The stable carbon isotopic composition of *Daphnia* ephippia in small, temperate lakes reflects in-lake methane availability

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Abstract

Daphnia can ingest methane-oxidizing bacteria and incorporate methanogenic carbon into their biomass, leading to low stable carbon isotope ratios (expressed as $\delta^{13}C$ values) of their tissue. Therefore, $\delta^{13}C$ analysis of *Daphnia* resting eggs (ephippia) in lake sediment records can potentially be used to reconstruct past in-lake availability of methane (CH_4). However, detailed multilake studies demonstrating that $\delta^{13}C$ values of recently deposited *Daphnia* ephippia ($\delta^{13}C_{ephippia}$) are systematically related to in-lake CH_4 concentrations ($[CH_4]_{aq}$) are still missing. We measured $\delta^{13}C_{ephippia}$ from surface sediments of 15 small lakes in Europe, and compared these values with late-summer $[CH_4]_{aq}$. $\delta^{13}C_{ephippia}$ ranged from $-51.6‰$ to $-25.9‰$, and was strongly correlated with $[CH_4]_{aq}$ in the surface water and above the sediment (r -0.73 and -0.77, respectively), whereas a negative rather than the expected positive correlation was found with $\delta^{13}C$ values of carbon dioxide (CO_2) (r -0.54), and no correlation was observed with $[CO_2]_{aq}$. At eight sites, offsets between $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{ephippia}$ exceeded offsets between $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{algae}$ reported in literature. $\delta^{13}C_{ephippia}$ was positively correlated with $\delta^{13}C$ values of sedimentary organic matter (r 0.54), but up to 20.7‰ lower in all except one of the lakes (average $-6.1‰$). We conclude that incorporation of methanogenic carbon prior to ephippia formation must have been widespread by *Daphnia* in our study lakes, especially those with high $[CH_4]_{aq}$. Our results suggest a systematic relationship between $\delta^{13}C_{ephippia}$ values and $[CH_4]_{aq}$ in small temperate lakes, and that $\delta^{13}C_{ephippia}$ analysis on sediment records may provide insights into past changes in in-lake $[CH_4]_{aq}$.

Lakes represent the second largest natural source of the greenhouse gas methane (CH_4) to the atmosphere, and future CH_4 emissions from lakes may be affected by changing climatic conditions such as increasing temperature (Yvon-Durocher et al. 2014) or variations in lake productivity associated with e.g., changes in nutrient concentrations in lakes (Juutinen et al. 2009). At present, however, long-term time series of carbon fluxes to and from lakes are rare (e.g., Lepistö et al. 2014). Paleolimnological approaches that provide insight into past variations of CH_4 availability in lakes would increase our understanding of the timing and degree of the response of lake CH_4 production and emissions to changing environmental conditions.

Biogenic CH_4 has a distinct stable carbon isotopic composition (expressed as $\delta^{13}C$ values), with values typically between $-80‰$ and $-50‰$ (Jedrysek 2005). CH_4 in lakes can be oxidized by methane-oxidizing bacteria (MOB), after

which the methanogenic carbon is once again bound in organic matter and available to the food web (Frenzel et al. 1990). MOB preferentially incorporate ^{12}C over ^{13}C, resulting in even lower $\delta^{13}C$ values of MOB biomass than recorded for CH_4 ($-100‰$ to $-55‰$) (Templeton et al. 2006). Field studies have demonstrated that $\delta^{13}C$ values of a number of aquatic invertebrate groups, including water fleas of the genus *Daphnia*, become lower if MOB form part of their diet (Kankaala et al. 2010; Ravinet et al. 2010). Low $\delta^{13}C$ values of fossil remains of these organism groups may therefore indicate periods in the past in which MOB were more abundant, or more accessible to these invertebrates, which in turn may be related to higher CH_4 availability in the examined lake ecosystems (van Hardenbroek et al. 2013).

Several recent studies have interpreted variations in $\delta^{13}C$ values of chitinous invertebrate remains in lake sediment records as an indication of changes in CH_4 availability in lakes (Wooller et al. 2012; van Hardenbroek et al. 2013; Frossard et al. 2014). Van Hardenbroek et al. (2013) reported a

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negative relationship between $\delta^{13}C$ values of the recently deposited resting eggs (ephippia) of *Daphnia* in lake surface sediments ($\delta^{13}C_{ephippia}$) and diffusive flux of CH_4 from the water surface to the atmosphere in boreal and tundra lakes. These results suggest that it may be possible to reconstruct past variations in CH_4 abundance (e.g., concentrations or fluxes) in lakes based on $\delta^{13}C_{ephippia}$ values. However, the lakes studied by van Hardenbroek et al. (2013) are located in south central Sweden and eastern Siberia, two regions which differ in their bedrock, catchment vegetation, and climatic conditions, and they represent two different lake types (boreal lakes of glacial origin and shallow thermokarst lakes). In their survey, van Hardenbroek et al. (2013) did not analyse in-lake CH_4 concentrations ($[CH_4]_{aq}$) to confirm the proposed relationship between $\delta^{13}C_{ephippia}$ values and in-lake CH_4 availability. Furthermore, no information about dissolved inorganic carbon (DIC) $\delta^{13}C$ values ($\delta^{13}C_{DIC}$) or those of CO_2 ($\delta^{13}C_{CO_2}$) in the lake water was available. Baseline $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CO_2}$ values are expected to have a strong influence on the $\delta^{13}C$ value of planktonic algae ($\delta^{13}C_{algae}$), the preferred food source of *Daphnia* (Lampert 2011; Smyntek et al. 2012). In addition, the concentration of CO_2 in the lake water ($[CO_2]_{aq}$) has a strong effect on the carbon isotope fractionation during the uptake of CO_2 by algae (e.g., Laws et al. 1995). Therefore, variations in $[CO_2]_{aq}$ and $\delta^{13}C_{DIC}$ between lakes, if covarying with CH_4 availability, could also potentially explain apparent relationships between diffusive CH_4 flux and $\delta^{13}C_{ephippia}$ values without the need to invoke MOB as a food source for these organisms. Van Hardenbroek et al. (2013) note that the $\delta^{13}C_{ephippia}$ values they present ($-37.8‰$ to $-27.0‰$) are within the range possible for algae growing within lakes. Conclusive data that show that the variability in $\delta^{13}C$ values of ephippia recovered from lake sediments is driven by $[CH_4]_{aq}$ rather than by variability in $\delta^{13}C$ values of photosynthetic primary producers are still lacking and necessary to demonstrate that $\delta^{13}C_{ephippia}$ values can be used as an indicator of past CH_4 availability in lakes.

Here, we present $\delta^{13}C_{ephippia}$ measurements from the surface sediments of 15 lakes in northern, western, and central Europe that differ widely in their $[CH_4]_{aq}$. In all of these lakes $[CH_4]_{aq}$, $[DIC]$, and $\delta^{13}C_{DIC}$ values were analyzed at different depths in the water column. Furthermore, basic limnological measurements and analyses of bulk sedimentary organic matter (SOM) $\delta^{13}C$ values ($\delta^{13}C_{SOM}$) were conducted. We use this dataset for a rigorous test of the hypothesis that $\delta^{13}C_{ephippia}$ values reflect $[CH_4]_{aq}$ in lakes. We also assess whether any apparent relationships with $[CH_4]_{aq}$ in our dataset can be explained by alternative hypotheses. Specifically, we examine whether $\delta^{13}C_{ephippia}$ values can be explained by variations in lake water $[DIC]$, $[CO_2]_{aq}$, $\delta^{13}C_{DIC}$, $\delta^{13}C_{CO_2}$, or $\delta^{13}C_{SOM}$, which are expected to be at least partly related to $\delta^{13}C$ values of suspended particulate organic matter (POM) available as food for *Daphnia* in the water column (Meyers 1994).

Methods

Sediment samples originate from a campaign studying lake sediments and limnological characteristics of 32 lakes in Finland, Sweden, Germany, the Netherlands, and Switzerland in August and September of 2010 and 2011 (Rinta et al. 2014). Here we present $\delta^{13}C_{ephippia}$ values from sediments within this survey that had high enough ephippia abundances to provide the sample weight necessary for our analyses (> 70 μg per sample).

Sediment sampling and treatment

On each lake, a gravity corer (UWITEC, Austria) was used to obtain replicate surface sediment samples (top 0-2 cm) in the deepest (typically central) part of the lake, in the nearshore waters (typically at 1-4 m water depth, just beyond emerging macrophytes), and, in all except one lake (Mekkojärvi), at an intermediate depth (just below the thermocline or, if the lake was not stratified, at a water depth halfway between the central and nearshore samples). These sediment samples were freeze dried and stored dry and dark until analysis. As deep-water sediments are most likely to represent organic matter produced by phytoplankton or other POM available for *Daphnia* (as opposed to terrestrial organic matter or littoral production), a small aliquot of the freeze dried sediments from the deep-water sediments was prepared for analysis of $\delta^{13}C_{SOM}$ values. It was exposed to 2.5% HCl solution for six hours (18 hours for the carbonate-rich Swiss lakes) and thoroughly rinsed, centrifuged (3000 rpm) and decanted five times to remove carbonates (Meyers and Teranes 2001) before being freeze dried again. Samples were then loaded into tin cups (8 × 5 mm; Elemental Microanalysis, United Kingdom) for $\delta^{13}C$ analysis. Additional sediment was deflocculated in 10% KOH for two hours at room temperature and sieved with a 100 μm mesh size. The sieving residue was then exposed to NH_4Cl solution (2 mol L^{-1}) buffered with NaOH (0.35 mol L^{-1}) for 20 h to remove carbonates at neutral pH (Verbruggen et al. 2010). Then, 70-120 μg of *Daphnia* ephippia (if present in sufficient numbers) were picked into silver cups (6 × 4 mm; Säntis, Switzerland) under a dissecting microscope (magnification x20–x50) for analysis of $\delta^{13}C_{ephippia}$ values. During this process any sediment attached to the ephippia was carefully removed with fine forceps. On a regular basis sample material was examined under a binocular with a polarisator to confirm there were no carbonate particles attached to the ephippia.

Gas sampling and water chemistry

The gas sampling and water chemistry results are described in detail in Rinta et al. (2014). In the deepest part of the lakes, a vertical profile of water temperature and oxygen concentrations ($[O_2]_{aq}$) was measured with an oxygen and temperature sensor (WTW CellOx® 325 oxi1970i, Germany). With a 5 L water sampler (UWITEC, Austria), water samples from the surface waters (0.7 m below the surface) and bottom waters (one meter above the sediment) were then taken. A 60 mL

subsample was injected into a prepepared 118 mL glass vial with a 10 mm thick massive butyl rubber stopper (Apodan, Denmark), leading to an overpressure in the vials. Before fieldwork, 0.2 mL H_3PO_4 was added to each of these vials in the laboratory after which they were evacuated and flushed with N_2 gas (Carbagas, Switzerland) three times to ensure a CO_2-free headspace. The H_3PO_4 was added as a preservative that also converts DIC in the sample to gaseous and dissolved CO_2. After evacuating and filling with N_2 gas for the third time, a needle was used to pierce the stopper and allow for pressure equilibration with the surrounding air (pressure recorded) by release of excess N_2. In the field, a 60 mL sample of water from approximately 10 cm above the sediment was taken from one of the sediment cores obtained at the deepest part of the lake using a small rubber tube attached to a syringe with a three-way valve. The water in the tube was flushed and care was taken to ensure no air remained in the tube and syringe before 60 mL of water was sampled and injected into 118 mL glass vials preprepared as described above. Gas samples were stored in the dark for 3-12 weeks, after which the CH_4 and CO_2 concentrations in the headspace of the vials were analyzed by gas chromatography using a flame ionization detector with a methanizer (GC-FID; Shimadzu GC-8, PoropackN column). Samples from the Finnish lakes were measured on a gas chromatograph with an autosampler (Agilent 6890 N, PlotQ capillary column, with FID for CH_4 and TCD for CO_2). Using Henry's law allowed us to back-calculate lake water $[CH_4]_{aq}$ and [DIC] using the CH_4 and CO_2 concentrations in the sample headspace. $[H_2CO_3^*]$, $[HCO_3^-]$, and $[CO_3^{2-}]$ were calculated following Stumm and Morgan (1996), based on DIC concentrations and lake water pH. $[H_2CO_3^*]$ is defined as $[H_2CO_3^{2-}] + [CO_2]_{aq}$ and, given the low $[H_2CO_3^{2-}]$ to $[CO_2]_{aq}$ ratio (Stumm and Morgan 1996), interpreted as $[CO_2]_{aq}$. The temperature dependency of the carbonate system equilibrium constants was accounted for by linearly interpolating the values for 10°C, 15°C, 20°C, and 25°C provided by Stumm and Morgan (1996). $\delta^{13}C_{CO_2}$ in the vial headspace was then analyzed to assess $\delta^{13}C_{DIC}$. $\delta^{13}C_{DIC}$ values were corrected to account for the fractionation between the aqueous and gaseous CO_2 in the vials (Assayag et al. 2006). As $\delta^{13}C_{CO_2}$ values are typically lower than $\delta^{13}C_{DIC}$ values, and CO_2 is the most important source of carbon for algae (Farquhar et al. 1989), $\delta^{13}C_{CO_2}$ was also calculated. When $\delta^{13}C_{DIC}$, $[H_2CO_3^*]$, $[HCO_3^-]$, $[CO_3^{2-}]$, and temperature are known, the $^{13}C : {}^{12}C$ ratio of $H_2CO_3^*$ can be calculated following Stumm and Morgan (1996), taking the temperature dependence of associated equilibrium constants into account (Deines et al. 1974).

Mass spectrometry

$\delta^{13}C_{SOM}$ was analyzed at the UC Davis Stable Isotope Facility (California, U.S.A.) on an Elementar Vario EL Cube or Micro Cube elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS). Ana-

lytical precision based on replicate standard (bovine liver, glutamic acid, and nylon) measurements was ± 0.05‰ ($n = 50$). $\delta^{13}C_{DIC}$ was measured at the Division of Climate and Environmental Physics of the Physics Institute of the University of Bern, Switzerland (Rinta et al. 2014). The amount of gas injected into a helium carrier gas stream was dependent on the CO_2 concentration, and ranged from 0.01 mL to 2.5 mL from the sample headspace. The sample was then dried in dry ice ethanol mixture (−78°C). CO_2 was cryogenically separated from the sample gas mixture by means of liquid nitrogen. Afterwards, CO_2 was volatilized and via a helium carrier gas stream transferred to the IRMS. The IRMS used was a ThermoFinnigan MAT Delta Plus XL with several special characteristics for low-volume trace gas isotope analysis described by Leuenberger et al. (2000a,b). Accuracy and precision of the isotope ratio measurements was better than 0.3‰.

$\delta^{13}C_{ephippia}$ was measured on a setup designed for triple stable isotope ratio measurements (C, O, H) at the Division of Climate and Environmental Physics of the Physics Institute of the University of Bern, Switzerland. A truly online system consisting of a High Temperature Elemental Analyzer (ThermoFinnigan) coupled to an Isoprime mass spectrometer was used. Pyrolysis temperature was set to 1450°C. Due to the instrumental setup and the relatively small (~ 70 μg to 120 μg) samples of fossil material, the precision associated with the $\delta^{13}C$ measurements is relatively low: The standard deviation of 56 replicate measurements of a chitin standard (Sigma Aldrich) and 54 measurements of a cellulose standard (Merck) was 1.1‰ and 1.2‰, respectively. All $\delta^{13}C$ values are reported in ‰ relative to V-PDB.

Numerical analyses

Pearson's correlation coefficient (r) and associated p values were calculated with the PAST software package, version 1.97 (Hammer et al. 2001). The dissolved gas concentration data were log-transformed to reduce skewness. The variance explained in $\delta^{13}C_{ephippia}$ by $[CH_4]_{aq}$ independent of the variance explained jointly with $\delta^{13}C_{SOM}$ was assessed following Borcard et al. (1992) using Redundancy Analysis with a single species variable ($\delta^{13}C_{ephippia}$) with CANOCO for Windows, version 4 (ter Braak and Šmilauer 1998; 9999 permutations to test for statistical significance). Following Ravinet et al. (2010), we used a two-source mixing model to provide a coarse estimate of the amount of methanogenic carbon in the diet of *Daphnia* that would be necessary to explain the observed offsets between $\delta^{13}C_{SOM}$ and $\delta^{13}C_{ephippia}$ in our dataset if *Daphnia* only fed on two sources: POM and MOB. For this purpose, $\delta^{13}C_{SOM}$ was assumed to represent an integrated sample of $\delta^{13}C$ values of POM ($\delta^{13}C_{POM}$) over the seasonal cycle (Meyers 1994). $\delta^{13}C_{SOM}$ was therefore used to represent $\delta^{13}C_{POM}$ for the mixing model. $\delta^{13}C$ values of CH_4 in the sediments of the study lakes were obtained from Rinta (2014). Values were modified by -16‰ to

Table 1. Names, locations (country and coordinates), areas, and maximum depth values of the 15 investigated lakes, and the results of the $\delta^{13}C_{ephippia}$ analyses. NA indicates no $\delta^{13}C_{ephippia}$ measurement was performed for that specific sediment sample. When more than one $\delta^{13}C_{ephippia}$ value is given for a sediment sample, these are replicate measurements from the same sediment sample.

| Lake name | Country | Longitude (decimal ° East) | Latitude (decimal ° North) | Lake area (km^2) | Max depth (m) | $\delta^{13}C_{ephippia}$ (‰) | | |
						Center	Intermediate	Nearshore
De Waay	the Netherlands	5.15	51.93	0.04	14.5	NA	NA	−40.0
								−40.4
								−39.8
								−37.1
Gerzensee	Switzerland	7.55	46.83	0.24	10.7	−42.3	NA	NA
Glimmingen	Sweden	15.57	57.93	1.67	31.5	NA	−31.5	NA
Grissjön	Sweden	15.14	58.77	0.23	16.0	NA	NA	−37.3
								−34.0
Hargsjön	Sweden	15.24	58.27	0.99	6.2	−33.0	−34.0	−33.3
Hijkermeer	the Netherlands	6.49	52.89	0.02	2.0	−25.9	NA	−26.8
Hinterburgsee	Switzerland	8.07	46.72	0.05	11.4	−45.2	−43.0	−44.6
						−47.4		
						−44.5		
Holzsee	Germany	10.18	54.16	0.19	6.9	−36.5	NA	NA
Illersjön	Sweden	15.00	58.58	0.07	12.1	−39.8	NA	−41.9
Jyväsjärvi	Finland	25.77	62.24	3.03	25.0	−35.1	NA	NA
Kisasjön (north basin)	Sweden	15.65	58.01	0.96	8.5	−35.3	−35.8	−35.8
						−33.7		
Mekkojärvi	Finland	25.14	61.23	0.003	4.0	−51.6	NA	−52.8
Plusssee	Germany	10.44	54.18	0.14	29.3	−45.3	−31.6	−31.9
Schöhsee	Germany	10.44	54.17	0.75	29.5	−34.5	NA	NA
Seealpsee	Switzerland	9.40	47.27	0.13	14.5	−35.5	NA	−35.1
								−36.5

account for carbon fractionation by MOB (Templeton et al. 2006) to represent $\delta^{13}C$ values that would be expected for MOB ($\delta^{13}C_{MOB}$).

Results

$\delta^{13}C_{ephippia}$ analysis

Sufficient *Daphnia* ephippia for $\delta^{13}C_{ephippia}$ analysis were recovered from 27 of the examined sediment samples (12 from the deep, central part of the lakes, 10 from nearshore sites and 5 from intermediate coring sites) originating from 15 of the 32 study lakes. $\delta^{13}C_{ephippia}$ values encompassed a wide range (−52.8‰ to −25.9‰, Table 1). For five sampling stations we were able to measure $\delta^{13}C_{ephippia}$ values of multiple (2-4) samples, in which case we present average $\delta^{13}C_{ephippia}$ values. For both sampling sites with more than two samples, the standard deviation of the $\delta^{13}C_{ephippia}$ values was 1.5‰. This variability is slightly higher than observed for the standards, but still relatively small compared with the overall range of our $\delta^{13}C_{ephippia}$ measurements. In most cases $\delta^{13}C_{ephippia}$ values from nearshore or intermediate sites were very similar to values observed in sediments taken in the

central part of the lakes, with exception of one site, Lake Plusssee (Fig. 1). Sediments from nearshore and intermediate sites yielded on average 0.5 ± 1.4 (standard deviation) ‰ lower $\delta^{13}C_{ephippia}$ values than central samples (1.9 ± 5.6‰ higher if Plusssee is included). For the three lakes for which $\delta^{13}C_{ephippia}$ values were only available for the nearshore and/ or intermediate sites, we therefore use the average of these values for further analyses, resulting in a dataset of 12 lakes with $\delta^{13}C_{ephippia}$ values from deep, central sediments, and three with estimates thereof based on nearshore and intermediate coring sites.

Comparison with gas concentrations and $\delta^{13}C_{SOM}$ values

Five of the 15 lakes that yielded $\delta^{13}C_{ephippia}$ values were characterized by oxic bottom waters ($[O_2]_{aq}$ 1.9-10.1 mg L^{-1}). These lakes had lower $[CH_4]_{aq}$ in the surface waters (0.1-1.1 μmol L^{-1}), bottom waters (0.04-1.3 μmol L^{-1}), and 10 cm above the sediment (0.1-3.2 μmol L^{-1}) than the lakes with bottom waters with $[O_2]_{aq}$ below 0.22 mg L^{-1} (surface 0.2-4.1 μmol L^{-1}, bottom 10.0-940.7 μmol L^{-1}, and 10 cm above the sediment 9.9-1238.6 μmol L^{-1}). $\delta^{13}C_{ephippia}$ values were not correlated with diffusive CH$_4$ flux from the water

surface to the atmosphere (data from Schilder et al. 2013; r −0.39, p 0.26; Fig. 2A). However, they were negatively correlated with surface water $[CH_4]_{aq}$ (r −0.64, p 0.01; Fig. 2B). We also found strong and statistically significant negative correlations between $δ^{13}C_{ephippia}$ values and $[CH_4]_{aq}$ in the deep-water layers (with $[CH_4]_{aq}$ one meter above the sediment r −0.74 p 0.002; with $[CH_4]_{aq}$ 10 cm above the sediment r −0.75 p 0.001), and that deep-water samples with high $[CH_4]_{aq}$ were associated with low $[O_2]_{aq}$ (Fig. 2C,D).

Fig. 1. $δ^{13}C_{ephippia}$ values from sediments from the lake center plotted vs. those from nearshore (circles) or intermediate (squares) coring sites for lakes for which measurements from multiple depths were available. The dashed line represents the 1 : 1 line.

Surface water [DIC] was variable but on average lower in the Fennoscandian lakes (68.2-1923 $μmol\ L^{-1}$, average 770 $μmol\ L^{-1}$) than in the other lakes (221.0-2475.0 $μmol\ L^{-1}$, average 1648.6 $μmol\ L^{-1}$), which is likely related to different bedrock type in Fennoscandia relative to the regions studied in central and northwest Europe. The range of $[CO_2]_{aq}$ in the surface waters, which ranged from 6.3 $μmol\ L^{-1}$ to 598.9 $μmol\ L^{-1}$, was similar in the different geographical areas. $δ^{13}C_{DIC}$ and $δ^{13}C_{CO_2}$ values in the surface water were on average lower in the Fennoscandian lakes ($-18.2 ± 1.9‰$ and $-23.8 ± 4.7‰$, respectively) than in the other lakes ($-7.3 ± 4.5‰$ and $-16.0 ± 4.6‰$). No significant correlation between $δ^{13}C_{DIC}$ and $δ^{13}C_{ephippia}$ values was apparent in our dataset (r −0.38, p 0.16; Fig. 3A), and $δ^{13}C_{CO_2}$ values correlated negatively with $δ^{13}C_{ephippia}$ (r −0.54, p 0.04; Fig. 3B). No significant correlation was found between $δ^{13}C_{ephippia}$ and $[CO_2]_{aq}$ (r −0.23, p 0.41; Fig. 3C).

$δ^{13}C_{SOM}$ values ranged from $-37.0‰$ to $-27.7‰$. The highest $δ^{13}C_{SOM}$ values were associated with higher atomic C : N ratios of bulk sediment organic matter, which ranged from 8.9 to 20.1. There was a positive correlation between $δ^{13}C_{SOM}$ values and $δ^{13}C_{ephippia}$ values in our data set (Fig. 3D; r 0.54 p 0.04), although $δ^{13}C_{ephippia}$ values were as much as 20‰ (average 6.1‰) lower than $δ^{13}C_{SOM}$ values.

Discussion

Daphnia are known to filter particles out of the water column, including algae, bacteria, and (terrestrial or aquatic) organic detritus, with algae usually forming the largest part of the diet (Lampert 2011; Smyntek et al. 2012). In our study sites, $δ^{13}C_{ephippia}$ values ranged from $-51.6‰$ to $-25.9‰$. Some $δ^{13}C_{ephippia}$ values were clearly lower than can be expected for algae in temperate and boreal lakes (-35 to $-25‰$) (France 1995b; Vuorio et al. 2006). Some of the observed values are also clearly lower than $δ^{13}C_{ephippia}$ values reported in lake surface sediments by van Hardenbroek et al.

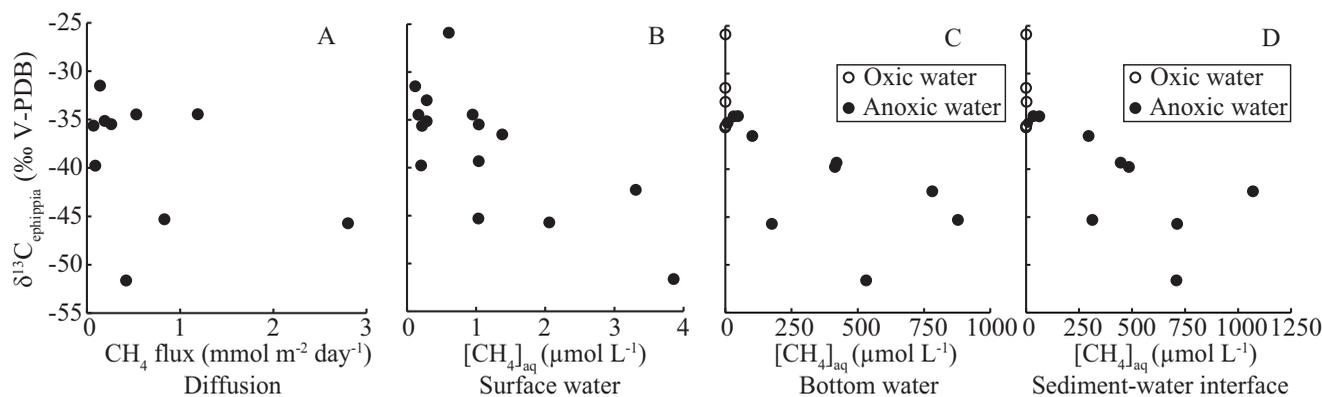**Fig. 2.** $δ^{13}C_{ephippia}$ values plotted vs. (A) diffusive CH_4 flux during fieldwork (data from Schilder et al. 2013), (B) surface water $[CH_4]_{aq}$, (C) bottom water $[CH_4]_{aq}$ and (D) $[CH_4]_{aq}$ 10 cm above the sediment. Lakes classifed as having oxic bottom water were characterized by $[O_2]_{aq} > 1\ mg\ L^{-1}$, lakes with anoxic bottom water by $[O_2]_{aq} < 1\ mg\ L^{-1}$.

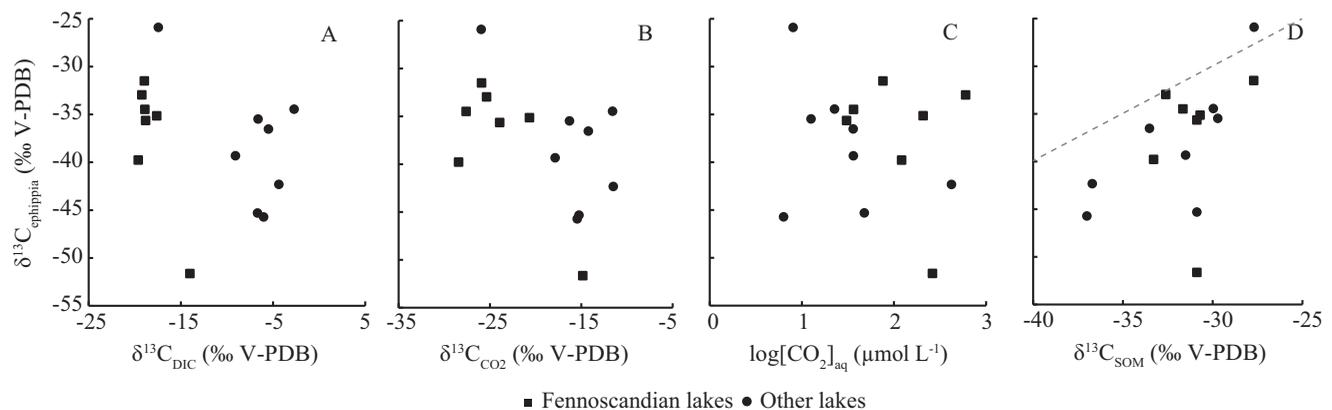**Fig. 3.** $\delta^{13}C_{ephippia}$ values plotted vs. (A) surface water $\delta^{13}C_{DIC}$ values, (B) surface water $\delta^{13}C_{CO_2}$ values, (C) surface water $[CO_2]_{aq}$, and (D) $\delta^{13}C_{SOM}$ values. The dashed line in (A) indicates the 1 : 1 line.

(2013), and in studies of older lake sediments by Wooller et al. (2012) and van Hardenbroek et al. (2013), which ranged from $-40‰$ to $-27.0‰$.

$\delta^{13}C_{ephippia}$ values are similar to the $\delta^{13}C$ values of *Daphnia* producing the resting eggs (Perga 2011). An important caveat for our study, however, is that $\delta^{13}C_{ephippia}$ is only representative of *Daphnia* living in the examined lakes during ephippia formation. *Daphnia* $\delta^{13}C$ values may vary considerably over the seasonal cycle, by as much as 10‰ in some lakes (Taipale et al. 2008; Smyntek et al. 2012). Ephippia formation is stimulated by external stimuli, such as food availability, photoperiod, and population density (Lampert 2011). Field observations have revealed that the timing of ephippia production varies between species, but that many of the investigated species have ephippia production peaks around April–June and September–November (e.g., Cáceres 1998). Our field campaigns occurred in August–September, which overlaps with the fall ephippia production peak. Furthermore, study lakes with high $[CH_4]_{aq}$ in the hypolimnion are expected to also have elevated $[CH_4]_{aq}$ in the photic zone during autumn overturning (e.g., Schubert et al. 2012). Our $[CH_4]_{aq}$ measurements are, therefore, likely representative for, or correlated with, CH_4 availability in the water column during the formation of a considerable part of the ephippia deposited in surface sediments.

Relationship of $\delta^{13}C_{ephippia}$ values with diffusive CH_4 flux and $[CH_4]_{aq}$

For 10 of our study lakes the diffusive CH_4 flux from the lake surface to the atmosphere has been measured during sampling (Schilder et al. 2013), whereas for the remaining sites calculating diffusive flux estimates was not possible due to high rates of gas bubbles escaping the sediment (ebullition). A weak negative relationship between $\delta^{13}C_{ephippia}$ values and diffusive flux estimates seems to exist (Fig. 2A). This relationship is clearly not statistically significant (r -0.39, p 0.26), in contrast to the field survey reported by van Harden-

broek et al. (2013). However, variable wind speeds can strongly affect short-term measurements of diffusive flux to the atmosphere at identical $[CH_4]_{aq}$ (Bade 2009), and the flux measurements from seven Swedish lakes presented by van Hardenbroek et al. (2013) integrate over much longer time periods (flux data collection during several weeks in total) than the the data presented by Schilder et al. (2013) (measurement during ca. six hours). Because long-term diffusive CH_4 flux is largely driven by $[CH_4]_{aq}$ in surface-water layers, the relationship between $\delta^{13}C_{ephippia}$ and surface water $[CH_4]_{aq}$ may provide a better indication of the long-term relationship between CH_4 flux and $\delta^{13}C_{ephippia}$ in our study lakes than the correlation with the flux data presented by Schilder et al. (2013). Therefore, the strong negative correlation between $\delta^{13}C_{ephippia}$ values and surface water $[CH_4]_{aq}$ (Fig. 2B), suggests a relationship between $\delta^{13}C_{ephippia}$ values and diffusive CH_4 flux from the water surface of our study lakes would have been apparent if longer flux measurements would have been available. Our data show that $\delta^{13}C_{ephippia}$ values in our study lakes are not only correlated with surface water $[CH_4]_{aq}$, but also with $[CH_4]_{aq}$ in the deeper water layers (Fig. 2C,D).

In contrast to van Hardenbroek et al. (2013), we observe $\delta^{13}C_{ephippia}$ values as low as $-51.6‰$ (Figs. 1-3), which is well below values reported for photosynthetically produced organic matter in lake ecosystems, such as algal biomass ($-35‰$ to $-25‰$) (France 1995b; Vuorio et al. 2006), macrophytes ($-30‰$ to $-10‰$) (France 1995a), and imported terrestrial plant detritus ($-30.5‰$ to $-10.8‰$) (Meyers and Teranes 2001). As MOB typically have very low $\delta^{13}C$ values ($-100‰$ to $-55‰$), this is an indication for a significant contribution of MOB, or other organisms feeding on MOB (e.g., ciliates), in the diet of *Daphnia* during ephippia formation in lakes with such low $\delta^{13}C_{ephippia}$ values, as has been reported e.g., for small Finnish lake ecosystems (e.g., Kankaala et al. 2010). Lampert (2011) describes vertical migrations of over 25 m by *Daphnia*, especially those carrying
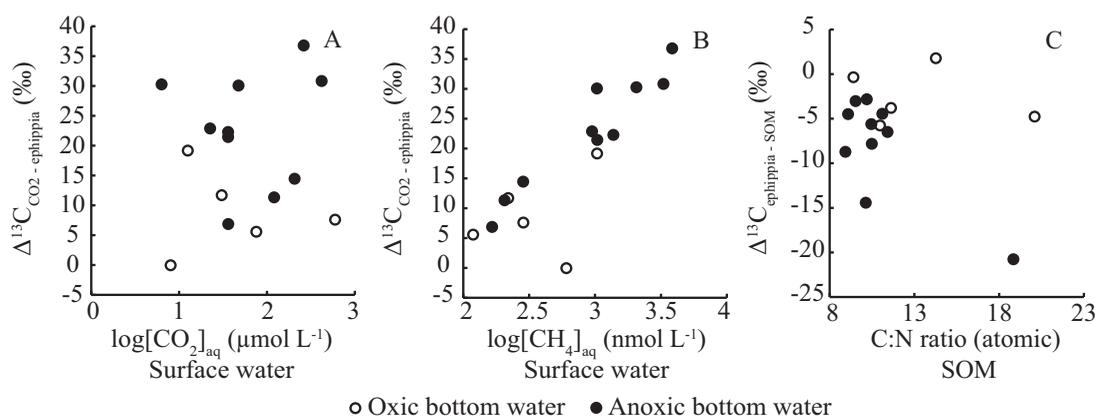
Fig. 4. $\Delta^{13}C_{CO_2 - ephippia}$ plotted vs. (A) surface water $[CO_2]_{aq}$, and (B) surface water $[CH_4]_{aq}$, and (C) $\Delta^{13}C_{ephippia - SOM}$ plotted vs. atomic C : N ratios of SOM. Lakes classifed as having oxic bottom water were characterized by $[O_2]_{aq} > 1$ mg L^{-1}, lakes with anoxic bottom water by $[O_2]_{aq} < 1$ mg L^{-1}.

ephippia in development, to well below the thermocline in a German lake. This behavior may explain how *Daphnia* in such stratified lakes can access MOB, which are expected to be most abundant near the oxycline (e.g., Rudd et al. 1976), or methanogenic carbon bound in POM in anoxic waters of stratified lakes (Lehmann et al. 2004). The lakes we studied had a maximum depth of 2-31.5 m (average 15 m), and the anoxic water layers were within the range of reported vertical migration by *Daphnia* at all localities.

Photosynthetic primary producers and the variability in $\delta^{13}C_{ephippia}$ values

The alternative mechanism to the uptake of methanogenic carbon that may potentially explain the apparent correlation between $\delta^{13}C_{ephippia}$ and $[CH_4]_{aq}$ in our dataset involves variability in the $\delta^{13}C$ of photosynthetic primary producers between lakes. $\delta^{13}C$ values of algal biomass can be influenced by variations in baseline $\delta^{13}C_{DIC}$ (and $\delta^{13}C_{CO_2}$) in the different study lakes (Bade et al. 2006), and by variable $[CO_2]_{aq}$ and algal growth rates which can lead to variations in carbon fractionation during the uptake of CO_2 by algae (e.g., Laws et al. 1995). Several algal groups can incorporate bicarbonate instead of CO_2 if CO_2 is limiting (e.g., Raven et al. 2011). As bicarbonate is characterized by distinctly higher $\delta^{13}C$ values than CO_2 (Stumm and Morgan 1996), this may also affect $\delta^{13}C_{algae}$. Furthermore, variable contributions to POM by phytoplankton and by organic matter from terrestrial sources, e.g., dissolved organic matter taken up by the bacterial foodweb (Lennon et al. 2006), could also have affected the $\delta^{13}C$ value of the diet of *Daphnia* in our study lakes. We examined these alternative hypotheses by (a) assessing whether $\delta^{13}C_{ephippia}$ values are related to $\delta^{13}C_{DIC}$, $\delta^{13}C_{CO_2}$ or $[CO_2]_{aq}$; (b) examining whether the observed $\delta^{13}C_{ephippia}$ values can be explained by carbon fractionation by algae; and (c) comparing $\delta^{13}C_{ephippia}$ values with the $\delta^{13}C_{SOM}$ values.

Geographical gradients in $\delta^{13}C_{DIC}$ values exist in our data set, which may be due to e.g., differences in bedrock types and land use, or different intensities of heterotrophic respiration in the study lakes (Lennon et al. 2006). If variations in $\delta^{13}C_{ephippia}$ values are strongly determined by between-lake variations in $\delta^{13}C_{DIC}$ values, we would expect to see a clear positive relationship between $\delta^{13}C_{DIC}$ or $\delta^{13}C_{CO_2}$ values and $\delta^{13}C_{ephippia}$ values. However, no significant correlation between $\delta^{13}C_{DIC}$ and $\delta^{13}C_{ephippia}$ values was detectable in our dataset (Fig. 3A). $\delta^{13}C$ values of CO_2, the carbon source for most algae (Farquhar et al. 1989), correlated negatively with $\delta^{13}C_{ephippia}$ (Fig. 3B), in contrast to the expected positive correlation if $\delta^{13}C_{ephippia}$ values primarily depended on those of algal carbon. Furthermore, no clear relationship is apparent between $[CO_2]_{aq}$ and $\delta^{13}C_{ephippia}$ values in our dataset (Fig. 3C), suggesting that bicarbonate uptake or $[CO_2]_{aq}$ limitation are not causing the observed variability in $\delta^{13}C_{ephippia}$ values. Laboratory studies typically report offsets between $\delta^{13}C$ values of CO_2 and algae ($\Delta^{13}C_{CO_2 - algae}$) up to 25‰ (e.g., Laws et al. 1995), with the highest values resulting from manipulating $[CO_2]_{aq}$ to be very high or growth rates to be very low, situations that are not necessarily representative of the natural situation. Field studies investigating the offset between $\delta^{13}C$ values of CO_2 and algae (or POM) in the surface water have show that algae (or POM) typically have $\delta^{13}C$ values 0-17‰ lower than that of CO_2, with some of the lowest reported values being representative of winter conditions in a large carbonate-rich lake (Hollander and McKenzie 1991; Bade et al. 2006; Marty and Planas 2008). In our lakes, the offset between $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{ephippia}$ values ($\Delta^{13}C_{CO_2 - ephippia}$) exceeded carbon fractionation values reported for algae in field studies in 8 of our 15 study sites, with offsets up to 36.8‰ (mean 17.5‰, standard deviation $\pm$ 10.9‰; Fig. 4A). We conclude that variable carbon fractionation by algae between our study lakes in combination with between-lake variability in baseline $\delta^{13}C_{DIC}$

Table 2. Results of the partitioning of the variance in $\delta^{13}C_{ephippia}$ into variance explained by $\delta^{13}C_{SOM}$ and $[CH_4]_{aq}$ in the surface water, bottom water, and at the sediment–water interface following Borcard et al. (1992). For each pair of variables the variance explained by each variable on its own as well as the variance explained independent of the second variable is provided.

	Explained variance (%)	*p*	Explained variance with the second variable partitioned out (%)	*p*
$\delta^{13}C_{SOM}$ (‰)	28.9	0.04	10.6	0.26
Surface water $[CH_4]_{aq}$ (µmol L^{-1})	54.8	0.002	43.1	0.03
$\delta^{13}C_{SOM}$ (‰)	28.9	0.04	15.7	0.16
Bottom water $[CH_4]_{aq}$ (µmol L^{-1})	53.7	0.001	45.2	0.02
$\delta^{13}C_{SOM}$ (‰)	28.9	0.04	0.8	0.76
Sediment-water $[CH_4]_{aq}$ (µmol L^{-1})	59.2	0.0005	43.1	0.01

cannot be the cause for the lowest values and large variability in $\delta^{13}C_{ephippia}$ we observed. Interestingly, $\Delta^{13}C_{CO_2 - ephippia}$ values show a strong relationship with surface water $[CH_4]_{aq}$ in our data set (r 0.85, p 0.00005; Fig. 4B), as well as with $[CH_4]_{aq}$ in de bottom water and at the sediment–water interface (r 0.73, p 0.002 and r 0.72, p 0.003, respectively) again suggesting that MOB may be a relevant alternative carbon source for *Daphnia* during ephippia formation in lakes with the largest offsets.

Our DIC data rely on spot measurements taken on a single sampling date during late summer. Concentrations and $\delta^{13}C$ values of DIC and CO_2 during *Daphnia* ephippia formation may therefore have differed to some extent from our reported DIC measurements. However, the between-lake gradient in $\delta^{13}C_{DIC}$ values (17‰) in our data is larger than the within-lake between-season variability that can be expected for most of our study sites ($< 5‰$; e.g., Hollander and McKenzie 1991; Lehmann et al. 2004), and there is a large gradient in $[CO_2]_{aq}$ (6.3-598.9 μmol L^{-1}) in our data set. Therefore, it seems unlikely that seasonal variability in CO_2 concentrations and $\delta^{13}C$ values are the reason why we do not observe a correlation between $\delta^{13}C_{ephippia}$ and $[CO_2]_{aq}$, $\delta^{13}C_{DIC}$ or $\delta^{13}C_{CO_2}$ measured in our study lakes.

Between-lake variability in algal $\delta^{13}C$ values may be strongly influenced by other variables than $\delta^{13}C$ values of DIC and CO_2 (Bade et al. 2006). In addition, other food sources unrelated to CH_4 such as bacteria growing on terrestrial or aquatic organic matter, or other heterotrophic microorganisms feeding on these organisms, can provide food for *Daphnia*. $\delta^{13}C_{SOM}$ values, especially in the deepest part of the lake basin, can be expected to provide an estimate of $\delta^{13}C_{POM}$ in our study lakes over the seasonal cycle (Meyers 1994), and to represent an integrated estimate of the $\delta^{13}C$ values of algal, bacterial, as well as terrestrial OM available for *Daphnia* in the water column. $\delta^{13}C_{SOM}$ values are positively correlated with $\delta^{13}C_{ephippia}$ values in our data set (Fig.

3D; r 0.54 $p = 0.04$), although there are very large differences between $\delta^{13}C_{SOM}$ and $\delta^{13}C_{ephippia}$ values, in individual lakes up to 20‰ (average 6.1‰). In all lakes except Lake Hijkermeer, $\delta^{13}C_{ephippia}$ values were lower than $\delta^{13}C_{SOM}$ values, suggesting that *Daphnia* assimilated carbon from at least one carbon source with lower $\delta^{13}C$ values than SOM. To some extent this could be explained by a significant contribution of terrestrial organic matter to SOM in some lakes. If terrestrial plant material, which often has higher $\delta^{13}C$ values than algae, contributes to the $\delta^{13}C_{SOM}$ value in our study lakes, then a selective feeding of *Daphnia* on POM of aquatic origin (e.g., based on particle size) could explain some of the observed difference between $\delta^{13}C_{ephippia}$ and $\delta^{13}C_{SOM}$ values. However, if the offset between $\delta^{13}C_{ephippia}$ and $\delta^{13}C_{SOM}$ values is examined relative to the atomic C : N ratio of SOM, typically interpreted as an indicator for whether SOM is predominantly of terrestrial or aquatic origin (Meyers and Teranes 2001), no relationship is apparent (Fig. 4C). It is important to note, however, that the deep water $\delta^{13}C_{POM}$ and $\delta^{13}C_{SOM}$ values in stratified lakes with anoxic hypolimnia may also be affected by the activity of MOB (e.g., Lehmann et al. 2004), potentially leading to lower $\delta^{13}C_{SOM}$ values in lakes with high hypolimnetic $[CH_4]_{aq}$. Therefore, the observed correlation between $\delta^{13}C_{SOM}$ and $\delta^{13}C_{ephippia}$ values in our study lakes may have been reinforced by organic matter produced by MOB in the lakes with the lowest $\delta^{13}C_{ephippia}$ values, which were also the ones with the highest $[CH_4]_{aq}$ throughout the water column. Nevertheless, as both $\delta^{13}C_{SOM}$ and $[CH_4]_{aq}$ correlate with $\delta^{13}C_{ephippia}$, the apparent relationship between $[CH_4]_{aq}$ and $\delta^{13}C_{ephippia}$ may also be a consequence of between-lake variability in $\delta^{13}C_{SOM}$ related to other processes than CH_4 oxidation and MOB availability. However, when we compare the variance explained in $\delta^{13}C_{ephippia}$ by $\delta^{13}C_{SOM}$ or $[CH_4]_{aq}$ with the variance these variables can explain if the effects of the second variable are partialled out (Table 2), it becomes apparent that
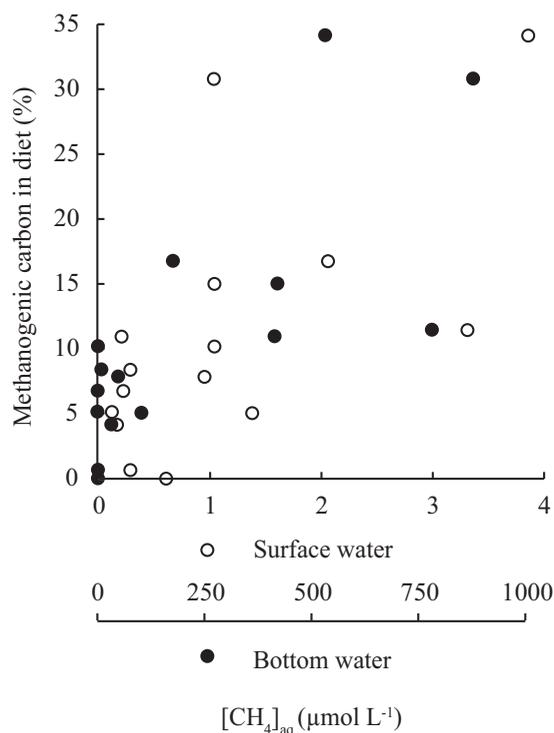
Fig. 5. Estimated proportion of methanogenic carbon in *Daphnia* diet necessary to change their $\delta^{13}C$ values from values typical for $\delta^{13}C_{SOM}$ to those observed for $\delta^{13}C_{ephippia}$ in the different study lakes. Calculations are based on a simple two-source mixing model based on $\delta^{13}C_{SOM}$ as an integrated estimate of $\delta^{13}C_{POM}$ in the study lakes and on $\delta^{13}C$ values for CH_4 reported in Rinta (2014). Values are plotted vs. $[CH_4]_{aq}$ in the surface water and the bottom water. See methods section for details on the mixing model calculations.

$[CH_4]_{aq}$ can explain a considerable part of the variability in $\delta^{13}C_{ephippia}$ independent of $\delta^{13}C_{SOM}$. In contrast, variations in $\delta^{13}C_{SOM}$ cannot explain significant variability in $\delta^{13}C_{ephippia}$ if the effects of $[CH_4]_{aq}$ are partialled out. This does not rule out that a large share of the variation in $\delta^{13}C_{ephippia}$ is related to variable $\delta^{13}C_{POM}$ or $\delta^{13}C_{algae}$ between the study lakes, and to variables such as the proportion of terrestrial and aquatic material in POM. However, it indicates that a relationship between CH_4 availability and $\delta^{13}C_{ephippia}$ in our study lakes exists independent of between-lake variability in $\delta^{13}C_{SOM}$ and $\delta^{13}C_{POM}$.

Implications for paleolimnology

Our survey demonstrates a relationship between $\delta^{13}C_{ephippia}$ values and $[CH_4]_{aq}$ in different parts of the water column in small temperate European lakes. As outlined above, the low $\delta^{13}C_{ephippia}$ values in some lakes of our study cannot be reconciled with a diet consisting purely of algae or terrestrial organic matter. Furthermore, we show that reduced discrimination against ^{13}C during photosynthesis due to limitation of CO_2, variable $\delta^{13}C_{POM}$ values, and differences in baseline $\delta^{13}C_{DIC}$ values are all very unlikely explanations for the

observed pattern in $\delta^{13}C_{ephippia}$ values and CH_4 concentrations. Instead, the very low $\delta^{13}C_{ephippia}$ values, the correlations between $\delta^{13}C_{ephippia}$ values and $[CH_4]_{aq}$, the large $\Delta^{13}C_{CO_2 \text{ - ephippia}}$ values, the strong correlation of these values with $[CH_4]_{aq}$, and the absence of a relationship between $\delta^{13}C_{ephippia}$ and surface water $[CO_2]_{aq}$, indicate that the diet of *Daphnia* during ephippia formation has been supplemented with ^{13}C-depleted biomass in most lakes, especially in those characterized by high $[CH_4]_{aq}$. MOB are a source of ^{13}C-depleted biomass *Daphnia* are known to feed on, and MOB, or other microorganisms feeding on MOB, as a supplementary component of *Daphnia* diet is the only way we can explain observed patterns in $\delta^{13}C_{ephippia}$ values in our dataset. Estimates based on a simple two-source mixing model indicate that the amount of methanogenic carbon in *Daphnia* diet necessary to decrease $\delta^{13}C$ values of *Daphnia* during ephippia formation from values close to $\delta^{13}C_{SOM}$ down to values observed for $\delta^{13}C_{ephippia}$ ranges from 0% to 20% for most lakes, but can reach up to 35% for some study sites (Fig. 5).

Considering the strength of the correlations we observed between $\delta^{13}C_{ephippia}$ and $[CH_4]_{aq}$ in different parts of the water column it seems likely that the relationship between in-lake CH_4 availability and $\delta^{13}C$ values of *Daphnia* is robust enough to allow inferences of past changes in lake water $[CH_4]_{aq}$ based on $\delta^{13}C_{ephippia}$ values, at least in relatively small lake basins situated in arctic, boreal, temperate and alpine climates, such as the ones examined in our study and by van Hardenbroek et al. (2013). Our dataset was characterized by a large between-lake variability in variables that can potentially influence $\delta^{13}C$ values of planktonic invertebrates, such as baseline $\delta^{13}C_{DIC}$ values, $[CO_2]_{aq}$, productivity, alkalinity, and catchment vegetation and land use. These variables can be expected to vary to a lesser degree within a single lake over time. Therefore the relationship between $[CH_4]_{aq}$ and $\delta^{13}C_{ephippia}$ may potentially be even more pronounced within individual lakes, and major variations in $[CH_4]_{aq}$ can be expected to be apparent in down-core $\delta^{13}C_{ephippia}$ records retrieved from them. Shifts in $\delta^{13}C_{ephippia}$ values up to 10‰ have been reported from lake sediment records (e.g., Wooller et al. 2012). Based on our data, variations in $\delta^{13}C_{ephippia}$ values of these magnitudes in small lakes may be explained by variations in in-lake CH_4 concentrations. Combining the results of down-core $\delta^{13}C_{ephippia}$ analyses with proxies that provide information on e.g., changes in past algal productivity or oxygen availability may strengthen the inferences regarding past in-lake CH_4 availability and help to separate changes in $\delta^{13}C_{ephippia}$ related to variations in $[CH_4]_{aq}$ from those related to other causes, such as changes in baseline $\delta^{13}C_{ephippia}$ or lake productivity.

There are some limits to the extent to which we can assess the potential of this approach for paleolimnological studies based on the presented data, however. First, we do not have a full matrix of $\delta^{13}C_{ephippia}$ values from deep-water

sediments and had to rely on ephippia isolated from shallower sediments for 3 of 15 sites. This issue is expected to weaken the relationship between $\delta^{13}C_{ephippia}$ values and deep water $[CH_4]_{aq}$ if ephippia deposited in nearshore areas are not representative for deep water $[CH_4]_{aq}$. However, there appears to be a good correspondence between $\delta^{13}C_{ephippia}$ values obtained from sediments from different coring sites in most lakes (Fig. 1). Further and more extensive studies based on a larger number of deep-water sediment samples are necessary to examine whether correlations between $[CH_4]_{aq}$ and $\delta^{13}C_{ephippia}$ values can be further improved by restricting analyses to the deepest sections of lakes. Second, within our study we were not able to determine the identity of the *Daphnia* species the ephippia belong to. *Daphnia* species may differ in the timing of ephippia production (e.g., Cáceres 1998), in size and, consequently, the size fraction they are able to filter from lake water (Lampert 2011). In future studies, screening of sediment samples for identifyable *Daphnia* remains (e.g., abdominal claws) may therefore provide information on which *Daphnia* species groups are susceptible to low $\delta^{13}C$ values at high $[CH_4]_{aq}$, and may explain some of the variability we observe in the relationship between $\delta^{13}C_{ephippia}$ values and $[CH_4]_{aq}$. Several processes may complicate the interpretation of $\delta^{13}C_{ephippia}$ values in down-core records, such as changes in $[CO_2]_{aq}$, baseline $\delta^{13}C_{DIC}$ values, as may be caused by shifts in catchment vegetation, algal productivity or rates of respiration in the water column (e.g., Lennon et al. 2006; Frossard et al. 2014), or changes in the seasonality of ephippia production. Finally, it is expected that the relationship between $\delta^{13}C_{ephippia}$ values and CH_4 availablity we describe is restricted to certain lake types (e.g., in respect to size, depth, and nutrient and oxygen availability) and it may break down if larger datasets are considered. Nevertheless the strength and robustness of the relationship between $\delta^{13}C_{ephippia}$ and $[CH_4]_{aq}$ we report suggests that major shifts in $\delta^{13}C_{ephippia}$ values in the sediments of small lakes in temperate, boreal, and arctic lakes (e.g., Wooller et al. 2012; van Hardenbroek et al. 2013), especially to values lower than -40‰, can be considered to be indicative of higher CH_4 availability in lakes. Our study suggests that such low $\delta^{13}C_{ephippia}$ values are restricted to lakes with high $[CH_4]_{aq}$ throughout the water column and hypoxic or anoxic conditions in the water column. This implies that $\delta^{13}C_{ephippia}$ values may not only provide insights into past changes in $[CH_4]_{aq}$, but also regarding oxygen depletion in stratified lakes.

References

Assayag, N., K. Rivé, M. Ader, D. Jézéquel, and P. Agrinier. 2006. Improved method for isotopic and quantitative analysis of dissolved inorganic carbon in natural water samples. Rapid Commun. Mass Spectrom. **20**: 2243–2251. doi:10.1002/rcm.2585

Bade, D. L. 2009. Gas exchange at the air-water interface, p. 70-78. In G. E. Likens [ed.], Encyclopedia of inland waters. Elsevier.

Bade, D. L., M. L. Pace, J. J. Cole, and S. R. Carpenter. 2006. Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models? Aquat. Sci. **68**: 142–153. doi:10.1007/s00027-006-0818-5

Borcard, D., P. Legendre, and P. Dapreau. 1992. Partialling out the spatial component of ecological variation. Ecology **73**: 1045–1055. doi:10.2307/1940179

Cáceres, C. E. 1998. Interspecific variation in the abundance, production and emergence of *Daphnia* diapausing eggs. Ecology **79**: 1699–1710. doi:10.1890/0012-9658(1998)079[1699:IVITAP]2.0.CO;2

Deines, P., D. Langmuir, and R. S. Harmon. 1974. Stable carbon isotope ratios and the existence of a gas phase in the evolution of carbonate ground waters. Geochim. Cosmochim. Acta **38**: 1147–1164. doi:10.1016/0016-7037(74)90010-6

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **40**: 503–537. doi:10.1146/annurev.pp.40.060189.002443

France, R. L. 1995a. Carbon-13 enrichment in benthic compared to planktonic algae: Foodweb implications. Mar. Ecol. Prog. Ser. **124**: 307–312. doi:10.3354/meps124307

France, R. L. 1995b. Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. Vegetatio **124**: 67–72. doi:10.1007/BF00045145

Frenzel, P., B. Thebrath, and R. Conrad. 1990. Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). FEMS Microbiol. Ecol. **73**: 149–158. doi:10.1111/j.1574-6968.1990.tb03935.x

Frossard, V., V. Verneaux, L. Millet, J.-P. Jenny, F. Arnaud, M. Magny, and M.-E. Perga. 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. doi:10.1111/fwb.12304

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological Statistics software package for education and data analysis. Paleontol. Electron. **4**: 9pp.

Hollander, D. J., and J. A. McKenzie. 1991. CO_2 control on carbon-isotope fractionation during aqueous photosynthesis: A paleo-pCO$_2$ barometer. Geology **19**: 929–932. doi:10.1130/0091-7613(1991)019<0929:CCOCIF>2.3.CO;2

Jedrysek, M. O. 2005. S–O–C isotopic picture of sulphate–methane–carbonate system in freshwater lakes from Poland. A review. Environ. Chem. Lett. **3**: 100–112. doi:10.1007/s10311-005-0008-z

Juutinen, S., and others. 2009. Methane dynamics in different boreal lake types. Biogeosciences **6**: 209–223. doi:10.5194/bg-6-209-2009

Kankaala, P., S. Taipale, L. Li, and R. I. Jones. 2010. Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. Aquat. Ecol. **44**: 781–795. doi:10.1007/s10452-010-9316-x

Lampert, W. 2011. *Daphnia*: Development of a model organism, p. 250. In Excellence in ecology. International ecology institute.

Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt, and S. A. Macko. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental results. Geochim. Cosmochim. Acta **59**: 1131–1138. doi:10.1016/0016-7037(95)00030-4

Lehmann, M. F., S. M. Bernasconi, J. A. Mckenzie, A. Barbieri, M. Simona, and M. Veronesi. 2004. Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. Limnol. Oceanogr. **49**: 415–429. doi:10.4319/lo.2004.49.2.0415

Lennon, J. T., A. M. Faiia, X. Feng, and K. L. Cottingham. 2006. Relative importance of CO_2 recycling and CH_4 pathways in lake food webs along a dissolved organic carbon gradient. Limnol. Oceanogr. **51**: 1602–1613. doi:10.4319/lo.2006.51.4.1602

Lepistö, A., M. N. Futter, and P. Kortelainen. 2014. Almost 50 years of monitoring shows that climate, not forestry, controls long-term organic carbon fluxes in a large boreal watershed. Glob. Chang. Biol. **20**: 1225–1237. doi:10.1111/gcb.12491

Leuenberger, M. C., P. Nyfeler, H. P. Moret, P. Sturm, and C. Huber. 2000a. A new gas inlet system for an isotope ratio mass spectrometer improves reproducibility. Rapid Commun. Mass Spectrom. **14**: 1543–1551. doi:10.1002/1097-0231(20000830)14:16<1543::AID-RCM62>3.0.CO;2-H

Leuenberger, M. C., P. Nyfeler, H. P. Moret, P. Sturm, A. Indermuhle, and J. Schwander. 2000b. $CO_{(2)}$ concentration measurements on air samples by mass spectrometry. Rapid Commun. Mass Spectrom. **14**: 1552–1557. doi:10.1002/1097-0231(20000830)14:16<1552::AID-RCM63>3.0.CO;2-C

Marty, J., and D. Planas. 2008. Comparison of methods to determine algal $\delta^{13}C$ in freshwater. Limnol. Oceanogr. Methods **6**: 51–63. doi:10.4319/lom.2008.6.51

Meyers, P. A. 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. **114**: 289–302. doi:10.1016/0009-2541(94)90059-0

Meyers, P. A., and J. L. Teranes. 2001. Sediment organic matter, p. 239-269. In W. M. Last and J. P. Smol [eds.], Tracking environmental change using lake sediments. V. 2: Physical and geochemical methods. Kluwer Academic Publishers.

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. doi:10.1007/s10933-011-9532-y

Raven, J. A., M. Giordano, J. Beardall, and S. C. Maberly. 2011. Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. Photosynthesis Res. **109**: 281–296. doi:10.1007/s11120-011-9632-6

Ravinet, M., J. Syväranta, R. I. Jones, and J. Grey. 2010. A trophic pathway from biogenic methane supports fish biomass in a temperate lake ecosystem. Oikos **119**: 409–416. doi:10.1111/j.1600-0706.2009.17859.x

Rinta, P. 2014. Lacustrine methane cycling in different environments, observations from a multi-lake survey and a sediment record. Ph.D. thesis. Univ. of Bern.

Rudd, J. W. M., A. Furutani, R. J. Flett, and R. J. Hamilton. 1976. Factors controlling methane oxidation in shield lakes: The role of nitrogen fixation and oxygen concentration. Limnol. Oceanogr. **21**: 357–364. doi:10.4319/lo.1976.21.3.0357

Schilder, J., D. Bastviken, M. van Hardenbroek, P. Kankaala, P. Rinta, T. Stötter, and O. Heiri. 2013. Spatial heterogeneity and lake morphology affect diffusive greenhouse gas emission estimates of lakes. Geophys. Res. Lett. **40**: 5752–5756. doi:10.1002/2013GL057669

Schubert, C. J., T. Diem, and W. Eugster. 2012. Methane emissions from a small wind shielded lake determined by eddy covariance, flux chambers, anchored funnels, and boundary model calculations: A comparison. Environ. Sci. Technol. **46**: 4515–4522. doi:10.1021/es203465x

Smyntek, P. M., S. C. Maberly, and J. Grey. 2012. Dissolved carbon dioxide concentration controls baseline stable carbon isotope signatures of a lake food web. Limnol. Oceanogr. **57**: 1292–1302. doi:10.4319/lo.2012.57.5.1292

Stumm, W., and J. J. Morgan. 1996. Aquatic chemistry: Chemical equilibria and rates in natural waters, 3rd ed. Wiley.

Taipale, S., P. Kankaala, M. Tiirola, and R. I. Jones. 2008. Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. Ecology **89**: 463–474. doi:10.1890/07-0702.1

Templeton, A. S., K.-H. Chu, L. Alvarez-Cohen, and M. E. Conrad. 2006. Variable carbon isotope fractionation expressed by aerobic CH_4-oxidizing bacteria. Geochim. Cosmochim. Acta **70**: 1739–1752. doi:10.1016/j.gca.2005.12.002

ter Braak, C. J. F., and P. Šmilauer. 1998. Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, New York.

van Hardenbroek, M., and others. 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. doi:10.1016/j.quascirev.2012.04.009

Verbruggen, F., O. Heiri, G.-J. Reichart, and A. F. Lotter. 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. doi:10.1016/j.quascirev.2010.05.030

Vuorio, K., M. Meili, and J. Sarvala. 2006. Taxon-specific variation in the stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of lake phytoplankton. Freshw. Biol. **51**: 807–822. doi:10.1111/j.1365-2427.2006.01529.x

Wooller, M. J., and others. 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. doi:10.1007/s10933-012-9591-8

Yvon-Durocher, G., and others. 2014. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. Nature **507**: 488–491. doi:10.1038/nature13164

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