Seasonality of cladoceran and bryozoan resting stage $\delta^{13}C$ values and implications for their use as palaeolimnological indicators of lacustrine carbon cycle dynamics

MA Morlock[1,*], J Schilder[1], M van Hardenbroek[1,2], S Szidat[3], MJ Wooller[4] & O Heiri[1]

[1]Institute of Plant Sciences & Oeschger Centre for Climate Change Research, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

[2]Geography and Environment, University of Southampton, Southampton SO17 1BJ, UK

[3]Department of Chemistry and Biochemistry & Oeschger Centre for Climate Change Research, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

[4]Alaska Stable Isotope Facility, University of Alaska Fairbanks, Fairbanks, Alaska, 99775, USA

* Corresponding author: marina.morlock@geo.unibe.ch

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

Abstract

The stable carbon isotope composition, expressed as $\delta^{13}C$ values, of chitinous resting stages of planktivorous invertebrates can provide information on past changes in carbon cycling in lakes. For example, the $\delta^{13}C$ values of cladoceran ephippia and bryozoan statoblasts have been used to estimate the past contribution of methane-derived carbon to lake food webs and variations in the $\delta^{13}C$ value of planktonic algae. Limited information, however, is available concerning seasonal variations in $\delta^{13}C$ values of these organisms and their resting stages. We measured the seasonal variation in $\delta^{13}C$ values of *Daphnia* (Branchiopoda: Cladocera: Daphniidae) and their floating ephippia over a 2-year period in small, dimictic Lake Gerzensee, Switzerland. Floating ephippia of *Ceriodaphnia* (Branchiopoda: Cladocera: Daphniidae) and statoblasts of *Plumatella* (Phylactolaemata: Plumatellida: Plumatellidae) were analysed during parts of this period. Furthermore, $\delta^{13}C$ values of remains from all three organism groups were analysed in a 62-cm-long sediment core. Throughout the year, *Daphnia* $\delta^{13}C$ values tracked the $\delta^{13}C$ values of particulate organic matter (POM), but were more negative than POM, indicating that *Daphnia* also utilize a relatively ^{13}C-depleted carbon source. *Daphnia* ephippia $\delta^{13}C$ values did not show any pronounced seasonal variation, suggesting that they are produced batch-wise in autumn and/or spring and float for several months. In contrast, $\delta^{13}C$ values of *Ceriodaphnia* ephippia and *Plumatella* statoblasts followed variations in $\delta^{13}C_{POM}$ values, *Ceriodaphnia* values being the most negative of the resting stages. Average cladoceran ephippia $\delta^{13}C$ values in the flotsam agreed well with ephippia values from Gerzensee surface sediments. In contrast, average *Plumatella* statoblast $\delta^{13}C$ values from the flotsam were 4 ‰ more negative than in the surface sediments. In the sediment core, $\delta^{13}C$ values of the two cladocerans remained low (mean -39.0 ‰ and -41.9 ‰) throughout the record. In contrast, *Plumatella* had distinctly less negative $\delta^{13}C$ values (mean -32.0 ‰). Our results indicate that in Gerzensee, *Daphnia* and *Ceriodaphnia* strongly relied on a ^{13}C-depleted food source throughout the past 150

years, most likely methane-oxidising bacteria, whereas this food source was not a major 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 δ^{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 δ^{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 δ^{13}C values (Perga 2009, 2011). It has recently been suggested that similar information may be gained from δ^{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 δ^{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 food web (Fry 2006; Taipale et al. 2007). Fundamental for such studies is that the carbon isotopic composition of an organism closely reflects the isotopic signature of its diet (DeNiro and Epstein 1978; Peterson and Fry 1987). For *Daphnia* and their ephippia, Perga (2011) and Schilder et al. (2015b) showed that ephippia $\delta^{13}C$ values closely reflect the $\delta^{13}C$ values of the living *Daphnia* at the time of ephippia production. Van Hardenbroek et al. (2016) recently demonstrated that the $\delta^{13}C$ values of bryozoan statoblasts are related to the $\delta^{13}C$ values of the colonies that produced them. More work is required, however, to show that $\delta^{13}C$ values of *Daphnia* ephippia and bryozoan statoblasts reflect the diet of the parent organisms during or shortly before resting stage formation.

Understanding the extent to which lakes change their capacity to sequester or release carbon in response to global warming and eutrophication is a key issue for palaeoecologists today (Seddon et al. 2014). New insights into lake carbon cycling can be gained from analysing $\delta^{13}C$ values of aquatic invertebrate remains. Recently, it was suggested that carbon from CH_4 may be an important additional transfer pathway of carbon in lake food webs, indicated by remarkably low $\delta^{13}C$ values of aquatic invertebrates when they incorporate CH_4-derived, ^{13}C-depleted carbon (Bastviken et al. 2003; Bunn and Boon 1993; Grey et al. 2016). The $\delta^{13}C$ values of biogenic CH_4 in small European lakes range between -86 and -61 ‰ (Rinta et al. 2015), which is markedly depleted in ^{13}C relative to algae with $\delta^{13}C$ values that typically fall in the range from -35 to -25 ‰ (Jones et al. 1999; Peterson and Fry 1987). The large difference in $\delta^{13}C$ values thus allows for differentiation between photosynthetically produced organic matter and MOB as potential carbon sources for primary consumers in lakes. Significant relationships have been observed between $\delta^{13}C$ of *Daphnia* ephippia in surface sediments and diffusive CH_4 flux (Van Hardenbroek et al. 2013) and within-lake CH_4 concentrations (Schilder et al. 2015a). Cladoceran remains may thus record changes in past diffusive CH_4 flux in lakes, though they do not provide direct information on other forms of CH_4 fluxes such as ebullition or plant-mediated transport.

Palaeoecological studies using $\delta^{13}C$ values of chitinous remains have largely overlooked the use of bryozoan statoblasts, which have been suggested as a resource for reconstructing $\delta^{13}C$ values of primary production (Turney 1999; van Hardenbroek et al. 2014, 2016). As filter feeders, Bryozoa rely on algae, particulate organic matter (POM) and associated microorganisms (e.g. bacteria) as their main food source (Kaminsky 1984). Bryozoa are mainly bound to their substrate and do not have access to food sources from deeper water layers and are therefore not, or only to a small degree, influenced by CH_4-derived carbon (van Hardenbroek et al. 2016). By combining $\delta^{13}C$ measurements of mobile filter feeders like *Daphnia* with sessile filter feeders like *Plumatella* (Phylactolaemata: Plumatellida: Plumatellidae), it is possible to separate changes in the importance of CH_4-derived carbon from changes in algal $\delta^{13}C$ values, or more generally, changes in lake productivity (van Hardenbroek et al. 2014; Rinta et al. 2016). At present, however, no information is available about seasonal changes in $\delta^{13}C$ values of cladoceran and bryozoan resting stages or about the extent to which this seasonality influences the $\delta^{13}C$ values of statoblast and ephippia remains in lake sediments. Furthermore, it is unknown whether the $\delta^{13}C$ values of deposited organism remains reflect integrated $\delta^{13}C$ values for statoblasts and ephippia that float on the lake surface, or whether the sedimentary assemblages are characterized by systematically higher or lower values. Such differences could appear as a consequence of degradation, transport processes, or production of resting stages in cryptic habitats that do not contribute to floating resting stages at the lake surface (e.g. from deeper littoral, lower epilimnetic or profundal habitats). These uncertainties currently hinder the development of $\delta^{13}C$ analysis of invertebrate resting stages as a proxy for reconstructing past variations in carbon cycling in lakes.

The main aim of this study was to assess the influence of seasonality on $\delta^{13}C$ values of zooplankton remains and evaluate their potential use as palaeoecological indicators for lacustrine carbon cycle dynamics. This study provides a first assessment of seasonal variations in $\delta^{13}C$ values of cladoceran and bryozoan resting stages in a small temperate European lake, with respect to physical, chemical and biological variables. In particular, the focus was on assessing seasonal variations in transfer pathways of carbon in the lake, and their influence on the $\delta^{13}C$ values of organisms in the water column, as well as their chitinous resting stages (i.e. planktonic Cladocera and their ephippia, bryozoan statoblasts). In a field campaign, $\delta^{13}C$ values of *Daphnia* and their ephippia were analysed over a two-year period, and $\delta^{13}C$ values of floating resting stages of *Ceriodaphnia* (Branchiopoda: Cladocera: Daphniidae) and *Plumatella* were analysed when present during this period. In a second step, we investigated the implications of seasonal changes for interpretation of $\delta^{13}C$ values in fossil invertebrate remains. To that end, ephippia of the cladocerans *Daphnia* and *Ceriodaphnia*, and statoblasts of the bryozoan *Plumatella* were analysed in the lake surface sediment and in a short sediment core covering roughly the past 150 years. Our study was conducted on Lake Gerzensee (7°33'E, 46°50'N, 606 m a.s.l.), a small temperate lake in the foreland of the Swiss Alps, about 20 km south of Bern (Fig. 1). The lake is characterised by exceptionally high lake water CH_4 concentrations (Rinta et al. 2015; Schilder et al. 2016). Lake Gerzensee has a surface area of 0.27 km^2, a total water volume of 0.16 km^3, and a maximum depth of 10 m. The catchment area (2.6 km^2) consists of 80 % agricultural land, 5 % wooded land, and 15 % urban areas (Lotter et al. 2000). The mean annual temperature is 8.8 °C (Bern Zollikofen 1981-2010, Bundesamt für Meteorologie und Klimatologie MeteoSchweiz 2014). Today, Lake Gerzensee is eutrophic, with summer anoxia in the hypolimnion (Zeh et al. 2004).

Materials and methods

Lake Gerzensee was sampled on 15 one-to-three-day visits between October 2012 and July 2014, throughout all seasons (Electronic Supplementary Material [ESM] Table S1). In the first period, between October 2012 and September 2013, measurements were taken every other month (6 visits). Based on the first year of data, the fieldwork protocol was adjusted to gain more detailed information about deeper-water POM and chlorophyll *a* concentrations (ESM Table S1). In the second year, intervals between field visits were reduced to two weeks from September 2013 to December 2013, to cover the destratification period (4 visits), and sampling continued with 2-4 week intervals after the lake ice thawed in February 2014, until July 2014 (5 visits). Data for March and July 2014 were collected along with other fieldwork activities, using a shortened protocol (ESM Table S1).

Sample collection

Vertical profiles of temperature and dissolved oxygen concentration ($[O_2]_{aq}$) were measured for every metre in the water column at the lake centre (location C1, Fig. 1), using a multi-sensor probe (WTW CellOx© 325 oxi1970i, Germany). *Daphnia* individuals were collected from the oxic part of the water column at the lake centre (location C1) in multiple vertical hauls, with a 40-µm mesh plankton net. Flotsam was collected from the lake surface with a hand net (mesh size ~120 µm). Water samples were collected at C1 in 0.7 m and 8 m water depth (top epilimnion and bottom hypolimnion, respectively), using a 5-L water sampler (UWITEC, Austria). For these water samples, pH was measured (Waterproof pHTestr 20, Oakton, USA), and 60 ml of water was injected with a syringe into a 118-ml glass vial through a 10-mm-thick butyl rubber stopper (Apodan, Denmark) to determine the abundance and $\delta^{13}C$ value of the dissolved inorganic carbon (DIC). The vials were prepared beforehand with 200 µl of H_3PO_4 (85 %), closed, and repeatedly vacuumed and flushed with N_2 to ensure that no CO_2 remained in the vials (Rinta et al. 2015; Schilder et al. 2015a). Samples for the $\delta^{13}C$ analysis of POM were collected from the lake water at C1 (0.7 m water depth) by passing the water through a 250-µm sieve before manually pushing water through a glass fibre filter (Whatman GF/C 25 mm, pore size 1.2 µm) with a syringe (water volume recorded in the field). A second sample of POM was obtained in a similar fashion, immediately put into 90 % undenatured ethanol, and covered with aluminium foil for chlorophyll *a* analysis. Immediately after returning from fieldwork, POM filters designated for chlorophyll *a* analysis were heated to 70 °C in 90 % undenatured ethanol for 10 min, put in an ultrasound bath for 5 min, and stored in a refrigerator for at least 48 hours. The samples were then filtered through a membrane filter to remove suspended particles. Chlorophyll *a* measurements were based on absorbance at characteristic wavelengths (Schwoerbel 1994). Samples were injected into glass cuvettes (1 cm light path) and absorbance at wavelengths (λ) 665 nm and 750 nm was measured with a spectrophotometer (Jenway, UK). Chlorophyll *a* content in the lake water was calculated following EAWAG (1995).

Isotope analysis

δ^{13}C analysis of DIC and POM

The concentration of CH_4 and CO_2 in the headspace of the DIC samples was measured by gas chromatography with a flame ionisation detector and methanizer (GC-FID; Shimadzu GC8, PoropackN column, see Rinta et al. 2015 for details). Lake water DIC concentrations and dissolved CH_4 concentrations ($[CH_4]_{aq}$) were back-calculated from measured headspace CO_2 and CH_4 concentrations. Dissolved CO_2 concentrations ($[CO_2]_{aq}$) (as the sum of dissolved CO_2 and H_2CO_3) were calculated following Stumm and Morgan (1996), accounting for lake water pH, temperature, and DIC concentrations.

Filters containing lake water POM were freeze-dried and a maximum amount of filter material was separated from the seston. The seston was then transferred into ultra-clean tin cups. For δ^{13}C analysis of DIC, 15-20 ml of gas from the headspace of the vial was allowed to escape into a 60-ml syringe. The gas was then injected into a pre-vacuumed 12-ml glass vial such that slight overpressure was applied. The procedure was repeated for $\delta^{13}CH_4$ measurements. Isotope samples of CO_2, CH_4, and POM were analysed at the Stable Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL Cube or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 isotope ratio mass spectrometer (IRMS). Analytical uncertainties for gas sample δ^{13}C values were < 0.1 ‰ (one standard deviation) for CO_2 for two to three replicate measurements of three laboratory standards (δ^{13}C = -40.73 ‰, -10.39 ‰, and -3.59 ‰) and < 0.1 ‰ (one standard deviation) for CH_4 for replicate measurements of a laboratory standard (δ^{13}C = -36.7 ‰, n = 6). Results are reported in conventional δ-notation relative to the international standard Vienna PeeDee

Belemnite (V-PDB).

δ^{13}C analysis of zooplankton

Living *Daphnia* were kept in approximately 1 L of unfiltered lake water for 1-2 days. They were then separated from other organisms under a dissecting microscope (magnification 20x–50x), and were frozen in demineralised water. Samples were freeze-dried and weighed into ultra clean tin cups (Lüdi Swiss AG, Switzerland). Because *Daphnia* ephippia cannot be identified to species level under the microscope, the species of living *Daphnia* was not determined for this analysis. Flotsam was sieved at 100 µm and examined under a dissecting microscope. Ephippia of the planktonic cladocerans *Daphnia* and *Ceriodaphnia*, and statoblasts of the bryozoan *Plumatella* were the only invertebrate resting stages found regularly and abundantly enough for δ^{13}C analysis. These resting stages were identified according to Vandekerkhove et al. (2004) (Cladocera) and Wood and Okamura (2005) (Bryozoa), exposed to 10 % potassium hydroxide (KOH) for 2 hours (van Hardenbroek et al. 2010), rinsed 5-10 times with demineralised water, and picked into ultra-clean tin cups. Isotope samples of *Daphnia* as well as ephippia and statoblasts were also analysed at the Stable Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL Cube or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 IRMS. Sample sizes were in the range of 120-250 individuals for *Daphnia* (150-500 µg), 150-200 for *Daphnia* ephippia (150-250 µg), 200-300 for *Plumatella* statoblasts (150-300 µg), and 450-600 (150-200 µg) for *Ceriodaphnia* ephippia. Analytical uncertainties for invertebrate δ^{13}C measurements were ≤ 0.6 ‰ (one standard deviation) for replicate measurements (n = 3-36) of five laboratory standards (Bovine Liver (δ^{13}C = -21.7 ‰), USGS-41 Glutamic Acid (δ^{13}C = 37.6 ‰), Nylon 5 (δ^{13}C = -27.7 ‰), Peach Leaves (δ^{13}C = -26.1 ‰), Glutamic Acid (δ^{13}C = -28.9 ‰)).

Sediment analysis

Sediment coring and chronology

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.

δ^{13}C analysis of sedimentary invertebrate remains

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_4Cl 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 were analysed at the Alaska Stable Isotope Facility of the University of Alaska, Fairbanks, on a Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo Delta V IRMS. Analytical uncertainties were < 0.1 ‰ for replicate measurements of a laboratory standard (peptone, $\delta^{13}C$ = -15.8 ‰, n = 24). Sample sizes were in the range of 20-55 remains for *Daphnia* ephippia and *Plumatella* statoblasts, and 100-200 for *Ceriodaphnia* ephippia. In addition to the sediment core, material from a sediment trap located close to the coring site at the centre of Gerzensee during 2012/13 was available from another study (C. Adolf, unpublished). The trap was placed 3 m above the lake floor and remained in the lake for 12 months. The material was processed in the same way as the sediment core, and one sample for *Ceriodaphnia* ephippia was analysed for its $\delta^{13}C$ value.

Statistical analysis

Statistical analyses on material collected alive and as floatsam were performed in R (R Development Core Team 2008). For Pearson correlation tests, normality of the variables was tested prior to analysis using the Shapiro-Wilk test. When a normal distribution of the data was rejected, Spearman's rank correlation was used to test for correlations. The average value reported for fieldwork parameters was calculated as a weighted mean of all samples collected during the campaign and each sample was weighted by the time interval for which it is most representative.

Results

Physical and chemical lake conditions

All variables showed characteristic seasonal variations throughout the two years of monitoring. Measured surface water temperatures ranged between 4.6 °C in December

2013 and 25.8 °C in July 2013, with a mean of 12.4 °C (Fig. 2a). The bottom water (8 m depth) was anoxic ($[O_2]_{aq}$ < 1 mg L^{-1}) from June to November (Fig. 2b). Chlorophyll *a* values 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 one measurement was taken during summer (July 2014). Deep-water chlorophyll *a* measured at 7 m depth in May and July 2014 was comparable (July, 14.7 μg chl *a* L^{-1}) or higher (May, 14.2 μg chl *a* L^{-1}) than surface water measurements (12.6 and 8.6 μg chl *a* L^{-1}, respectively). $[CO_2]_{aq}$ in the epilimnion ranged from peak values just after autumn mixing and in spring (96.7-192.8 μM) to 9.6 μM in September (ESM Fig. S1). $[CH_4]_{aq}$ in the water column reached high values (1082 μM) in the hypolimnion during summer stratification. Surface water $[CH_4]_{aq}$ was highest in November during autumn turnover (49.8 μM, ESM Fig. S1).

Stable carbon isotopes

With the exception of *Daphnia* ephippia, all measured variables showed seasonal variation in $\delta^{13}C$ values, with the lowest values right after autumn lake mixing and throughout winter, and the highest values in summer (Fig. 3a).

The isotopic composition of $[CO_2]_{aq}$ ($\delta^{13}C_{CO2}$) for location C1 at the lake surface was most ^{13}C-depleted in February and March ($\delta^{13}C_{CO2}$ ~-21 ‰), whereas the highest $\delta^{13}C_{CO2}$ value of -12.1 ‰ was recorded in July 2013 (Fig. 3a). The seasonal cycle in $\delta^{13}C_{CO2}$ values was less pronounced in the hypolimnion (data not shown). During summer, a vertical gradient in $\delta^{13}C_{CO2}$ values developed between the epilimnion and the hypolimnion, which was eliminated at lake mixing in autumn. At the lake centre, the $\delta^{13}C$ value of surface water POM ($\delta^{13}C_{POM}$) was highest during summer (-28.5 ‰), and reached its minimum in March 2013 (-41.6 ‰, mean = -34.7 ‰; Fig. 3a). Bottom water $\delta^{13}C_{POM}$, measured at 7 m depth, was in the range of surface water $\delta^{13}C_{POM}$ for measurements in April and May 2014 (ESM Fig. S2d). However, during summer stratification in July 2014, bottom water $\delta^{13}C_{POM}$ values were distinctly more negative than surface water $\delta^{13}C_{POM}$ (-38.0 ‰ and -30.0 ‰, respectively). Surface water $\delta^{13}C_{POM}$ was on average 17.0 ‰ (range 13.2 to 21.0 ‰) more negative than surface water $\delta^{13}C_{CO2}$, with the largest differences measured in March 2013 and May 2014 (Fig. 3a).

Daphnia $\delta^{13}C$ values ($\delta^{13}C_{Daph}$) in the lake centre were on average 3.4 ‰ more negative than $\delta^{13}C_{POM}$ values (Fig. 3a). The average $\delta^{13}C_{Daph}$ value was -39.4 ‰. Values ranged from -44.2 ‰ in early spring to -29.8 ‰ in summer. $\delta^{13}C_{Daph}$ values were positively correlated with chlorophyll *a* concentrations in surface water POM (Pearson correlation $r = 0.86$, $p < 0.05$, $n = 6$; ESM Fig. S2a), and $[CH_4]_{aq}$ in bottom waters (log-transformed, Pearson correlation $r = 0.86$, $p < 0.01$, $n = 13$; ESM Fig. S2b). Moreover, bottom water $[CH_4]_{aq}$ showed a negative correlation with the offset between $\delta^{13}C_{POM}$ and $\delta^{13}C_{Daph}$ ($\Delta^{13}C_{POM-Daph}$) (Spearman's rank correlation $r = 0.67$, $p < 0.05$, $n = 13$; ESM Fig. S2c).

In contrast to $\delta^{13}C_{Daph}$ values, the $\delta^{13}C$ values of floating *Daphnia* ephippia ($\delta^{13}C_{DaphFlot}$) did not show seasonal variations, but instead remained relatively constant around -39.5 ‰ (range -41.7 to -38.8 ‰, Fig. 3a and b). Interestingly, floating *Ceriodaphnia* ephippia $\delta^{13}C$ values ($\delta^{13}C_{CerioFlot}$) showed changes over time, with the lowest $\delta^{13}C$ values down to -50.1 ‰ in winter (Fig. 3b). On average, $\delta^{13}C_{CerioFlot}$ was more ^{13}C-depleted (mean = -42.9 ‰, range -50.1 to -34.3 ‰) than $\delta^{13}C_{DaphFlot}$, whereas *Plumatella* statoblast $\delta^{13}C$ ($\delta^{13}C_{PluFlot}$) was least ^{13}C-depleted (mean = -36.3 ‰, range -40.0 to -34.6 ‰). In-lake abundance of the floating remains could not be assessed by the applied sampling technique, and no structural degradation of the remains was visible under the microscope on any of the fieldwork dates. *Ceriodaphnia* ephippia from the sediment trap had a $\delta^{13}C$ value of -42.4 ‰.

Sediment core and chronology

For core GER12, activity of unsupported ^{210}Pb was transformed into an age-depth model for the upper 48 cm using the constant rate of supply (CRS) model (Appleby 2001; Appleby and Oldfield 1978) (Fig. 4). Activity measurements of ^{137}Cs showed a distinct peak at 28.5 cm and a second smaller peak at 20.5 cm. Many European lakes show two maxima in ^{137}Cs

activity, which are associated with aboveground nuclear bomb tests in AD 1963 and the Chernobyl reactor accident in AD 1986 (Appleby 2001). For Swiss lakes, the latter peak is usually greater than the first (Albrecht et al. 1998; Lotter et al. 1997). This was not the case in Gerzensee sediments where only one measurement point defined the anticipated Chernobyl peak. The Chernobyl peak may thus not be fully revealed in the record because of the relatively low sampling resolution, i.e. every 4 cm. Therefore the ^{210}Pb model was not modified to fit the ^{137}Cs profile. The lower peak in ^{137}Cs activity, expected to coincide with the nuclear bomb peak in AD 1963, was confirmed by ^{241}Am and agrees well with the ^{210}Pb chronology (Fig. 4).

The abundance of invertebrate remains in the sediments of Gerzensee allowed for the analysis of eight samples of *Daphnia* ephippia covering the upper 27 cm, nine samples of *Plumatella* statoblasts (0-43 cm), and 15 samples of *Ceriodaphnia* ephippia (0-62 cm). The δ^{13}C values of all three invertebrate genera showed only small variations throughout the record (*Daphnia* ephippia -39.8 to -37.9 ‰, *Ceriodaphnia* ephippia -43.3 to -39.2 ‰, and *Plumatella* statoblasts -33.3 to -29.7 ‰; Fig. 5). δ^{13}C values of *Plumatella* statoblasts were on average distinctly less negative (-32.0 ‰) than the remains of the two mobile filter feeders *Daphnia* and *Ceriodaphnia* (-39.0 ‰ and -41.9 ‰, respectively). The most negative δ^{13}C values were measured for *Ceriodaphnia* ephippia with the minimum of -43.3 ‰ at 41-42 cm depth (AD ~1920). Remains of *Daphnia* and *Ceriodaphnia* showed a very similar pattern, with one distinct peak of less negative δ^{13}C values at about 18 cm depth (AD ~1980). In contrast, δ^{13}C values of *Plumatella* statoblasts remained constant during this period, but show a 3 ‰ increase around 30 cm (AD ~1955). Besides differences in δ^{13}C values, *Plumatella* statoblasts had a lower C:N ratio (mean = 4.3) compared to values for *Daphnia* and *Ceriodaphnia* ephippia (6.3 and 6.5, respectively).

Discussion

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

Daphnia $\delta^{13}C$ values were consistently below -38.5 ‰ and reached minimum values, less than -44 ‰, in the period from autumn to spring. This is well below commonly reported values for algae, which usually lie above -35 ‰ (France 1995; Peterson and Fry 1987; Vuorio et al. 2006). Nonetheless, $\delta^{13}C_{Daph}$ values closely track the seasonal cycle of surface water $\delta^{13}C_{POM}$ values (Fig. 3a), but with an average difference of 3.4 ‰ between $\delta^{13}C_{POM}$ and $\delta^{13}C_{Daph}$. Hence, $\Delta^{13}C_{POM\text{-}Daph}$ was slightly higher than values reported by del Giorgio and France (1996) for zooplankton in Canadian lakes and other published values discussed by these authors (mean difference 2.6 ‰). A positive correlation between $\delta^{13}C_{Daph}$ values and surface water chlorophyll *a* concentrations, which can serve as a proxy for algal concentrations (Sartory and Grobbelaar 1984), was observed between autumn and spring (ESM Fig. S2a). Hence, low $\delta^{13}C_{Daph}$ values coincide with relatively low concentrations of algae in surface water POM. During times when algae are less abundant, other food sources may contribute to the carbon uptake of *Daphnia* (Taipale et al. 2008). Several studies have shown that *Daphnia* and other invertebrates can incorporate CH_4-derived carbon to supplement their diet (Bastviken et al. 2003; Devlin et al. 2015; Kankaala et al. 2006). In a study of a small Finnish lake, Taipale et al. (2008) found that MOB contributed to *Daphnia*'s diet throughout the year, comprising up to 50 % of the diet in autumn. Considering the high CH_4 concentrations in Gerzensee, uptake of ^{13}C-depleted carbon by feeding on MOB seems the likely explanation for the exceptionally low $\delta^{13}C_{Daph}$ values measured in Gerzensee.

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

Field studies that compared the carbon isotopic composition of invertebrate remains in surface sediment samples with in-lake CH_4 abundance suggest that a relationship exists between the $\delta^{13}C$ values of some invertebrate groups and lake water CH_4 concentrations (Schilder et al. 2015a; van Hardenbroek et al. 2013). Within our two-year measurement period at Gerzensee, $\delta^{13}C_{Daph}$ was positively correlated with $[CH_4]_{aq}$ in the bottom waters, i.e. we recorded $\delta^{13}C_{Daph}$ values more similar to $\delta^{13}C_{POM}$ during times of high bottom water $[CH_4]_{aq}$ (ESM Fig. S2b). This is also indicated by the negative correlation between $[CH_4]_{aq}$ in the bottom waters and $\Delta^{13}C_{POM\text{-}Daph}$ (ESM Fig. S2c). Hence, during lake stratification, when $[CH_4]_{aq}$ is increasing in the hypolimnion, *Daphnia* utilise carbon whose isotopic signature is more similar to $\delta^{13}C_{POM}$ than at times when the water column is mixed. This suggests that during lake stratification, algae are the main food source of *Daphnia*, whereas *Daphnia* may rely more heavily on other food sources when the lake is mixed. In a multi-lake study, Schilder et al. (2015a) found a negative correlation between late summer $[CH_4]_{aq}$ in both bottom and surface waters, and $\delta^{13}C$ values of *Daphnia* ephippia isolated from surface sediment samples, indicating that *Daphnia* are more likely to incorporate CH_4-derived carbon in lakes with high CH_4 abundance. With regard to this relationship, our results imply that in lakes with high CH_4 accumulation during summer stratification, more ^{13}C-depleted carbon becomes accessible to the food web upon mixing and oxygenation of CH_4 in the water column, and this signal is seen in the floating ephippia. Other processes, e.g. increased transport of allochthonous organic carbon and higher associated CH_4 production may also contribute to the importance of CH_4-derived carbon for *Daphnia* in the autumn months.

Floating cladoceran and bryozoan remains

In a field study, Perga (2011) showed that there is no significant carbon isotope fractionation between *Daphnia* and their ephippia. This was confirmed in a laboratory experiment by Schilder et al. (2015b), which showed that *Daphnia* ephippia $\delta^{13}C$ values closely resemble those of *Daphnia* during ephippia production under different environmental conditions (-0.2 ±0.4 ‰ for 12°C, 1.3 ±0.3 ‰ for 20°C). In Gerzensee, $\delta^{13}C_{DaphFlot}$ remained very similar across the annual cycle (Figs. 3a, b). If no fractionation is assumed during ephippia production, this suggests that the ephippia were produced batch-wise and then floated on the lake for several months. In the data set presented here, periods when $\delta^{13}C_{DaphFlot}$

coincided with $\delta^{13}C_{Daph}$ include December 2012, June 2013, November 2013, and April 2014. This is in line with the observation that ephippia production commonly occurs in early summer and late autumn (Cáceres 1998), when environmental conditions deteriorate, e.g. there is temperature decline, crowding, oxygen depletion, or limited food availability (Korhola and Rautio 2001). Hence, in Gerzensee, changes in $\delta^{13}C_{DaphFlot}$ reflect $\delta^{13}C_{Daph}$ during ephippia production, i.e. in late autumn and/or early spring, but $\delta^{13}C_{DaphFlot}$ does not reflect seasonal changes in the diet of *Daphnia*. This observation differs from the findings of Schilder (2014) at Dutch Lake De Waay, where *Daphnia* ephippia followed the seasonal variation of *Daphnia* $\delta^{13}C$, indicating several production periods per year. The reason for the different timing of ephippia production is unclear, although it could be explained by differences in species composition between the two lakes. Regardless of the cause, our results indicate that different production intervals, and possibly switching between different production intervals over time, potentially influences *Daphnia* ephippia $\delta^{13}C$ values in sediment records.

Floating *Ceriodaphnia* ephippia show larger variability in $\delta^{13}C$ values than *Daphnia* ephippia (Fig. 3b). To our knowledge, no prior study has assessed the fractionation between maternal *Ceriodaphnia* and the chitinous structure of their resting eggs. Thus, following the simplest assumption of no (or constant) fractionation, as is observed for *Daphnia*, our results suggest that *Ceriodaphnia* ephippia are produced at several times or continuously throughout the year. No living *Ceriodaphnia* were analysed in this study, but the broad range of $\delta^{13}C_{CerioFlot}$ values (-34.3 to -50.1 ‰), which is comparable in span to the seasonal cycle of living *Daphnia* (-29.8 to -44.2 ‰), may be an indication that the changes in $\delta^{13}C_{CerioFlot}$ represent (a part of) the seasonal cycle of *Ceriodaphnia* $\delta^{13}C$.

Floating *Plumatella* statoblast $\delta^{13}C$ values also show more pronounced seasonal changes than $\delta^{13}C_{DaphFlot}$, but the variability is not as large as for $\delta^{13}C_{CerioFlot}$ (Fig. 3b). Since no living *Plumatella* zooids were analysed, no pattern of statoblast production can be determined. However, a recent study by van Hardenbroek et al. (2016) suggests that the $\delta^{13}C$ values of *Plumatella* statoblasts collected from colonies are significantly correlated with

Plumatella zooid δ^{13}C. Okamura and Hatton-Ellis (1995) found that production of statoblasts may start in mid-summer, but highest production occurs in late summer and early autumn. Overwintering statoblasts are released when colonies collapse in late autumn. Following these findings, δ^{13}C values of floating *Plumatella* statoblasts may reflect zooid δ^{13}C values during statoblast production from late summer to late autumn.

Linking δ^{13}C values of floating resting stages to those of fossil invertebrate remains

The average δ^{13}C value of floating *Daphnia* ephippia (-39.5 ‰ ± 0.9 [one standard deviation]) is in excellent agreement with the δ^{13}C value of *Daphnia* ephippia in the uppermost 1-3 cm of the sediment at the centre of the lake (-39.8 ‰; Fig. 5), and with the average δ^{13}C value of living *Daphnia* collected during the two-year fieldwork period (-39.4 ‰ ± 4.6). This confirms that δ^{13}C values of *Daphnia* ephippia in the sediment of Gerzensee reflect the δ^{13}C signal of floating *Daphnia* ephippia during the year. Seasonal changes in $\delta^{13}C_{Daph}$, however, are not recorded by floating or deposited *Daphnia* ephippia δ^{13}C values in the lake (see previous sections). This has implications for the interpretation of the sediment core record of *Daphnia* ephippia δ^{13}C values. In Gerzensee, nearly constant $\delta^{13}C_{DaphFlot}$ values throughout the year indicate batch-wise production of ephippia in autumn and/or early spring. Changes in the δ^{13}C value of sedimentary *Daphnia* ephippia may thus record changes in the importance of MOB in the diet of *Daphnia* (only) during spring and/or autumn. The latter period is indirectly coupled with the accumulation of $[CH_4]_{aq}$ during summer stratification, which is the reason for high CH_4 abundance in the water column during autumn lake mixing. Hence δ^{13}C values of sedimentary *Daphnia* ephippia may indicate the accumulation of $[CH_4]_{aq}$ during summer stratification in small European lakes like Lake Gerzensee, as suggested by Schilder et al. (2015a).

The *Ceriodaphnia* ephippia δ^{13}C value recorded in the sediment trap during 2012/13 (-42.5 ‰) is in excellent agreement with the surface sediment measurement presented here (-42.9 ‰, 1-3 cm; Fig. 5). The average flotsam δ^{13}C value (-42.9 ‰ ± 4.8) also agrees very well with the surface sediment measurement, although no full annual cycle is covered by the flotsam measurements (October to May only, Fig. 3b). Our study is the first to assess and confirm that the $\delta^{13}C$ values of *Ceriodaphnia* ephippia in the sediment can provide information about the $\delta^{13}C$ value of floating ephippia at the time of deposition.

The *Plumatella* statoblast $\delta^{13}C$ value in the surface sediment (-32.7 ‰, pooled 1-3 and 5-7 cm, this study) was distinctly less negative than the average $\delta^{13}C$ value of floating statoblasts (-36.3 ‰ ± 1.7; Fig. 5). It should, however, be noted here that summer measurements of $\delta^{13}C_{PluFlot}$ are underrepresented in our dataset, with only one measurement in July 2013 (Fig. 3b). It therefore remains uncertain whether a better representation of the summer period may resolve this mismatch between average flotsam values and the surface sediment measurement.

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

Daphnia ephippia $\delta^{13}C$ values in our sediment record (-39.8 to -37.9 ‰) were continuously below -35 ‰, whereas algae are commonly reported to have $\delta^{13}C$ values above -35 ‰ (France 1995; Peterson and Fry 1987; Vuorio et al. 2006). Our values are comparable to sedimentary *Daphnia* ephippia $\delta^{13}C$ values found in Lake De Waay, the Netherlands (Schilder 2014), but are distinctly more negative than cladoceran $\delta^{13}C$ values reported from sediment records of Lake Strandsjön, Sweden (van Hardenbroek et al. 2014) and Lake Annecy, France (Frossard et al. 2014). In sediment records covering longer time periods, van Hardenbroek et al. (2013) and Wooller et al. (2012) found $\delta^{13}C$ values over the range of all of the studies mentioned above. In a recent study in Lake Mekkojärvi, Finland, Rinta et al. (2016) showed that *Daphnia* ephippia $\delta^{13}C$ values changed abruptly from values below -45 ‰ to values greater than -40 ‰, shifts that these authors interpreted as representing changes in the availability of CH_4 in this lake. As discussed earlier, $\delta^{13}C$ values in *Daphnia* ephippia have been shown to correlate negatively with CH_4 abundance in the hypolimnia of small European lakes (Schilder et al. 2015a). Therefore, the observed *Daphnia* ephippia

$\delta^{13}C$ values below -35 ‰ suggest that $[CH_4]_{aq}$ remained high at Gerzensee throughout the record. Only the least negative $\delta^{13}C$ values, around 20 cm (AD ~1970-1980), could indicate a short period with a reduced influence of ^{13}C-depleted carbon in the diet of *Daphnia*, but $\delta^{13}C$ values remained clearly more negative than commonly reported algal $\delta^{13}C$ values. Lower *Daphnia* $\delta^{13}C$ values observed for the sediments of eutrophic and stratified lakes Gerzensee and De Waay can potentially be explained by a greater importance of CH_4-derived carbon compared to that in less nutrient-rich Lake Annecy and non-stratified Lake Strandsjön, where algae are the main carbon source of *Daphnia.* Mekkojärvi is a very small, stratified, humic lake with low oxygen concentrations within 1 m of the lake surface (Rinta et al. 2015), which may explain the high relevance of CH_4-derived carbon in its planktonic food web.

Ceriodaphnia ephippia were not analysed in any of the above-mentioned studies. *Ceriodaphnia* ephippia, however, had even more negative $\delta^{13}C$ values than *Daphnia* in the Gerzensee sediment (range -43.3 to -39.4 ‰; Fig. 5). This indicates a similar, but potentially more dominant source of ^{13}C-depleted carbon for *Ceriodaphnia* ephippia. The systematic ^{13}C-depletion of *Ceriodaphnia* ephippia relative to *Daphnia* ephippia may be caused by a difference in the average particle size that these taxa filter from the water. *Ceriodaphnia* feed on smaller particles than *Daphnia* (Geller and Müller 1981), potentially containing a higher proportion of MOB and other bacteria. The relative ^{13}C-depletion of *Ceriodaphnia* could also be caused by differences in body composition, e.g. lipid content, between taxa, as has been suggested by Matthews and Mazumder (2005) for other zooplankton species. However, C:N ratios, which may be an indicator of lipid content (Matthews and Mazumder 2005), do not show large differences between *Daphnia* and *Ceriodaphnia* ephippia (mean = 6.3 and 6.4 for the uppermost 27 cm of the sediment record, respectively). Therefore, low $\delta^{13}C$ values throughout the record suggest that, similar to *Daphnia*, *Ceriodaphnia* rather consistently incorporated a ^{13}C-depleted carbon source over the past 150+ years, at least during the season(s) of ephippia production.

Plumatella statoblast $\delta^{13}C$ values in our sediment record were distinctly less negative compared to *Daphnia* and *Ceriodaphnia* ephippia (-33.3 to -29.7 ‰; Fig. 5). This suggests that *Plumatella* zooids accessed carbon sources that were less ^{13}C-depleted compared to those on which the two cladoceran taxa relied. Similar δ^{13}C values were reported by van Hardenbroek et al. (2014), for *Plumatella* statoblasts in shallow Lake Strandsjön in Sweden (-33.1 to -28.0 ‰). Our δ^{13}C values for *Plumatella* statoblasts are well in the range of commonly reported δ^{13}C values for algae. Hence, it appears that δ^{13}C values of sedimentary *Plumatella* statoblasts can potentially provide information about the temporal evolution of algal δ^{13}C, and thus help track past changes in the lake's carbon cycle. However, a single statoblast flotsam sample from our study, collected during autumn mixing in November 2013 (Fig. 3b), yielded a δ^{13}C value of -40.0 ‰, well below the values expected for algae in Lake Gerzensee. Similarly, individual bryozoan samples with very negative δ^{13}C values have been reported in previous studies (e.g. Rinta et al. 2016; van Hardenbroek et al. 2016). This suggests that these organisms may also ingest ^{13}C-depleted carbon sources such as MOB under circumstances when they are abundant in their habitats.

In the sediment core from Gerzensee, *Daphnia* and *Ceriodaphnia* ephippia show very similar variations in their δ^{13}C values in the part of the core where both groups are present (0-27 cm; Fig. 5). This suggests that *Daphnia* and *Ceriodaphnia* were subject to similar changes in their diet over time. In contrast, *Plumatella* statoblasts show a peak in δ^{13}C values that does not coincide with the peak in the ephippia of *Daphnia* and *Ceriodaphnia*. The two groups also differ in the range of their δ^{13}C values, which points towards different food sources for *Plumatella* and the cladoceran taxa. Access to different carbon sources may occur because of differences in both habitat and particle-size preference. *Plumatella* are sessile organisms in shallow areas of lakes, whereas free-living invertebrates like *Daphnia* and *Ceriodaphnia* can reach deep-water food sources. Zooplankton in deeper water layers may feed directly on MOB, or on other organisms that feed on MOB (e.g. ciliates), which can lead to distinctly negative zooplankton δ^{13}C values (Jones and Grey 2011). In addition, Kaminski (1984) estimated that *Plumatella* can ingest particles between 5 and 17 μm in diameter, whereas *Daphnia* are able to filter particles between 0.5 and 30 μm in diameter (Geller and Müller 1981). Hence, bacteria (< 2 µm) that are not attached to larger particles may be too small to be caught in the lophophores of *Plumatella*, but may be accessible as a food source for *Daphnia*. An alternative explanation for the observed differences between cladoceran and bryozoan remains may be that both groups rely on the same carbon sources (e.g. algae, detritus, heterotrophic bacteria, and possibly MOB), but their relative importance in the diet is different. Whether one, both or more reasons are causing the observed differences in δ^{13}C values cannot be conclusively answered by this study.

Conclusions

In Gerzensee, *Daphnia* δ^{13}C values closely follow the seasonal cycle of POM δ^{13}C values, with the most negative values of *Daphnia*, down to -44.2 ‰, measured in early spring. Interestingly, *Daphnia* ephippia δ^{13}C values did not show any pronounced seasonal variations. Seasonal changes in *Daphnia* δ^{13}C values, and hence potential seasonal changes in the diet of *Daphnia*, are therefore not recorded in the δ^{13}C values of *Daphnia* ephippia in the sediments of Gerzensee. Very low δ^{13}C values of *Ceriodaphnia* ephippia in the flotsam, especially in winter (-50.1 ‰), confirm that there is a contribution of CH_4-derived carbon to the diet of the crustacean zooplankton in Gerzensee. *Plumatella* statoblasts are less depleted in ^{13}C than the two cladoceran taxa, indicating little or no influence of CH_4-derived carbon in the diet of the bryozoan, at least during the time(s) when statoblasts are produced.

The average *Ceriodaphnia* and *Daphnia* ephippia δ^{13}C values in the flotsam agree very well with the values in surface sediments, and for *Ceriodaphnia*, with measurements from a sediment trap. This shows that in Gerzensee the δ^{13}C values of ephippia in the surface sediment, and potentially in general at a certain sediment depth, reflect an integrated value for ephippia floating on the lake during a particular time span, possibly over one to two years. In contrast, average flotsam *Plumatella* statoblast δ^{13}C values were about 4 ‰ more negative than the surface sediment measurement. The reason for this mismatch may be an under-representation of the summer period in our data set. Nonetheless, this issue deserves further investigation in Gerzensee and other lakes.

Low δ^{13}C values of the two cladocerans throughout the 62-cm-long sediment core, comparable to those measured in the flotsam, indicate that in Gerzensee, *Daphnia* and *Ceriodaphnia* relied on a ^{13}C-depleted carbon source to supplement their diet throughout the past 150 years. *Daphnia* and *Ceriodaphnia* ephippia δ^{13}C values showed similar variations, with the highest values for the two taxa around the same depth. This suggests that *Daphnia* and *Ceriodaphnia* have been subject to similar changes in their diet over time. In contrast, *Plumatella* statoblast δ^{13}C values in the sediment record were again distinctly less negative, and showed a 3 ‰ increase at a different depth than the δ^{13}C values of the two cladocerans. This difference might be explained, in part, by the feeding behaviour and different habitats of the organism groups, which influences the extent to which they can incorporate CH_4-derived carbon. Our study confirms the findings of earlier studies, which indicated that taxon-specific δ^{13}C values measured on aquatic invertebrate remains can provide insights into long-term changes in the relative importance of different carbon sources. This technique can be applied to better understand the impacts of, for example, land-use change, eutrophication, and climate change on the carbon cycling in lakes. Additional studies similar to the one presented here will be needed to further constrain the effects of seasonality on the δ^{13}C values of fossil statoblast and ephippia samples, and to assess the extent to which seasonal patterns observed in Gerzensee are representative of those in other lake ecosystems.

Acknowledgements

We thank Clemens Stampfli, Studienzentrum Gerzensee and the Stiftung der Schweizerischen Nationalbank for granting access to Gerzensee, Daniel Steiner for assistance with chlorophyll *a* measurements and Carole Adolf for providing the sediment trap material. This research was supported by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. 239858 (RECONMET).

References

Albrecht A, Reiser R, Lück A, Stoll J-MA, Giger W (1998) Radiocesium dating of sediments from lakes and reservoirs of different hydrological regimes. Environ Sci Technol 32:1882-1887

Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 1: Basin analysis, coring, and chronological techniques. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 171-203

Appleby PG, Oldfield F (1978) The calculation of lead-210 dates assuming a constant rate of supply of unsupported ^{210}Pb to the sediment. Catena 5:1-8

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

Belle S, Parent C, Frossard V, Verneaux V, Millet L, Chronopoulou P-M, Sabatier P, Magny M (2014) Temporal changes in the contribution of methane-oxidizing bacteria to the biomass of chironomid larvae determined using stable carbon isotopes and ancient DNA. J Paleolimnol 52:215-228

Bundesamt für Meteorologie und Klimatologie MeteoSchweiz (2014) Homogene Monatsdaten. Available at: http://www.meteoschweiz.admin.ch/home/klima/vergangenheit/homogene-monatsdaten.html (2015)

Bunn SE, Boon PI (1993) What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96:85-94

Cáceres CE (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79:1699-1710

Del Giorgio PA, France RL (1996) Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. Limnol Oceanogr 41:359-365

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495-506

Devlin SP, Saarenheimo J, Syväranta J, Jones RI (2015) Top consumer abundance influences lake methane efflux. Nat Commun 6:8787

EAWAG (1995) Protokoll zur Bestimmung von Chlorophyll *a* mit Photometrie. Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dübendorf, Switzerland

Edmondson WT (1957) Trophic relations of the zooplankton. Trans Am Microsc Soc 76:225-245

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40:1310-1313

Francis DR (2001) Bryozoan statoblasts. In Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments, Volume 4: Zoological Indicators. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 105-123

Frossard V, Verneaux V, Millet L, Jenny J-P, Arnaud F, Magny M, Perga M-E (2014) Reconstructing long-term changes (150 years) in the carbon cycle of a clear-water lake based on the stable carbon isotope composition ($\delta^{13}C$) of chironomid and cladoceran subfossil remains. Freshw Biol 59:789-802

Fry B (2006) Stable Isotope Ecology, Springer, New York, USA

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

Grey J (2016) The incredible lightness of being methane-fuelled: stable isotopes reveal alternative energy pathways in aquatic ecosystems and beyond. Front Ecol Evol 4(8):1-14

Heiri O, Schilder J, van Hardenbroek M (2012) Stable isotopic analysis of fossil chironomids as an approach to environmental reconstruction: state of development and future challenges. Fauna Nor 31:7-18

Jones RI, Grey J (2011) Biogenic methane in freshwater food webs. Freshw Biol 56:213-229

Jones RI, Grey J, Sleep D, Arvola L (1999) Stable isotope analysis of zooplankton carbon nutrition in humic lakes. OIKOS 86:97-104

Kaminski M (1984) Food composition of three bryozoan species (Bryozoa, Phylactolaemata) in a mesotrophic lake. Pol Arch Hydrobiol 31:45-53

Kankaala P, Taipale S, Grey J, Sonninen E, Arvola L, Jones RI (2006) Experimental $\delta^{13}C$ evidence for a contribution of methane to pelagic food webs in lakes. Limnol Oceanogr 51:2821-2827

Korhola A, Rautio M (2001) Cladocera and other branchiopod crustaceans. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments, Volume 4: Zoological Indicators. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 5-41

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

Lotter AF, Sturm M, Teranes JL, Wehrli B (1997) Varve formation since 1885 and high-resolution varve analyses in hypertrophic Baldeggersee (Switzerland). Aquat Sci 59:304-325

Lotter AF, Birks HJB, Eicher U, Hofmann W, Schwander J, Wick L (2000) Younger Dryas and Allerød summer temperatures at Gerzensee (Switzerland) inferred from fossil pollen and cladoceran assemblages. Palaeogeogr Palaeoclimatol Palaeoecol 159:349-361

Matthews B, Mazumder A (2005) Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton δ^{13}C. Freshw Biol 50:502-515

Okamura B, Hatton-Ellis T (1995) Population biology of bryozoans: correlates of sessile, colonial life histories in freshwater habitats. Experientia 51:510-525

Perga M-E (2009) Potential of δ^{13}C and δ^{15}N of cladoceran subfossil exoskeletons for paleo-ecological studies. J Paleolimnol 44:387-395

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

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293-320

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: http://www.r-project.org

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 δ^{13}C values of methane and dissolved inorganic carbon in small European lakes. Aquat Sci 77:667-680

Rinta P, van Hardenbroek M, Jones RI, Kankaala P, Rey F, Szidat S, Wooller MJ, Heiri O (2016) Land use affects carbon sources to the pelagic food web in a small boreal lake. PLoS ONE 11(8):e0159900

Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 14:177-187

Schilder J (2014) Methanogenic carbon in *Daphnia* tissue as an indicator of lacustrine methane budgets. Observations from the field, experiments and fossil records. PhD-Thesis, University of Bern

Schilder J, Bastviken D, van Hardenbroek M, Heiri O (2016) Spatiotemporal patterns in methane flux and gas transfer velocity at low wind speeds: Implications for upscaling studies on small lakes. J Geophys Res Biogeosci 121:1-12

Schilder J, Bastviken D, van Hardenbroek M, Leuenberger M, Rinta P, Stötter T, Heiri O (2015a) The δ^{13}C values of *Daphnia* ephippia reflect in-lake methane availability. Limnol Oceanogr 60:1064-1075

Schilder J, Tellenbach C, Möst M, Spaak P, van Hardenbroek M, Wooller M, Heiri O (2015b) The stable isotopic composition of *Daphnia* ephippia reflects changes in δ^{13}C and δ^{18}O values of food and water. Biogeosciences 12:3819-3830

Schwoerbel J (1994) Methoden der Hydrobiologie, Süßwasserbiologie. Gustav Fischer Verlag, Stuttgart, Germany (UTB 979).

Seddon AW, Mackay AW, Baker AG, Birks HJB, Breman E, Buck CE, Ellis EC, Froyd CA, Gill JL, Gillson L (2014) Looking forward through the past: Identification of 50 priority research questions in palaeoecology. J Ecol 101(1):256-267

Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural waters. Wiley, New York, USA

Taipale S, Kankaala P, Jones RI (2007) Contributions of different organic carbon sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: Results from mesocosm DI^{13}C-additions. Ecosystems 10:757-772

Taipale S, Kankaala P, Tiirola M, Jones RI (2008) Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. Ecology 89:463-474

Turney CSM (1999) Lacustrine bulk organic δ^{13}C in the British Isles during the last glacial – Holocene transition (14-9 ka ^{14}C BP). Arct, Antarc, Alp Res 31:71-81

Vandekerkhove J, Declerck S, Vanhove M, Brendonck L, Jeppesen E, Porcuna Conde JM, De Meester L (2004) Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls. J Limnol 63 (Suppl. 1):75-84

Van Hardenbroek M, Leuenberger M, Hartikainen H, Okamura B, Heiri O (2016) Bryozoan stable carbon and hydrogen isotopes: relationships between the isotopic composition of zooids, statoblasts and lake water. Hydrobiologia 765:209-223

Van Hardenbroek M, Lotter AF, Bastviken D, Andersen TJ, Heiri O (2014) Taxon-specific $\delta^{13}C$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden. J Paleolimnol 52:95-105

Van Hardenbroek M, Heiri O, Grey J, Bodelier PLE, Verbruggen F, Lotter AF (2010) Fossil chironomid $\delta^{13}C$ as a proxy for past methanogenic contribution to benthic food webs in lakes? J Paleolimnol, 43:235-245

Van Hardenbroek M, Heiri O, Parmentier FJW, Bastviken D, Ilyashuk BP, Wiklund JA, Hall RI, Lotter AF (2013) Evidence for past variations in methane availability in a Siberian thermokarst lake based on $\delta^{13}C$ of chitinous invertebrate remains. Quat Sci Rev 66:74-84

Verbruggen F, Heiri O, Reichart G-J, Lotter AF (2010) Chironomid $\delta^{18}O$ as a proxy for past lake water $\delta^{18}O$: A lateglacial record from Rotsee (Switzerland). Quat Sci Rev 29:2271-2279

Vuorio K, Meili M, Sarvala J (2006) Taxon-specific variation in the stable isotopic signatures (d13C and d15N) of lake phytoplankton. Freshw Biol 51:807-822

Wood TS, Okamura B (2005) A new key to the freshwater bryozoans of Britain, Ireland and Continental Europe, with notes on their ecology. Freshwater Biological Association, London, UK

Wooller MJ, Pohlman JW, Gaglioti BV, Langdon P, Jones M, Walter Anthony KM, Becker KW, Hinrichs K-U, Elvert M (2012) Reconstruction of past methane availability in an Arctic Alaska wetland indicates climate influenced methane release during the past ~12,000 years. J Paleolimnol 48:27-42

Zeh M, Scheidegger D, Hoehn E, Zbären D, Maurer V (2004) Kleinseen 2003. Amt für Gewässerschutz und Abfallwirtschaft des Kantons Bern, Gewässer- und Bodenschutzlabor (GBL) (eds), Bern, Switzerland

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 $\delta^{13}C$

analysis. Measurements may represent remains from up to 6 cm of sediment (see text for details.

Figures

Figure 1

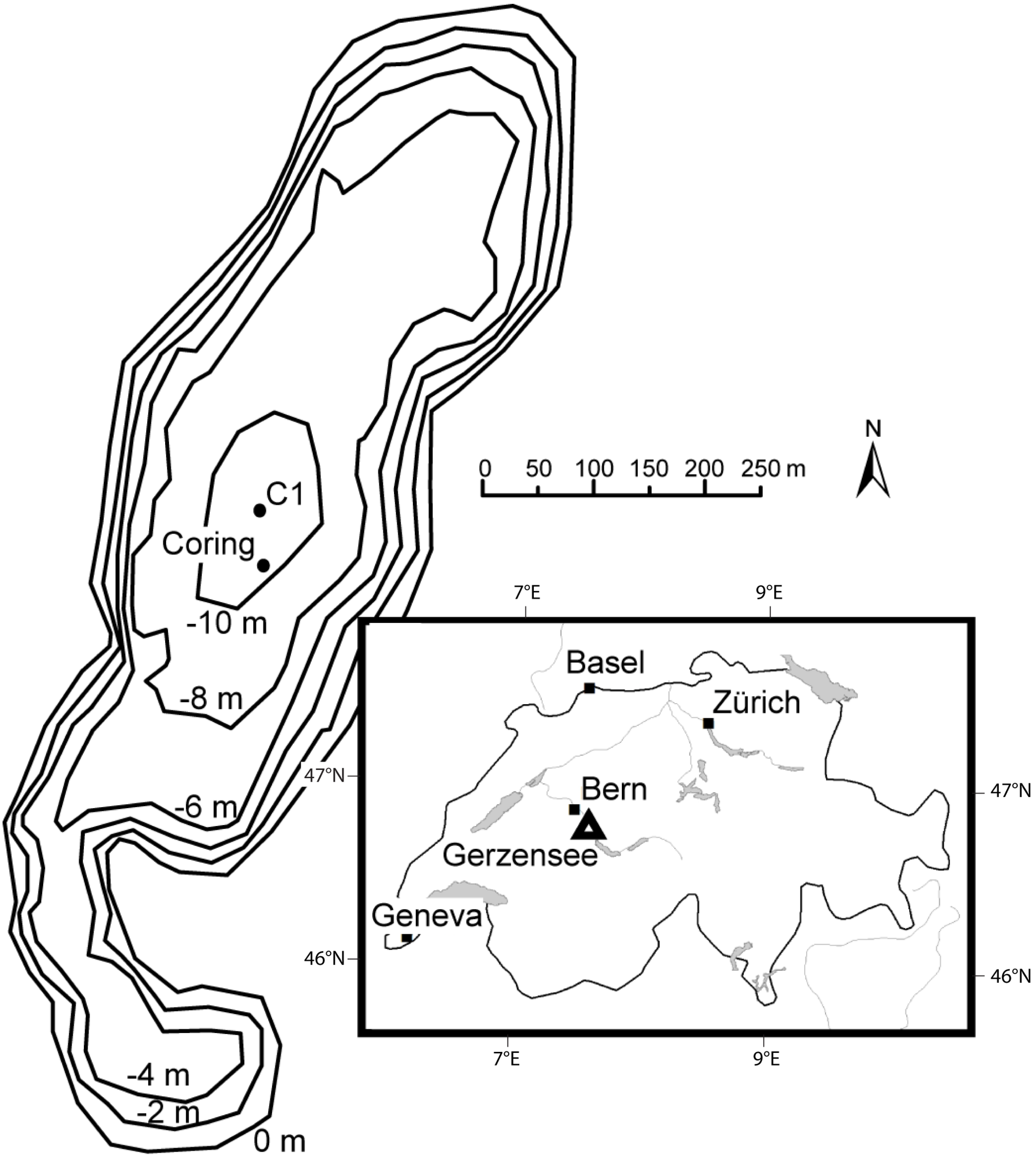


Figure 2

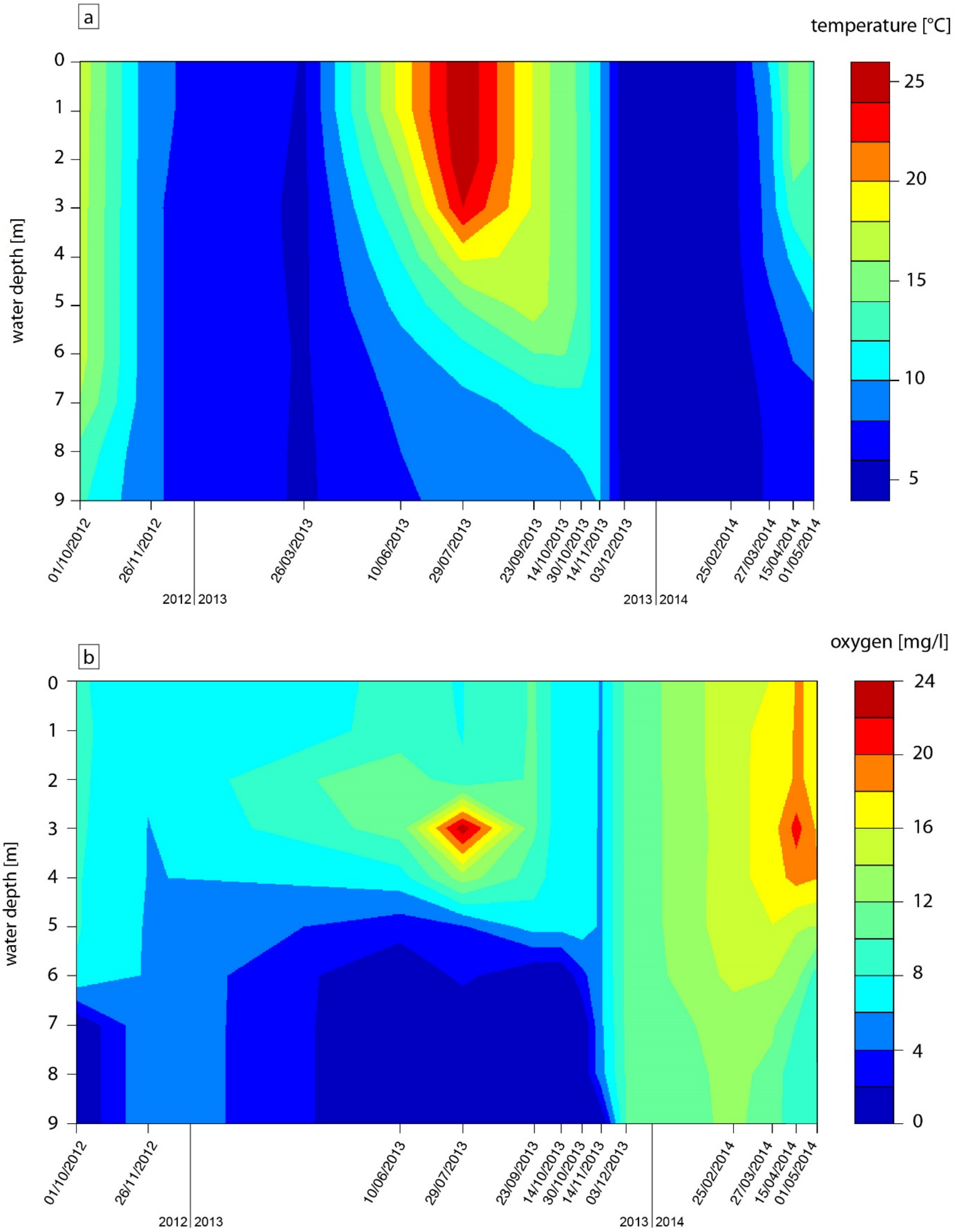

a
temperature [°C]
25
20
15
10
5
water depth [m]
0
1
2
3
4
5
6
7
8
9
01/10/2012
26/11/2012
26/03/2013
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
2012 | 2013
2013 | 2014
b
oxygen [mg/l]
24
20
16
12
8
4
0
water depth [m]
0
1
2
3
4
5
6
7
8
9
01/10/2012
26/11/2012
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
2012 | 2013
2013 | 2014

Figure 3

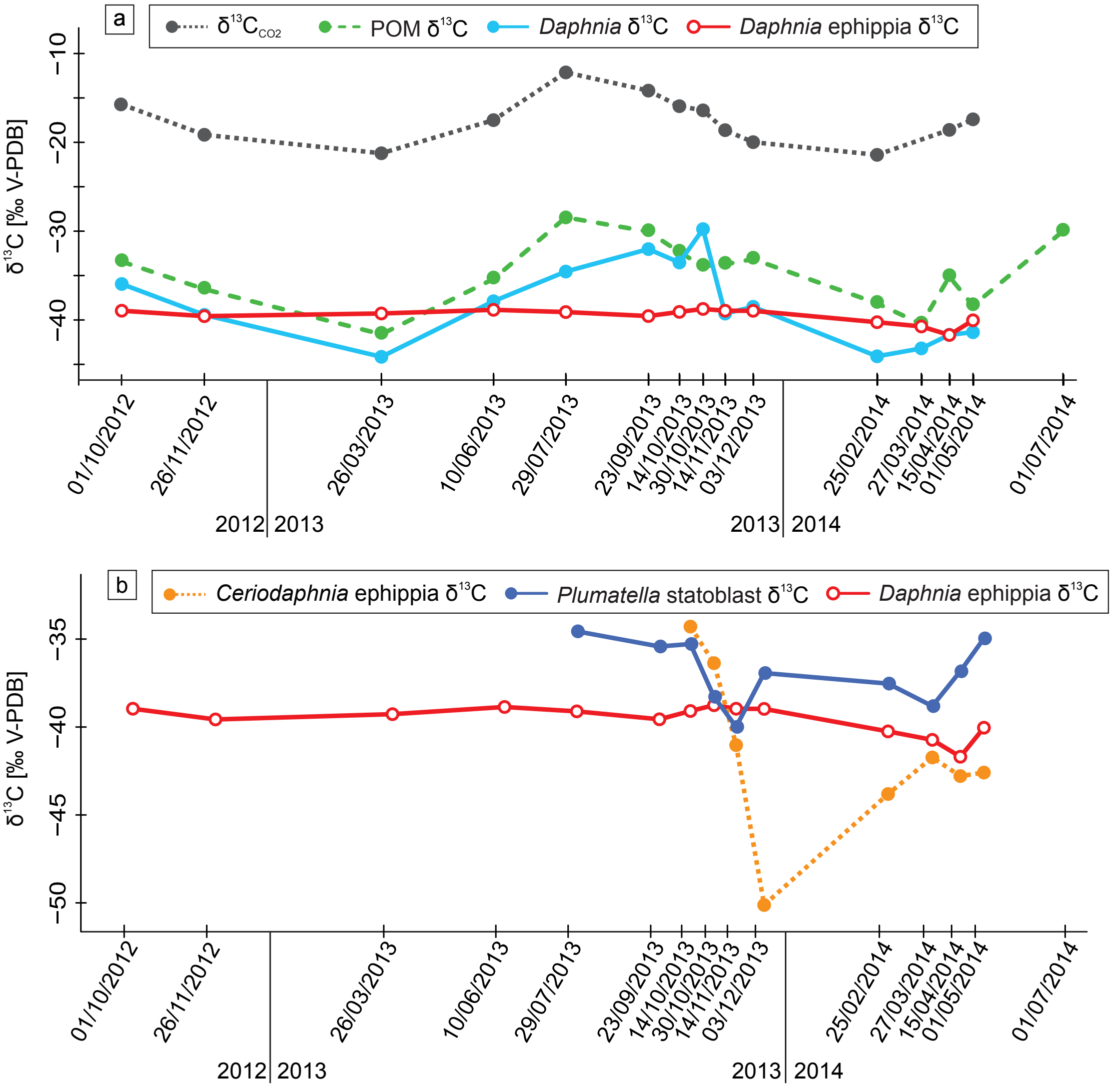

a
δ13CCO2
POM δ13C
Daphnia δ13C
Daphnia ephippia δ13C
δ13C [‰ V-PDB]
−10
−20
−30
−40
01/10/2012
26/11/2012
26/03/2013
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
01/07/2014
2012
2013
2013
2014
b
Ceriodaphnia ephippia δ13C
Plumatella statoblast δ13C
Daphnia ephippia δ13C
δ13C [‰ V-PDB]
−35
−40
−45
−50
01/10/2012
26/11/2012
26/03/2013
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
01/07/2014
2012
2013
2013
2014

Figure 4

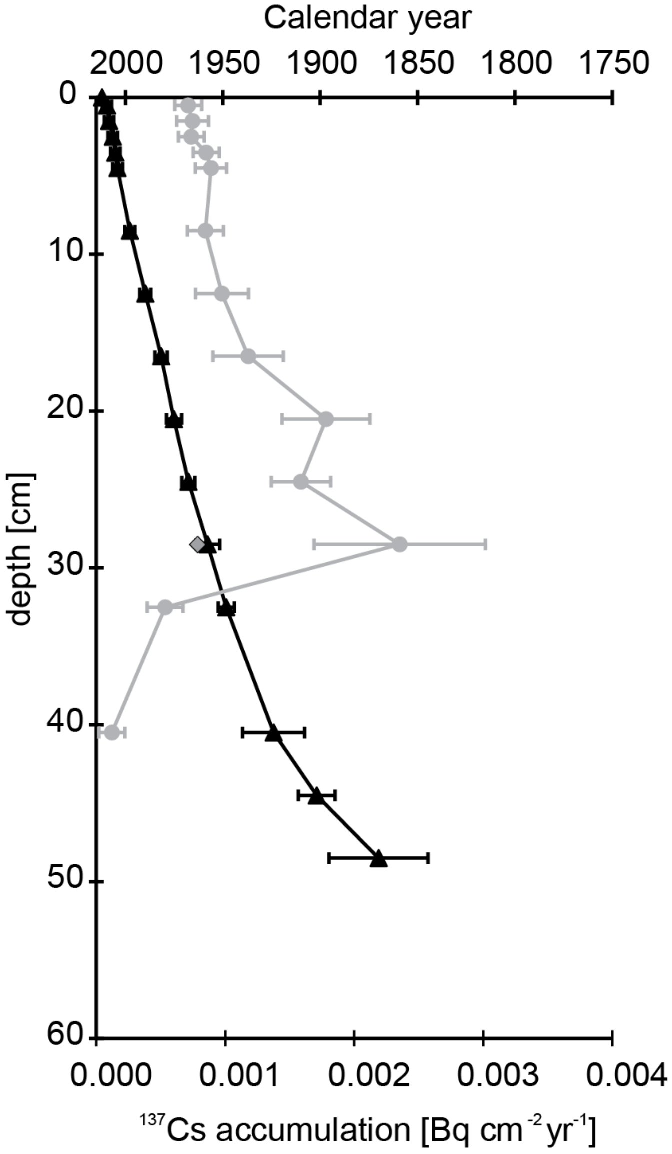
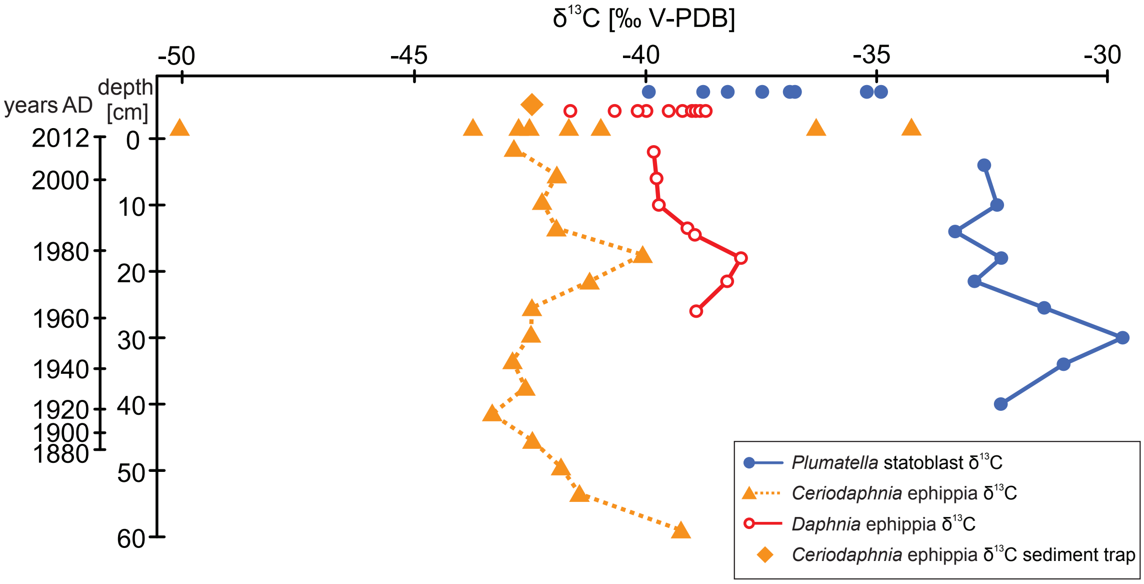

Calendar year
2000
1950
1900
1850
1800
1750
0
10
20
30
40
50
60
depth [cm]
0.000
0.001
0.002
0.003
0.004
^{137}Cs accumulation [Bq cm^{-2} yr^{-1}]

Figure 5


δ13C [‰ V-PDB]
-50
-45
-40
-35
-30
years AD
depth [cm]
2012
2000
1980
1960
1940
1920
1900
1880
0
10
20
30
40
50
60
Plumatella statoblast δ13C
Ceriodaphnia ephippia δ13C
Daphnia ephippia δ13C
Ceriodaphnia ephippia δ13C sediment trap

Electronic Supplementary Material

Figure Captions

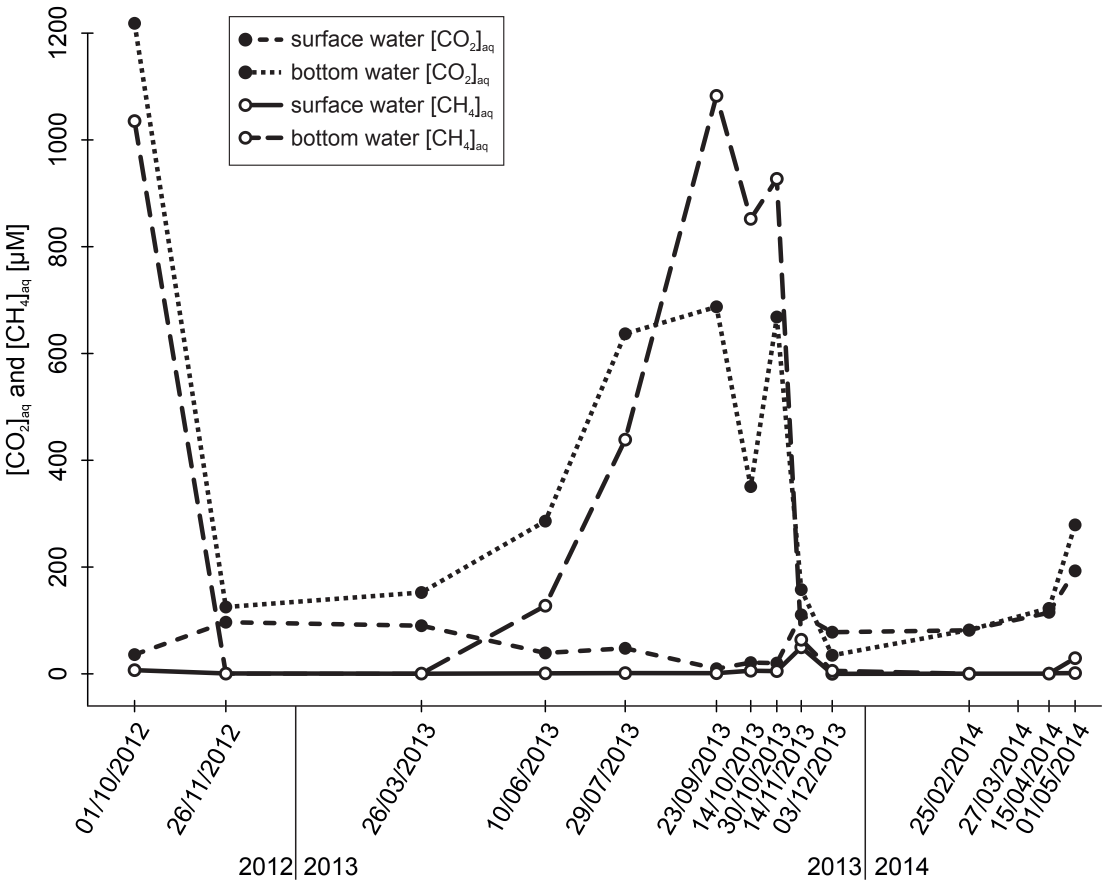
Fig. S1

Dissolved CO_2 ($[CO_2]_{aq}$) and CH_4 ($[CH_4]_{aq}$) concentrations for surface (0.7 m water depth) and bottom waters (8 m water depth) at the lake centre (location C1) over the fieldwork period in 2012-2014

Fig. S2

(a) Relationship between surface water chlorophyll *a* concentrations (0.7 m water depth) and *Daphnia* $\delta^{13}C$ at the lake centre (location C1); (b) relationship between log-transformed bottom water dissolved CH_4 concentrations ($[CH_4]_{aq}$) (8 m water depth) and *Daphnia* $\delta^{13}C$ at the lake centre (location C1); (c) relationship of the difference between particulate organic matter (POM) $\delta^{13}C$ and *Daphnia* $\delta^{13}C$ in the surface water ($\Delta^{13}C_{POM\text{-}Daph}$) and bottom water (8 m water depth) $[CH_4]_{aq}$; (d) surface (0.7 m water depth) and bottom water (8 m water depth) POM $\delta^{13}C$ at the lake centre (location C1)

Figure S1



surface water $[CO_2]_{aq}$
bottom water $[CO_2]_{aq}$
surface water $[CH_4]_{aq}$
bottom water $[CH_4]_{aq}$
$[CO_2]_{aq}$ and $[CH_4]_{aq}$ [µM]
0
200
400
600
800
1000
1200
01/10/2012
26/11/2012
26/03/2013
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
2012
2013
2013
2014

Figure S2

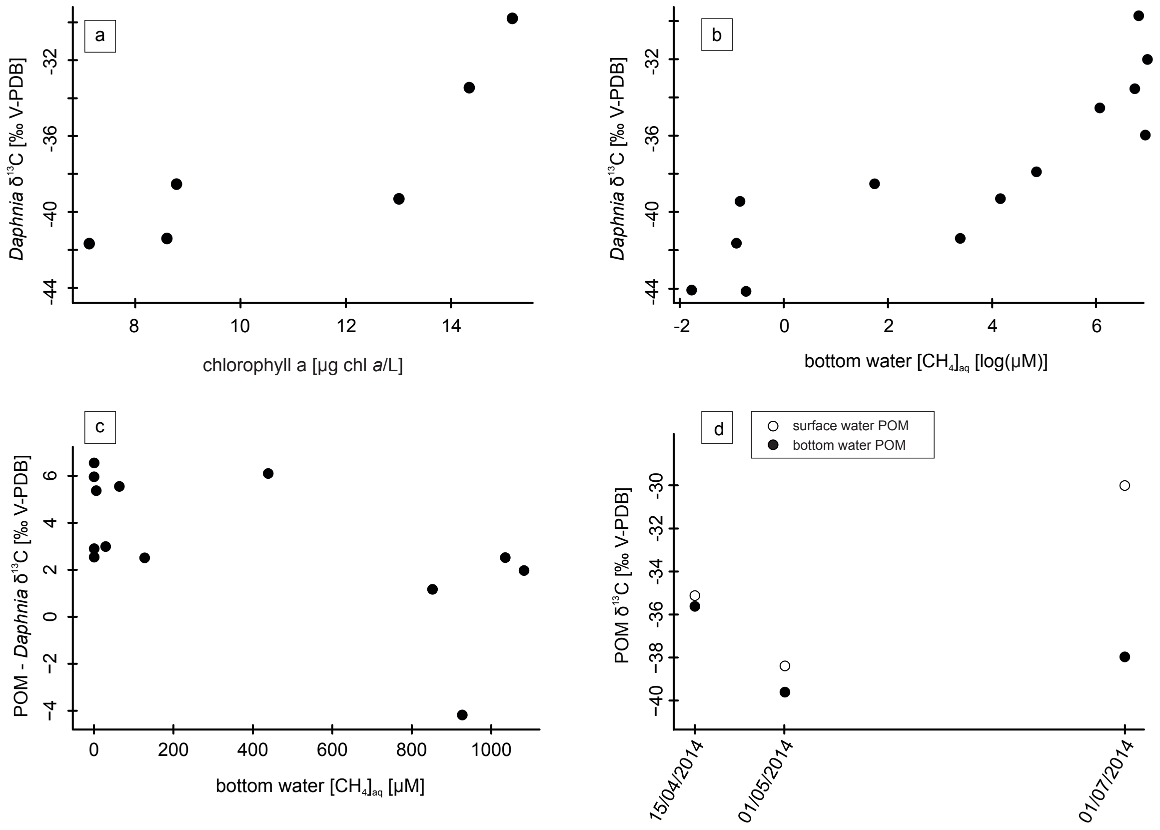

a
Daphnia δ13C [‰ V-PDB]
-32
-36
-40
-44
8
10
12
14
chlorophyll a [µg chl a/L]
b
Daphnia δ13C [‰ V-PDB]
-32
-36
-40
-44
-2
0
2
4
6
bottom water [CH4]aq [log(µM)]
c
POM - Daphnia δ13C [‰ V-PDB]
6
4
2
0
−2
−4
0
200
400
600
800
1000
bottom water [CH4]aq [µM]
d
○ surface water POM
● bottom water POM
POM δ13C [‰ V-PDB]
−30
−32
−34
−36
−38
−40
15/04/2014
01/05/2014
01/07/2014

Table S1 Samples collected and analysed in the course of repeated fieldwork on Gerzensee between October 2012 and July 2014. “x” denotes that a sample was collected, “-“ shows where no sample was collected

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