed to 10 % potassium hydroxide (KOH) for 2 hours (van Hardenbroek et al.

230   2010), rinsed 5-10 times with demineralised water, and picked into ultra-clean tin cups.

231   Isotope samples of *Daphnia* as well as ephippia and statoblasts were also analysed at the

232   Stable Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL

233   Cube or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 IRMS. Sample

234   sizes were in the range of 120-250 individuals for *Daphnia* (150-500 μg), 150-200 for

235   *Daphnia* ephippia (150-250 μg), 200-300 for *Plumatella* statoblasts (150-300 μg), and 450-

236   600 (150-200 μg) for *Ceriodaphnia* ephippia. Analytical uncertainties for invertebrate δ^{13}C

237   measurements were ≤ 0.6 ‰ (one standard deviation) for replicate measurements (n = 3-36)

238   of five laboratory standards (Bovine Liver (δ^{13}C = -21.7 ‰), USGS-41 Glutamic Acid (δ^{13}C =

239   37.6 ‰), Nylon 5 (δ^{13}C = -27.7 ‰), Peach Leaves (δ^{13}C = -26.1 ‰), Glutamic Acid (δ^{13}C = -

240   28.9 ‰)).

241

242   Sediment analysis

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Sediment coring and chronology

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In October 2012, a 62-cm-long sediment core (GER12) was recovered from the centre of Lake Gerzensee (9.5 m water depth) using a gravity corer (UWITEC, Austria). Upon arrival in the laboratory, core GER12 was sampled at 1-cm intervals and freeze-dried until further analysis. For ^{210}Pb and ^{137}Cs dating, a total of 15 freeze-dried samples from the upper 48 cm of the core were analysed using gamma spectrometry at the Department of Chemistry and Biochemistry at the University of Bern, Switzerland. ^{210}Pb (46.5 keV), ^{241}Am (59.5 keV), ^{226}Ra progenies (351.9 and 609.3 keV), and ^{137}Cs (661.7 keV) were measured using a Broad Energy Germanium (BEGe) Canberra detector with low background and high absolute full-energy peak efficiencies for close on-top geometries of > 20 % and ~ 5 % for ^{210}Pb and ^{137}Cs, respectively.

256

δ^{13}C analysis of sedimentary invertebrate remains

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For invertebrate δ^{13}C analysis, core GER12 was sampled every fourth centimetre. When sample mass of the invertebrate remains was not sufficient for δ^{13}C analysis, the lower adjacent centimetre was added to the sample. Nonetheless, some samples in the lower half of the core had to be pooled with the next regular-interval sample to obtain a sufficient number of remains for analysis. Hence, invertebrate δ^{13}C values represent remains from up to 6 cm of sediment. For each sample, 50 % by weight of the freeze-dried material was deflocculated in 10 % KOH for 2 hours and sieved at 100 μm (van Hardenbroek et al. 2010). *Daphnia* ephippia, *Ceriodaphnia* ephippia, and *Plumatella* statoblasts were identified according to Vandekerkhove et al. (2004) for Cladocera and Wood and Okamura (2005) for Bryozoa and separated from the sediment. Remains were treated with 2M NH$_4$Cl solution buffered with 0.35 NaOH for 20 hours to remove carbonates (Verbruggen et al. 2010), and picked into pre-weighed silver cups (6 x 4 mm; Säntis, Switzerland). All fossil invertebrates

271   were analysed at the Alaska Stable Isotope Facility of the University of Alaska, Fairbanks,

272   on a Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo

273   Delta V IRMS. Analytical uncertainties were < 0.1 ‰ for replicate measurements of a

274   laboratory standard (peptone, $\delta^{13}C$ = -15.8 ‰, n = 24). Sample sizes were in the range of

275   20-55 remains for *Daphnia* ephippia and *Plumatella* statoblasts, and 100-200 for

276   *Ceriodaphnia* ephippia. In addition to the sediment core, material from a sediment trap

277   located close to the coring site at the centre of Gerzensee during 2012/13 was available

278   from another study (C. Adolf, unpublished). The trap was placed 3 m above the lake floor

279   and remained in the lake for 12 months. The material was processed in the same way as the

280   sediment core, and one sample for *Ceriodaphnia* ephippia was analysed for its $\delta^{13}C$ value.

281

282   Statistical analysis

283

284   Statistical analyses on material collected alive and as floatsam were performed in R (R

285   Development Core Team 2008). For Pearson correlation tests, normality of the variables

286   was tested prior to analysis using the Shapiro-Wilk test. When a normal distribution of the

287   data was rejected, Spearman's rank correlation was used to test for correlations. The

288   average value reported for fieldwork parameters was calculated as a weighted mean of all

289   samples collected during the campaign and each sample was weighted by the time interval

290   for which it is most representative.

291

292   **Results**

293

294   Physical and chemical lake conditions

295

296   All variables showed characteristic seasonal variations throughout the two years of

297   monitoring. Measured surface water temperatures ranged between 4.6 °C in December

298   2013 and 25.8 °C in July 2013, with a mean of 12.4 °C (Fig. 2a). The bottom water (8 m

299   depth) was anoxic ($[O_2]_{aq}$ < 1 mg L^{-1}) from June to November (Fig. 2b). Chlorophyll *a* values

300   ranged from 15.2 µg chl *a* L^{-1} in autumn 2013 to 7.2 µg chl *a* L^{-1} in February 2014, but only

301   one measurement was taken during summer (July 2014). Deep-water chlorophyll *a*

302   measured at 7 m depth in May and July 2014 was comparable (July, 14.7 µg chl *a* L^{-1}) or

303   higher (May, 14.2 µg chl *a* L^{-1}) than surface water measurements (12.6 and 8.6 µg chl *a* L^{-1},

304   respectively). $[CO_2]_{aq}$ in the epilimnion ranged from peak values just after autumn mixing and

305   in spring (96.7-192.8 µM) to 9.6 µM in September (ESM Fig. S1). $[CH_4]_{aq}$ in the water

306   column reached high values (1082 µM) in the hypolimnion during summer stratification.

307   Surface water $[CH_4]_{aq}$ was highest in November during autumn turnover (49.8 µM, ESM Fig.

308   S1).

309

310   Stable carbon isotopes

311

312   With the exception of *Daphnia* ephippia, all measured variables showed seasonal variation

313   in $\delta^{13}C$ values, with the lowest values right after autumn lake mixing and throughout winter,

314   and the highest values in summer (Fig. 3a).

315   The isotopic composition of $[CO_2]_{aq}$ ($\delta^{13}C_{CO2}$) for location C1 at the lake surface was

316   most ^{13}C-depleted in February and March ($\delta^{13}C_{CO2}$ ~-21 ‰), whereas the highest $\delta^{13}C_{CO2}$

317   value of -12.1 ‰ was recorded in July 2013 (Fig. 3a). The seasonal cycle in $\delta^{13}C_{CO2}$ values

318   was less pronounced in the hypolimnion (data not shown). During summer, a vertical

319   gradient in $\delta^{13}C_{CO2}$ values developed between the epilimnion and the hypolimnion, which

320   was eliminated at lake mixing in autumn. At the lake centre, the $\delta^{13}C$ value of surface water

321   POM ($\delta^{13}C_{POM}$) was highest during summer (-28.5 ‰), and reached its minimum in March

322   2013 (-41.6 ‰, mean = -34.7 ‰; Fig. 3a). Bottom water $\delta^{13}C_{POM}$, measured at 7 m depth,

323   was in the range of surface water $\delta^{13}C_{POM}$ for measurements in April and May 2014 (ESM

324   Fig. S2d). However, during summer stratification in July 2014, bottom water $\delta^{13}C_{POM}$ values

325   were distinctly more negative than surface water $\delta^{13}C_{POM}$ (-38.0 ‰ and -30.0 ‰,

326   respectively). Surface water $\delta^{13}C_{POM}$ was on average 17.0 ‰ (range 13.2 to 21.0 ‰) more

327   negative than surface water $\delta^{13}C_{CO2}$, with the largest differences measured in March 2013

328   and May 2014 (Fig. 3a).

329   *Daphnia* $\delta^{13}C$ values ($\delta^{13}C_{Daph}$) in the lake centre were on average 3.4 ‰ more

330   negative than $\delta^{13}C_{POM}$ values (Fig. 3a). The average $\delta^{13}C_{Daph}$ value was -39.4 ‰. Values

331   ranged from -44.2 ‰ in early spring to -29.8 ‰ in summer. $\delta^{13}C_{Daph}$ values were positively

332   correlated with chlorophyll *a* concentrations in surface water POM (Pearson correlation r =

333   0.86, p < 0.05, n = 6; ESM Fig. S2a), and $[CH_4]_{aq}$ in bottom waters (log-transformed,

334   Pearson correlation r = 0.86, p < 0.01, n = 13; ESM Fig. S2b). Moreover, bottom water

335    $[CH_4]_{aq}$ showed a negative correlation with the offset between $\delta^{13}C_{POM}$ and $\delta^{13}C_{Daph}$ ($\Delta^{13}C_{POM-}$

336    $_{Daph}$) (Spearman's rank correlation r = 0.67, p < 0.05, n = 13; ESM Fig. S2c).

337   In contrast to $\delta^{13}C_{Daph}$ values, the $\delta^{13}C$ values of floating *Daphnia* ephippia ($\delta^{13}C_{DaphFlot}$)

338   did not show seasonal variations, but instead remained relatively constant around -39.5 ‰

339   (range -41.7 to -38.8 ‰, Fig. 3a and b). Interestingly, floating *Ceriodaphnia* ephippia $\delta^{13}C$

340   values ($\delta^{13}C_{CerioFlot}$) showed changes over time, with the lowest $\delta^{13}C$ values down to -50.1 ‰

341   in winter (Fig. 3b). On average, $\delta^{13}C_{CerioFlot}$ was more ^{13}C-depleted (mean = -42.9 ‰, range -

342   50.1 to -34.3 ‰) than $\delta^{13}C_{DaphFlot}$, whereas *Plumatella* statoblast $\delta^{13}C$ ($\delta^{13}C_{PluFlot}$) was least

343   ^{13}C-depleted (mean = -36.3 ‰, range -40.0 to -34.6 ‰). In-lake abundance of the floating

344   remains could not be assessed by the applied sampling technique, and no structural

345   degradation of the remains was visible under the microscope on any of the fieldwork dates.

346   *Ceriodaphnia* ephippia from the sediment trap had a $\delta^{13}C$ value of -42.4 ‰.

347

348   Sediment core and chronology

349

350   For core GER12, activity of unsupported ^{210}Pb was transformed into an age-depth model for

351   the upper 48 cm using the constant rate of supply (CRS) model (Appleby 2001; Appleby and

352   Oldfield 1978) (Fig. 4). Activity measurements of ^{137}Cs showed a distinct peak at 28.5 cm

353   and a second smaller peak at 20.5 cm. Many European lakes show two maxima in ^{137}Cs

354   activity, which are associated with aboveground nuclear bomb tests in AD 1963 and the

355   Chernobyl reactor accident in AD 1986 (Appleby 2001). For Swiss lakes, the latter peak is

356   usually greater than the first (Albrecht et al. 1998; Lotter et al. 1997). This was not the case

357   in Gerzensee sediments where only one measurement point defined the anticipated

358   Chernobyl peak. The Chernobyl peak may thus not be fully revealed in the record because

359   of the relatively low sampling resolution, i.e. every 4 cm. Therefore the ^{210}Pb model was not

360   modified to fit the ^{137}Cs profile. The lower peak in ^{137}Cs activity, expected to coincide with the

361   nuclear bomb peak in AD 1963, was confirmed by ^{241}Am and agrees well with the ^{210}Pb

362   chronology (Fig. 4).

363   The abundance of invertebrate remains in the sediments of Gerzensee allowed for the

364   analysis of eight samples of *Daphnia* ephippia covering the upper 27 cm, nine samples of

365   *Plumatella* statoblasts (0-43 cm), and 15 samples of *Ceriodaphnia* ephippia (0-62 cm). The

366   δ^{13}C values of all three invertebrate genera showed only small variations throughout the

367   record (*Daphnia* ephippia -39.8 to -37.9 ‰, *Ceriodaphnia* ephippia -43.3 to -39.2 ‰, and

368   *Plumatella* statoblasts -33.3 to -29.7 ‰; Fig. 5). δ^{13}C values of *Plumatella* statoblasts were

369   on average distinctly less negative (-32.0 ‰) than the remains of the two mobile filter

370   feeders *Daphnia* and *Ceriodaphnia* (-39.0 ‰ and -41.9 ‰, respectively). The most negative

371   δ^{13}C values were measured for *Ceriodaphnia* ephippia with the minimum of -43.3 ‰ at 41-

372   42 cm depth (AD ~1920). Remains of *Daphnia* and *Ceriodaphnia* showed a very similar

373   pattern, with one distinct peak of less negative δ^{13}C values at about 18 cm depth (AD ~1980).

374   In contrast, δ^{13}C values of *Plumatella* statoblasts remained constant during this period, but

375   show a 3 ‰ increase around 30 cm (AD ~1955). Besides differences in δ^{13}C values,

376   *Plumatella* statoblasts had a lower C:N ratio (mean = 4.3) compared to values for *Daphnia*

377   and *Ceriodaphnia* ephippia (6.3 and 6.5, respectively).

378

379   **Discussion**

380

381   Seasonal variation in *Daphnia* $\delta^{13}C$ values

382

383   *Daphnia* $\delta^{13}C$ values were consistently below -38.5 ‰ and reached minimum values, less

384   than -44 ‰, in the period from autumn to spring. This is well below commonly reported

385   values for algae, which usually lie above -35 ‰ (France 1995; Peterson and Fry 1987;

386   Vuorio et al. 2006). Nonetheless, $\delta^{13}C_{POM}$ values closely track the seasonal cycle of surface

387   water $\delta^{13}C_{POM}$ values (Fig. 3a), but with an average difference of 3.4 ‰ between $\delta^{13}C_{POM}$

388   and $\delta^{13}C_{Daph}$. Hence, $\Delta^{13}C_{POM-Daph}$ was slightly higher than values reported by del Giorgio and

389   France (1996) for zooplankton in Canadian lakes and other published values discussed by

390   these authors (mean difference 2.6 ‰). A positive correlation between $\delta^{13}C_{Daph}$ values and

391   surface water chlorophyll *a* concentrations, which can serve as a proxy for algal

392   concentrations (Sartory and Grobbelaar 1984), was observed between autumn and spring

393   (ESM Fig. S2a). Hence, low $\delta^{13}C_{Daph}$ values coincide with relatively low concentrations of

394   algae in surface water POM. During times when algae are less abundant, other food sources

395   may contribute to the carbon uptake of *Daphnia* (Taipale et al. 2008). Several studies have

396   shown that *Daphnia* and other invertebrates can incorporate CH_4-derived carbon to

397   supplement their diet (Bastviken et al. 2003; Devlin et al. 2015; Kankaala et al. 2006). In a

398   study of a small Finnish lake, Taipale et al. (2008) found that MOB contributed to *Daphnia*'s

399   diet throughout the year, comprising up to 50 % of the diet in autumn. Considering the high

400   CH_4 concentrations in Gerzensee, uptake of ^{13}C-depleted carbon by feeding on MOB seems

401   the likely explanation for the exceptionally low $\delta^{13}C_{Daph}$ values measured in Gerzensee.

402

403   The relationship between *Daphnia* $\delta^{13}C$ values and $[CH_4]_{aq}$

404

405   Field studies that compared the carbon isotopic composition of invertebrate remains in

406   surface sediment samples with in-lake CH_4 abundance suggest that a relationship exists

407   between the $\delta^{13}C$ values of some invertebrate groups and lake water CH_4 concentrations

408   (Schilder et al. 2015a; van Hardenbroek et al. 2013). Within our two-year measurement

409   period at Gerzensee, $\delta^{13}C_{Daph}$ was positively correlated with $[CH_4]_{aq}$ in the bottom waters, i.e.

410   we recorded $\delta^{13}C_{Daph}$ values more similar to $\delta^{13}C_{POM}$ during times of high bottom water

411     $[CH_4]_{aq}$ (ESM Fig. S2b). This is also indicated by the negative correlation between $[CH_4]_{aq}$ in

412   the bottom waters and $\Delta^{13}C_{POM-Daph}$ (ESM Fig. S2c). Hence, during lake stratification, when

413     $[CH_4]_{aq}$ is increasing in the hypolimnion, *Daphnia* utilise carbon whose isotopic signature is

414   more similar to $\delta^{13}C_{POM}$ than at times when the water column is mixed. This suggests that

415   during lake stratification, algae are the main food source of *Daphnia*, whereas *Daphnia* may

416   rely more heavily on other food sources when the lake is mixed. In a multi-lake study,

417   Schilder et al. (2015a) found a negative correlation between late summer $[CH_4]_{aq}$ in both

418   bottom and surface waters, and $\delta^{13}C$ values of *Daphnia* ephippia isolated from surface

419   sediment samples, indicating that *Daphnia* are more likely to incorporate CH_4-derived carbon

420   in lakes with high CH_4 abundance. With regard to this relationship, our results imply that in

421   lakes with high CH_4 accumulation during summer stratification, more ^{13}C-depleted carbon

422   becomes accessible to the food web upon mixing and oxygenation of CH_4 in the water

423   column, and this signal is seen in the floating ephippia. Other processes, e.g. increased

424   transport of allochthonous organic carbon and higher associated CH_4 production may also

425   contribute to the importance of CH_4-derived carbon for *Daphnia* in the autumn months.

426

427   Floating cladoceran and bryozoan remains

428

429   In a field study, Perga (2011) showed that there is no significant carbon isotope fractionation

430   between *Daphnia* and their ephippia. This was confirmed in a laboratory experiment by

431   Schilder et al. (2015b), which showed that *Daphnia* ephippia $\delta^{13}C$ values closely resemble

432   those of *Daphnia* during ephippia production under different environmental conditions (-0.2

433   ±0.4 ‰ for 12°C, 1.3 ±0.3 ‰ for 20°C). In Gerzensee, $\delta^{13}C_{DaphFlot}$ remained very similar

434   across the annual cycle (Figs. 3a, b). If no fractionation is assumed during ephippia

435   production, this suggests that the ephippia were produced batch-wise and then floated on

436   the lake for several months. In the data set presented here, periods when $\delta^{13}C_{DaphFlot}$

437 coincided with $\delta^{13}C_{Daph}$ include December 2012, June 2013, November 2013, and April 2014.

438 This is in line with the observation that ephippia production commonly occurs in early

439 summer and late autumn (Cáceres 1998), when environmental conditions deteriorate, e.g.

440 there is temperature decline, crowding, oxygen depletion, or limited food availability (Korhola

441 and Rautio 2001). Hence, in Gerzensee, changes in $\delta^{13}C_{DaphFlot}$ reflect $\delta^{13}C_{Daph}$ during

442 ephippia production, i.e. in late autumn and/or early spring, but $\delta^{13}C_{DaphFlot}$ does not reflect

443 seasonal changes in the diet of *Daphnia*. This observation differs from the findings of

444 Schilder (2014) at Dutch Lake De Waay, where *Daphnia* ephippia followed the seasonal

445 variation of *Daphnia* $\delta^{13}C$, indicating several production periods per year. The reason for the

446 different timing of ephippia production is unclear, although it could be explained by

447 differences in species composition between the two lakes. Regardless of the cause, our

448 results indicate that different production intervals, and possibly switching between different

449 production intervals over time, potentially influences *Daphnia* ephippia $\delta^{13}C$ values in

450 sediment records.

451 Floating *Ceriodaphnia* ephippia show larger variability in $\delta^{13}C$ values than *Daphnia*

452 ephippia (Fig. 3b). To our knowledge, no prior study has assessed the fractionation between

453 maternal *Ceriodaphnia* and the chitinous structure of their resting eggs. Thus, following the

454 simplest assumption of no (or constant) fractionation, as is observed for *Daphnia*, our results

455 suggest that *Ceriodaphnia* ephippia are produced at several times or continuously

456 throughout the year. No living *Ceriodaphnia* were analysed in this study, but the broad range

457 of $\delta^{13}C_{CerioFlot}$ values (-34.3 to -50.1 ‰), which is comparable in span to the seasonal cycle of

458 living *Daphnia* (-29.8 to -44.2 ‰), may be an indication that the changes in $\delta^{13}C_{CerioFlot}$

459 represent (a part of) the seasonal cycle of *Ceriodaphnia* $\delta^{13}C$.

460 Floating *Plumatella* statoblast $\delta^{13}C$ values also show more pronounced seasonal

461 changes than $\delta^{13}C_{DaphFlot}$, but the variability is not as large as for $\delta^{13}C_{CerioFlot}$ (Fig. 3b). Since

462 no living *Plumatella* zooids were analysed, no pattern of statoblast production can be

463 determined. However, a recent study by van Hardenbroek et al. (2016) suggests that the

464 $\delta^{13}C$ values of *Plumatella* statoblasts collected from colonies are significantly correlated with

18

465   *Plumatella* zooid $\delta^{13}C$. Okamura and Hatton-Ellis (1995) found that production of statoblasts

466   may start in mid-summer, but highest production occurs in late summer and early autumn.

467   Overwintering statoblasts are released when colonies collapse in late autumn. Following

468   these findings, $\delta^{13}C$ values of floating *Plumatella* statoblasts may reflect zooid $\delta^{13}C$ values

469   during statoblast production from late summer to late autumn.

470

471   Linking $\delta^{13}C$ values of floating resting stages to those of fossil invertebrate remains

472

473   The average $\delta^{13}C$ value of floating *Daphnia* ephippia (-39.5 ‰ ± 0.9 [one standard

474   deviation]) is in excellent agreement with the $\delta^{13}C$ value of *Daphnia* ephippia in the

475   uppermost 1-3 cm of the sediment at the centre of the lake (-39.8 ‰; Fig. 5), and with the

476   average $\delta^{13}C$ value of living *Daphnia* collected during the two-year fieldwork period (-39.4 ‰

477   ± 4.6). This confirms that $\delta^{13}C$ values of *Daphnia* ephippia in the sediment of Gerzensee

478   reflect the $\delta^{13}C$ signal of floating *Daphnia* ephippia during the year. Seasonal changes in

479      $\delta^{13}C_{Daph}$, however, are not recorded by floating or deposited *Daphnia* ephippia $\delta^{13}C$ values in

480   the lake (see previous sections). This has implications for the interpretation of the sediment

481   core record of *Daphnia* ephippia $\delta^{13}C$ values. In Gerzensee, nearly constant $\delta^{13}C_{DaphFlot}$

482   values throughout the year indicate batch-wise production of ephippia in autumn and/or early

483   spring. Changes in the $\delta^{13}C$ value of sedimentary *Daphnia* ephippia may thus record

484   changes in the importance of MOB in the diet of *Daphnia* (only) during spring and/or autumn.

485   The latter period is indirectly coupled with the accumulation of $[CH_4]_{aq}$ during summer

486   stratification, which is the reason for high CH_4 abundance in the water column during autumn

487   lake mixing. Hence $\delta^{13}C$ values of sedimentary *Daphnia* ephippia may indicate the

488   accumulation of $[CH_4]_{aq}$ during summer stratification in small European lakes like Lake

489   Gerzensee, as suggested by Schilder et al. (2015a).

490   The *Ceriodaphnia* ephippia $\delta^{13}C$ value recorded in the sediment trap during 2012/13 (-

491   42.5 ‰) is in excellent agreement with the surface sediment measurement presented here (-

492   42.9 ‰, 1-3 cm; Fig. 5). The average flotsam $\delta^{13}C$ value (-42.9 ‰ ± 4.8) also agrees very

493　well with the surface sediment measurement, although no full annual cycle is covered by the

494　flotsam measurements (October to May only, Fig. 3b). Our study is the first to assess and

495　confirm that the $\delta^{13}C$ values of *Ceriodaphnia* ephippia in the sediment can provide

496　information about the $\delta^{13}C$ value of floating ephippia at the time of deposition.

497　　　The *Plumatella* statoblast $\delta^{13}C$ value in the surface sediment (-32.7 ‰, pooled 1-3 and

498　5-7 cm, this study) was distinctly less negative than the average $\delta^{13}C$ value of floating

499　statoblasts (-36.3 ‰ ± 1.7; Fig. 5). It should, however, be noted here that summer

500　measurements of $\delta^{13}C_{PluFlot}$ are underrepresented in our dataset, with only one measurement

501　in July 2013 (Fig. 3b). It therefore remains uncertain whether a better representation of the

502　summer period may resolve this mismatch between average flotsam values and the surface

503　sediment measurement.

504

505　Interpreting fossil invertebrate $\delta^{13}C$ values

506

507　*Daphnia* ephippia $\delta^{13}C$ values in our sediment record (-39.8 to -37.9 ‰) were continuously

508　below -35 ‰, whereas algae are commonly reported to have $\delta^{13}C$ values above -35 ‰

509　(France 1995; Peterson and Fry 1987; Vuorio et al. 2006). Our values are comparable to

510　sedimentary *Daphnia* ephippia $\delta^{13}C$ values found in Lake De Waay, the Netherlands

511　(Schilder 2014), but are distinctly more negative than cladoceran $\delta^{13}C$ values reported from

512　sediment records of Lake Strandsjön, Sweden (van Hardenbroek et al. 2014) and Lake

513　Annecy, France (Frossard et al. 2014). In sediment records covering longer time periods,

514　van Hardenbroek et al. (2013) and Wooller et al. (2012) found $\delta^{13}C$ values over the range of

515　all of the studies mentioned above. In a recent study in Lake Mekkojärvi, Finland, Rinta et al.

516　(2016) showed that *Daphnia* ephippia $\delta^{13}C$ values changed abruptly from values below -

517　45 ‰ to values greater than -40 ‰, shifts that these authors interpreted as representing

518　changes in the availability of CH_4 in this lake. As discussed earlier, $\delta^{13}C$ values in *Daphnia*

519　ephippia have been shown to correlate negatively with CH_4 abundance in the hypolimnia of

520　small European lakes (Schilder et al. 2015a). Therefore, the observed *Daphnia* ephippia

521 δ¹³C values below -35 ‰ suggest that $[CH_4]_{aq}$ remained high at Gerzensee throughout the

522 record. Only the least negative δ¹³C values, around 20 cm (AD ~1970-1980), could indicate

523 a short period with a reduced influence of ¹³C-depleted carbon in the diet of *Daphnia*, but

524 δ¹³C values remained clearly more negative than commonly reported algal δ¹³C values.

525 Lower *Daphnia* δ¹³C values observed for the sediments of eutrophic and stratified lakes

526 Gerzensee and De Waay can potentially be explained by a greater importance of CH_4-

527 derived carbon compared to that in less nutrient-rich Lake Annecy and non-stratified Lake

528 Strandsjön, where algae are the main carbon source of *Daphnia.* Mekkojärvi is a very small,

529 stratified, humic lake with low oxygen concentrations within 1 m of the lake surface (Rinta et

530 al. 2015), which may explain the high relevance of CH_4-derived carbon in its planktonic food

531 web.

532 *Ceriodaphnia* ephippia were not analysed in any of the above-mentioned studies.

533 *Ceriodaphnia* ephippia, however, had even more negative δ¹³C values than *Daphnia* in the

534 Gerzensee sediment (range -43.3 to -39.4 ‰; Fig. 5). This indicates a similar, but potentially

535 more dominant source of ¹³C-depleted carbon for *Ceriodaphnia* ephippia. The systematic

536 ¹³C-depletion of *Ceriodaphnia* ephippia relative to *Daphnia* ephippia may be caused by a

537 difference in the average particle size that these taxa filter from the water. *Ceriodaphnia* feed

538 on smaller particles than *Daphnia* (Geller and Müller 1981), potentially containing a higher

539 proportion of MOB and other bacteria. The relative ¹³C-depletion of *Ceriodaphnia* could also

540 be caused by differences in body composition, e.g. lipid content, between taxa, as has been

541 suggested by Matthews and Mazumder (2005) for other zooplankton species. However, C:N

542 ratios, which may be an indicator of lipid content (Matthews and Mazumder 2005), do not

543 show large differences between *Daphnia* and *Ceriodaphnia* ephippia (mean = 6.3 and 6.4 for

544 the uppermost 27 cm of the sediment record, respectively). Therefore, low δ¹³C values

545 throughout the record suggest that, similar to *Daphnia*, *Ceriodaphnia* rather consistently

546 incorporated a ¹³C-depleted carbon source over the past 150+ years, at least during the

547 season(s) of ephippia production.

548 *Plumatella* statoblast δ¹³C values in our sediment record were distinctly less negative

549   compared to *Daphnia* and *Ceriodaphnia* ephippia (-33.3 to -29.7 ‰; Fig. 5). This suggests

550   that *Plumatella* zooids accessed carbon sources that were less ^{13}C-depleted compared to

551   those on which the two cladoceran taxa relied. Similar δ^{13}C values were reported by van

552   Hardenbroek et al. (2014), for *Plumatella* statoblasts in shallow Lake Strandsjön in Sweden

553   (-33.1 to -28.0 ‰). Our δ^{13}C values for *Plumatella* statoblasts are well in the range of

554   commonly reported δ^{13}C values for algae. Hence, it appears that δ^{13}C values of sedimentary

555   *Plumatella* statoblasts can potentially provide information about the temporal evolution of

556   algal δ^{13}C, and thus help track past changes in the lake's carbon cycle. However, a single

557   statoblast flotsam sample from our study, collected during autumn mixing in November 2013

558   (Fig. 3b), yielded a δ^{13}C value of -40.0 ‰, well below the values expected for algae in Lake

559   Gerzensee. Similarly, individual bryozoan samples with very negative δ^{13}C values have

560   been reported in previous studies (e.g. Rinta et al. 2016; van Hardenbroek et al. 2016). This

561   suggests that these organisms may also ingest ^{13}C-depleted carbon sources such as MOB

562   under circumstances when they are abundant in their habitats.

563   In the sediment core from Gerzensee, *Daphnia* and *Ceriodaphnia* ephippia show very

564   similar variations in their δ^{13}C values in the part of the core where both groups are present

565   (0-27 cm; Fig. 5). This suggests that *Daphnia* and *Ceriodaphnia* were subject to similar

566   changes in their diet over time. In contrast, *Plumatella* statoblasts show a peak in δ^{13}C

567   values that does not coincide with the peak in the ephippia of *Daphnia* and *Ceriodaphnia*.

568   The two groups also differ in the range of their δ^{13}C values, which points towards different

569   food sources for *Plumatella* and the cladoceran taxa. Access to different carbon sources

570   may occur because of differences in both habitat and particle-size preference. *Plumatella*

571   are sessile organisms in shallow areas of lakes, whereas free-living invertebrates like

572   *Daphnia* and *Ceriodaphnia* can reach deep-water food sources. Zooplankton in deeper

573   water layers may feed directly on MOB, or on other organisms that feed on MOB (e.g.

574   ciliates), which can lead to distinctly negative zooplankton δ^{13}C values (Jones and Grey

575   2011). In addition, Kaminski (1984) estimated that *Plumatella* can ingest particles between 5

576   and 17 µm in diameter, whereas *Daphnia* are able to filter particles between 0.5 and 30 µm

577 in diameter (Geller and Müller 1981). Hence, bacteria (< 2 μm) that are not attached to

578 larger particles may be too small to be caught in the lophophores of *Plumatella*, but may be

579 accessible as a food source for *Daphnia*. An alternative explanation for the observed

580 differences between cladoceran and bryozoan remains may be that both groups rely on the

581 same carbon sources (e.g. algae, detritus, heterotrophic bacteria, and possibly MOB), but

582 their relative importance in the diet is different. Whether one, both or more reasons are

583 causing the observed differences in δ^{13}C values cannot be conclusively answered by this

584 study.

585

586 **Conclusions**

587

588 In Gerzensee, *Daphnia* δ^{13}C values closely follow the seasonal cycle of POM δ^{13}C values,

589 with the most negative values of *Daphnia*, down to -44.2 ‰, measured in early spring.

590 Interestingly, *Daphnia* ephippia δ^{13}C values did not show any pronounced seasonal

591 variations. Seasonal changes in *Daphnia* δ^{13}C values, and hence potential seasonal

592 changes in the diet of *Daphnia*, are therefore not recorded in the δ^{13}C values of *Daphnia*

593 ephippia in the sediments of Gerzensee. Very low δ^{13}C values of *Ceriodaphnia* ephippia in

594 the flotsam, especially in winter (-50.1 ‰), confirm that there is a contribution of CH_4-derived

595 carbon to the diet of the crustacean zooplankton in Gerzensee. *Plumatella* statoblasts are

596 less depleted in ^{13}C than the two cladoceran taxa, indicating little or no influence of CH_4-

597 derived carbon in the diet of the bryozoan, at least during the time(s) when statoblasts are

598 produced.

599   The average *Ceriodaphnia* and *Daphnia* ephippia δ^{13}C values in the flotsam agree

600 very well with the values in surface sediments, and for *Ceriodaphnia*, with measurements

601 from a sediment trap. This shows that in Gerzensee the δ^{13}C values of ephippia in the

602 surface sediment, and potentially in general at a certain sediment depth, reflect an

603 integrated value for ephippia floating on the lake during a particular time span, possibly over

604 one to two years. In contrast, average flotsam *Plumatella* statoblast δ^{13}C values were about

605 4 ‰ more negative than the surface sediment measurement. The reason for this mismatch

606 may be an under-representation of the summer period in our data set. Nonetheless, this

607 issue deserves further investigation in Gerzensee and other lakes.

608   Low δ^{13}C values of the two cladocerans throughout the 62-cm-long sediment core,

609 comparable to those measured in the flotsam, indicate that in Gerzensee, *Daphnia* and

610 *Ceriodaphnia* relied on a ^{13}C-depleted carbon source to supplement their diet throughout the

611 past 150 years. *Daphnia* and *Ceriodaphnia* ephippia δ^{13}C values showed similar variations,

612 with the highest values for the two taxa around the same depth. This suggests that *Daphnia*

613 and *Ceriodaphnia* have been subject to similar changes in their diet over time. In contrast,

614 *Plumatella* statoblast δ^{13}C values in the sediment record were again distinctly less negative,

615 and showed a 3 ‰ increase at a different depth than the δ^{13}C values of the two cladocerans.

616 This difference might be explained, in part, by the feeding behaviour and different habitats of

617 the organism groups, which influences the extent to which they can incorporate CH_4-derived

618 carbon. Our study confirms the findings of earlier studies, which indicated that taxon-specific

619 δ^{13}C values measured on aquatic invertebrate remains can provide insights into long-term

620 changes in the relative importance of different carbon sources. This technique can be

621 applied to better understand the impacts of, for example, land-use change, eutrophication,

622 and climate change on the carbon cycling in lakes. Additional studies similar to the one

623 presented here will be needed to further constrain the effects of seasonality on the δ^{13}C

624 values of fossil statoblast and ephippia samples, and to assess the extent to which seasonal

625 patterns observed in Gerzensee are representative of those in other lake ecosystems.

626

627 **Acknowledgements**

628

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Figure Captions

Fig. 1 Map of Switzerland showing the location of Lake Gerzensee in the Swiss foreland of
the Alps (triangle) and the bathymetry of the lake. Black circles indicate the fieldwork
measuring station (C1) and coring site

Fig. 2 (a) Temperature and (b) dissolved oxygen concentrations ($[O_2]_{aq}$) measured at 1-m
depth intervals in the water column at the lake centre (location C1) over the fieldwork period
in 2012-2014. Colour figure online.

Fig. 3 (a) $\delta^{13}C$ values of CO_2, particulate organic matter (POM), *Daphnia* for location C1
(pelagic zone) and *Daphnia* ephippia (whole lake) over the fieldwork period. (b) $\delta^{13}C$ values
of *Daphnia* ephippia, *Ceriodaphnia* ephippia, and *Plumatella* statoblasts (whole lake) over
the fieldwork period. *Daphnia* and flotsam $\delta^{13}C$ values represent single measurements
except for 25 February 2014 and 27 March 2014 when enough material was available for
three measurements of *Daphnia* $\delta^{13}C$. In those cases average values are presented

Fig. 4 Age-depth model (black line, triangles) for core GER12 based on ^{210}Pb activity. The
grey line shows the accumulation of ^{137}Cs activity in the record (based on the ^{210}Pb-inferred
accumulation rate), with the diamond showing the location of the lower activity peak of ^{137}Cs
in the ^{210}Pb-based age model. This peak is correlated with atmospheric nuclear bomb testing
in AD 1963 and coincides with the only measurable ^{241}Am activity in the core (1.9±0.2 Bq/kg).

Fig. 5 $\delta^{13}C$ values of fossil invertebrate remains of *Daphnia*, *Ceriodaphnia* and *Plumatella* in
sediment core GER12 and from the flotsam of the lake (flotsam values plotted above 0 cm
sediment core depth). Symbols in the downcore record represent the average depth of the
measurements in cases for which samples were pooled to obtain sufficient weight for $\delta^{13}C$

809   analysis. Measurements may represent remains from up to 6 cm of sediment (see text for
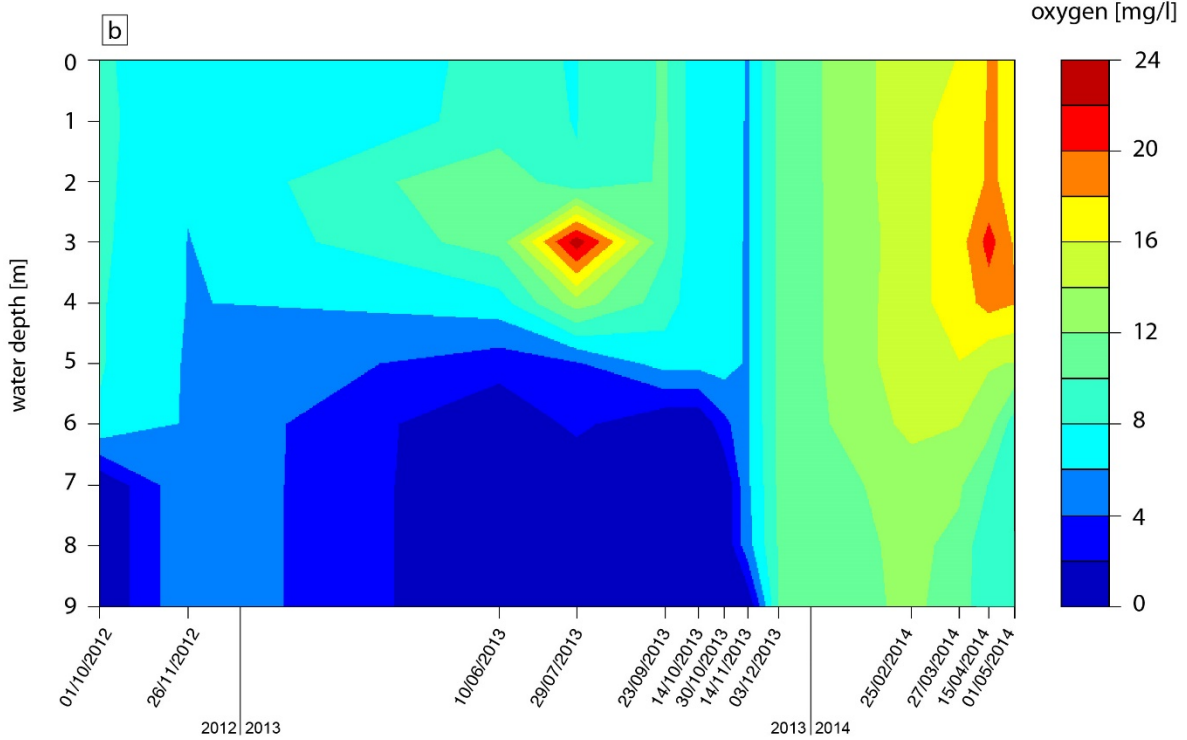
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Figures
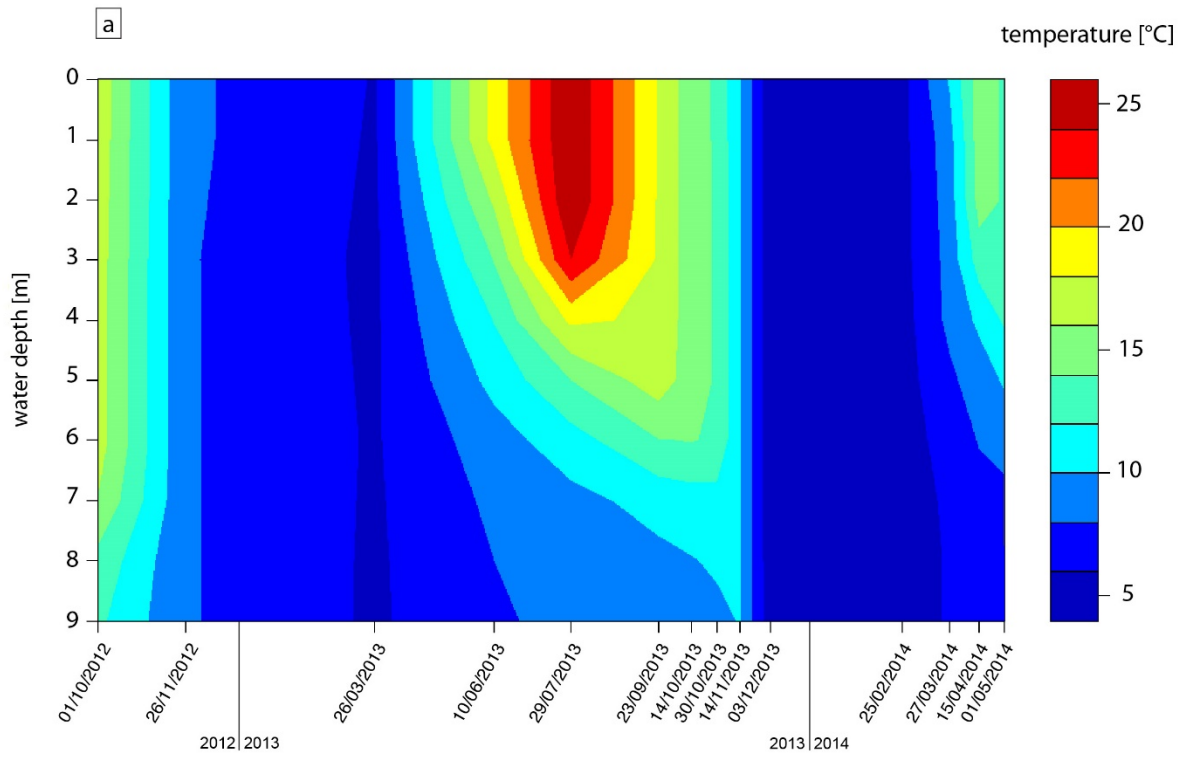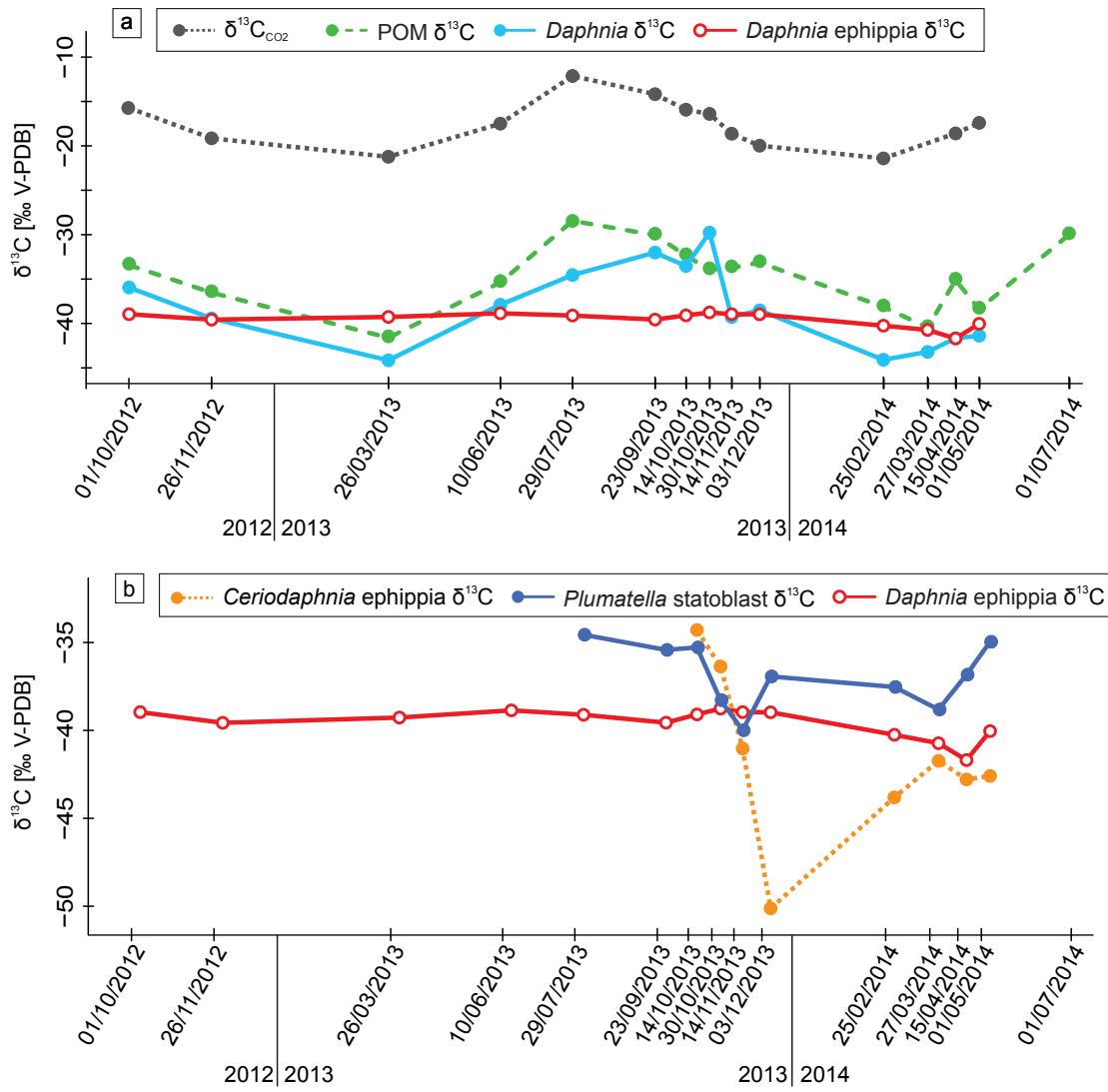
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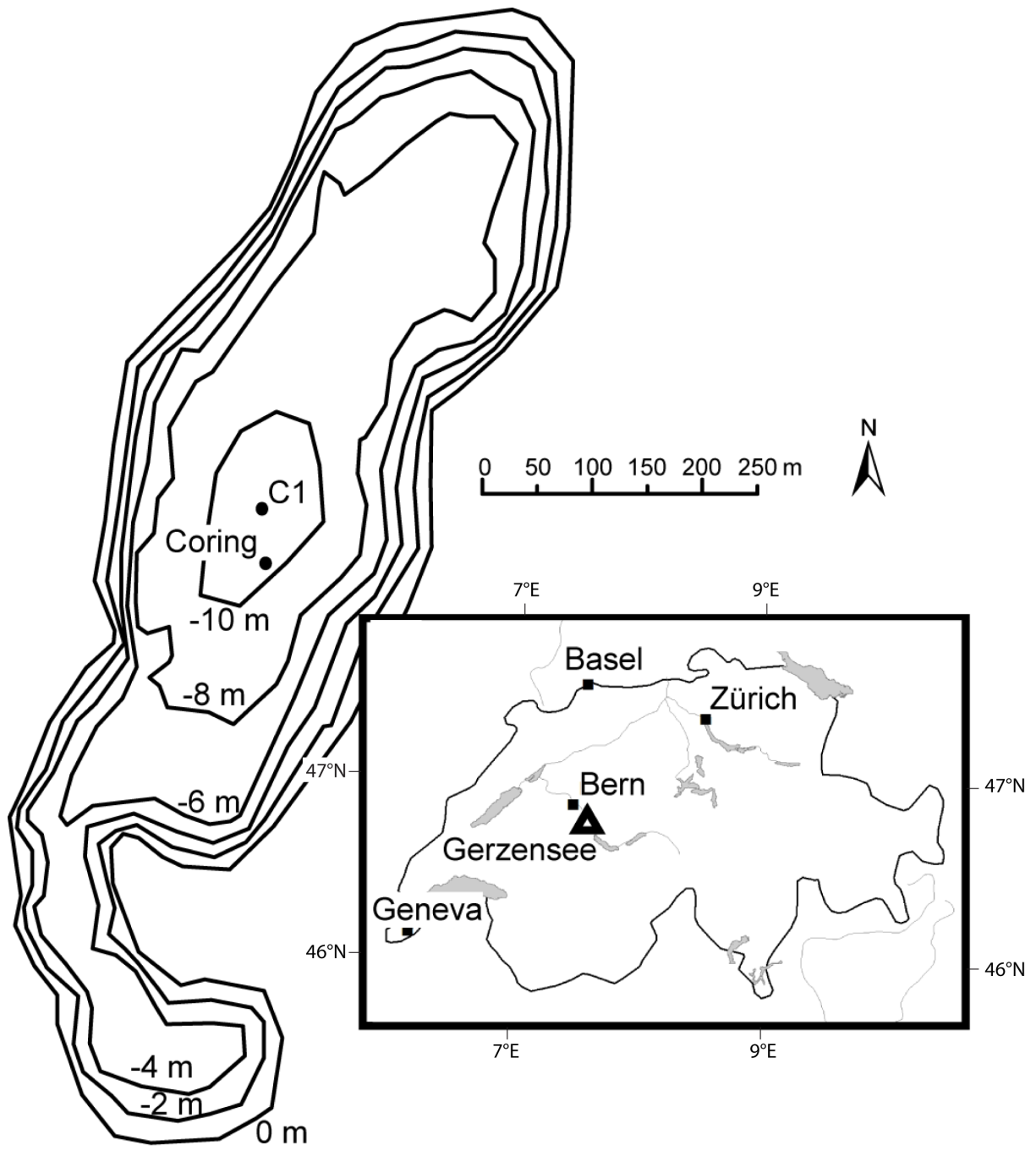
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815   Figure 2

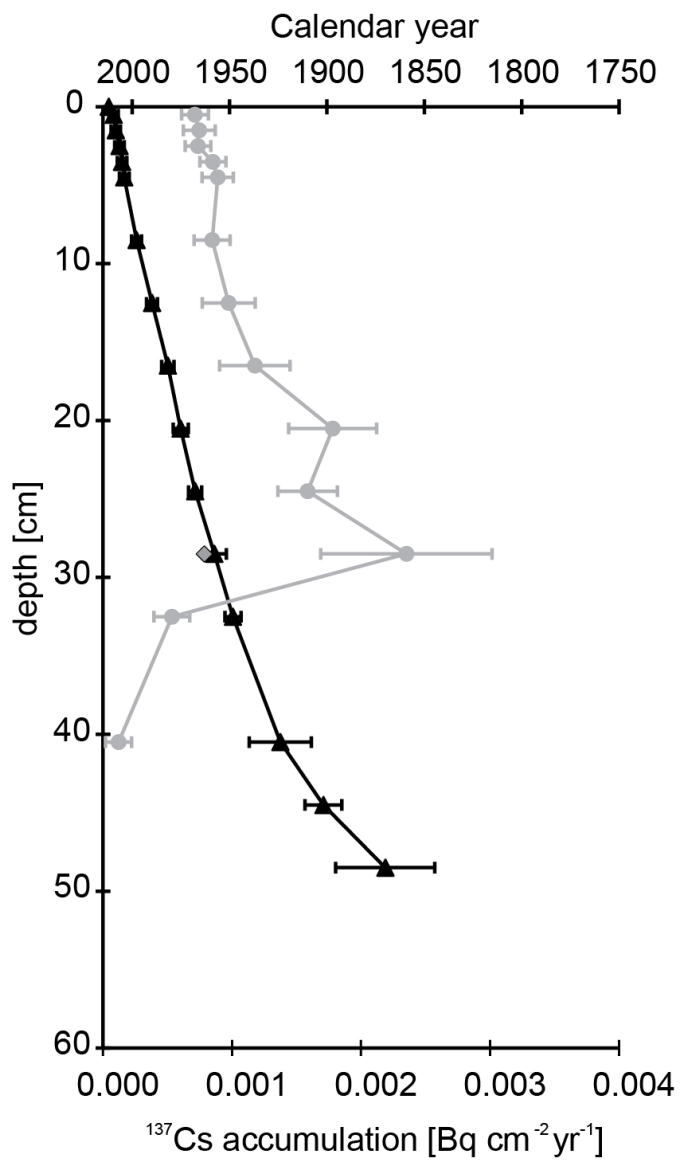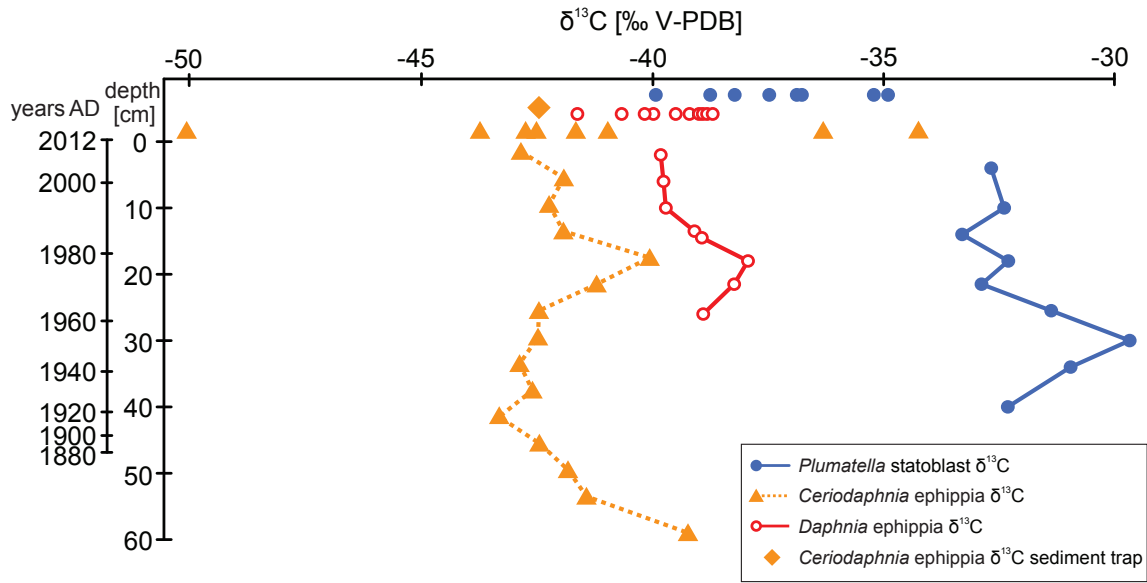
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818   Figure 3

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821   Figure 4

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Figure 5

Electronic Supplementary Material

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829 **Figure Captions**

830

831 **Fig. S1**

832 Dissolved CO_2 ($[CO_2]_{aq}$) and CH_4 ($[CH_4]_{aq}$) concentrations for surface (0.7 m water depth)

833 and bottom waters (8 m water depth) at the lake centre (location C1) over the fieldwork

834 period in 2012-2014

835

836 **Fig. S2**

837 (a) Relationship between surface water chlorophyll *a* concentrations (0.7 m water depth) and

838 *Daphnia* $\delta^{13}C$ at the lake centre (location C1); (b) relationship between log-transformed

839 bottom water dissolved CH_4 concentrations ($[CH_4]_{aq}$) (8 m water depth) and *Daphnia* $\delta^{13}C$ at

840 the lake centre (location C1); (c) relationship of the difference between particulate organic

841 matter (POM) $\delta^{13}C$ and *Daphnia* $\delta^{13}C$ in the surface water ($\Delta^{13}C_{POM-Daph}$) and bottom water (8

842 m water depth) $[CH_4]_{aq}$; (d) surface (0.7 m water depth) and bottom water (8 m water depth)

843 POM $\delta^{13}C$ at the lake centre (location C1)
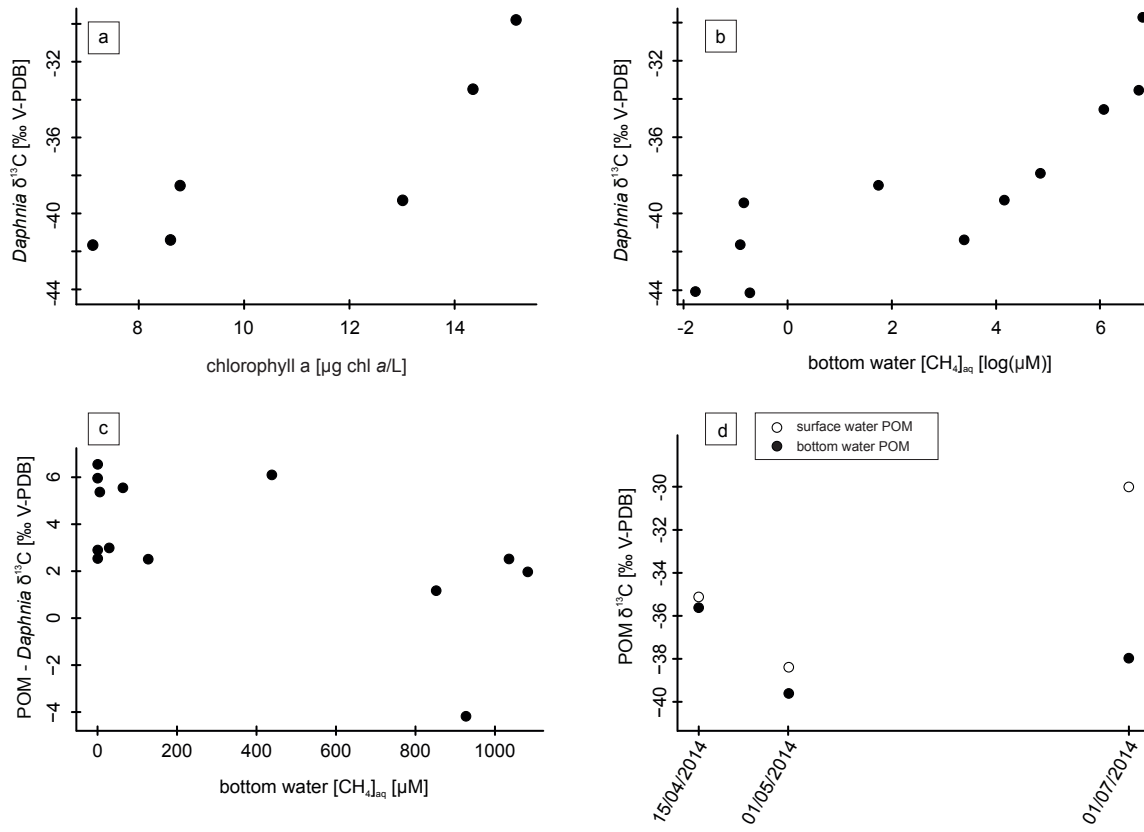
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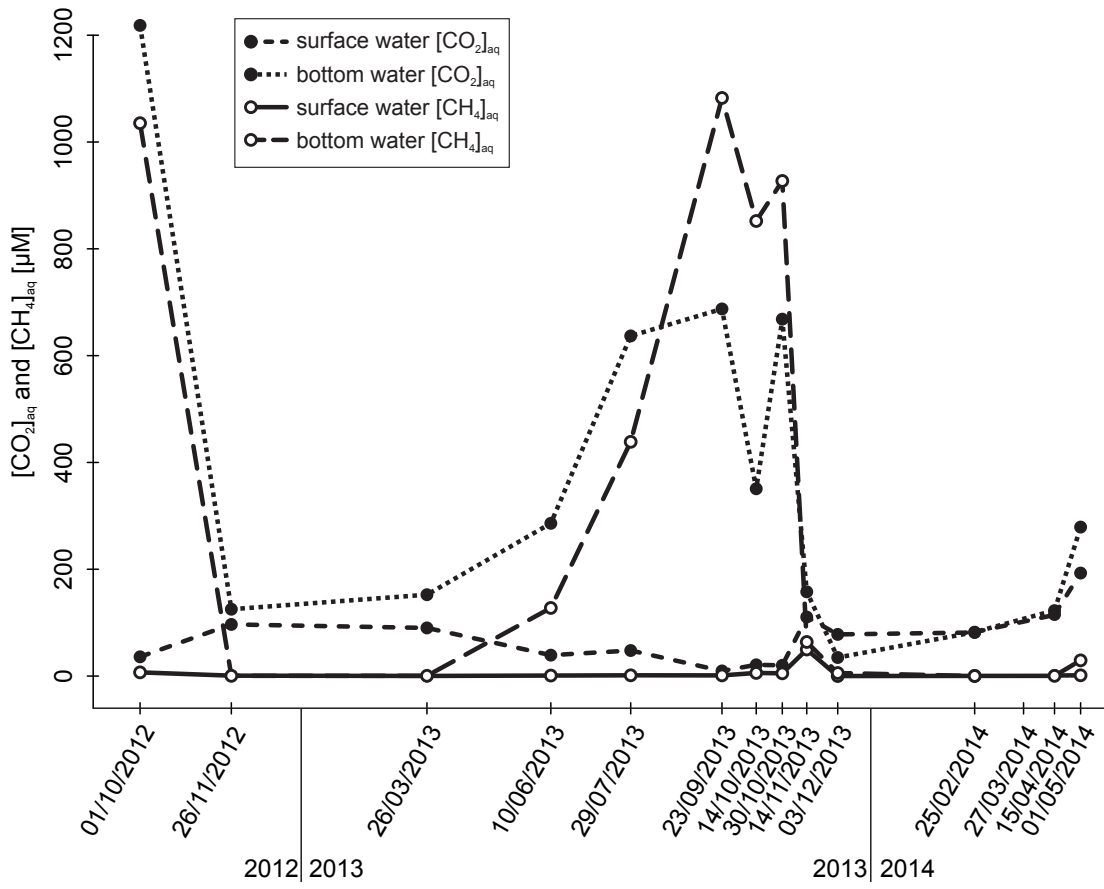
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848   Figure S2

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851 **Table S1** Samples collected and analysed in the course of repeated fieldwork on Gerzensee between October 2012 and July 2014. "x" denotes

852 that a sample was collected, "-" shows where no sample was collected

853

Fieldwork dates [d.m.yr]	Temp	$[O_2]_{aq}$	Daphnia $\delta^{13}C$	Daphnia ephippia $\delta^{13}C$	Ceriodaphnia ephippia $\delta^{13}C$	Plumatella statoblast $\delta^{13}C$	DIC	$\delta^{13}C_{POM}$ (0.7 m)	$\delta^{13}C_{POM}$ (7 m)	Chl a [µg/l]	$[CH_4]$ & $[CO_2]$
01.-03.10.2012	x	x	x	x	-	-	x	x	-	-	x
26.-28.11.2012	x	x	x	x	-	-	x	x	-	-	x
26.-28.03.2013	x	x	x	x	-	-	x	x	-	-	x
10.-13.06.2013	x	x	x	x	-	-	x	x	-	-	x
29.-31.07.2013	x	x	x	x	-	x	x	x	-	-	x
23.-25.09.2013	x	x	x	x	x	x	x	x	-	-	x
14.10.2013	x	x	x	x	x	x	x	x	-	x	x
30.10.2013	x	x	x	x	x	x	x	x	-	x	x
14.11.2013	x	x	x	x	x	x	x	x	-	x	x
03.12.2013	x	x	x	x	x	x	x	x	-	x	x
25.02.2014	x	x	x	x	x	x	x	x	-	-	x
26.03.2014	x	-	-	x	x	x	-	-	-	-	x
15.04.2014	x	x	x	x	x	x	x	x	x	x	x
01.05.2014	x	x	x	x	x	x	x	x	x	x	x
01.07.2014	-	-	-	-	-	-	-	x	x	x	x

854