



**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Schilder, Johannes; van Roij, Linda; Reichart, Gert-Jan; Sluijs, Appy; Heiri, Oliver

**Title:** Variability in  $\delta^{13}\text{C}$  values between individual Daphnia ephippia : Implications for palaeo-studies

**Year:** 2018

**Version:** Accepted version (Final draft)

**Copyright:** © 2018 Elsevier Ltd.

**Rights:** CC BY-NC-ND 4.0

**Rights url:** <https://creativecommons.org/licenses/by-nc-nd/4.0/>

**Please cite the original version:**

Schilder, J., van Roij, L., Reichart, G.-J., Sluijs, A., & Heiri, O. (2018). Variability in  $\delta^{13}\text{C}$  values between individual Daphnia ephippia : Implications for palaeo-studies. *Quaternary Science Reviews*, 189, 127-133. <https://doi.org/10.1016/j.quascirev.2018.04.007>

1 Variability in  $\delta^{13}\text{C}$  values between individual *Daphnia* ephippia: Implications for  
2 palaeo-studies

3 Jos Schilder<sup>1,2</sup>, Linda van Rijj<sup>3</sup>, Gert-Jan Reichart<sup>3,4</sup>, Appy Sluijs<sup>3</sup> and Oliver Heiri<sup>1</sup>

4

5 This manuscript was published in Quaternary Science Reviews 189 (2018), 127-133  
6 on April 24, 2018. <https://doi.org/10.1016/j.quascirev.2018.04.007>

7

8    **Variability in  $\delta^{13}\text{C}$  values between individual *Daphnia* ephippia: Implications for**  
9    **palaeo-studies**

10    **Jos Schilder<sup>1,2</sup>, Linda van Rij<sup>3</sup>, Gert-Jan Reichart<sup>3,4</sup>, Appy Sluijs<sup>3</sup> and Oliver Heiri<sup>1</sup>**

11    <sup>1</sup>Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of  
12    Bern, Altenbergrain 21, 3013 Bern, Switzerland

13    <sup>2</sup>Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35,  
14    40014 Jyväskylä, Finland

15    <sup>3</sup>Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Heidelberglaan  
16    2, 3584 CS Utrecht, the Netherlands

17    <sup>4</sup>Royal Netherlands Institute for Sea Research (NIOZ), Landsdiep 4, 1797 SZ 't Horntje  
18    (Texel), the Netherlands

19

20    Corresponding author: Jos Schilder, j.c.schilder@gmail.com

21

22    **Keywords:** *Daphnia* ephippia, Stable carbon isotopes, Laser ablation, Lakes, Seasonality,  
23    Present, Palaeolimnology, Europe, Stable isotopes

24

25    **Highlights:** (3-5, 85 chars)

26    \*Laser ablation-based techniques can be used to measure single ephippium  $\delta^{13}\text{C}$  values

27    \*Bulk ephippia  $\delta^{13}\text{C}$  values may represent strong within-sample variability

28    \*This variability may be caused by seasonal or species-specific differences in diet

29    \*This technique can be used to assess seasonal patterns behind shifts in bulk samples

30    \*Larger numbers of ephippia in samples can increase the precision of reconstructions

31 ABSTRACT

32 The stable carbon isotope ratio ( $\delta^{13}\text{C}$  value) of *Daphnia* spp. resting egg shells (ephippia)  
33 provides information on past changes in *Daphnia* diet. Measurements are typically performed  
34 on samples of  $\geq 20$  ephippia, which obscures the range of values associated with individual  
35 ephippia. Using a recently developed laser ablation-based technique, we perform multiple  
36  $\delta^{13}\text{C}$  analyses on individual ephippia, which show a high degree of reproducibility (standard  
37 deviations 0.1 to 0.5 ‰). We further measured  $\delta^{13}\text{C}$  values of 13 ephippia from surface  
38 sediments of three Swiss lakes. In the well-oxygenated lake with low methane concentrations,  
39  $\delta^{13}\text{C}$  values are close to values typical for algae (-31.4 ‰) and the range in values is  
40 relatively small (5.8 ‰). This variability is likely driven by seasonal (or inter-annual)  
41 variability in algae  $\delta^{13}\text{C}$  values. In two seasonally anoxic lakes with higher methane  
42 concentrations, average values were lower (-41.4 and -43.9 ‰, respectively) and the ranges  
43 much larger (10.7 and 20.0 ‰). We attribute this variability to seasonal variation in  
44 incorporation of methane-derived carbon. In one lake we identify two statistically distinct  
45 isotopic populations, which may reflect separate production peaks. The potentially large  
46 within-sample variability should be considered when interpreting small-amplitude, short-  
47 lived isotope excursions based on samples consisting of few ephippia. We show that  
48 measurements on single ephippia can be performed using laser ablation, which allows for  
49 refined assessments of past *Daphnia* diet and carbon cycling in lake food webs. Furthermore,  
50 our study provides a basis for similar measurements on other chitinous remains (e.g. from  
51 chironomids, bryozoans).

53     1. Introduction

54     The use of the stable isotopic composition of organic remains in lake sediments has been  
55     emerging as a means to reconstruct changes in past ecological and climatological conditions  
56     in and around lakes (Leng and Henderson, 2013). Because they are often abundantly found  
57     in sediments and are resistant to degradation (Verbrugge et al., 2010), the chitinous remains  
58     of aquatic invertebrates have a strong potential as a proxy for such reconstructions (Heiri et  
59     al., 2012). For example, the stable carbon isotopic composition (expressed as  $\delta^{13}\text{C}$  values) of  
60     the shells (ephippia) of the resting eggs of water fleas (*Daphnia* spp.) has been shown to  
61     reflect that of the maternal *Daphnia* and its diet (Schilder et al., 2015b). This allows for the  
62     use of  $\delta^{13}\text{C}$  values of ephippia to investigate the  $\delta^{13}\text{C}$  value of *Daphnia* diet in the past (see  
63     e.g. Morlock et al., 2016; Rinta et al., 2016; Schilder et al., 2017). Chitinous remains of other  
64     organisms, most notably chironomid head capsules, bryozoan statoblasts and *Ceriodaphnia*  
65     ephippia have also been used to investigate past changes in the stable carbon isotopic  
66     composition of aquatic invertebrates in lake food webs, often in combination with *Daphnia*  
67     ephippia (Belle et al., 2014; Frossard et al., 2013; Morlock et al., 2016; Rinta et al., 2016; van  
68     Hardenbroek et al., 2013; Wooller et al., 2012).

69                 *Daphnia* feed on suspended organic particles, predominantly algae, in the water  
70     column of lakes (Lampert, 2011). In addition, *Daphnia* may ingest methanotrophic bacteria  
71     (MOB) or other microorganisms feeding on MOB. This leads to  $\delta^{13}\text{C}$  values in *Daphnia* that  
72     are much lower (Kankaala et al., 2006) than typical for freshwater algae (-35 to -25 ‰,  
73     (Vuorio et al., 2006)), because the  $\delta^{13}\text{C}$  values of biogenic methane in lake ecosystems (-85  
74     to -50 ‰, (Jedrysek, 2005; Rinta et al., 2015; Whiticar et al., 1986)) and MOB metabolizing  
75     this methane (Templeton et al., 2006) are exceptionally low. Schilder et al. (2015a) found that  
76      $\delta^{13}\text{C}$  values of ephippia from surface sediments were lower in small temperate lakes with  
77     high methane concentrations in the water column than in those with low methane

78 concentrations in the water column. This suggests that the amount of methane-derived carbon  
79 in these ephippia (and consequently their  $\delta^{13}\text{C}$  values) are systematically related to in-lake  
80 methane concentrations. The  $\delta^{13}\text{C}$  values of ephippia deposited in lake sediments may thus  
81 provide an indication of past availability of methane in lakes. Given the importance of lakes  
82 (and other freshwater bodies) in the global carbon cycle as producers and emitters of methane  
83 (Bastviken et al., 2011; Battin et al., 2009), this method can provide valuable insight into the  
84 past response of lakes to environmental and climatic change in terms of methane productivity  
85 and carbon cycling in general (e.g Perga et al., 2016; van Hardenbroek et al., 2014, 2013;  
86 Wooller et al., 2012). This information, in turn, can be used to better predict future responses  
87 of the lacustrine carbon cycle, and particularly of lacustrine methane production and  
88 emission, to changes in the environment and in the climate.

89 Seasonal fluctuations in  $\delta^{13}\text{C}$  values of *Daphnia* exceed 10 ‰ in some lakes  
90 (Morlock et al., 2016; Perga and Gerdeaux, 2006; Smyntek et al., 2012) and are partly driven  
91 by changes in the  $\delta^{13}\text{C}$  value of phytoplankton. For example, the seasonal succession of  
92 dominant algae species with different carbon fractionation rates can drive seasonal shifts in  
93 algal  $\delta^{13}\text{C}$  values (e.g Hollander and McKenzie, 1991) and therefore the stable carbon  
94 isotopic composition of food available to *Daphnia*. Additionally, changes in CO<sub>2</sub>  
95 concentrations (due to e.g. uptake of CO<sub>2</sub> by algae, CO<sub>2</sub> produced as a product of  
96 decomposition of organic matter or changes in pH that shift the equilibrium between the  
97 various dissolved carbonate species) can also influence both the baseline  $\delta^{13}\text{C}$  value of CO<sub>2</sub>  
98 and algal carbon fractionation rates (Laws et al., 1995; Smyntek et al., 2012), forming  
99 another source of seasonal variability in *Daphnia*  $\delta^{13}\text{C}$  values. As a consequence, higher  $\delta^{13}\text{C}$   
100 values of algae are typically observed during (late) spring and summer and lower values  
101 during fall and winter (Hollander and McKenzie, 1991; Morlock et al., 2016; Schilder et al.,  
102 2017; Smyntek et al., 2012). However, incorporation of MOB by *Daphnia* also likely occurs

103 mostly in fall when algal abundance declines and hypolimnetic methane is exposed to oxygen  
104 upon lake mixing (Morlock et al., 2016; Taipale et al., 2007). This can promote large  
105 seasonal fluctuations in *Daphnia*  $\delta^{13}\text{C}$  values in dimictic lakes with high methane production,  
106 leading to *Daphnia*  $\delta^{13}\text{C}$  values much lower than reported for algae (-40 ‰ and lower, see e.g.  
107 Kankaala et al. (2010) and Schilder et al. (2017)). In addition, there is a seasonality in the  
108 production of ephippia themselves: Ephippia may be produced throughout the year, but there  
109 typically are distinct production peaks in spring and in fall (Cáceres, 1998; Cáceres and  
110 Tessier, 2004).

111 The extent to which these sources of (potential) seasonal variability affect  
112 down-core variations in  $\delta^{13}\text{C}$  values of *Daphnia* ephippia is poorly constrained. In part, this is  
113 because  $\delta^{13}\text{C}$  analysis of single (sub)fossil ephippia shells has to date been impossible.  $\delta^{13}\text{C}$   
114 measurements on a large number of shells from a sample (hereafter called bulk  
115 measurements) represent a weighted average of  $\delta^{13}\text{C}$  values of individual ephippia which  
116 may originate from populations of very uniform or highly variable  $\delta^{13}\text{C}$  values.  
117 Measurements on individual ephippia would provide insights into this within-sample  
118 variation. Furthermore, they could provide information on whether ephippia in a sample  
119 represent one or more isotopically distinct ephippia production peaks or *Daphnia* populations  
120 (e.g. spring and autumn production peaks of the same or different *Daphnia* species), and how  
121 food sources different from algae (e.g. MOB) contributed to the diet of *Daphnia* producing  
122 these ephippia.

123 The amount of *Daphnia* ephippia needed for down-core ephippia  $\delta^{13}\text{C}$  analysis  
124 constrains the resolution of presently available records, often resulting in records with a  
125 relatively low level of taxonomic and temporal resolution (e.g. Morlock et al., 2016; Schilder  
126 et al., 2017). The number of ephippia needed is in turn strongly constrained by the size  
127 (mass) of the ephippia found in a sediment record and the lower limit of sample mass that can

128 be measured with a given analytical setup. For standard total combustion isotope ratio mass  
129 spectrometry (TC/IRMS), 20 or more individual ephippia are typically measured (Morlock et  
130 al., 2016; Rinta et al., 2016; Schilder et al., 2017). The  $\delta^{13}\text{C}$  analysis on invertebrate remains  
131 is at times performed on samples as small as 20  $\mu\text{g}$  (see e.g. Belle et al., 2017) and given the  
132 potentially large weight of *Daphnia* ephippia (0.5 to 5  $\mu\text{g}$  per ephippium, with very large  
133 ephippia even exceeding 5  $\mu\text{g}$ ; Van Hardenbroek et al., this issue) measurements could be  
134 performed on samples containing only 5 to 10 individual ephippia (or even less). However, if  
135 the variability in  $\delta^{13}\text{C}$  values is high in a sediment sample, measurements on bulk samples  
136 containing a low number of ephippia may also lead to high apparent variability in down-core  
137 records, which can complicate their interpretation. Here we apply a novel analytical setup  
138 capable of analysing  $\delta^{13}\text{C}$  values of individual ephippia which we use to investigate within-  
139 bulk sample variability.

140 Recent advances in analytical procedures (e.g. approaches based on laser-  
141 ablation or Spooling Wire Microcombustion) now allow the measurement of the isotopic  
142 composition of samples considerably smaller than those conventionally measured in isotope  
143 studies of lacustrine invertebrate remains (see e.g. Eek et al., 2007; Moran et al., 2011;  
144 Pearson et al., 2016), potentially allowing single or even multiple measurements on  
145 individual microfossils (e.g. Nelson et al., 2007; van Roij et al., 2017; Zhao et al., 2017) . We  
146 use a laser-ablation based setup for measuring the  $\delta^{13}\text{C}$  values of individual ephippia. The  
147 setup has been developed for  $\delta^{13}\text{C}$  analysis of small organic particles, such as organic  
148 microfossils, and has been shown to produce high accuracy and precision data for an  
149 international standard, as well as for single grains of pollen and dinoflagellate cysts (Sluijs et  
150 al., 2018; van Roij et al., 2017). We analyse individual *Daphnia* ephippia from surface  
151 sediments from three Central European lakes that vary in their geographical and elevational  
152 setting (subalpine versus lowland), occurrence of anoxia in deeper water layers, and the

153 extent to which methane enters the open water column. We aim to explore the potential of  
154 this approach to investigate the variability behind  $\delta^{13}\text{C}$  values based on bulk *Daphnia*  
155 ephippia samples such as presented by Schilder et al. (2015a). For each lake we present  
156 multiple  $\delta^{13}\text{C}$  measurements on the same ephippium to constrain the reproducibility of the  
157 approach and assess whether individual ephippia were homogeneous in their stable carbon  
158 isotopic composition. Furthermore, for each lake we analysed another 13 individual ephippia  
159 from the same surface sediment sample once to investigate the variability in *Daphnia*  
160 ephippia  $\delta^{13}\text{C}$  values within a fossil ephippia assemblage. We expected to find larger  
161 variability in ephippia  $\delta^{13}\text{C}$  values in the stratified lakes with high methane concentrations  
162 than in the well-mixed lake with low methane concentrations, since it can be expected that in  
163 the lakes with high methane concentrations *Daphnia* diet may be supplemented by strongly  
164  $^{13}\text{C}$ -depleted, methane-derived carbon during part of the seasonal cycle (e.g. Morlock et al.,  
165 2016; Rinta et al., 2016; Schilder et al., 2017; Taipale et al., 2007).

166

## 167 2. Methods

### 168 2.1 Description of sites

169 For this study the surface sediments of three dimictic Swiss lakes, Burgäschisee (BUR),  
170 Hinterburgsee (HIN) and Seetalpsee (SEE) were investigated. HIN and SEE are mountain  
171 lakes (1516 and 1141 m above sea level (asl), respectively), whereas BUR is situated in the  
172 lowlands of the Swiss plateau (434 m asl). At the time of sampling (late summer 2011), all  
173 three lakes were thermally stratified. BUR and HIN were characterized by anoxic bottom  
174 waters, whereas the water column of SEE was fully oxygenated. Surface water methane  
175 concentrations were 1.15 (BUR), 2.80 (HIN) and 1.04 (SEE)  $\mu\text{mol l}^{-1}$ , and the bottom water  
176 methane concentrations were 226.63 (BUR), 13.40 (HIN) and 1.26 (SEE)  $\mu\text{mol l}^{-1}$  (see Table

177 1 for further details on the lakes and Rinta et al. (2015) for full details on the trace gas  
178 measurements and information on the lakes).

179

	Seepsee (SEE)	Hinterburgsee (HIN)	Burgäschisee (BUR)
<b>Longitude</b> (WGS 1984, decimal)	9.4000	8.0675	7.6686
<b>Latitude</b> (WGS 1984, decimal)	47.2689	46.7173	47.1690
<b>Altitude</b> (m asl)	1141	1516	434
<b>Max depth</b> (m)	14.5	11.4	30
<b>Area</b> (ha)	13.5	4.5	20.4
<b>pH surface water</b>	8.7	8.9	8.6
<b>TN surface water</b> ( $\mu\text{g l}^{-1}$ )	550	800	1200
<b>TP surface water</b> ( $\mu\text{g l}^{-1}$ )	10	11	14
<b>[O<sub>2</sub>] surface water</b> (mg l <sup>-1</sup> )	10.33	10.83	9.92
<b>[O<sub>2</sub>] bottom water</b> (mg l <sup>-1</sup> )	10.08	0.13	0.05
<b>[CH<sub>4</sub>] surface water</b> ( $\mu\text{M}$ )	1.04	2.8	1.15
<b>[CH<sub>4</sub>] bottom water</b> ( $\mu\text{M}$ )	1.26	13.40	226.63

180 **Table 1:** Location and limnological characteristics of the three study lakes.

181

182 2.2 Surface sediment ephippia

183 In the deepest part of the lakes, surface sediment (the top 2 cm) was retrieved using a gravity  
184 corer (UWITECH, Austria). The sediment was treated chemically (2 h 10% KOH and 20 h  
185 2M NH<sub>4</sub>Cl buffered in 0.35 M NaOH) to deflocculate the sediment and remove carbonates  
186 (following Verbruggen et al. (2010)), sieved (100  $\mu\text{m}$ ) and ephippia were isolated from the  
187 sieving residue using forceps and a binocular (20 - 50 x magnification) as described by  
188 Schilder et al. (2015a). For each sample, 25 ephippia were transferred to a glass vial  
189 containing demineralized water for short-term (2 months) dark storage and transport. NaCl

190 was added to each vial to prevent biological degradation during this period. Later, these  
191 ephippia were rinsed in demineralized water to remove the salt. From each lake, the  $\delta^{13}\text{C}$   
192 value of one ephippium was determined three to four times to assess whether a single  
193 measurement is representative of the whole specimen. From 13 further ephippia the  $\delta^{13}\text{C}$   
194 value was measured once.

195

### 196 2.3 Mass Spectrometry

197 Individual ephippia  $\delta^{13}\text{C}$  values were measured using the laser ablation, nano combustion gas  
198 chromatography and isotope ratio mass spectrometry (LA/nC/GC/IRMS) setup described by  
199 van Roij et al. (2017). In short, 2 to 4 ephippia were placed between two nickel discs (6 mm  
200 diameter) and pressed with a hydraulic press ( $3.5 \text{ ton cm}^{-2}$ ) to attach the ephippia to the nickel  
201 discs. A nickel disc with ephippia was then introduced to a small ablation chamber positioned  
202 under a 193 nm ArF laser system (COMPex 102; Lambda Physik, Göttingen, Germany).  
203 Using the laser, a small section of one ephippium (100  $\mu\text{m}$  diameter hole: Fig. 1) was ablated  
204 and carried to a GC combustion III interface (ThermoFinnigan, Bremen, Germany) coupled  
205 on line to a Delta V Advantage isotope ratio mass spectrometer (ThermoFinnigan) by a  
206 helium flow. A microscope equipped with a camera was used to aim the laser beam. Aside  
207 from avoiding the edges of the ephippia, no particular area of the ephippia was targeted  
208 specifically. The analytical precision (based on the results of 2 to 3 analyses of the laboratory  
209 standard IAEA CH-7 (polyethylene, PE) per sequence) ranged from  $\pm 0.05$  to  $\pm 0.5 \text{ \textperthousand}$ . 2 to 4  
210 ephippium measurements were performed per sequence. A small fragment of the PE standard  
211 was placed on the same nickel disc as the ephippia and ablated at the beginning and end of  
212 each measurement sequence.

213

214 2.4 Statistical analyses

215 Shapiro-Wilk tests were used to test for normal distribution of the results. To compare the  
216 variance of *Daphnia* ephippia  $\delta^{13}\text{C}$  values retrieved from the three lake sediment samples the  
217 Brown-Forsyth test for homogeneity of variance was applied. Hartigan's dip-test was applied  
218 to test for unimodality of the distributions of  $\delta^{13}\text{C}$  values within a sediment sample. All  
219 statistics were performed using the R software package (R Core Team, 2013).

220

### 221 3. Results

222 Repeated measurements on a single ephippium from each lake show very consistent results  
223 (Figure 2), with a standard deviation of 0.1 ‰ (SEE, n = 3) and 0.5 ‰ (HIN and BUR, n = 4  
224 and 3, respectively). This is comparable to the standard deviation observed for the laboratory  
225 standard, implying that a *Daphnia* ephippium is as homogeneous in its  $\delta^{13}\text{C}$  value as the PE  
226 standard. Repeated measurements on PE and on single ephippia therefore both indicate an  
227 analytical uncertainty and repeatability of our measurements of  $\leq 0.5$  ‰ (expressed as 1  
228 standard deviation).

229 There is a considerable amount of variation in  $\delta^{13}\text{C}$  values of individual  
230 *Daphnia* ephippia within each surface sediment sample, as well as a large difference in  
231 variability between the lakes. Ephippia  $\delta^{13}\text{C}$  values from SEE ranged from -37.3 to -31.5 ‰  
232 (range 5.8 ‰, standard deviation 1.8 ‰), values from HIN ranged from -45.9 to -35.2 ‰  
233 (range 10.8 ‰, standard deviation 4.0 ‰) and those from BUR ranged from -54.1 to -34.1 ‰  
234 (range 20.0 ‰, standard deviation 6.8 ‰). The average values are in line with Schilder et al.  
235 (2015a) who reported much lower bulk *Daphnia* ephippia  $\delta^{13}\text{C}$  values in HIN (-45.2 and -  
236 47.4 ‰) than in SEE (-35.5 ‰) isolated from different surface sediment samples than  
237 analysed in this study. The Shapiro-Wilk tests indicate that the data from HIN are not

238 normally distributed ( $p < 0.05$ ), whereas those of SEE and BUR are. The Brown-Forsyth test  
239 confirms a strong dissimilarity in the variance of the  $\delta^{13}\text{C}$  values between the lakes ( $p <$   
240 0.0005). Furthermore, Hartigan's dip-test suggests that the distribution of ephippia  $\delta^{13}\text{C}$   
241 values from BUR was not unimodal (i.e. at least bimodal,  $p < 0.05$ ).

242

243 4. Discussion

244 4.1 Within-sample variability in  $\delta^{13}\text{C}$  values

245 The repeated measurements on single ephippia demonstrate that the setup used in our study  
246 provides measurements with a high degree of replicability for ephippia analyses. They also  
247 show very low heterogeneity in  $\delta^{13}\text{C}$  values within ephippia, which means that results from  
248 different ephippia based on single measurements may be compared meaningfully.

249 In SEE, the variability between individual ephippia is relatively small (standard  
250 deviation 1.8 ‰) and the  $\delta^{13}\text{C}$  values (-37.3 to -31.5 ‰) typically are in the range of those  
251 reported for algae (-35 to -25 ‰, (Vuorio et al., 2006)). We consider it likely that this  
252 variability represents seasonal (or inter-annual) changes in  $\delta^{13}\text{C}$  values of algae that  
253 dominated in diet of *Daphnia* in this lake. Considering the low concentrations of methane in  
254 this lake it is very unlikely that MOB formed a major part of *Daphnia* diet and this is  
255 reflected in the absence of low *Daphnia* ephippia  $\delta^{13}\text{C}$  values (Figure 2).

256 In HIN, the standard deviation of  $\delta^{13}\text{C}$  values (4.0 ‰) is more than twice as  
257 large as in SEE and 8 out of 14 ephippia have a value of -41.9 ‰ or lower, much lower than  
258 can be expected for algae (Vuorio et al., 2006). We therefore interpret these low ephippia  
259  $\delta^{13}\text{C}$  values as indicating that these remains originate from *Daphnia* that incorporated  
260 methane-derived carbon. It appears that the variability is caused by both variation in algal

261  $\delta^{13}\text{C}$  values and variation in the importance of MOB as a dietary component. We do note that  
262 because the top 2 cm of sediments in our studied lakes likely represents multiple seasonal  
263 cycles, the observed variability may reflect both seasonal and inter-annual variations. MOB  
264 incorporation by *Daphnia* occurs predominantly in fall and winter (Schilder et al., 2017;  
265 Taipale et al., 2007). The  $\delta^{13}\text{C}$  values may hint at more than one production peak (one during  
266 fall and one during e.g. spring). However, the test for multimodality was not significant so we  
267 cannot state with confidence that timing of ephippia production was a causal factor in the  
268 variation of ephippia  $\delta^{13}\text{C}$  values we observed. Additional measurements may reveal  
269 evidence for multimodality of ephippia  $\delta^{13}\text{C}$  values within this sample.

270 The distribution of ephippia  $\delta^{13}\text{C}$  values from BUR was identified as  
271 multimodal and a clear separation can be seen between very low values (-54 to -45 ‰), much  
272 lower than can be expected from algae, and values more in line with those expected from  
273 algal primary productivity (-37 to -34 ‰). This pattern of  $\delta^{13}\text{C}$  values can be most easily  
274 explained by two or more seasonal production peaks of *Daphnia* ephippia during periods in  
275 the seasonal cycle when *Daphnia* either preferentially feed on algal material or on a mixed  
276 diet of phytoplankton and organisms incorporating methane-derived carbon (e.g. MOB or  
277 micro-organisms feeding on MOB). In Central European lakes such periods would include,  
278 e.g., early summer and autumn overturning, periods in which at least some *Daphnia* species  
279 are known to produce ephippia (Cáceres, 1998; Cáceres and Tessier, 2004).

280

## 281 4.2 Implications for palaeo-studies

282 The sample from BUR demonstrates that the range of seasonal and/or inter-annual variability  
283 in  $\delta^{13}\text{C}$  values of ephippia-producing *Daphnia* is preserved and reflected in the fossil record.  
284 Furthermore, it demonstrates that the distinct imprint of multiple production peaks remains

285 preserved in such samples. We interpret this variability as mainly reflecting seasonal changes  
286 in the relative importance and  $\delta^{13}\text{C}$  values of food sources ingested by *Daphnia*. Because of  
287 the peak-wise production of ephippia in many lakes (Cáceres, 1998) and the observation that  
288 *Daphnia* and ephippia tend to have very low  $\delta^{13}\text{C}$  values indicative of incorporation of  
289 methane-derived carbon typically during autumn and winter (Schilder et al., 2017; Taipale et  
290 al., 2007), it is likely that seasonal variation in ephippium production is the main contributor  
291 to the observed inter-ephippium variability. We regard seasonal variability especially relevant  
292 as a source for inter-ephippium variability in lakes where methane-derived carbon enters the  
293 pelagic food web. However, other sources of variation, such as species-dependent differences  
294 in feeding strategy or timing of ephippium production may also contribute to this variability.  
295 We also note that in lakes where ephippia are only produced once per year during a short  
296 period, strong seasonal variability in *Daphnia*  $\delta^{13}\text{C}$  values may not be faithfully recorded by  
297 the ephippia preserved in the sediment.

298 Analyses of within-sample distribution patterns of *Daphnia* ephippia  $\delta^{13}\text{C}$   
299 values may provide new avenues for reconstructing past environmental and ecological  
300 changes based on geochemical analyses of *Daphnia* remains. For example, measuring the  
301 stable oxygen isotope ratios and/or Mg/Ca values of individual marine foraminifera shells  
302 (Ganssen et al., 2011; Wit et al., 2010) to reveal changes in the range of seasonal sea surface  
303 temperatures allowed to further refine reconstructions of past (average) sea surface  
304 temperatures, which normally target mean annual values only. Moreover, individual marine  
305 dinoflagellate cyst  $\delta^{13}\text{C}$  analyses not only showed species-specific ecological variations but  
306 also yielded information on the ecological flexibility or fitness of populations (Sluijs et al.,  
307 2018). Similarly, down-core changes in the variability of within-sample  $\delta^{13}\text{C}$  variation of  
308 ephippia and or extreme (particularly minimum) values may provide information on changes  
309 in carbon sources for *Daphnia* ephippia in lakes. For example, is the relative abundance of

310 ephippia containing methane-derived carbon in the sample changing, or is the amount of  
311 methane-derived carbon in these ephippia changing, thereby altering the mean  $\delta^{13}\text{C}$  values of  
312 these ephippia? Measuring individual ephippia  $\delta^{13}\text{C}$  values may therefore allow for more  
313 sensitive assessments as to whether methane-derived carbon contributed to the planktonic  
314 food web of lakes. In addition, such information may potentially reveal changes in the  
315 production period of *Daphnia* ephippia in the past.

316 Our findings also allow an assessment of the expected variability of fossil  
317 ephippia  $\delta^{13}\text{C}$  values of bulk ephippia samples that originates from between-ephippium  
318 variability of  $\delta^{13}\text{C}$  values. Low ephippia numbers in such bulk samples may lead to  
319 substantial down-core variability in records due to an overrepresentation of ephippia with  
320 relatively (compared to the sample's average) low or high  $\delta^{13}\text{C}$  values in some samples. This  
321 is especially of concern for lake systems in which strong influences of methane-derived  
322 carbon in *Daphnia* diet may be expected (given the large variability in BUR and to a lesser  
323 degree HIN as opposed to SEE). To illustrate this, we used our 14 data points per lake (under  
324 the assumption that these properly reflect the variability within the lake's sediments, and that  
325 the ephippia all have equal mass) to randomly generate 40 bulk ephippia samples for each of  
326 17 different sample sizes between 1 and 150 ephippia, for each lake. Figure 3 shows the  
327 standard deviation from the average for those 40 samples for each sample size and lake. The  
328 calculations clearly show that for SEE, with relatively low between-ephippium variability,  
329 bulk samples with 20 ephippia are much more likely to return values within 1 % of the true  
330 average than for HIN and BUR. This implies that especially in lakes with a high variability in  
331 ephippia  $\delta^{13}\text{C}$  values, short (i.e. single sample), low-amplitude isotope excursions should not  
332 be over-interpreted in bulk *Daphnia* ephippia  $\delta^{13}\text{C}$  records, unless they are based on a very  
333 large amount of ephippia or well-supported by corroborating evidence. Measuring one or  
334 more secondary, independent proxies can help to separate such minor variations in bulk

335 *Daphnia*  $\delta^{13}\text{C}$  values which represent true changes in aquatic food webs and past  
336 environments from those which are due to large heterogeneity of ephippia  $\delta^{13}\text{C}$  values within  
337 samples. For example, earlier studies have used geochemical analyses, diatom-inferred total  
338 phosphorus (Schilder et al., 2017),  $\delta^{13}\text{C}$  measurements on other invertebrate taxa (e.g.  
339 chironomids, bryozoans, *Ceriodaphnia*) or bulk sediment organic matter  $\delta^{13}\text{C}$  analyses  
340 (Morlock et al., 2016; van Hardenbroek et al., 2013), or have analysed invertebrate remains  
341 from a second core from the same lake (Frossard et al., 2014) to support the interpretation of  
342 down-core invertebrate  $\delta^{13}\text{C}$  records based on the analysis of bulk samples.

343 In sediments that contain two or more distinct groups or clusters of ephippia  
344  $\delta^{13}\text{C}$  values (such as is the case in BUR), down-core changes in bulk ephippia  $\delta^{13}\text{C}$  values  
345 may reflect changes in one or two of these groups. For example, variations may be driven  
346 only by changes in fall ephippia  $\delta^{13}\text{C}$  values. In such cases changes in bulk ephippia  $\delta^{13}\text{C}$   
347 values may be effectively dampened by stability of ephippia produced during other parts of  
348 the seasonal cycle, for example ephippia from spring / early summer *Daphnia* that feed  
349 predominantly on algae. Such mechanisms may need to be taken into account when  
350 interpreting down-core bulk ephippia  $\delta^{13}\text{C}$  records. Further investigations of the main  
351 ephippia production peaks in our study lakes and of their stable carbon isotopic composition  
352 (e.g. with the help of sediment trap studies) would help to further constrain the effects of  
353 between-year variability in *Daphnia* ephippia  $\delta^{13}\text{C}$  values and multiple production peaks on  
354 reconstructed *Daphnia*  $\delta^{13}\text{C}$  values.

355

356

357 5. Conclusion

358 The data we present show that LA/nC/GC/IRMS analysis of *Daphnia* ephippia is possible  
359 and a feasible approach to investigate ephippia  $\delta^{13}\text{C}$  values on individual epihippia or even  
360 investigate the variability within the same ephippium with a high degree of repeatability. Our  
361 results imply that the within-sample variability of epihippia  $\delta^{13}\text{C}$  values, related to e.g.  
362 seasonal variability in *Daphnia*  $\delta^{13}\text{C}$  values and timing of ephippia production peaks, can  
363 have an impact on a fossil bulk ephippia  $\delta^{13}\text{C}$  record. This has some implications for  
364 preparing samples for down-core records based on bulk ephippia  $\delta^{13}\text{C}$  analysis and  
365 interpreting the results, but also opens up new avenues for future research.

366 Our results indicate that bulk samples with low  $\delta^{13}\text{C}$  values may represent  
367 averages of ephippium assemblages with a high between-ephippium variability in  $\delta^{13}\text{C}$   
368 values. Bulk ephippia samples therefore ideally consist of sufficiently high numbers of  
369 ephippia to increase the likelihood of properly reflecting the true average  $\delta^{13}\text{C}$  value of the  
370 deposited ephippia during a certain period of time. The optimal amount of ephippia depends  
371 strongly on the (expected) variability within the sample and the desired accuracy of the  
372 reconstruction (Figure 3). Especially when dealing with low amounts of ephippia, the use of  
373 secondary, independent proxies can produce necessary constraints to interpretations of down-  
374 core records. Measuring individual ephippia for certain parts of a record, for example for  
375 sections with relatively high or low rates of incorporation of methane-derived carbon, can  
376 also provide an avenue for constraining and improving interpretations. Moreover, this  
377 approach can potentially also be used to investigate how the range of ephippia  $\delta^{13}\text{C}$  values  
378 produced during different seasons and/or by different *Daphnia* populations changed over time.

379 Finally, our results show that  $\delta^{13}\text{C}$  analyses of individual chitinous microfossils  
380 in lake sediments are possible and produce reproducible results. This opens the opportunity

381 for applying this method to chitinous fossils of other invertebrate groups such as chironomid  
382 head capsules, cladoceran (e.g. bosminid or chydorid) head shields and carapaces or  
383 bryozoan statoblasts. Possible research questions which could be explored include, e.g. the  
384 within assemblage variability of  $\delta^{13}\text{C}$  values for individual taxa and fossil structures, but also  
385 inter taxon variability for fossil groups that produce too small amounts of fossil chitinous  
386 material to measure with conventional bulk measurement techniques.

387

## 388 ACKNOWLEDGEMENTS

389 We thank Mat Wooller and one anonymous reviewer for their constructive comments on this  
390 manuscript. This research received funding from the European Research Council under the  
391 European Union's Seventh Framework Programme (FP/2007- 2013) / ERC Grant Agreement  
392 n. 239858 (RECONMET) to O.H. The Netherlands Organization for Scientific Research  
393 supported this research through grant ALWOP.223 to A.S. and G.-J.R. This work benefitted  
394 from European Research Council Starting Grant 259627 awarded to A.S. This research was  
395 carried out under the program of the Netherlands Earth System Science Centre, financially  
396 supported by the Ministry of Education, Culture and Science.

397

## 398 REFERENCES

399 Bastviken, D., Tranvik, L.J., Downing, J.A., Crill, P.M., Enrich-prast, A., 2011. Freshwater  
400 methane emissions offset the continental carbon sink. *Science* (80). 331, 50.  
401 doi:10.1126/science.1196808

402 Battin, T.J., Luyssaert, S., Kaplan, L.A., Aufdenkampe, A.K., Richter, A., Tranvik, L.J.,  
403 2009. The boundless carbon cycle. *Nat. Geosci.* 2, 598–600. doi:10.1038/ngeo618

- 404 Belle, S., Parent, C., Frossard, V., Verneaux, V., Millet, L., Chronopoulou, P.-M., Sabatier,  
405 P., Magny, M., 2014. Temporal changes in the contribution of methane-oxidizing  
406 bacteria to the biomass of chironomid larvae determined using stable carbon isotopes  
407 and ancient DNA. *J. Paleolimnol.* 52, 215–228. doi:10.1007/s10933-014-9789-z
- 408 Belle, S., Verneaux, V., Mariet, A.L., Millet, L., 2017. Impact of eutrophication on the  
409 carbon stable-isotopic baseline of benthic invertebrates in two deep soft-water lakes.  
410 *Freshw. Biol.* 1105–1115. doi:10.1111/fwb.12931
- 411 Cáceres, C.E., 1998. Interspecific variation in the abundance, production and emergence of  
412 *Daphnia* diapausing eggs. *Ecology* 79, 1699–1710.
- 413 Cáceres, C.E., Tessier, A.J., 2004. Incidence of diapause varies among populations of  
414 *Daphnia pulicaria*. *Oecologia* 141, 425–31. doi:10.1007/s00442-004-1657-5
- 415 Eek, K.M., Sessions, A.L., Lies, D.P., 2007. Carbon-isotopic analysis of microbial cells  
416 sorted by flow cytometry. *Geobiology* 5, 85–95. doi:10.1111/j.1472-4669.2006.00094.x
- 417 Frossard, V., Millet, L., Verneaux, V., Jenny, J.-P., Arnaud, F., Magny, M., Poulenard, J.,  
418 Perga, M.-E., 2013. Chironomid assemblages in cores from multiple water depths reflect  
419 oxygen-driven changes in a deep French lake over the last 150 years. *J. Paleolimnol.* 50,  
420 257–273. doi:10.1007/s10933-013-9722-x
- 421 Frossard, V., Verneaux, V., Millet, L., Jenny, J.-P., Arnaud, F., Magny, M., Perga, M.-E.,  
422 2014. Reconstructing long-term changes (150 years) in the carbon cycle of a clear-water  
423 lake based on the stable carbon isotope composition ( $\delta^{13}\text{C}$ ) of chironomid and  
424 cladoceran subfossil remains. *Freshw. Biol.* 59, 789–802. doi:10.1111/fwb.12304
- 425 Ganssen, G.M., Peeters, F.J.C., Metcalfe, B., Anand, P., Jung, S.J.A., Kroon, D., Brummer,  
426 G.J.A., 2011. Quantifying sea surface temperature ranges of the Arabian Sea for the past

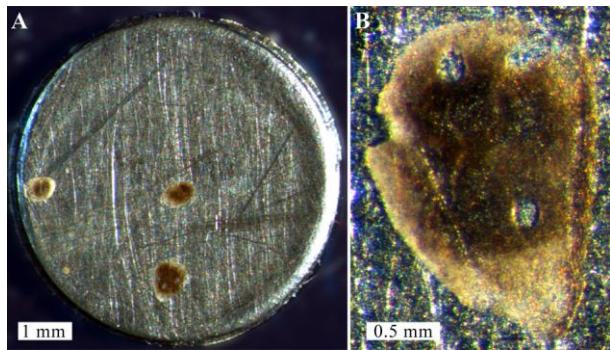
- 427 20000 years. *Clim. Past* 7, 1337–1349. doi:10.5194/cp-7-1337-2011
- 428 Heiri, O., Schilder, J., Hardenbroek, M. van, 2012. Stable isotopic analysis of fossil  
429 chironomids as an approach to environmental reconstruction: state of development and  
430 future challenges. *Fauna Nor.* 31, 7–18. doi:10.5324/fn.v31i0.1436.
- 431 Hollander, D.J., McKenzie, J.A., 1991. CO<sub>2</sub> control on carbon-isotope fractionation during  
432 aqueous photosynthesis: A paleo-pCO<sub>2</sub> barometer. *Geology* 19, 929–932.
- 433 Jedrysek, M.O., 2005. S–O–C isotopic picture of sulphate–methane–carbonate system in  
434 freshwater lakes from Poland. A review. *Environ. Chem. Lett.* 3, 100–112.  
435 doi:10.1007/s10311-005-0008-z
- 436 Kankaala, P., Taipale, S., Grey, J., Sonninen, E., Arvola, L., Jones, R.I., 2006. Experimental  
437 δ<sup>13</sup>C evidence for a contribution of methane to pelagic food webs in lakes. *Limnol.*  
438 *Oceanogr.* 51, 2821–2827.
- 439 Kankaala, P., Taipale, S., Li, L., Jones, R.I., 2010. Diets of crustacean zooplankton, inferred  
440 from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous  
441 dissolved organic carbon content. *Aquat. Ecol.* 44, 781–795. doi:10.1007/s10452-010-  
442 9316-x
- 443 Lampert, W., 2011. *Daphnia*: Development of a model organism, in: Excellence in Ecology.  
444 International ecology institute, Oldendorf/Luhe, p. 250.
- 445 Laws, E.A., Popp, B.N., Bidigare, R.R., Kennicutt, M.C., Macko, S.A., 1995. Dependence of  
446 phytoplankton carbon isotopic composition on growth rate and [CO<sub>2</sub>]<sub>aq</sub>: Theoretical  
447 considerations and experimental results. *Geochim. Cosmochim. Acta* 59, 1131–1138.
- 448 Leng, M.J., Henderson, A.C.G., 2013. Recent advances in isotopes as palaeolimnological

- 449 proxies. *J. Paleolimnol.* 49, 481–496. doi:10.1007/s10933-012-9667-5
- 450 Moran, J.J., Newburn, M.K., Alexander, M.L., Sams, R.L., Kelly, J.F., Kreuzer, H.W., 2011.
- 451 Laser ablation isotope ratio mass spectrometry for enhanced sensitivity and spatial
- 452 resolution in stable isotope analysis. *Rapid Commun. Mass Spectrom.* 25, 1282–1290.
- 453 doi:10.1002/rcm.4985
- 454 Morlock, M.A., Schilder, J., van Hardenbroek, M., Szidat, S., Wooller, M.J., Heiri, O., 2016.
- 455 Seasonality of cladoceran and bryozoan resting stage  $\delta^{13}\text{C}$  values and implications for
- 456 their use as palaeolimnological indicators of lacustrine carbon cycle dynamics. *J.*
- 457 *Paleolimnol.* 57, 141–156. doi:10.1007/s10933-016-9936-9
- 458 Nelson, D.M., Hu, F.S., Mikucki, J.A., Tian, J., Pearson, A., 2007. Carbon-isotopic analysis
- 459 of individual pollen grains from C3 and C4 grasses using a spooling-wire
- 460 microcombustion interface. *Geochim. Cosmochim. Acta* 71, 4005–4014.
- 461 doi:10.1016/j.gca.2007.06.002
- 462 Pearson, A., Hurley, S.J., Walter, S.R.S., Kusch, S., Lichtin, S., Zhang, Y.G., 2016. Stable
- 463 carbon isotope ratios of intact GDGTs indicate heterogeneous sources to marine
- 464 sediments. *Geochim. Cosmochim. Acta* 181, 18–35. doi:10.1016/j.gca.2016.02.034
- 465 Perga, M.-E., Maberly, S.C., Jenny, J.-P., Alric, B., Pignol, C., Naffrechoux, E., 2016. A
- 466 century of human-driven changes in the carbon dioxide concentration of lakes. *Glob.*
- 467 *biogeochem. Cycles* 30, 93–104. doi:10.1002/2015GB005286. Received
- 468 Perga, M.E., Gerdeaux, D., 2006. Seasonal variability in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values
- 469 of the zooplankton taxa in two alpine lakes. *Acta Oecologica* 30, 69–77.
- 470 doi:10.1016/j.actao.2006.01.007
- 471 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation

- 472 for Statistical Computing, Vienna, Austria.
- 473 Rinta, P., Bastviken, D., van Hardenbroek, M., Kankaala, P., Leuenberger, M., Schilder, J.,
- 474 Stötter, T., Heiri, O., 2015. An inter-regional assessment of concentrations and  $\delta^{13}\text{C}$
- 475 values of methane and dissolved inorganic carbon in small European lakes. *Aquat. Sci.*
- 476 77, 667–680. doi:10.1007/s00027-015-0410-y
- 477 Rinta, P., Van Hardenbroek, M., Jones, R.I., Kankaala, P., Rey, F., Szidat, S., Wooller, M.J.,
- 478 Heiri, O., 2016. Land use affects carbon sources to the pelagic food web in a small
- 479 boreal lake. *PLoS One* 11, 1–18. doi:10.1371/journal.pone.0159900
- 480 Schilder, J., Bastviken, D., van Hardenbroek, M., Leuenberger, M., Rinta, P., Stötter, T.,
- 481 Heiri, O., 2015a. The stable carbon isotopic composition of *Daphnia* ephippia in small,
- 482 temperate lakes reflects in-lake methane availability. *Limnol. Oceanogr.* 60, 1064–1075.
- 483 doi:10.1002/lno.10079
- 484 Schilder, J., Hardenbroek, M. Van, Bodelier, P., Lotter, F., Heiri, O., Kirilova, E.P.,
- 485 Leuenberger, M., 2017. Trophic state changes can affect the importance of methane-
- 486 derived carbon in aquatic food webs. *Proc. R. Soc. B-Biological Sci.* 284, 1–8.
- 487 doi:10.1098/rspb.2017.0278
- 488 Schilder, J., Tellenbach, C., Möst, M., Spaak, P., van Hardenbroek, M., Wooller, M.J., Heiri,
- 489 O., 2015b. The stable isotopic composition of *Daphnia* ephippia reflects changes in  $\delta^{13}\text{C}$
- 490 and  $\delta^{18}\text{O}$  values of food and water. *Biogeosciences* 12, 3819–3830. doi:10.5194/bg-12-
- 491 3819-2015
- 492 Sluijs, A., Van Roij, L., Frieling, J., Laks, J., Reichart, G.-J., 2018. Single Species
- 493 Dinoflagellate Cyst Carbon Isotope Ecology across the Paleocene-Eocene Thermal
- 494 Maximum. *Geology* 46, 77–82.

- 495 Smyntek, P.M., Maberly, S.C., Grey, J., 2012. Dissolved carbon dioxide concentration  
496 controls baseline stable carbon isotope signatures of a lake food web. Limnol. Oceanogr.  
497 57, 1292–1302. doi:10.4319/lo.2012.57.5.1292
- 498 Taipale, S., Kankaala, P., Jones, R.I., 2007. Contributions of different organic carbon sources  
499 to *Daphnia* in the pelagic foodweb of a small polyhumic lake: results from mesocosm  
500 DI13C-additions. Ecosystems 10, 757–772. doi:10.1007/s10021-007-9056-5
- 501 Templeton, A.S., Chu, K.-H., Alvarez-Cohen, L., Conrad, M.E., 2006. Variable carbon  
502 isotope fractionation expressed by aerobic CH<sub>4</sub>-oxidizing bacteria. Geochim.  
503 Cosmochim. Acta 70, 1739–1752. doi:10.1016/j.gca.2005.12.002
- 504 van Hardenbroek, M., Heiri, O., Parmentier, F.J.W., Bastviken, D., Ilyashuk, B.P., Wiklund,  
505 J.A., Hall, R.I., Lotter, A.F., 2013. Evidence for past variations in methane availability  
506 in a Siberian thermokarst lake based on  $\delta^{13}\text{C}$  of chitinous invertebrate remains. Quat.  
507 Sci. Rev. 66, 74–84. doi:10.1016/j.quascirev.2012.04.009
- 508 van Hardenbroek, M., Lotter, A.F., Bastviken, D., Andersen, T.J., Heiri, O., 2014. Taxon-  
509 specific  $\delta^{13}\text{C}$  analysis of chitinous invertebrate remains in sediments from Strandsjön,  
510 Sweden. J. Paleolimnol. 52, 95–105. doi:10.1007/s10933-014-9780-8
- 511 van Roij, L., Sluijs, A., Laks, J.J., Reichart, G.J., 2017. Stable carbon isotope analyses of  
512 nanogram quantities of particulate organic carbon (pollen) with laser ablation nano  
513 combustion gas chromatography/isotope ratio mass spectrometry. Rapid Commun. Mass  
514 Spectrom. 31, 47–58. doi:10.1002/rcm.7769
- 515 Verbruggen, F., Heiri, O., Reichart, G.-J., De Leeuw, J.W., Nierop, K.G.J., Lotter, A.F.,  
516 2010. Effects of chemical pretreatments on  $\delta^{18}\text{O}$  measurements, chemical composition,  
517 and morphology of chironomid head capsules. J. Paleolimnol. 43, 857–872.

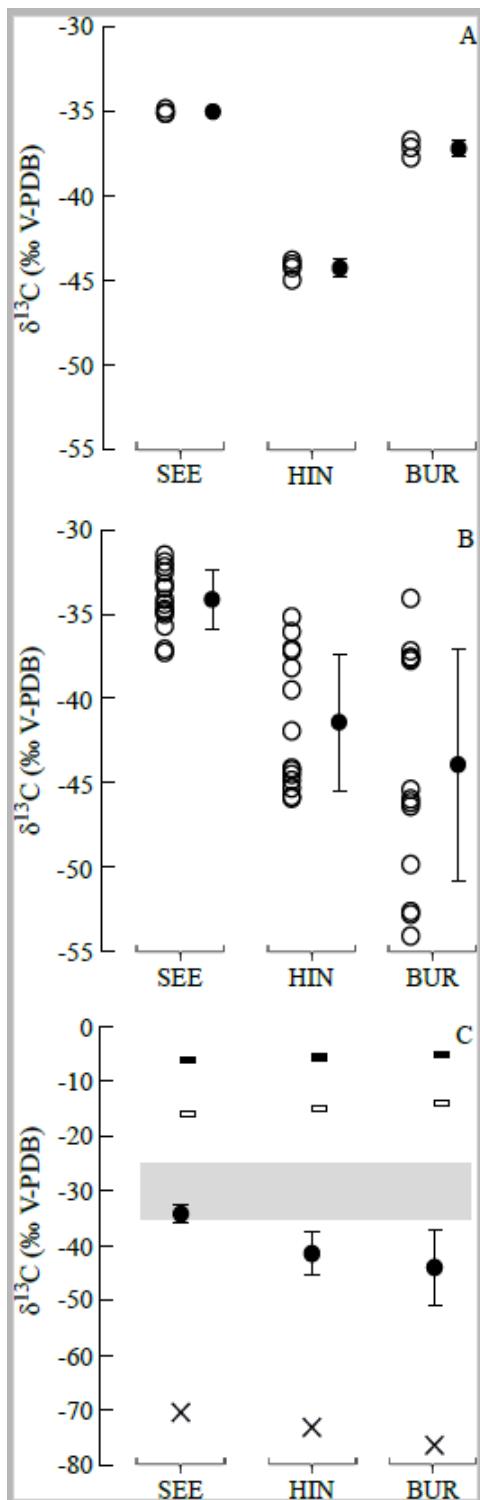
- 518 Vuorio, K., Meili, M., Sarvala, J., 2006. Taxon-specific variation in the stable isotopic  
519 signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of lake phytoplankton. *Freshw. Biol.* 51, 807–822.  
520 doi:10.1111/j.1365-2427.2006.01529.x
- 521 Whiticar, M.J., Faber, E., Schoel, M.L., 1986. Biogenic methane formation in marine and  
522 freshwater environments : CO<sub>2</sub> reduction vs. acetate fermentation-Isotope evidence.  
523 *Geochim. Cosmochim. Acta* 50, 693–709.
- 524 Wit, J.C., Reichart, G.J., A Jung, S.J., Kroon, D., 2010. Approaches to unravel seasonality in  
525 sea surface temperatures using paired single-specimen foraminiferal  $\delta^{18}\text{O}$  and Mg/Ca  
526 analyses. *Paleoceanography* 25, 1–15. doi:10.1029/2009PA001857
- 527 Wooller, M.J., Pohlman, J.W., Gaglioti, B. V, Langdon, P., Jones, M., Walter Anthony,  
528 K.M., Becker, K.W., Hinrichs, K.-U., Elvert, M., 2012. Reconstruction of past methane  
529 availability in an Arctic Alaska wetland indicates climate influenced methane release  
530 during the past ~12,000 years. *J. Paleolimnol.* 48, 27–42. doi:10.1007/s10933-012-9591-  
531 8
- 532 Zhao, Y., Nelson, D.M., Clegg, B.F., An, C.-B., Hu, F.S., 2017. Isotopic analysis on  
533 nanogram quantities of carbon from dissolved insect cuticle: a method for  
534 paleoenvironmental inferences. *Rapid Commun. Mass Spectrom.* doi:10.1002/rcm.7965
- 535
- 536



537

538 **Figure 1: Laser-ablated ephippia.** (A) Photograph showing a 6 mm nickel plate with three  
539 ephippia pressed onto it. (B) An ephippium with two holes resulting from the laser ablation.

540

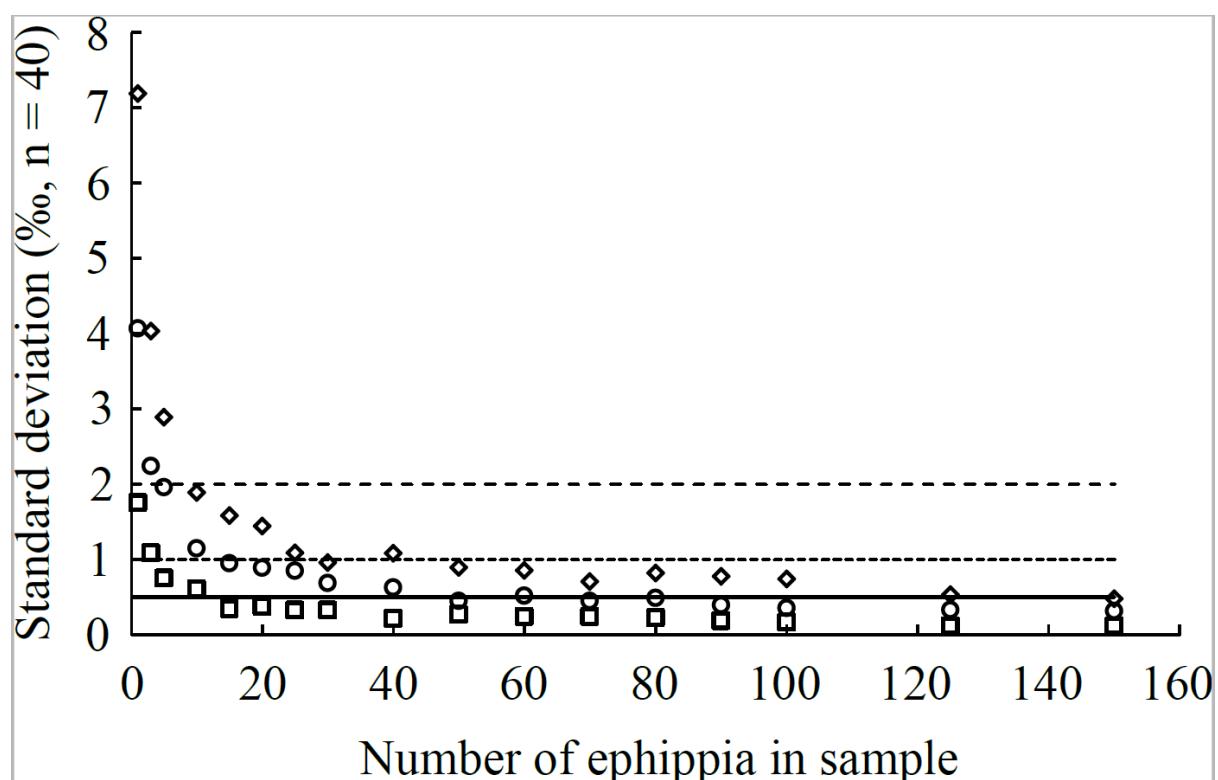


541

542 **Figure 2: Individual ephippia  $\delta^{13}\text{C}$  values.** A:  $\delta^{13}\text{C}$  values of repeated measurement of a  
 543 single ephippium from the sediments of SEE (n=3), HIN (n=4) and BUR (n=3). Open circles  
 544 represent the measurements and the closed circles represent the average, the error bars  
 545 indicate the standard deviation. B: The result of measurements on 14 individual ephippia

546 from the same sediments. Open circles indicate individual measurements and the closed  
547 circles the average value (error bars represent the standard deviation). C:  $\delta^{13}\text{C}$  values of  
548 dissolved inorganic carbon (black bars), dissolved carbon dioxide (open bars) and dissolved  
549 methane (cross marks) measured in the lakes (for details see Rinta et al., (2015) and Schilder  
550 et al. (2015a)). The grey area indicates  $\delta^{13}\text{C}$  values typical for algae (-35 to -25 ‰, Vuorio et  
551 al. (2006)) and the black circles with error bars indicate the average and standard deviation of  
552 the 14 individual ephippium  $\delta^{13}\text{C}$  values from each lake (as in B).

553



554  
555 **Figure 3: Sample size and standard deviation.** The standard deviation of 40 randomly  
556 generated ephippia samples (see main text for details) for various sample sizes (between 1  
557 and 150 ephippia) based on our 14 measurements on ephippia from BUR (diamonds), HIN  
558 (circles) and SEE (squares). The lines indicate a standard deviation of 0.5, 1 and 2 ‰.