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

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Abstract

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

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

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

Introduction

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

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

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

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

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

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

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

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

Methods

Field survey

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

Between one and six replicate colonies were measured for each site (Table 1 and 3). Colonies were kept cool and dissected within 24 hours of collection. Gut evacuation was often incomplete when colonies died soon after detachment from their substrate. Extraneous material such as wood, algae and silt, was removed from the zooids with lancet and forceps to minimize contamination, but complete removal was not always possible due to the disintegration of fragile zooid tissues. Zooid material 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{18}O$. For five sites only $\delta^{18}O$ of lake water was measured.
160 For these five lakes δD was estimated based on $\delta^{18}O$ and the relationship between $\delta^{18}O$
161 and δD observed for Swiss lakes: First, the difference ($\Delta\delta^{18}O$) between measured $\delta^{18}O$
162 in lake water and estimated $\delta^{18}O$ in precipitation (Bowen & Revenaugh, 2003; Bowen,
163 2014) was calculated for each of our sampling locations. Similarly, ΔδD 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 ΔδD as a function of $\Delta\delta^{18}O$ ($n = 21$, $r = 0.99$). This relationship was
167 used to calculate ΔδD from $\Delta\delta^{18}O$ for sites without lake water δD measurements and to
168 estimate δD based on the δD of precipitation and ΔδD. 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 $\delta^{13}C$ values of their zooids and statoblasts and how
174 this relates to the $\delta^{13}C$ of their diet, particulate organic matter (POM). A microcosm for

175 culturing bryozoans was established at constant 18 °C (± 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 ±0.1‰.
208
209 *Statistical analyses*
210 To examine the relationship between $\delta^{13}C$ 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 $\delta^{13}C$
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 $\delta^{13}C$
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,

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

Results

Field survey

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

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

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

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

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

δD values of zooids and statoblasts

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

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

δD values of bryozoans and lake water

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

Laboratory study

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

Discussion

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

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

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

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

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

In a study on the River Rhine, colonies of *Plumatella repens* and *P. fungosa* were characterized by $\delta^{13}C$ values of -31.1‰ and -28.8‰, respectively (van Riel et al., 2006). These values were substantially lower than $\delta^{13}C$ values observed in the same study for POM (-24.27‰), which contrasts with the results of our experiments. In the same study on the River Rhine, however, van Riel et al. also found that *Plumatella* $\delta^{13}C$ was only 0.9-3.3‰ higher than $\delta^{13}C$ values of phytoplankton (-32‰) that they estimated based on the $\delta^{13}C$ values of dissolved inorganic carbon. The $\delta^{13}C$ offset between *Plumatella* and phytoplankton reported by van Riel et al. was therefore 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 $\delta^{13}C$ 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 ±0.3‰ (Schilder et al., 2015a). In our laboratory study,
380 the 1.7‰ higher $\delta^{13}C$ values of zooids of *Plumatella* and *Lophopus* compared with the
381 $\delta^{13}C$ values of their food are of similar magnitude, suggesting that zooid $\delta^{13}C$ values
382 provide a direct indication of the $\delta^{13}C$ values of bryozoan diet.
383 In our laboratory study we found very small offsets between $\delta^{13}C$ values of
384 bryozoan zooids and statoblasts, based on a diet with constant $\delta^{13}C$ values. We
385 observed no significant offset between $\delta^{13}C$ 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 $\delta^{13}C$ values of the
389 ephippia of *Daphnia* from Lake Geneva were indistinguishable (± 0.1‰) from $\delta^{13}C$
390 values of whole body tissue. Similarly, a culturing experiment by Schilder et al. (2015a)
391 indicated that $\delta^{13}C$ values of *Daphnia* ephippia were on average 0.2 ± 0.4‰ higher
392 than whole body tissue. Head capsules of 4th instar *Chironomus riparius* (Meigen 1804)
393 larvae were on average 1.2 ± 0.9‰ and 0.9 ± 0.2‰ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values we observed in the 23 sites of our field survey
403 exceeds this 1.2‰ range. Other studies also indicated larger variability of $\delta^{13}C$ values
404 in ecosystem studies and in down core records. For example, Vander Zanden &
405 Rasmussen (1999) report a range of 6‰ for $\delta^{13}C$ values of primary consumers in
406 modern lake ecosystems, and Van Hardenbroek et al. (2014) report a range of 5‰ for
407 $\delta^{13}C$ values of bryozoan statoblasts in a sediment record. The majority of this variation
408 in $\delta^{13}C$ values can thus be interpreted in terms of changing carbon sources, or changing
409 $\delta^{13}C$ values of these carbon sources.
410
411 *Zooid and statoblast $\delta^{13}C$ values under field conditions*
412 We observed a strong correlation between the median $\delta^{13}C$ values of zooids and
413 associated statoblasts at the different study sites (Fig. 3), which confirms that $\delta^{13}C$
414 values of statoblasts are systematically related to $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ was more than 10‰ lower than $\delta^{13}C$ 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

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

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

Taxonomic differences in δD values

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

One explanation for the differences between *Cristatella* and *Plumatella* may be 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

δD offsets between zooids and statoblasts

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

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

Relationship between δD of lake water and Bryozoa

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

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

Conclusions

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

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

Acknowledgements

We thank Michiel van der Waaij for collecting samples in Dutch lakes and for useful information on the habitat and ecology of several freshwater bryozoan species (www.bryozoans.nl). Winfried Lampert, Peter Hammond, Alex Gruhl, and Elena Brand greatly helped during an exploratory field trip. Robert Dünner is kindly acknowledged for suggesting locations in a number of Swiss lakes. Peter Nyfeler's work analysing the stable isotope data has been invaluable. We thank four anonymous reviewers for their comments on earlier versions of this manuscript. This study was funded by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement no. 239858 (RECONMET).

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Figure captions

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

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

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

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

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

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

Figure 1
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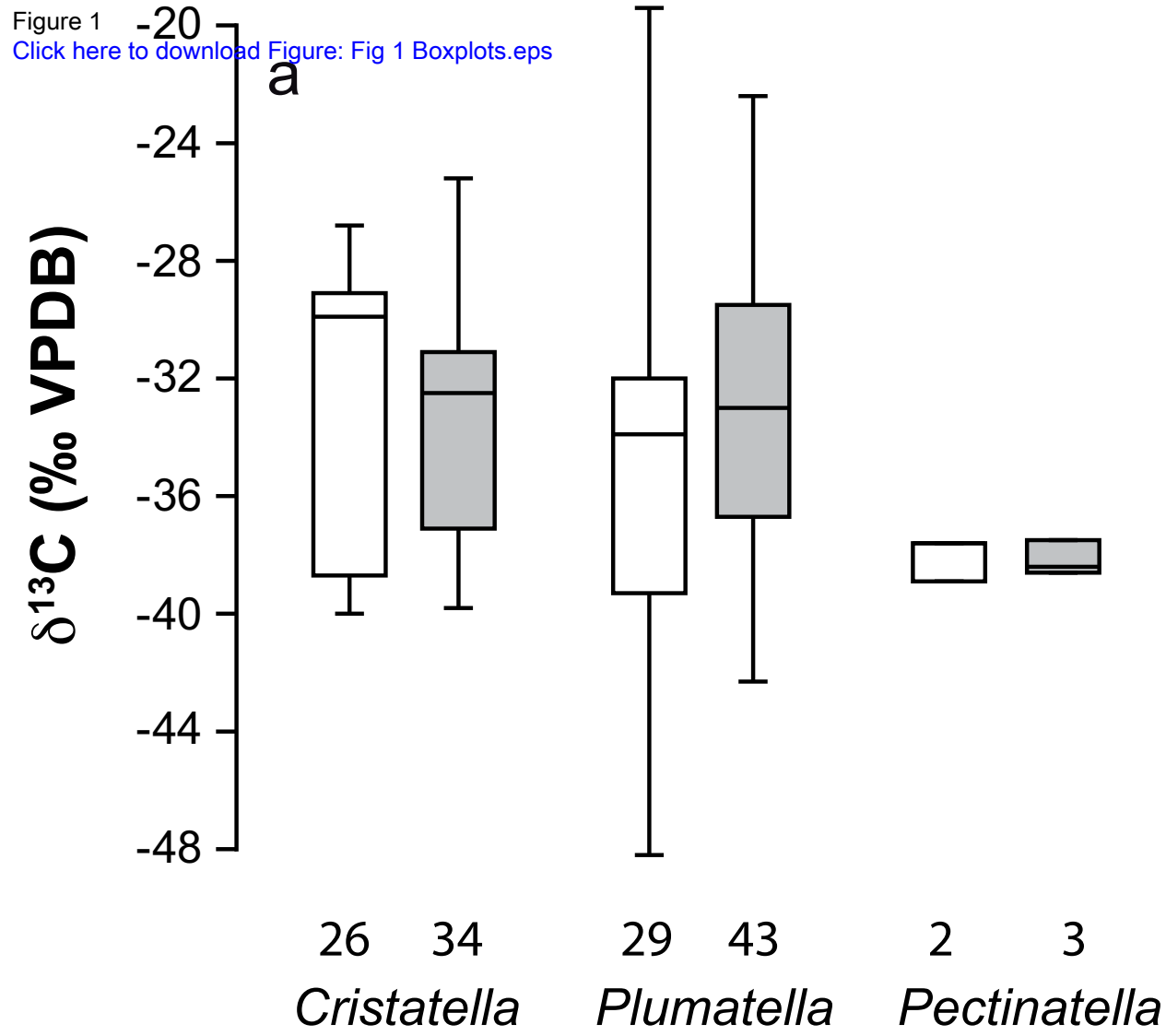

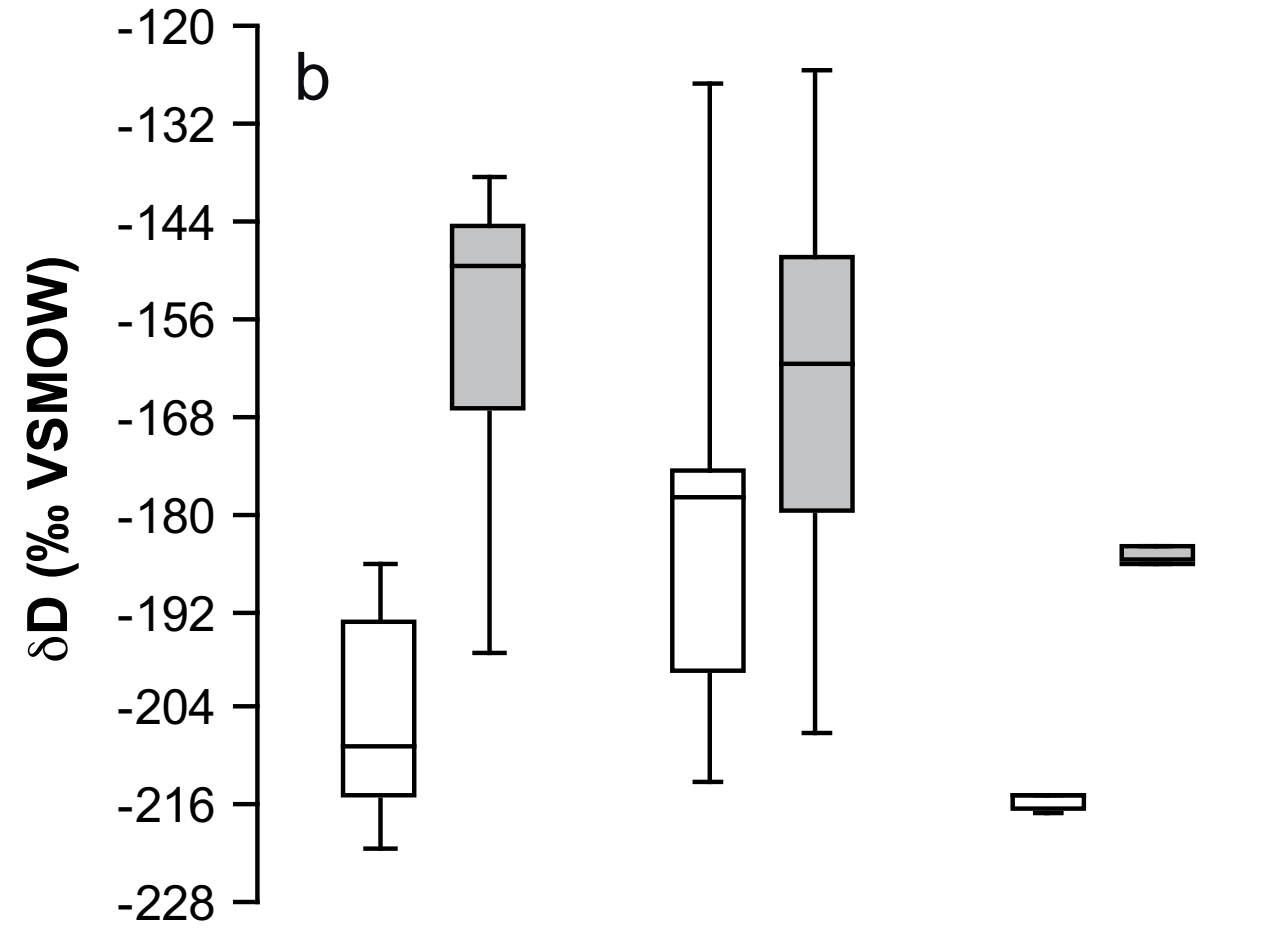

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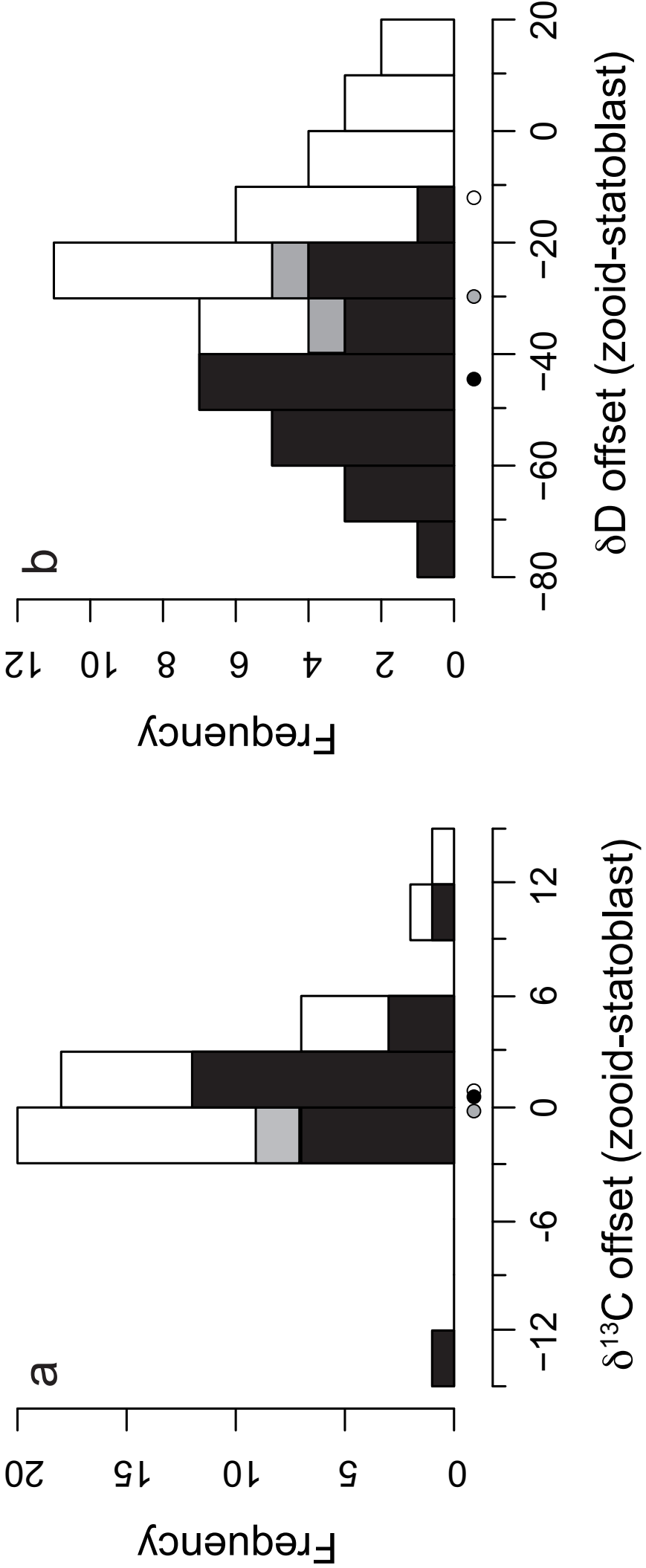

Figure 3
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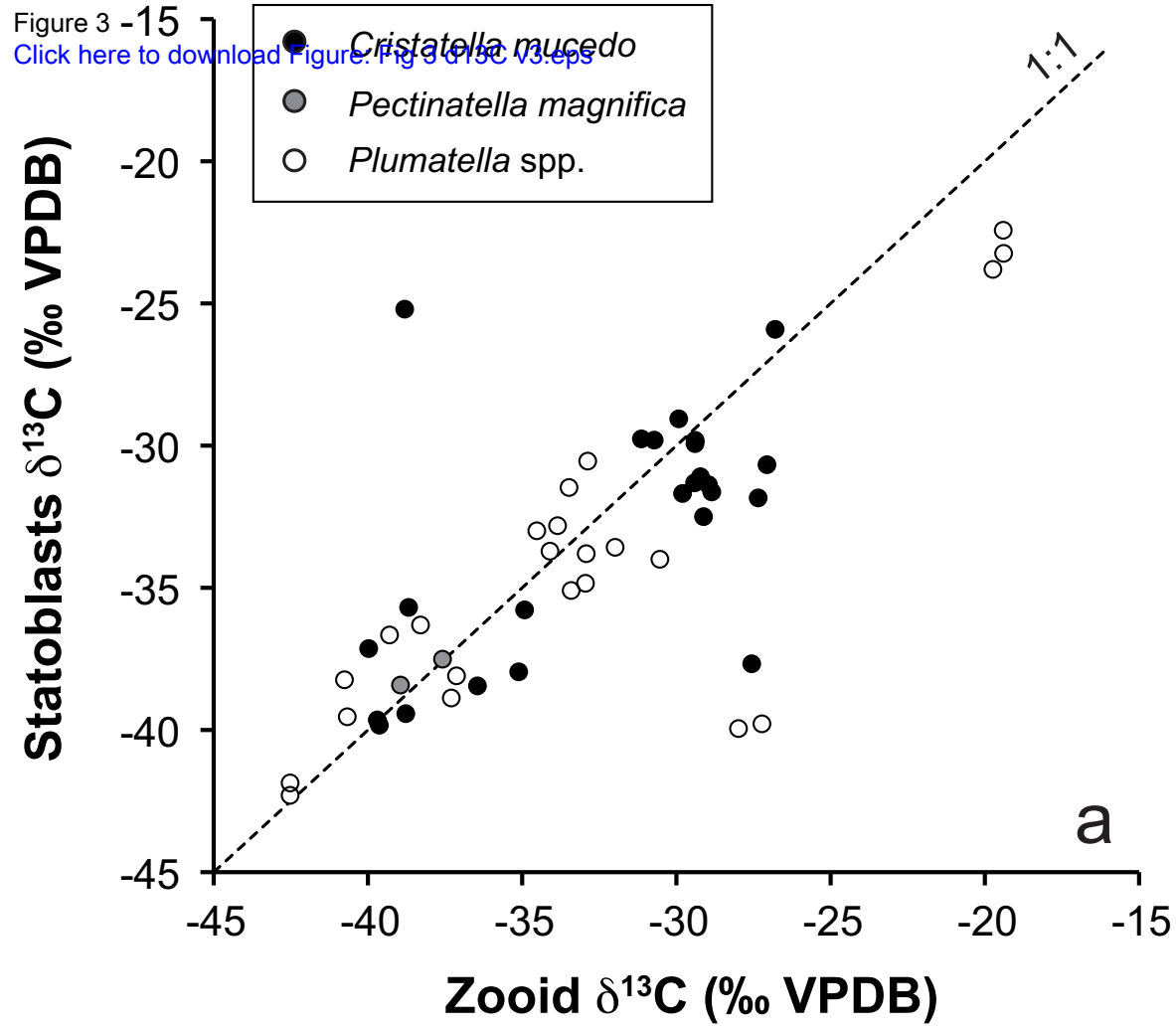

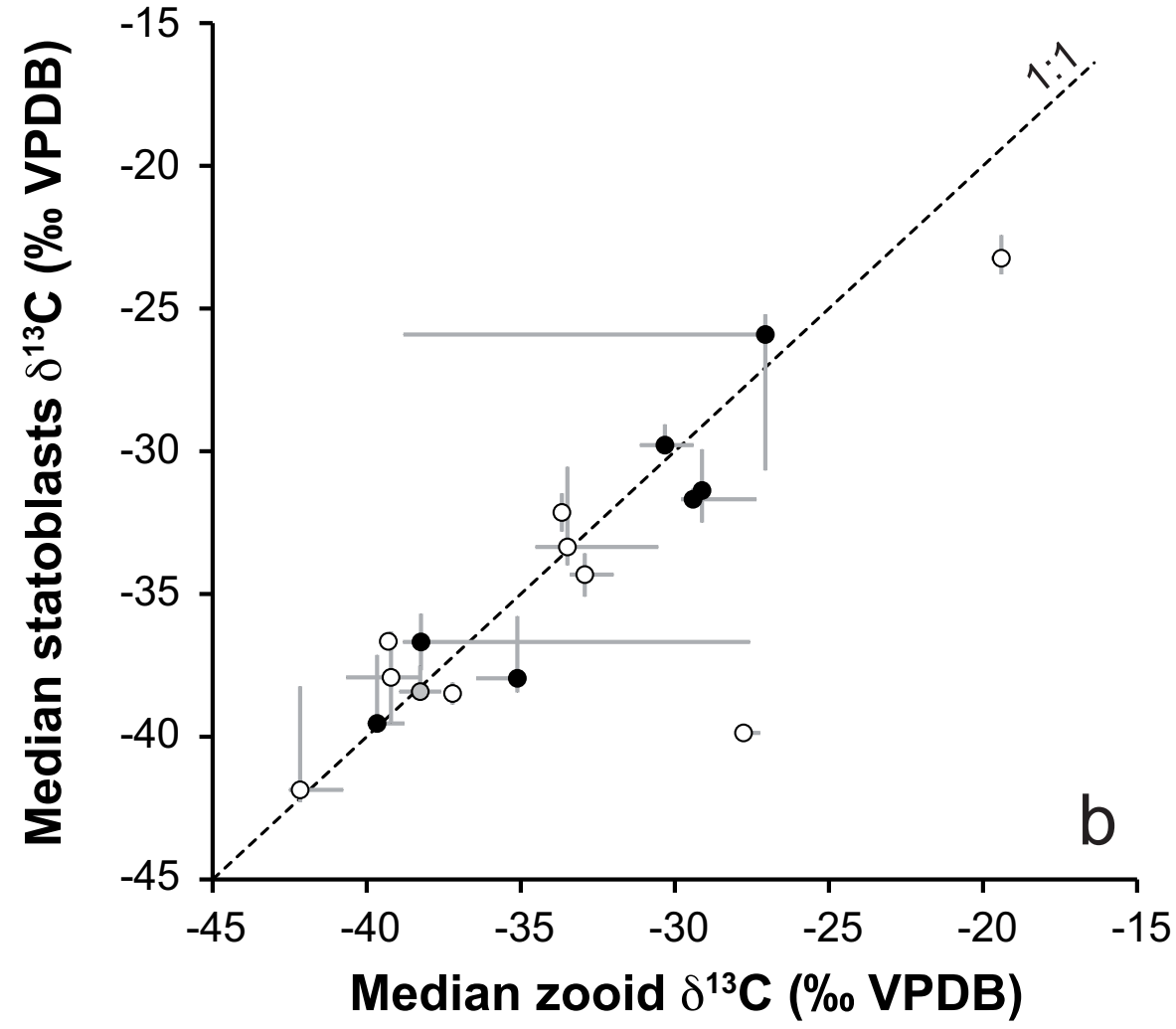

Figure 4

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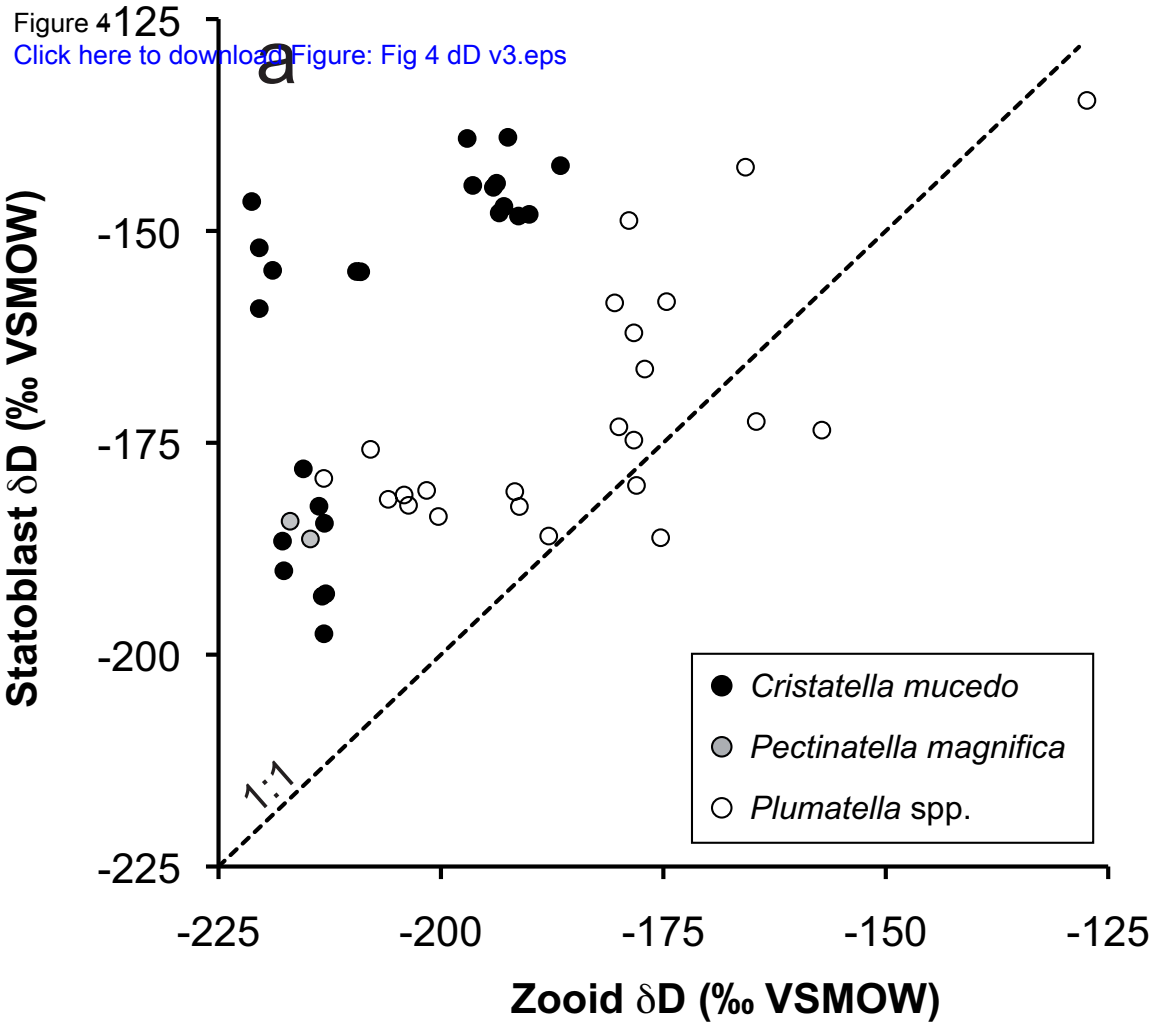

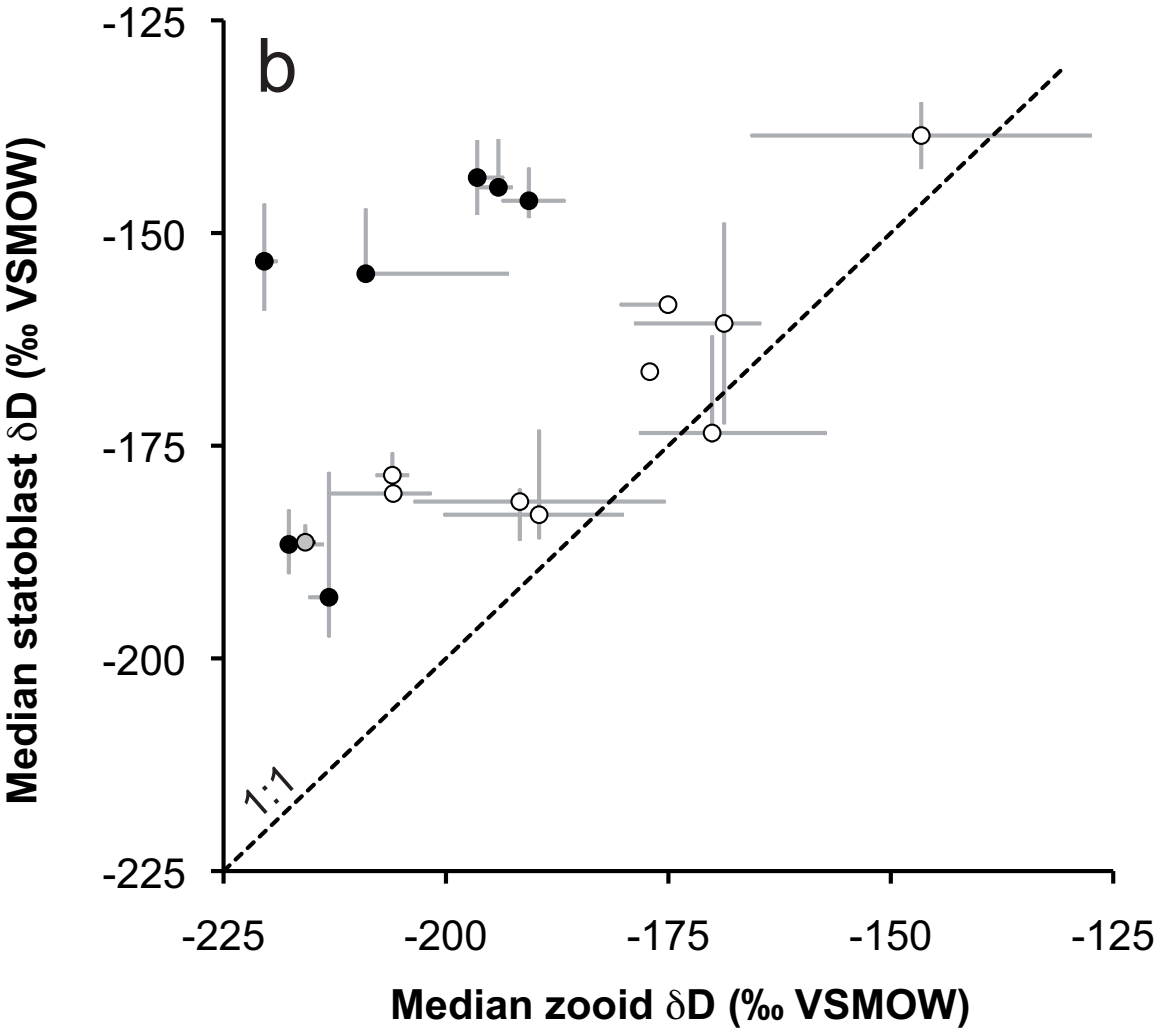

Figure 5

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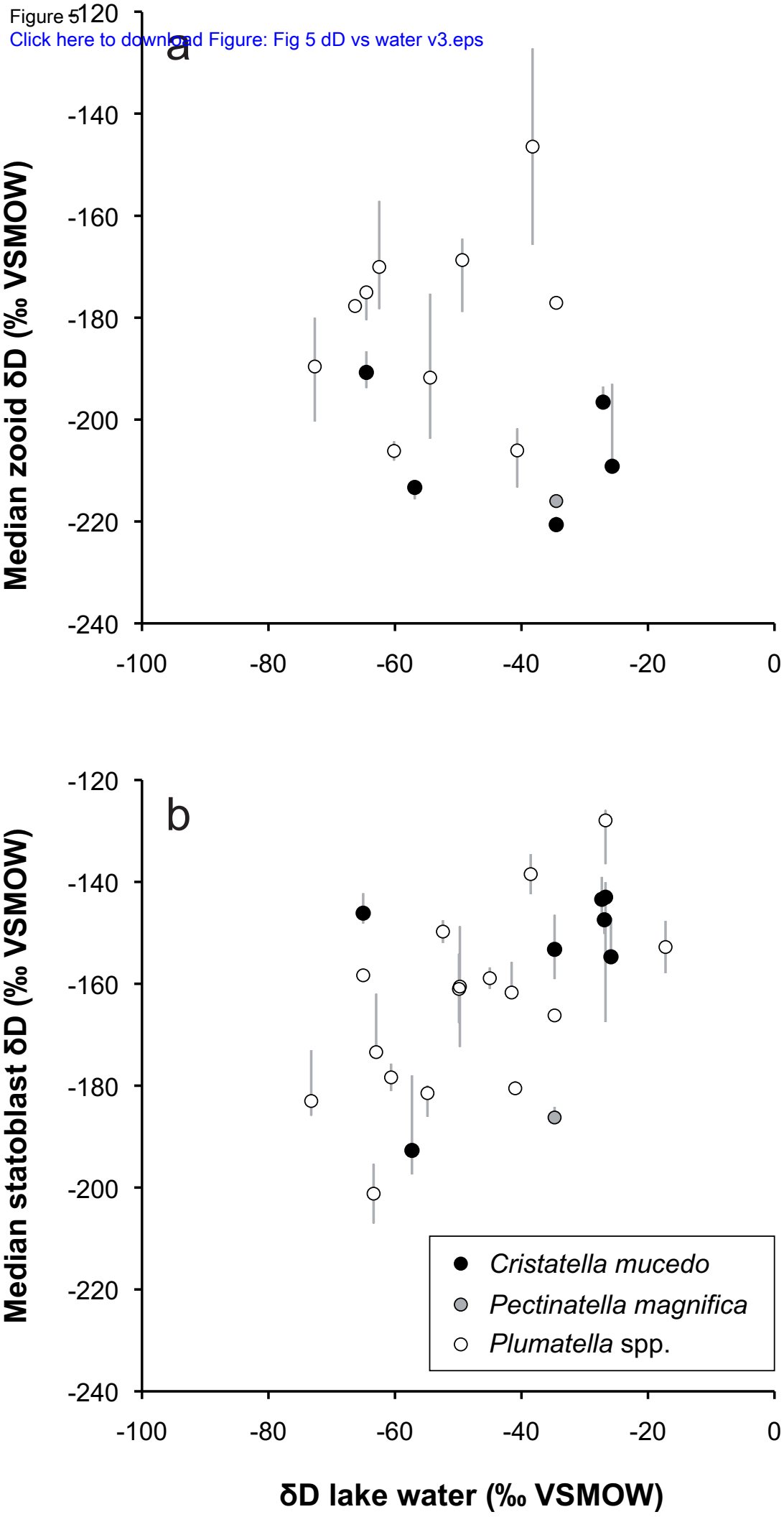

Figure 6

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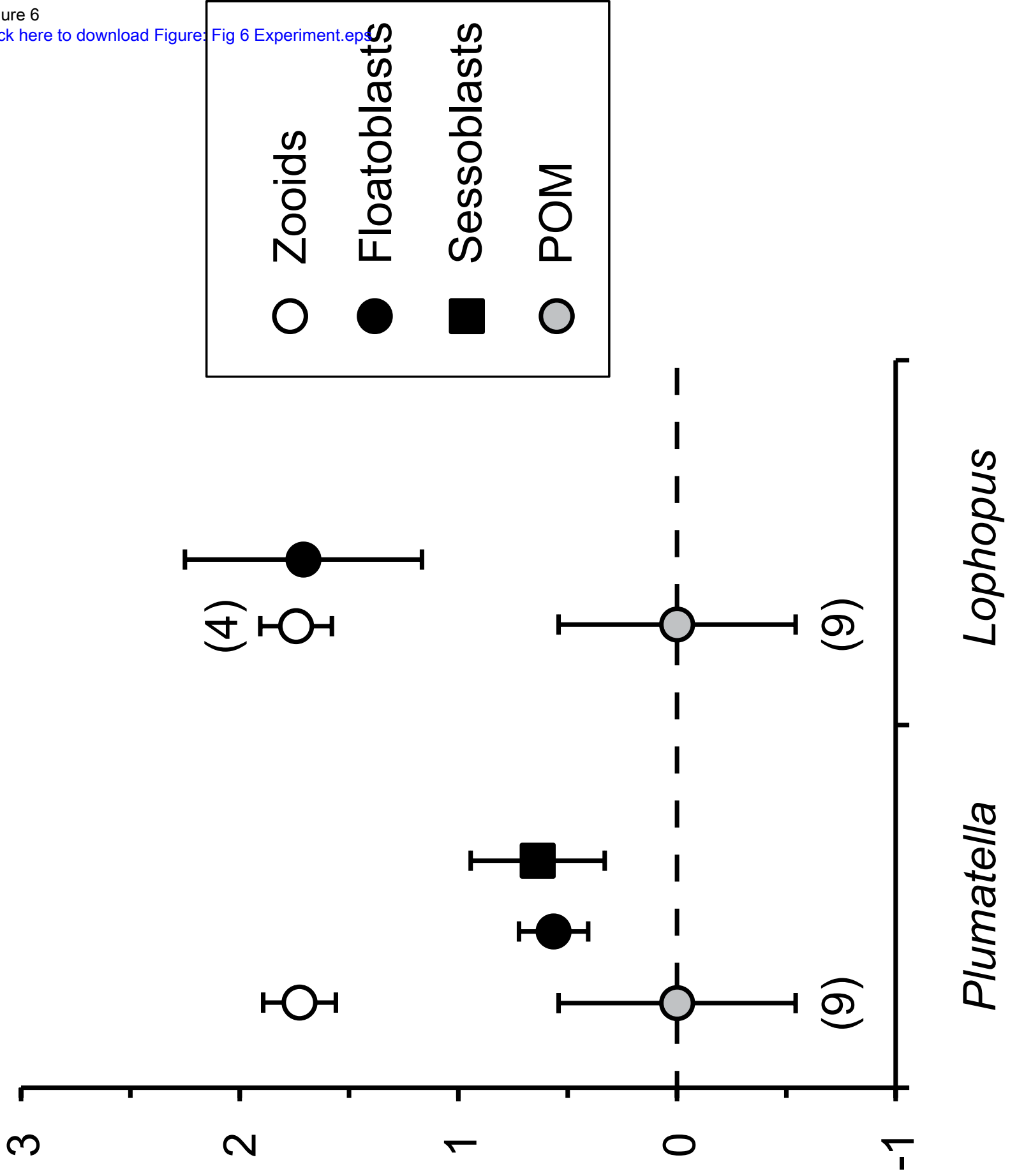

Tables 1, 2, and 3

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

	Cristatella		*Plumatella*		*Pectinatella*		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: $\delta^{13}C$ 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 $\delta^{13}C$	SD	n	mean statoblast $\delta^{13}C$	SD	n	mean zooid δD	SD	n	mean statoblast δD	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
Pluβsee	C				-32.7	1.6	5				-148.2	11.5	5
Schöhsee	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	Pl				-30.2		2				-201.3		2
Aatalweiher	Pl	-27.7	0.4	3	-39.9		2	-176.7	3.3	3	-158.4		2
Chli Golihübweiher	Pl	-33.7		2	-32.1		2	-146.6		2	-138.5		2
Chli Moossee	Pl	-47.7		2				-177.7		2			
Golihübweiher	Pl	-41.9	0.8	4	-40.8	2.2	3	-168.9	11.0	4	-170.1	7.0	3
Greifensee	Pl	-32.8	0.5	5	-34.3	0.8	4	-190.0	13.0	5	-182.3	2.8	4
Hinterburgsee	Pl	-33.0	1.8	4	-32.8	1.6	4	-189.8	8.4	4	-181.3	5.7	4
Holzsee	Pl				-32.5	1.0	3				-159.8	3.5	3
Inkwylersee	Pl				-34.9		2				-149.8		2
Lobsigensee	Pl	-39.4	1.2	3	-37.9		2	-170.7	7.4	3	-160.6		2
Moossee	Pl	-37.2		2	-38.5		2	-206.0		2	-178.5		2
Piepertkolk '11	Pl	-38.0		2				-171.7		2			
Pluβsee	Pl				-29.1	0.6	6				-128.9	3.9	6
Sempachersee	Pl				-28.2	0.6	3				-161.0	6.8	3
Sisselenweiher	Pl				-39.6		2				-159.0		2
De Waay	Pl	-19.5	0.2	3	-23.2	0.7	3	-206.9	5.8	3	-180.5	1.2	3