

1 **Bryozoan stable carbon and hydrogen isotopes: Relationships between the**
2 **isotopic composition of zooids, statoblasts and lake water**

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20
21 **Abstract**

22 We explored the extent to which $\delta^{13}\text{C}$ and δD values of freshwater bryozoan statoblasts
23 can provide information about the isotopic composition of zooids, bryozoan food and
24 surrounding water. Bryozoan samples were collected from 23 sites and encompassed
25 ranges of nearly 30‰ for $\delta^{13}\text{C}$ and 100‰ for δD values. $\delta^{13}\text{C}$ offsets between zooids

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26 and statoblasts generally ranged from -3 to +4.5‰, with larger offsets observed in four
27 samples. However, a laboratory study with *Plumatella emarginata* and *Lophopus*
28 *crystallinus* demonstrated that, in controlled settings, zooids had only 0 to 1.2‰ higher
29 $\delta^{13}\text{C}$ values than statoblasts, and 1.7‰ higher values than their food. At our field sites,
30 we observed a strong positive correlation between median $\delta^{13}\text{C}$ values of zooids and
31 median $\delta^{13}\text{C}$ values of corresponding statoblasts. We also observed a positive
32 correlation between median δD values of zooids and statoblasts for *Plumatella*, and a
33 positive correlation between median δD values of statoblasts and δD values of lake
34 water for *Plumatella* and when all bryozoan taxa were examined together. Our results
35 suggest that isotope measurements on statoblasts collected from flotsam or sediment
36 samples can provide information on the feeding ecology of bryozoans and the H
37 isotopic composition of lake water.

38

39 **Keywords:** freshwater Bryozoa; stable isotopes; statoblasts; lakes; feeding ecology;
40 palaeoecology

41

42 **Introduction**

43

44 Moss animals (Bryozoa) are a common element of freshwater invertebrate
45 assemblages, but have received relatively little attention in ecological and
46 palaeoecological studies compared with other invertebrate taxa in lakes, e.g. insects or
47 crustaceans. Bryozoans are sessile colonial suspension feeders that grow on
48 submerged substrates (Wood & Okamura, 2005). Colonies are composed of asexually
49 produced modules, called zooids, that use ciliated tentacles to create feeding currents
50 to capture suspended food particles, including phytoplankton and bacteria (Kaminski,

51 1984; Wood and Okamura, 2005). Collecting bryozoan colonies can be challenging as
52 they can be difficult to locate. Hence, bryozoans are generally not collected by standard
53 sampling methods (e.g. kick-sampling). An alternative way to assess bryozoan
54 presence and abundance is to collect their dormant stages, or statoblasts, which have
55 robust, chitinous outer valves that are regularly found in flotsam, flood debris, and lake
56 sediments (Hill et al., 2007). Statoblasts are commonly found in lake sediment records,
57 and can therefore be analysed in palaeoecological studies to infer past dynamics of
58 invertebrate assemblages (Francis, 2001; Okamura et al., 2013).

59 In modern ecosystem studies, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope
60 analyses on aquatic invertebrates can provide information on food sources and on the
61 length and structure of food webs of lakes (Post, 2002). For invertebrates that produce
62 fossilizing chitinous structures, $\delta^{13}\text{C}$ analysis of fossil remains can also provide
63 information on past changes in the structure and carbon sources of lacustrine food
64 webs (Wooller et al., 2008; Van Hardenbroek et al., 2014). For example, $\delta^{13}\text{C}$ analyses
65 on *Daphnia* and chironomid larvae have recently been used to reconstruct the
66 relevance of methane-derived carbon in benthic and planktonic food webs in the past
67 (Wooller et al., 2012; Van Hardenbroek et al. 2013a, Belle et al. 2014).

68 Climate strongly influences the H and O isotopic composition of lake water,
69 which in turn determines the δD and $\delta^{18}\text{O}$ values of lacustrine invertebrates. The stable
70 isotopic composition of H and O in aquatic invertebrate fossils reflects the δD and $\delta^{18}\text{O}$
71 value of lake water at the time when these invertebrates were alive, and δD and $\delta^{18}\text{O}$
72 values of invertebrate fossils can thus provide information about past climatic change.
73 For example, $\delta^{18}\text{O}$ and δD values of fossil remains of aquatic insects have been
74 identified as proxies for reconstructing past variations in lake water $\delta^{18}\text{O}$ and δD
75 values (e.g. Wooller et al., 2004; Verbruggen et al., 2010; Van Hardenbroek et al.,

76 2013b). However, $\delta^{18}\text{O}$ and δD values of aquatic invertebrates are also influenced by
77 the $\delta^{18}\text{O}$ and δD values of food (Wang et al., 2009; Soto et al., 2013; Schilder et al.,
78 2015b). Reconstructions may therefore also be affected by variations in food sources
79 available to aquatic invertebrates and in the isotopic composition of these food sources.

80 Despite their ubiquity and preservation in lake sediments, the potential use of
81 statoblasts in stable isotope studies has been largely unexplored. Here we present an
82 exploratory study of the carbon and hydrogen isotopic composition of bryozoan zooids
83 and statoblasts collected at 23 sites in Northwest and Central Europe. We provide
84 information on the range of bryozoan $\delta^{13}\text{C}$ and δD values, as well as on the offsets
85 between zooids and statoblasts under field conditions. We focused on stable carbon
86 isotopes since invertebrate $\delta^{13}\text{C}$ analyses are widely used in modern food web studies
87 (Vander Zanden & Rasmussen, 1999; Grey et al., 2004a) and are increasingly analysed
88 for palaeoecological reconstructions of carbon cycling in lakes (Frossard et al., 2014;
89 Van Hardenbroek et al., 2014). Because our analytical set-up allowed us to
90 simultaneously measure δD and $\delta^{13}\text{C}$ values on relatively small bryozoan samples, we
91 analysed hydrogen rather than oxygen isotopes. We also present the results of a
92 laboratory study designed to characterise the offset between $\delta^{13}\text{C}$ values of bryozoan
93 zooids and statoblasts under controlled conditions.

94 Our study first investigates the relationship between zooids and statoblasts
95 regarding their $\delta^{13}\text{C}$ and δD values and secondly the relationship between bryozoans and
96 their food/surrounding water. Specifically, we focus on the following questions: (1)
97 How do $\delta^{13}\text{C}$ values of bryozoan zooids relate to those of statoblasts under field
98 conditions? (2) How do δD values of zooids relate to δD values of statoblasts under
99 field conditions? (3) How are $\delta^{13}\text{C}$ values of zooids/statoblasts related to $\delta^{13}\text{C}$ values of
100 their food under laboratory conditions? (4) How do δD values of zooids/statoblasts

101 reflect lake water δD values? Our study provides a basis for a future use of $\delta^{13}C$ and δD
102 values of bryozoan statoblasts in palaeo food web studies and for inferring past
103 variations in lake water δD values based on δD analyses of fossil bryozoan remains.

104

105

106 **Methods**

107

108 *Field survey*

109 Bryozoan colonies with statoblasts were collected from 23 sites in the littoral zone of
110 lakes and ponds and from one stream. Species collected were *Cristatella mucedo*
111 (Cuvier, 1798) (8 sites), *Pectinatella magnifica* (Leidy, 1851) (1 site), and
112 representatives of the genus *Plumatella*, which were not identified to species (16 sites).
113 Sites were visited from 2010-2012 and included locations in the Netherlands, Germany,
114 and Switzerland (Table 1). Sufficient material to measure stable isotopes of 'paired'
115 samples of both zooids and statoblasts was collected from most sites, but occasionally
116 only zooids or statoblasts were available (Table 2). Simultaneously, water samples for
117 stable isotope analysis were collected at all 23 sites in sealed containers and stored
118 cool and dark until analysis within 2 months of collection.

119 Between one and six replicate colonies were measured for each site (Table 1
120 and 3). Colonies were kept cool and dissected within 24 hours of collection. Gut
121 evacuation was often incomplete when colonies died soon after detachment from their
122 substrate. Extraneous material such as wood, algae and silt, was removed from the
123 zooids with lancet and forceps to minimize contamination, but complete removal was
124 not always possible due to the disintegration of fragile zooid tissues. Zooid material
125 was freeze-dried and transferred into silver cups.

126 Mature statoblasts were identified with a dissection microscope (4-40x
127 magnification) and opened to remove the mass of yolk granules and germinal tissue
128 using lancet and forceps. Statoblasts were then treated with 10% KOH for 2 hours at
129 room temperature to remove remaining attached soft tissue. This KOH treatment is
130 commonly used in palaeolimnological studies of chitinous invertebrate remains and
131 has been shown to have negligible effect on $\delta^{13}\text{C}$ values of chitinous sheaths and
132 exoskeletons (van Hardenbroek et al., 2010; Schilder et al., 2015a). Samples were then
133 rinsed with deionised water, freeze-dried, and transferred into silver cups for stable
134 carbon and hydrogen isotope analysis.

135 Zooid tissue and matching statoblast samples from the field survey were
136 measured on a high temperature elemental analyzer (ThermoFinnigan, Bremen,
137 Germany) coupled to a mass spectrometer (Isoprime, Cheadle, UK). Pyrolysis
138 temperature was set to 1450 °C. Since we attempted to measure stable isotope ratios
139 for C and H simultaneously on small (60 to 160 μg) samples, the precision associated
140 with the $\delta^{13}\text{C}$ and δD measurements is relatively low. Replicate measurements ($n = 37$)
141 on a chitin standard (Sigma Aldrich, Zwijndrecht, The Netherlands) had a standard
142 deviation of 1.1‰ for $\delta^{13}\text{C}$ and 3.1‰ for δD . Replicate measurements ($n = 35$) of a
143 cellulose standard (Merck, Darmstadt, Germany) had a standard deviation of 1.0‰ for
144 $\delta^{13}\text{C}$ and 10.8‰ for δD . Stable carbon isotopes are reported relative to VPDB and
145 stable hydrogen isotopes relative to V-SMOW. δD values of bryozoan samples were
146 corrected for exchangeable hydrogen using the method described by Filot et al. (2006):
147 In short, exchangeable hydrogen in the samples was equilibrated with standard water
148 vapour of known isotopic composition. δD of bryozoan samples was calculated based
149 on the measured δD after equilibration, the δD of the standard water vapour, and an

150 estimated percentage of 23.9% exchangeable H in the sample, assuming all samples
151 have the same percentage of exchangeable H atoms.

152 Stable H and O isotopes of water samples from the field survey were analysed
153 on a Finnigan MAT 250 mass spectrometer (Finnigan MAT, San Jose, CA) after
154 equilibration of the water samples with a standard carbon dioxide using an
155 equilibration device developed at the Physics Institute (University of Bern, Bern,
156 Switzerland). Four small-volume samples were measured on a Picarro L1102-i
157 analyser (Picarro Inc., Sunnyvale, CA) at the same laboratory. Standard deviations of
158 measurements on water standards of known isotopic composition were better than
159 0.5‰ for δD and 0.1‰ for $\delta^{18}\text{O}$. For five sites only $\delta^{18}\text{O}$ of lake water was measured.
160 For these five lakes δD was estimated based on $\delta^{18}\text{O}$ and the relationship between $\delta^{18}\text{O}$
161 and δD observed for Swiss lakes: First, the difference ($\Delta\delta^{18}\text{O}$) between measured $\delta^{18}\text{O}$
162 in lake water and estimated $\delta^{18}\text{O}$ in precipitation (Bowen & Revenaugh, 2003; Bowen,
163 2014) was calculated for each of our sampling locations. Similarly, $\Delta\delta\text{D}$ was calculated
164 as the difference between measured lake water δD and estimated δD of precipitation
165 for those sites where we had measured lake water δD . A linear regression was then
166 used to estimate $\Delta\delta\text{D}$ as a function of $\Delta\delta^{18}\text{O}$ ($n = 21$, $r = 0.99$). This relationship was
167 used to calculate $\Delta\delta\text{D}$ from $\Delta\delta^{18}\text{O}$ for sites without lake water δD measurements and to
168 estimate δD based on the δD of precipitation and $\Delta\delta\text{D}$. Table 1 specifies the method
169 used to derive δD for each water sample.

170

171 *Laboratory study*

172 Colonies of *Lophopus crystallinus* (Pallas, 1768) and *Plumatella emarginata* (Allman,
173 1844) were grown to evaluate the $\delta^{13}\text{C}$ values of their zooids and statoblasts and how
174 this relates to the $\delta^{13}\text{C}$ of their diet, particulate organic matter (POM). A microcosm for

175 culturing bryozoans was established at constant 18 °C (\pm 1–2 °C) as in Hartikainen &
176 Okamura (2012). The microcosm contained deionized water spiked periodically with
177 natural pond water. The system comprised two 16-litre side tanks, housing the
178 bryozoan colonies, connected to a 30-litre main tank containing 2 goldfish. A
179 fluorescent light tube above the main tank (Tropic Sun 5500 K, ZooMed, Ekeren,
180 Belgium) and fish excretions promoted algal and bacterial production and hence food
181 for bryozoans. Water was continuously circulated between the main and side tanks via
182 airlifts and U-tubes.

183 Bryozoa collected from three UK sites (Barton Blow Wells in Lincolnshire, the
184 Norfolk Broads in Norfolk, and Padworth in Berkshire) were allowed to grow for at
185 least 30 days in the laboratory. Zooids grown *de novo* in the laboratory were
186 transparent, allowing the exclusion of sediment-covered zooids and statoblasts formed
187 in the field. This allowed us to select laboratory-grown material, which had only
188 incorporated carbon from the POM under the laboratory conditions. After 30 days,
189 colonies were transferred to artificial pond water for 24 hours to allow gut evacuation.
190 Zooids and mature statoblasts were separated with forceps and a lancet under a
191 stereomicroscope (4-40x magnification). Two types of statoblasts were collected from
192 *Plumatella* colonies: sessoblasts, which remain attached inside colonies, and
193 floatoblasts, which are released into the water. All zooid material per taxon was
194 combined, freeze-dried, and homogenized. From this homogenized material, 3-4
195 replicate samples were weighed into tin capsules, and stored in a desiccator until
196 stable isotope analysis. The same procedure was followed for statoblast material per
197 taxon.

198 In addition, POM was collected at the start of the culture, at 14 days, and at 30
199 days to assess if $\delta^{13}\text{C}$ values of POM changed during the study. POM was filtered onto

200 pre-combusted filters (Whatman GF/C), freeze-dried, weighed into tin capsules, and
201 stored in a desiccator until stable isotope analysis.

202 Because of their low weight (32-78 μg), zooid and statoblast samples from the
203 culturing study could be only analyzed for $\delta^{13}\text{C}$ values. This was done on a Fisons NA
204 1500 NCS Elemental Analyzer coupled to a Thermo Electron Delta plus IRMS at the
205 Geochemistry laboratory, Utrecht University, The Netherlands. Repeated
206 measurements (n=10) of an internal laboratory standard (NAXOS carbonate) yielded
207 an analytical precision better than $\pm 0.1\text{‰}$.

208

209 *Statistical analyses*

210 To examine the relationship between $\delta^{13}\text{C}$ and δD values of bryozoan soft tissue
211 and statoblasts in the field study, we compared median values of replicate
212 measurements per field site using nonparametric Spearman's rank correlation
213 coefficient (ρ) and associated significance tests ('Hmisc' package, R core team, 2013).
214 Correlations were calculated for *Cristatella* and *Plumatella* separately and for all
215 Bryozoa combined to see if similar relationships can be observed at genus level and for
216 freshwater Bryozoa as a group. This provides useful information for
217 palaeoenvironmental applications, where statoblasts of different genera might need to
218 be pooled to retrieve enough material for stable isotope analyses. Nonparametric
219 correlation coefficients and tests were selected since offsets between bryozoan soft
220 tissues and statoblasts suggested some unusual outlier values in the $\delta^{13}\text{C}$
221 measurements (see results). The same median values of replicate measurements per
222 field site for zooids and statoblasts were used to test for significant differences in $\delta^{13}\text{C}$
223 and δD values between zooids and statoblasts, using a paired-samples t-test. This was
224 done for all bryozoan samples together and for *Cristatella* and *Plumatella* samples,

225 using Past software version 2.14 (Hammer et al., 2001). We tested for significant
226 differences between mean $\delta^{13}\text{C}$ values of POM, zooids, and statoblast in the laboratory
227 study using 1-way ANOVA and pairwise comparisons with Tukey-HSD test (Past
228 software version 2.14, Hammer et al., 2001).

229

230 **Results**

231

232 *Field survey*

233 In total 88 colonies from 21 sites were sampled (Table 1). Because of small sample
234 quantities in some sites, reliable stable isotope measurements were only available for
235 80 statoblast samples and 57 zooid samples. Table 2 shows that paired samples of
236 zooids and statoblasts from the same colony were found for *Plumatella* at 9 sites (23
237 paired samples), and for *Cristatella* at 7 sites (24 paired samples). *Pectinatella* was only
238 found at 1 site (2 paired samples).

239

240 *$\delta^{13}\text{C}$ values of zooids and statoblasts*

241 A remarkably large range of $\delta^{13}\text{C}$ values of nearly 30‰ characterised bryozoans at the
242 study sites (Fig. 1a). *Plumatella* zooids ranged from -48.2 to -19.4‰ compared with
243 values of -42.3 to -22.4‰ measured on statoblasts. $\delta^{13}\text{C}$ ranges of *Cristatella* were -
244 40.0 to -26.8‰ for zooids and -39.8 to -25.2‰ for statoblasts. Offsets in $\delta^{13}\text{C}$ between
245 zooids and statoblasts generally ranged from -3.0 to +4.5‰. However, very large
246 offsets were observed for four individual samples, ranging from -13.6‰ (one sample
247 of *Cristatella* in Schöhsee) to +12.6‰ (one sample of *Cristatella* in Veenmeer and two
248 samples of *Plumatella* in Aatalweiher). The overall mean of differences between
249 median zooid $\delta^{13}\text{C}$ values and median statoblast $\delta^{13}\text{C}$ values per site was relatively

250 small ($1.4\text{‰} \pm 4.4\text{‰}$ SD for *Plumatella*, $1.0\text{‰} \pm 1.9\text{‰}$ SD for *Cristatella*, and -0.2‰
251 for *Pectinatella*, Fig. 2a). Differences between zooid and statoblast median $\delta^{13}\text{C}$ values
252 were not statistically significant for *Plumatella*, for *Cristatella*, or for all Bryozoa pooled
253 (paired-samples t-test).

254 Median $\delta^{13}\text{C}$ values of zooids and statoblasts were strongly and positively
255 correlated ($\rho = 0.70$, $P = 0.0019$, $n = 17$) when all paired samples from bryozoans were
256 examined together. Considering the taxa separately, a similar correlation was found for
257 *Cristatella* ($\rho = 0.85$, $P = 0.012$, $n = 7$), but not for *Plumatella* due to the two samples in
258 Aatalweiher with unusually low statoblast $\delta^{13}\text{C}$ values (Fig. 3a). Without the
259 Aatalweiher site the correlation would also have been strongly positive for *Plumatella*
260 ($\rho = 0.81$, $P = 0.022$, $n = 8$) and even stronger for all bryozoan samples ($\rho = 0.90$, $P <$
261 0.0001 , $n = 16$).

262

263 *δD values of zooids and statoblasts*

264 The observed range of bryozoan δD values from our sites is nearly 100‰ (Fig. 1b). δD
265 values of *Plumatella* zooids ranged from -213.2 to -127.4‰ , compared with values of $-$
266 207.2 to -125.9‰ measured on statoblasts. The ranges of *Cristatella* were -221.3 to $-$
267 186.6‰ for zooids and -197.5 to -139.0‰ for statoblasts. Offsets in δD values
268 between zooids and statoblasts ranged from -75 to $+16\text{‰}$ (Fig. 2b). Statoblast δD
269 values appeared higher than δD values of zooids (Fig. 1b and 2b). The mean difference
270 between median statoblast δD values and median zooid δD values per site was -44.8‰
271 $\pm 15.5\text{‰}$ SD for *Cristatella*, and $-12.2\text{‰} \pm 14.7\text{‰}$ SD for *Plumatella*. These differences
272 were statistically significant for *Cristatella* (two-sample t-test, $t = -7.783$, $p < 0.001$),
273 but not for *Plumatella*. For *Pectinatella* the median offset was -29.5‰ , with too few
274 samples for significance testing.

275 Only for *Plumatella* did we observe a positive correlation between median δD
276 values of statoblasts and median δD values of zooids ($\rho = 0.75$, $P = 0.0025$, $n = 9$; Fig. 4),
277 whereas the relationships between median values were not significant for *Cristatella*
278 or for all bryozoan samples combined. A visual examination of the scatter plots (Fig. 4)
279 suggests that this lack of significance may be associated with more variable offsets
280 between statoblast and zooid tissue in *Cristatella* than observed for the other bryozoan
281 groups.

282

283 *δD values of bryozoans and lake water*

284 Sample pairs of zooids and lake water were available for *Plumatella* from 10 sites (29
285 paired samples), for *Cristatella* from 5 sites (20 paired samples), and for *Pectinatella*
286 from 1 site (2 paired samples). However, when comparing median zooid δD values per
287 site with lake water δD values based on Spearman correlation coefficients no
288 systematic relationships between lake water and zooid δD values were observed (Fig.
289 5a). Sample pairs of statoblasts and lake water were available for *Plumatella* from 16
290 sites (43 paired samples), for *Cristatella* from 7 sites (26 paired samples), and for
291 *Pectinatella* from 1 site (3 paired samples). A positive correlation was observed (Fig.
292 5b) between median statoblast δD values from the same location and lake water δD
293 values when considering all bryozoan samples combined ($\rho = 0.56$, $P = 0.005$, $n = 24$)
294 and when considering *Plumatella* ($\rho = 0.55$, $P = 0.027$, $n = 16$), but this was not
295 apparent for *Cristatella*. The lack of significant relationship for *Cristatella* may partly be
296 a consequence of the lower number of localities for which samples of this species were
297 measured.

298

299 *Laboratory study*

300 The laboratory study yielded three and four replicate samples of homogenized zooid
301 tissue for *Plumatella* and *Lophopus*, respectively. For *Plumatella*, three replicate
302 samples were available for both sessoblasts and floatoblasts, and for *Lophopus* three
303 replicate samples of floatoblasts were collected. Measured $\delta^{13}\text{C}$ values of zooids were
304 in general very similar to $\delta^{13}\text{C}$ values of sessoblasts and floatoblasts, as well as to
305 values observed for POM (Fig. 6). ANOVA indicated statistically significant differences
306 between POM, zooids and statoblasts for both *Plumatella* and *Lophopus*. For *Lophopus*
307 pairwise comparisons with Tukey-HSD tests indicated no significant differences
308 between zooids and statoblasts. For *Plumatella*, however, Tukey-HSD tests confirmed
309 that the observed mean differences of 1.2‰ between zooids and floatoblasts (Tukey-
310 HSD, $Q = 5.14$, $P = 0.023$) and the 1.2‰ mean difference between zooids and
311 sessoblasts ($Q = 5.48$, $P = 0.014$) were significant. No significant difference was
312 observed between floatoblasts and sessoblasts of *Plumatella* (Tukey-HSD).
313 Furthermore, the mean differences between zooids and POM of 1.7‰ were significant
314 for both *Lophopus* (Tukey-HSD, $Q = 8.22$, $P < 0.001$) and *Plumatella* ($Q = 8.14$, $P <$
315 0.001). The mean 1.7‰ difference in $\delta^{13}\text{C}$ values between POM and *Lophopus*
316 statoblasts was significant (Tukey-HSD, $Q = 8.26$, $P < 0.001$), but no significant
317 difference was found between POM and *Plumatella* sessoblasts or floatoblasts (Tukey-
318 HSD).

319

320

321 **Discussion**

322

323 *Large range of bryozoan $\delta^{13}\text{C}$ values*

324 The nearly 30‰-range of $\delta^{13}\text{C}$ values observed for freshwater bryozoan tissues in this
325 study is much larger than range of $\delta^{13}\text{C}$ values previously reported by Turney (1999),
326 Van Riel et al. (2006), and Van Hardenbroek et al. (2014). These earlier studies found
327 $\delta^{13}\text{C}$ values between -35 and -20‰ that largely overlap with reported ranges for
328 phytoplankton and POM (France, 1995; Vuorio et al., 2006). At 10 sites we found $\delta^{13}\text{C}$
329 values that were lower than -35‰ and at one site, Chli Moossee, values measured for
330 zooids were as low as -48.2 and -47.2‰. Planktonic algae can in some situations be
331 characterized by $\delta^{13}\text{C}$ values lower than -35‰. For example, $\delta^{13}\text{C}$ values of -41 to -
332 37‰ were reported by Jones et al. (1999) and Kankaala et al. (2010) for three small
333 Finnish brown water lakes with low phytoplankton growth rates. Such low
334 phytoplankton $\delta^{13}\text{C}$ values, however, are very unusual for eutrophic lakes like Chli
335 Moossee, and an additional source of ^{13}C -depleted carbon must have been available to
336 bryozoans. Methane-derived carbon is strongly ^{13}C -depleted and it has been shown
337 that different groups of freshwater invertebrates can incorporate carbon of methane-
338 oxidizing bacteria (MOB) (Jones & Grey, 2011; Schilder et al., 2015b), leading to
339 observed $\delta^{13}\text{C}$ values as low as -70‰ in some invertebrate groups. The availability of
340 MOB is especially high at the anoxic-oxic interface (Jones & Grey, 2011) and, in lakes
341 with anoxic bottom waters, planktonic filter feeders have been observed to incorporate
342 methanogenic carbon, leading to $\delta^{13}\text{C}$ values lower than -50‰ in their biomass
343 (Taipale et al., 2007; Schilder et al., 2015b). Bryozoans are sessile filter feeders, and all
344 colonies obtained in this study originate from shallow parts of lakes down to a depth of
345 2 m. Richelle et al. (1994) have demonstrated that bryozoans can feed on microbial
346 biomass. Our results suggest that, in some lakes, MOB may form a relevant part of POM
347 in the shallow littoral zone and that bryozoans may incorporate carbon from MOB
348 under these circumstances. Feeding partly on MOB would explain the extremely low

349 $\delta^{13}\text{C}$ values of Bryozoa found at Aatalweiher, Sisselenweiher, Chli Moossee,
350 Golihübweiher, Lobsigensee, and Piepertkolk (Table 3). However, more detailed
351 measurements of $\delta^{13}\text{C}$ values of bryozoans and POM, and of the abundance of MOB in
352 POM in littoral habitats would be necessary to confirm this hypothesis.

353

354 *$\delta^{13}\text{C}$ offsets between POM, zooids, and statoblasts*

355 Freshwater consumers are usually very similar in their $\delta^{13}\text{C}$ values compared to their
356 diet, with consumer $\delta^{13}\text{C}$ values on average 0 to 1.3‰ higher than those of their diet
357 (DeNiro & Epstein, 1978; McCutchan et al., 2003; Peters et al., 2012). It has therefore
358 been suggested that $\delta^{13}\text{C}$ values of freshwater bryozoans reflect the $\delta^{13}\text{C}$ values of
359 phytoplankton or POM in the water column (Van Hardenbroek et al., 2014). This idea is
360 supported by the results of our laboratory study. Although the 1.7‰ offset we
361 observed between $\delta^{13}\text{C}$ values of POM and cultured bryozoan zooids was statistically
362 significant, it was small relative to the 30‰ range of $\delta^{13}\text{C}$ values observed for zooids in
363 the field survey.

364 In a study on the River Rhine, colonies of *Plumatella repens* and *P. fungosa* were
365 characterized by $\delta^{13}\text{C}$ values of -31.1‰ and -28.8‰, respectively (van Riel et al.,
366 2006). These values were substantially lower than $\delta^{13}\text{C}$ values observed in the same
367 study for POM (-24.27‰), which contrasts with the results of our experiments. In the
368 same study on the River Rhine, however, van Riel et al. also found that *Plumatella* $\delta^{13}\text{C}$
369 was only 0.9-3.3‰ higher than $\delta^{13}\text{C}$ values of phytoplankton (-32‰) that they
370 estimated based on the $\delta^{13}\text{C}$ values of dissolved inorganic carbon. The $\delta^{13}\text{C}$ offset
371 between *Plumatella* and phytoplankton reported by van Riel et al. was therefore
372 apparently similar to the offsets we report between POM and zooids in our laboratory

373 study, suggesting that bryozoans were selectively feeding on phytoplankton, and that
374 POM collected by van Riel et al. contained organic matter not assimilated by bryozoans.

375 Other culturing experiments with planktonic filter feeders are in keeping with
376 the results obtained in our laboratory study. For example, cultured specimens of
377 *Daphnia magna* (Straus, 1820) were characterized by $\delta^{13}\text{C}$ values 1.7 to 3.1‰ higher
378 than their food (Power et al., 2003). In another study with *Daphnia pulicaria* (Forbes,
379 1893) this difference was $0.5 \pm 0.3\text{‰}$ (Schilder et al., 2015a). In our laboratory study,
380 the 1.7‰ higher $\delta^{13}\text{C}$ values of zooids of *Plumatella* and *Lophopus* compared with the
381 $\delta^{13}\text{C}$ values of their food are of similar magnitude, suggesting that zooid $\delta^{13}\text{C}$ values
382 provide a direct indication of the $\delta^{13}\text{C}$ values of bryozoan diet.

383 In our laboratory study we found very small offsets between $\delta^{13}\text{C}$ values of
384 bryozoan zooids and statoblasts, based on a diet with constant $\delta^{13}\text{C}$ values. We
385 observed no significant offset between $\delta^{13}\text{C}$ values of zooids and statoblasts for
386 *Lophopus*, and a small but significant 1.2‰ offset for *Plumatella*. This is in agreement
387 with differences reported between whole body tissue of other aquatic invertebrates
388 and their fossilizing, chitinous body parts. Perga (2011) showed that $\delta^{13}\text{C}$ values of the
389 ephippia of *Daphnia* from Lake Geneva were indistinguishable ($\pm 0.1\text{‰}$) from $\delta^{13}\text{C}$
390 values of whole body tissue. Similarly, a culturing experiment by Schilder et al. (2015a)
391 indicated that $\delta^{13}\text{C}$ values of *Daphnia* ephippia were on average $0.2 \pm 0.4\text{‰}$ higher
392 than whole body tissue. Head capsules of 4th instar *Chironomus riparius* (Meigen 1804)
393 larvae were on average $1.2 \pm 0.9\text{‰}$ and $0.9 \pm 0.2\text{‰}$ lower than whole body tissue in
394 culturing experiments by Heiri et al. (2012) and Frossard et al. (2013), respectively.

395 Our laboratory study suggests that the $\delta^{13}\text{C}$ offset between food and zooids
396 (1.7‰) does not vary greatly between colonies, at least for the two taxa investigated.
397 In contrast, the offset between body tissue and fossilizing structure can vary between 0

398 and 1.2‰. Variations <1.2‰ in $\delta^{13}\text{C}$ values of statoblasts from sediment samples could
399 therefore be the result of natural variability in the offset between zooids and
400 statoblasts. Variations >1.2‰ can thus be interpreted as a colony-independent signal
401 that has ecological or environmental significance. Certainly the large between-lake
402 variability in bryozoan $\delta^{13}\text{C}$ values we observed in the 23 sites of our field survey
403 exceeds this 1.2‰ range. Other studies also indicated larger variability of $\delta^{13}\text{C}$ values
404 in ecosystem studies and in down core records. For example, Vander Zanden &
405 Rasmussen (1999) report a range of 6‰ for $\delta^{13}\text{C}$ values of primary consumers in
406 modern lake ecosystems, and Van Hardenbroek et al. (2014) report a range of 5‰ for
407 $\delta^{13}\text{C}$ values of bryozoan statoblasts in a sediment record. The majority of this variation
408 in $\delta^{13}\text{C}$ values can thus be interpreted in terms of changing carbon sources, or changing
409 $\delta^{13}\text{C}$ values of these carbon sources.

410

411 *Zooid and statoblast $\delta^{13}\text{C}$ values under field conditions*

412 We observed a strong correlation between the median $\delta^{13}\text{C}$ values of zooids and
413 associated statoblasts at the different study sites (Fig. 3), which confirms that $\delta^{13}\text{C}$
414 values of statoblasts are systematically related to $\delta^{13}\text{C}$ values of zooids. We observed a
415 clearly greater variability in offsets between zooids and statoblasts, however, in the
416 field survey than in the laboratory. In general, offsets in $\delta^{13}\text{C}$ values between zooids
417 and statoblasts ranged between -3 and +4.5‰ (Fig. 2a), with average offsets of 1.0‰,
418 1.4‰, and -0.2‰ for *Cristatella*, *Plumatella*, and *Pectinatella*, respectively. However, in
419 three cases statoblast $\delta^{13}\text{C}$ was more than 10‰ lower than $\delta^{13}\text{C}$ of zooids and the
420 opposite was observed in one instance. These four data points clearly fall outside the
421 regular range for offsets (Fig. 2a), and at two of these four extreme sites we also
422 collected paired samples with offsets of only 0.9 to 3.6‰. This suggests that the

423 availability of food sources that differ >10‰ can be a very localized phenomenon in
424 time and space, possibly occurring in particular microhabitats. Differences in $\delta^{13}\text{C}$
425 values of similar magnitude (10 to 20‰) in chironomid larvae were linked to local
426 oxygen depletion in lakes and localized incorporation of methane-derived carbon
427 (Grey et al., 2004b; Agasild et al., 2013).

428 In addition to spatial and temporal variability in the carbon sources available to
429 bryozoans other factors may have contributed to the large range of $\delta^{13}\text{C}$ offsets in the
430 field survey. Examination of colonies collected during fieldwork revealed that some of
431 them were partly covered or interspersed by periphyton and that the guts of the
432 bryozoans still contained variable amounts of material. Because zooids rapidly
433 disintegrated during dissection, it is likely that non-bryozoan material was not
434 completely removed from zooids. Variable amounts of non-bryozoan material in our
435 samples might also explain the relatively large variability in $\delta^{13}\text{C}$ values of replicate
436 colonies from the same location (Table 3). Without additional isotopic analyses of POM,
437 gut content, and periphyton at the different sites, however, we cannot draw firm
438 conclusions about the causes for the observed variability in $\delta^{13}\text{C}$ values of bryozoan
439 colonies.

440

441 *Taxonomic differences in δD values*

442 Our data suggest that *Plumatella* statoblast δD values are clearly related to δD values of
443 associated zooids and to δD values of lake water, whereas this is not observed for
444 *Cristatella* statoblasts (Fig. 4 and 5). This might simply be explained by the low number
445 of data points for *Cristatella*, but other explanations could also be considered.

446 One explanation for the differences between *Cristatella* and *Plumatella* may be
447 the difference in food particles ingested by these two groups, because δD values of

448 aquatic invertebrates are strongly influenced by δD values of their food (Solomon et al.,
449 2009; Wang et al., 2009; Soto et al., 2013). At most of our study lakes bryozoans can be
450 expected to feed predominantly on planktonic algae or microorganisms feeding on
451 them. The δD values of this food source can be expected to be closely related to lake
452 water δD values and therefore relatively constant for a given site. However, for some
453 organism groups, such as MOB, extremely low δD values have been reported (Whiticar,
454 1999; Deines et al., 2009). Furthermore, organisms feeding predominantly on
455 terrestrial organic matter may be characterized by δD values that differ from algal
456 organic matter produced within lakes (Karlsson et al., 2012). Kaminski (1984)
457 demonstrated that *Cristatella mucedo* selects small seston (<7 μm in diameter), which
458 can include bacteria, whereas *Plumatella repens* prefers slightly larger particles
459 (ranging from 5 to 17 μm in diameter). *Cristatella* may therefore feed on small
460 organisms with a more variable isotopic composition, such as chemoautotrophic or
461 methane-oxidizing bacteria, which are less abundant in the larger particles than
462 *Plumatella* feeds on.

463 Another explanation could be that *Plumatella* is firmly attached to substrates,
464 whereas *Cristatella* is mobile and has been found at water depths of up to 20 m
465 (Lacourt, 1968), both on hard and soft substrates. As lake water δD values can vary
466 within the water column (Gat, 1995) and *Cristatella* colonies are capable of limited
467 movement to different microhabitats, *Cristatella* could potentially incorporate different
468 food sources and be exposed to water with different δD values than the immobile
469 *Plumatella*. Even if the mechanism behind this observation is not fully understood, our
470 results indicate that δD values of *Plumatella* statoblasts reflect lake water δD more
471 closely than δD of *Plumatella* zooids and *Cristatella* tissues.

472

473 *δD offsets between zooids and statoblasts*

474 In contrast to the $\delta^{13}\text{C}$ values, which were similar for zooids and statoblasts if median
475 values were examined, we found that zooid δD was substantially lower than statoblast
476 δD for most of the paired samples examined in the field survey (Fig. 2). A visual
477 examination of Fig. 4 reveals that this is largely due to δD values for *Cristatella*
478 obtained from 5 sites (i.e. Alte Aare, Piepertkolk, Schöhsee, Veenmeer in 2010, and
479 Veenmeer in 2012), which are characterized by higher offsets between zooid and
480 statoblast values and fall outside the scatter of other data points. These large offsets
481 between δD of zooids and statoblasts might be linked to differences in food type and
482 mobility as discussed above.

483 In addition, fractionation during the synthesis of different compounds can result
484 in different δD values between tissues. For example, lipids are especially D-depleted
485 compared with other tissues (Hobson et al., 1999; Soto et al., 2013). The higher atomic
486 carbon content of zooids (mean 44%) compared to statoblasts (mean 30%) in our
487 culture supports the idea of a higher lipid content in zooids. Further experiments with
488 controlled δD values of food and environmental water, and analysis of the chemical
489 composition of bryozoan tissues, will be necessary to further constrain the reasons for
490 the unexpectedly large offset in δD values between zooids and statoblasts, especially
491 for *Cristatella*.

492

493 *Relationship between δD of lake water and Bryozoa*

494 Lake water δD values are more clearly related to the δD values of statoblasts than to
495 the δD values of zooids (Fig. 5). One explanation for this may be that zooid samples are
496 more easily affected by contamination with attached organic material and undigested
497 particles in the guts, as discussed above. Secondly, we assumed a constant proportion

498 of exchangeable H in our samples based on chitin and cellulose reference materials.
499 However, the proportion of exchangeable H may differ between tissues (Wassenaar &
500 Hobson, 2000; Schimmelmann et al., 2006). Zooid tissues are more diverse in chemical
501 composition, leading to additional variability in δD values after correcting for
502 exchangeable H, especially if compounded by contamination with non-bryozoan
503 organic matter. Thirdly, differences in turnover rates between zooid and statoblast
504 biomass might lead to incorporation of H into zooids that is different in δD from the
505 material incorporated into statoblasts. δD values of lake water can change seasonally
506 (Gat, 1995; Schürch et al., 2003), leading to temporal changes in δD of the water and
507 food available to bryozoans. However, controlled experiments are required to estimate
508 turnover rates in different bryozoan tissues and to investigate how quickly changes in
509 δD values of water and diet are recorded in different bryozoan tissues.

510

511

512 **Conclusions**

513

514 Our results demonstrate that the C isotopic composition of freshwater bryozoan
515 statoblasts is systematically related to the isotopic composition of the zooids over a
516 large range of $\delta^{13}C$ values. Offsets in $\delta^{13}C$ values between zooids and statoblasts were
517 considerably more variable in the field survey than in our laboratory study, with very
518 large offsets observed for some of the sampled colonies. However, median estimates,
519 based on statoblast $\delta^{13}C$ values from several colonies per sampling site, were strongly
520 related with zooid $\delta^{13}C$ values. Similarly, median statoblast δD values based on
521 material from several colonies per site showed a robust relationship with lake water
522 δD and, for *Plumatella*, with δD values of zooids.

523 Statoblasts obtained in flotsam and lake sediment samples typically originate
524 from numerous bryozoan colonies within an examined lake. Our results therefore
525 suggest that C and H isotopic analyses on such samples can provide insights into
526 variations of $\delta^{13}\text{C}$ values of bryozoan zooids in lakes and in situations where bryozoans
527 predominantly feed on algal organic matter, on variations in lake water δD . The robust
528 nature of chitinous statoblasts makes them particularly suitable for studying the
529 isotopic composition of lacustrine primary consumers over long time scales, using
530 statoblasts preserved in lake sediment records. Since statoblasts also include N, O, and
531 S, the stable isotopic composition of these elements may provide further valuable
532 information of a distinct ecosystem component near the base of aquatic food webs.

533

534

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536

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546

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725 **Figure captions**

726

727

728 **Fig. 1.** Boxplots of $\delta^{13}\text{C}$ values **(a)** and δD values **(b)** of the three bryozoan
729 genera investigated in this study. Values for zooids are shown in white and for
730 statoblasts in grey. Numbers indicate how many data points constitute each
731 boxplot.

732

733 **Fig. 2.** Stacked histograms representing the offsets between $\delta^{13}\text{C}$ values **(a)** of
734 zooids and statoblasts for *Cristatella* (black), *Pectinatella* (grey), and *Plumatella*
735 (white). Average offsets calculated from the median value per site for each genus
736 are indicated by circles of the same colour. In **(b)** the same is shown for δD
737 values.

738

739 **Fig. 3.** $\delta^{13}\text{C}$ values of statoblasts plotted against $\delta^{13}\text{C}$ values of zooids for
740 *Cristatella*, *Pectinatella*, and *Plumatella*. The dotted line indicates the 1:1 line. All
741 data points shown in **(a)**; Median values for each sampling location are shown in
742 **(b)** with grey lines representing the range of replicate δD values for each
743 location.

744

745 **Fig. 4.** δD values of statoblasts plotted against δD values of zooids for *Cristatella*,
746 *Pectinatella*, and *Plumatella*. The dotted line indicates the 1:1 line. All data points
747 shown in **(a)**; Median values for each sampling location are shown in **(b)** with
748 grey lines representing the range of replicate δD values for each location.

749

750 **Fig. 5.** Median δD values (with ranges indicated by grey lines) of *Cristatella*,
751 *Pectinatella*, and *Plumatella* zooids **(a)** and statoblasts **(b)** plotted against δD of
752 lake water. Grey lines represent the range of replicate δD values for each location.

753

754 **Fig. 6** Average offsets between $\delta^{13}C$ values of food (particulate organic matter,
755 POM, grey circles) and $\delta^{13}C$ values of *Plumatella* and *Lophopus* zooids (open
756 circles), floatoblasts (closed circles), and sessoblasts (closed squares) in the
757 culturing experiment. Error bars indicate the standard deviation of three
758 replicate measurements, unless indicated otherwise (in brackets). The standard
759 deviation of the POM samples is shown to provide an indication of the variability
760 of $\delta^{13}C$ of POM in the culturing experiment.

Figure 1
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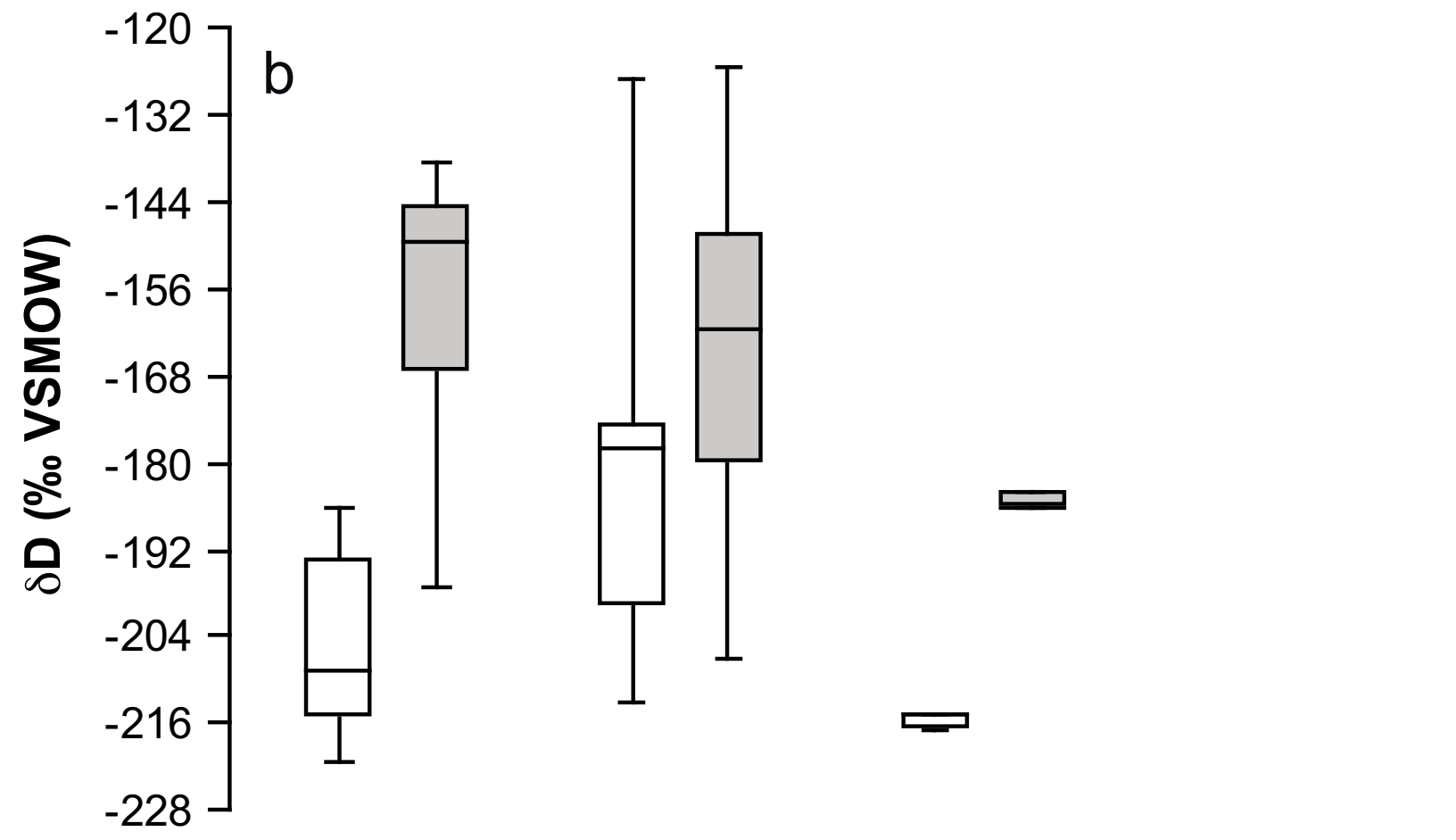
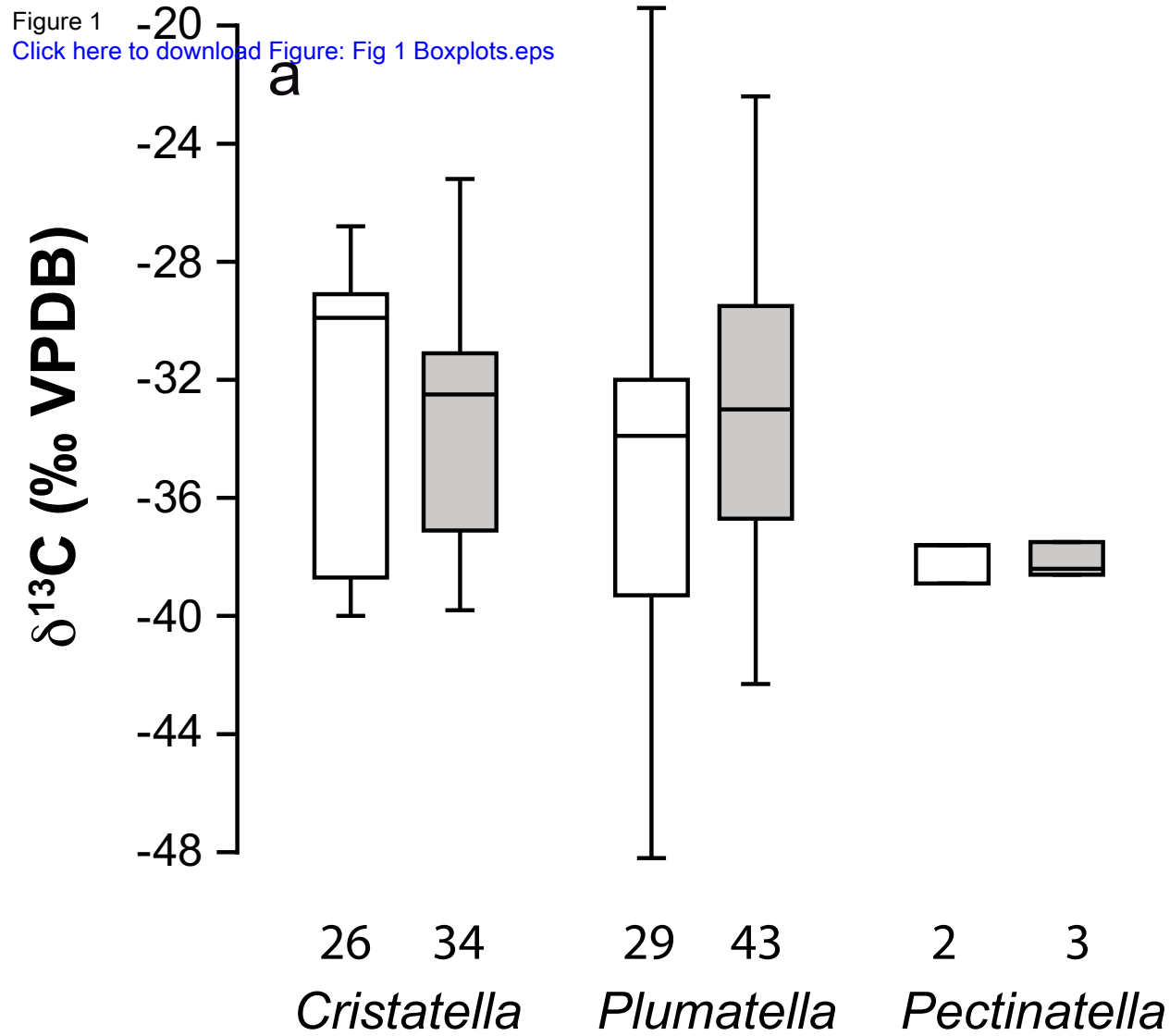


Figure 2

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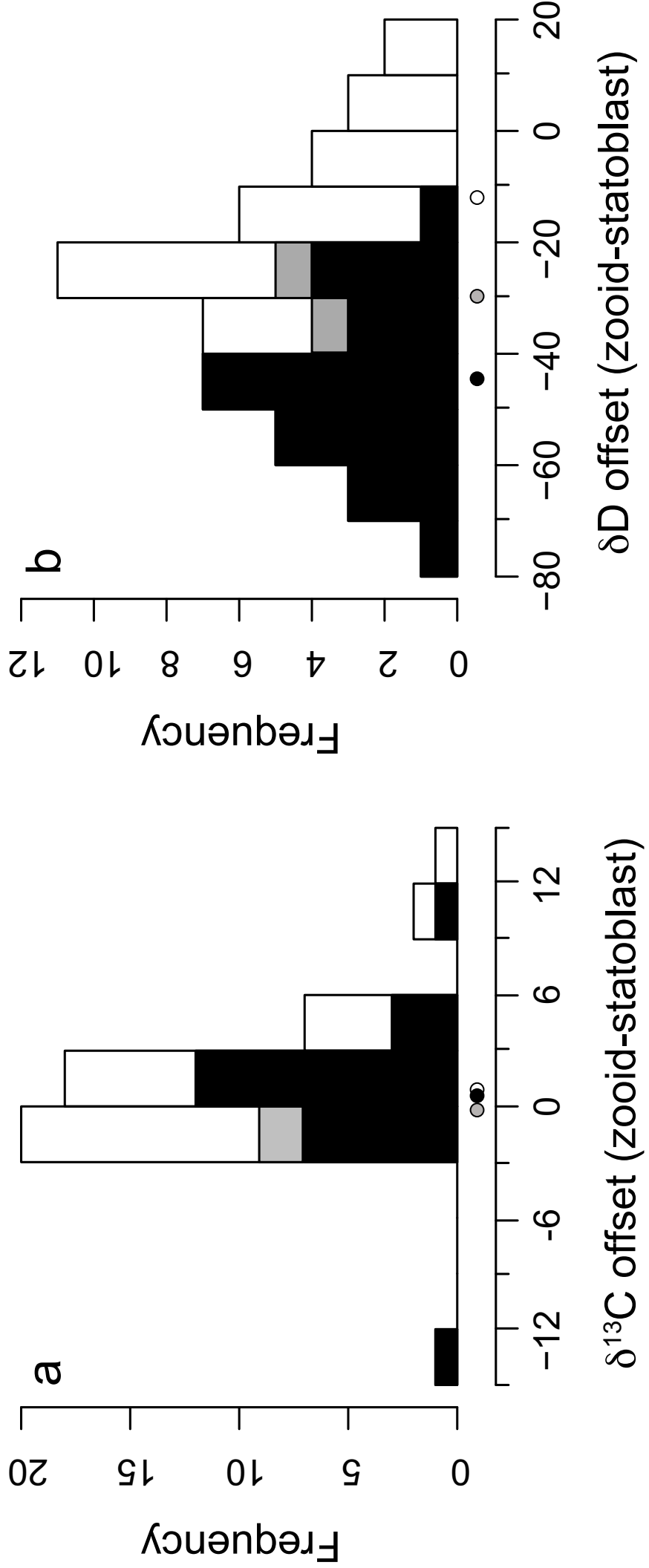
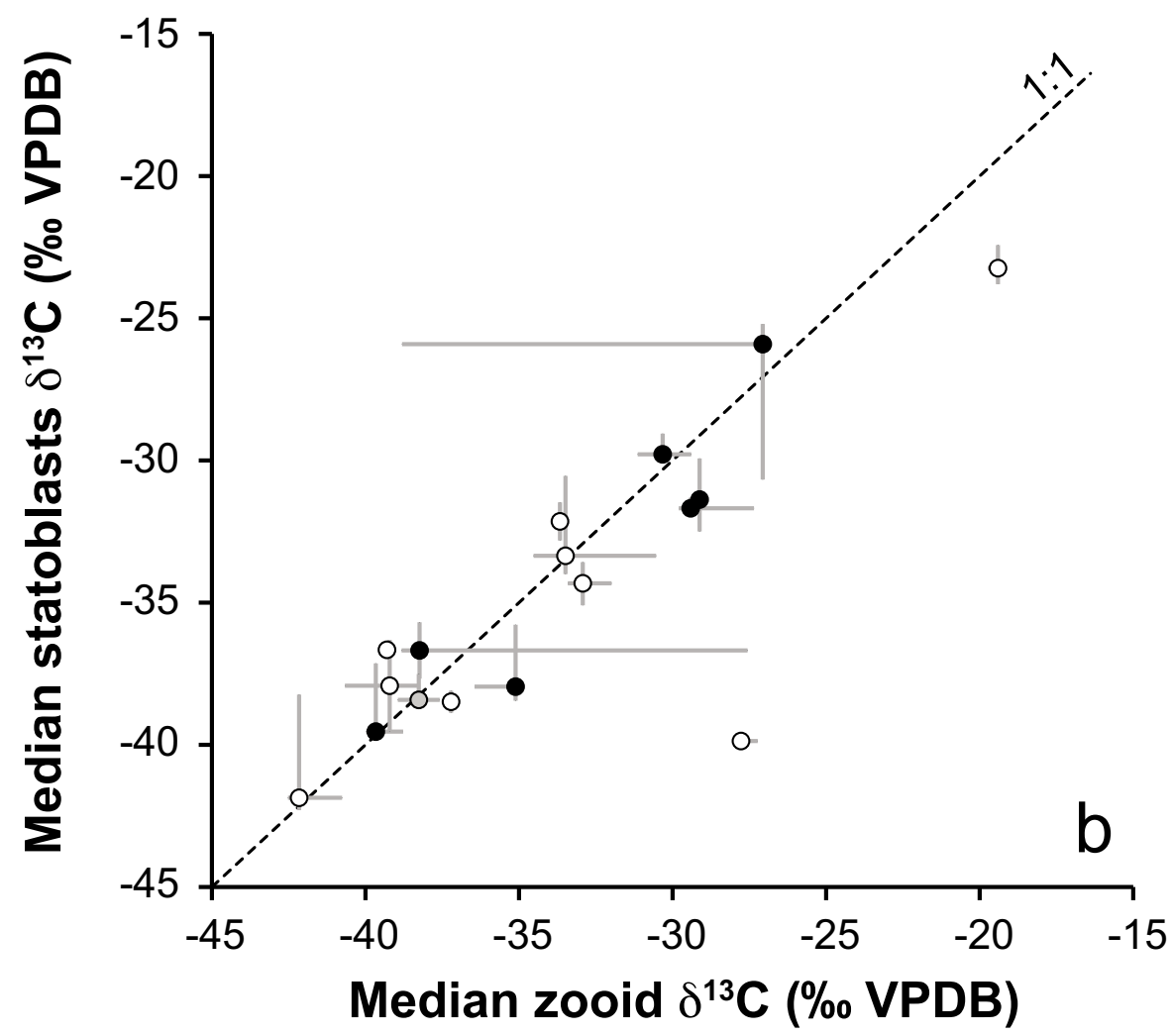
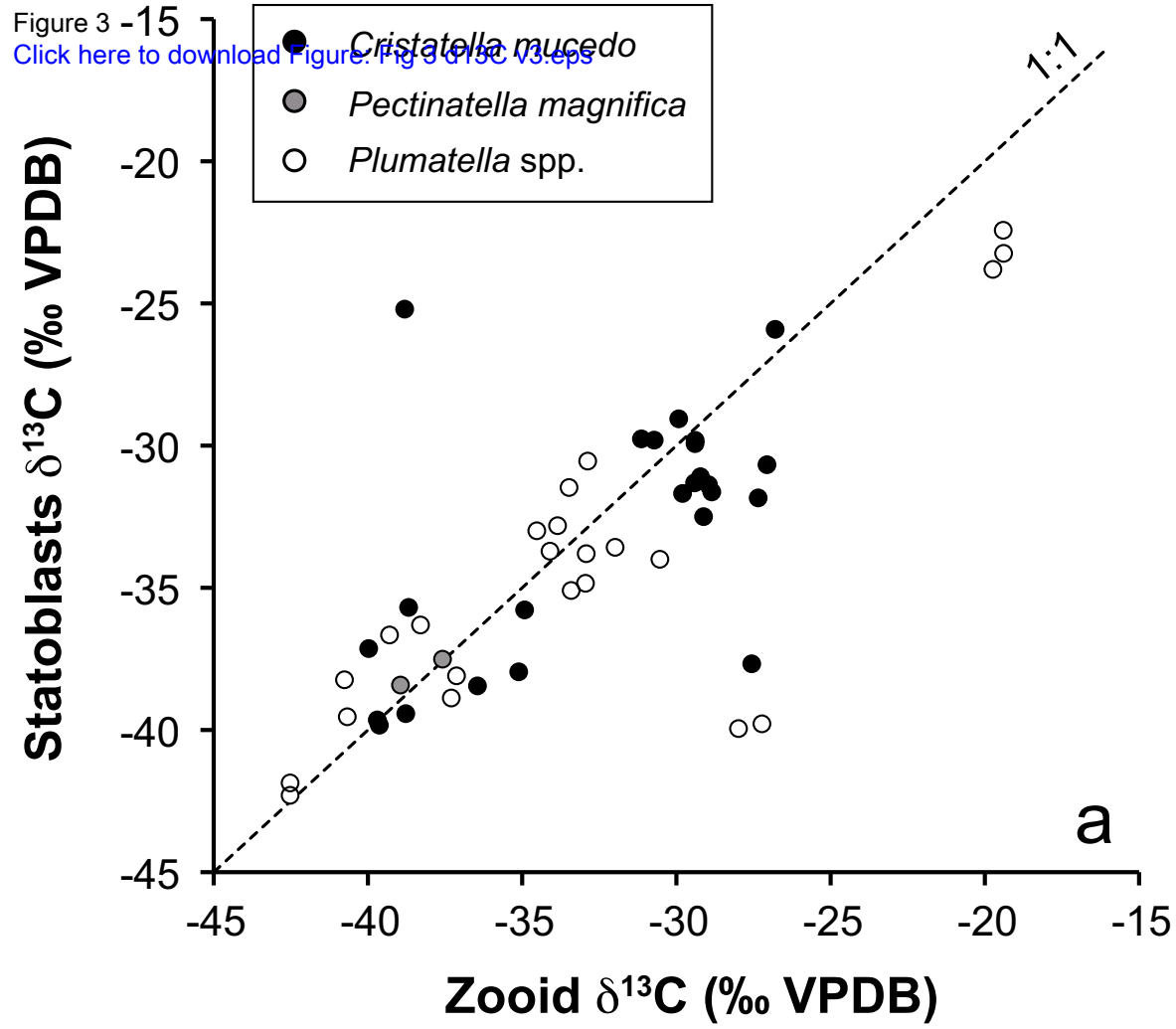
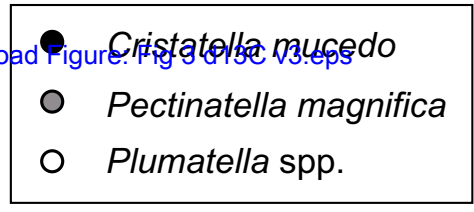
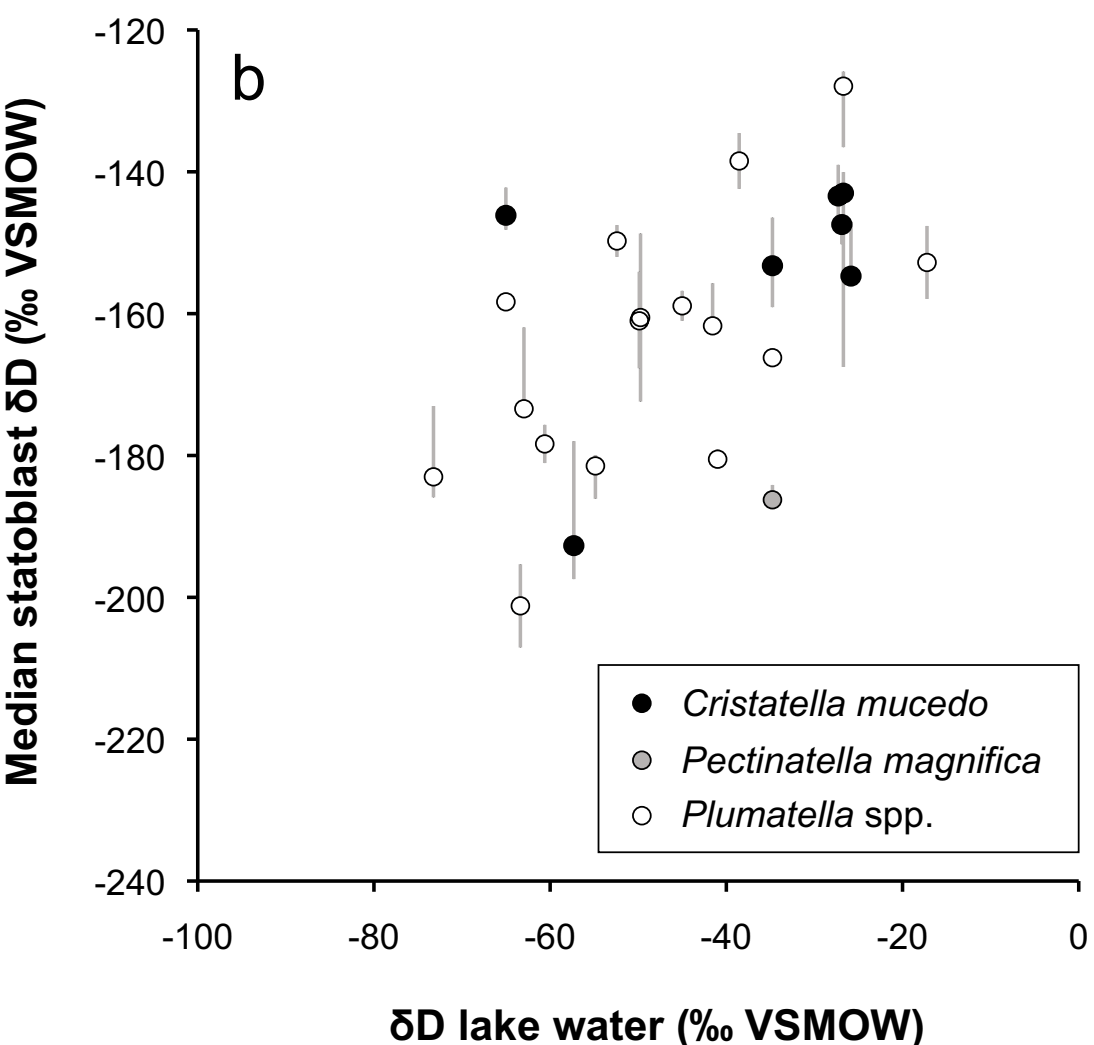
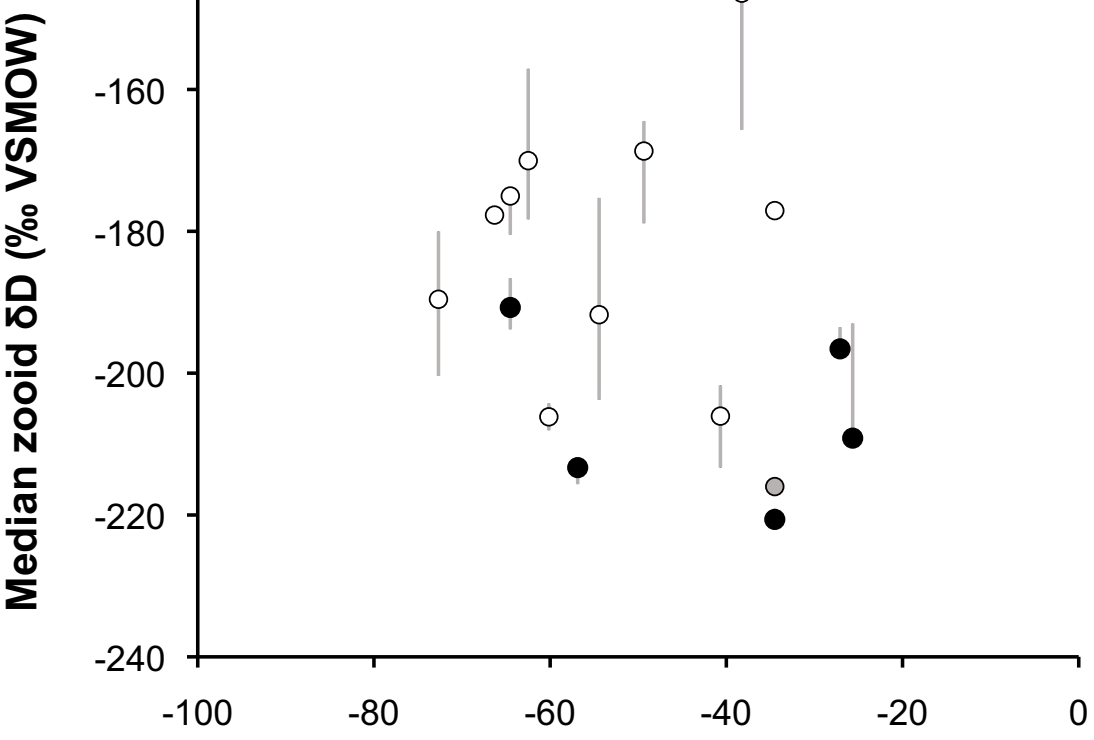


Figure 3 -15

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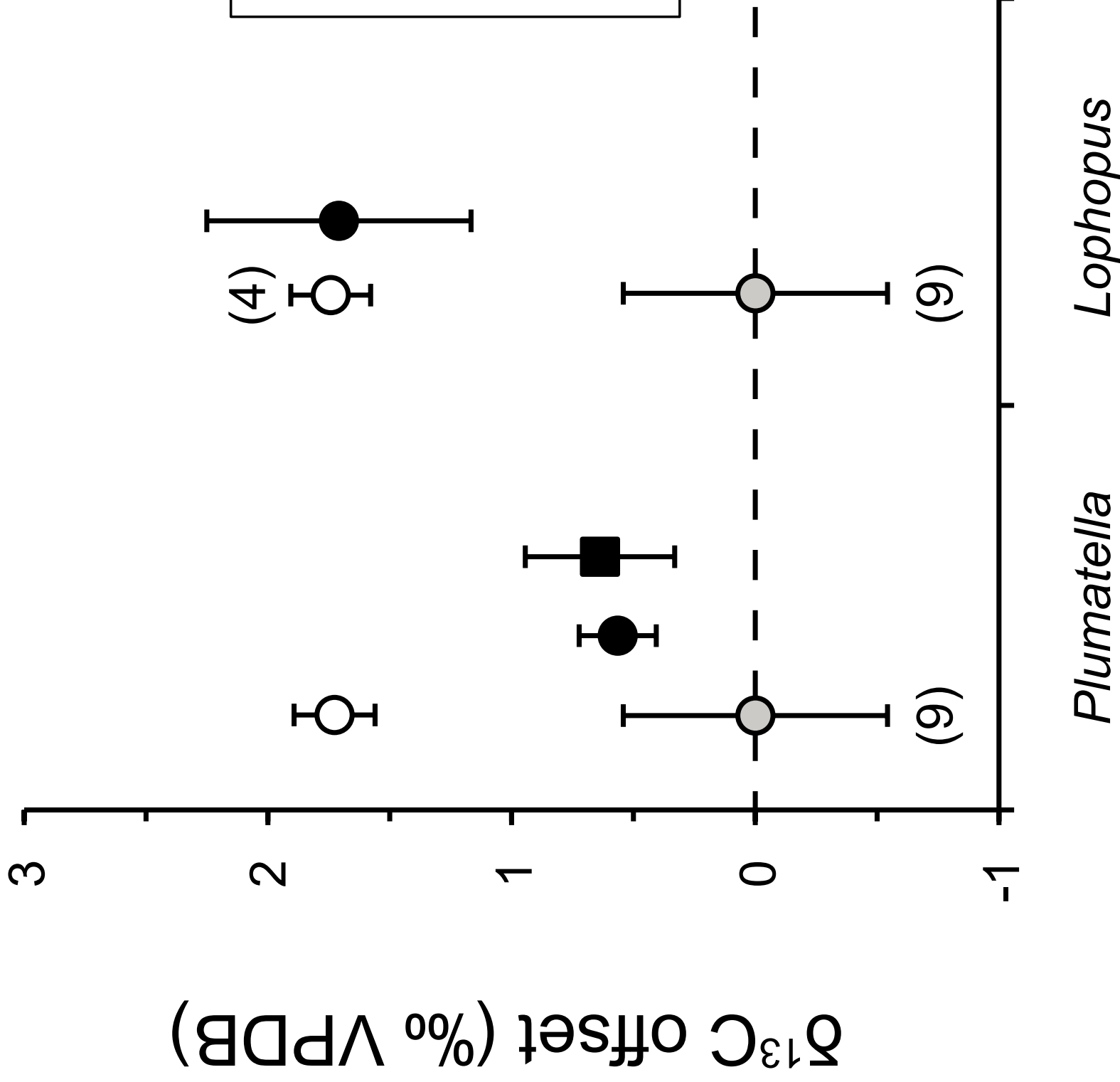


Table 1. Location, date, and substrate of sampled Bryozoa. For each location the number of zooid samples (n_z), statoblast samples (n_s), and how many of those are paired samples (n_p) with zooids and statoblast. Stable isotope values of lake water are also given.

Lake	Coordinates	Date	Sampled substrate	Taxon	n_z	n_s	n_p	δD_{water}	$\delta^{18}O_{\text{water}}$
Aarbergerweiher, CH	47°3'20"N / 7°17'4"E	29-09-12	submerged vegetation	<i>Plumatella</i>	0	2	0	-63.0	-8.28
Aatalweiher, CH	47°14'23"N / 8°57'9"E	10-09-11	underside of stones	<i>Plumatella</i>	3	2	2	-64.6 [#]	-9.83
Ägerisee 0.5m deep, CH	47°6'10"N / 8°38'7"E	10-09-11	breakwater 0.5m depth	<i>Cristatella mucedo</i>	3	3	3	NA	NA
Ägerisee 2.0m deep, CH	47°6'10"N / 8°38'7"E	10-09-11	breakwater 2.0m depth	<i>Cristatella mucedo</i>	5	5	5	-56.9 [#]	-8.39
Alte Aare, CH	47°6'42"N / 7°19'2"E	04-10-12	submerged branch	<i>Cristatella mucedo</i>	4	4	4	-64.6 [#]	-9.83
Chli Golihübweiher, CH	47°4'33"N / 7°22'31"E	04-10-12	submerged wood of jetty	<i>Plumatella</i>	2	2	2	-38.3	-4.27
Chli Moossee, CH	47°1'36"N / 7°28'12"E	04-10-12	submerged branch	<i>Plumatella</i>	2	0	0	-66.4	-9.41
De Waay, NL	51°55'55"N / 5°8'55"E	09-08-11	submerged branch	<i>Plumatella geimermassardi</i>	3	3	3	-40.7	-4.90 §
Golihübweiher, CH	47°4'47"N / 7°22'26"E	04-10-12	submerged branch	<i>Plumatella</i>	4	3	3	-62.6	-9.00
Greifensee, CH	47°21'40"N / 8°40'46"E	10-09-11	submerged branch	<i>Plumatella</i>	5	4	4	-54.5 [#]	-8.15
Hinterburgsee, CH	46°43'5"N / 8°4'1"E	16-09-11	underside of old log	<i>Plumatella</i>	4	4	4	-72.8 [#]	-10.76
Holzsee, D	54°9'35"N / 10°11'13"E	05-08-11	metal poles of jetty	<i>Plumatella</i>	0	3	0	-41.3	-5.03 §
Inkwylsersee, CH	47°11'50"N / 7°39'39"E	15-09-12	rootlets	<i>Plumatella</i>	0	2	0	-52.1	-6.73
Lobsigensee, CH	47°1'49"N / 7°17'55"E	29-09-12	submerged metal / vegetation	<i>Plumatella</i>	3	2	2	-49.4	-6.31
Moossee, CH	47°1'11"N / 7°29'10"E	04-10-12	submerged branch	<i>Plumatella</i>	2	2	2	-60.2	-8.26
Picardhofplas, NL	53°11'16"N / 6°32'23"E	01-08-11	submerged branch	<i>Cristatella mucedo</i>	0	3	0	-26.7	-3.63
Piepertkolk, NL	52°38'43"N / 6°3'56"E	07-08-11	submerged branch	<i>Plumatella fruticosa</i>	1	1	1	-34.5	-4.69
		07-08-11	submerged branch	<i>Cristatella mucedo</i>	4	4	4	-34.5	-4.69
		07-08-11	submerged branch	<i>Pectinatella magnifica</i>	2	3	2	-34.5	-4.69
		05-08-12	submerged branch	<i>Cristatella mucedo</i>	0	2	0	NA	NA
Plussee, D	54°10'58"N / 10°26'47"E	06-08-11	boat	<i>Plumatella</i>	0	6	0	-26.5	-2.10 §
			boat	<i>Cristatella mucedo</i>	0	5	0	-26.5	-2.10 §
Steenbergen, NL	53°06'31 "N/6°23'33"E	08-08-12	submerged vegetation	<i>Plumatella</i>	0	2	0	-17.1	-1.50
Schöhsee, D	54°9'36"N / 10°26'8"E	07-08-11	submerged branch	<i>Cristatella mucedo</i>	3	3	3	-25.7	-2.72 §
Sempachersee, CH	47°9'48"N / 8°8'42"E	15-09-12	old wooden board/rudder	<i>Plumatella</i>	0	3	0	-49.5	-6.30
Siselenweiher, CH	47°1'38"N / 7°12'16"E	29-09-12	submerged branch	<i>Plumatella</i>	0	2	0	-44.7	-5.32
Veenmeer, NL	53°5'5"N / 6°38'6"E	28-11-10	submerged vegetation	<i>Cristatella mucedo</i>	4	2	2	-27.1	-3.33
		03-06-12	submerged vegetation	<i>Cristatella mucedo</i>	3	3	3	NA	NA
TOTAL					57	80	49		

CH = Switzerland, D = Germany, NL = The Netherlands

Water samples were analysed on a Finnigan MAT 250, except samples marked with § that were measured on a Picarro L1102-i

[#] Estimated from linear regression between $\Delta\delta^{18}O$ (lake water $\delta^{18}O$ – estimated precipitation $\delta^{18}O$) and $\Delta\delta D$ (lake water δD – estimated precipitation δD)

Table 2: Number of sampled colonies and number of sites for which median stable isotope values are calculated. ‘Paired samples’ indicates for how many sites there are with paired samples of zooid and statoblast, or lake water and zooids, or lake water and statoblasts. Figure numbers refer to the figures that show the respective row of data.

	<i>Cristatella</i>		<i>Plumatella</i>		<i>Pectinatella</i>		All Bryozoa		Figure
	colonies	sites	colonies	sites	colonies	sites	colonies	sites	
Colonies sampled	36	8	49	17	3	1	88	23	Fig 1 & 2
Zooid samples	26	6	29	10	2	1	57	15	Fig 1 & 2
Statoblasts samples	34	8	43	16	3	1	80	22	Fig 1 & 2
Paired samples: zooid + statoblast	24	7	23	9	2	1	49	14	Fig 3 & 4
Paired samples: water + zooid	20	5	29	10	2	1	51	15	Fig 5a
Paired samples: water + statoblast	26	7	43	16	3	1	72	21	Fig 5b

Table 3: $\delta^{13}\text{C}$ and δD values (mean and standard deviation) of zooid and statoblasts of the three taxa studied: *Cristatella mucedo* (C), *Pectinatella magnifica* (Pe), and *Plumatella* (Pl).

Site	Taxon	mean zooid $\delta^{13}\text{C}$			mean statoblast $\delta^{13}\text{C}$			mean zooid δD			mean statoblast δD		
		SD	n		SD	n		SD	n		SD	n	
Ägerisee 0.5m depth	C	-28.6	1.3	3	-31.6	0.3	3	-216.4	2.3	3	-184.4	3.8	3
Ägerisee 2.0m depth	C	-29.1	0.2	5	-31.3	0.9	5	-213.6	1.1	5	-189.2	7.8	5
Alte Aare	C	-30.3	0.8	4	-29.6	0.4	4	-190.4	3.0	4	-145.7	2.9	4
Picardhofplas	C				-37.4	1.3	3				-148.2	1.9	3
Piepertkolk '11	C	-39.5	0.5	4	-39.0	1.3	4	-220.3	1.0	4	-153.1	5.3	4
Piepertkolk '12	C				-36.5		2				-161.9		2
Plußsee	C				-32.7	1.6	5				-148.2	11.5	5
Schönsee	C	-30.9	6.9	3	-27.3	3.0	3	-203.8	9.4	3	-152.2	4.4	3
Veenmeer '10	C	-35.7	5.5	4	-36.7		2	-195.9	1.6	4	-143.5		2
Veenmeer '12	C	-35.5	0.8	3	-37.4	1.4	3	-194.3	2.0	3	-142.8	3.3	3

Piepertkolk '11	Pe	-38.3	2		-38.2	0.6	3		-215.8	2		-185.7	1.2	3
Aarbergerweiher	PI				-30.2		2					-201.3		2
Aatalweiher	PI	-27.7	0.4	3	-39.9		2		-176.7	3.3	3	-158.4		2
Chli Golihübweiher	PI	-33.7		2	-32.1		2		-146.6		2	-138.5		2
Chli Moossee	PI	-47.7		2					-177.7		2			
Golihübweiher	PI	-41.9	0.8	4	-40.8	2.2	3		-168.9	11.0	4	-170.1	7.0	3
Greifensee	PI	-32.8	0.5	5	-34.3	0.8	4		-190.0	13.0	5	-182.3	2.8	4
Hinterburgsee	PI	-33.0	1.8	4	-32.8	1.6	4		-189.8	8.4	4	-181.3	5.7	4
Holzsee	PI				-32.5	1.0	3					-159.8	3.5	3
Inkwylensee	PI				-34.9		2					-149.8		2
Lobsigensee	PI	-39.4	1.2	3	-37.9		2		-170.7	7.4	3	-160.6		2
Moossee	PI	-37.2		2	-38.5		2		-206.0		2	-178.5		2
Piepertkolk '11	PI	-38.0		2					-171.7		2			
Plußsee	PI				-29.1	0.6	6					-128.9	3.9	6
Sempachersee	PI				-28.2	0.6	3					-161.0	6.8	3
Sisselenweiher	PI				-39.6		2					-159.0		2
De Waay	PI	-19.5	0.2	3	-23.2	0.7	3		-206.9	5.8	3	-180.5	1.2	3