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

M. van Hardenbroek1,2, M. Leuenberger3, H. Hartikainen4,5, B. Okamura4, O. Heiri1

1 Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland
2 Geography and Environment, University of Southampton, Southampton SO17 1BJ, United Kingdom
3 Physics Institute and Oeschger Centre for Climate Change Research, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland
4 Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom
5 EAWAG, Department of Aquatic Ecology and ETH-Zurich, Institute of Integrative Biology (IBZ), Überlandstrasse 133, CH-8600, Dübendorf, Switzerland

Corresponding author: M. van Hardenbroek, vanhardenbroek@soton.ac.uk, T: +44 023 8059 2218, F: +44 023 8059 3295

Abstract
We explored the extent to which δ13C 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 δ13C and 100‰ for δD values. δ13C offsets between zooids

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and statoblasts generally ranged from -3 to +4.5‰, with larger offsets observed in four samples. However, a laboratory study with *Plumatella emarginata* and *Lophopus crystallinus* demonstrated that, in controlled settings, zooids had only 0 to 1.2‰ higher δ¹³C values than statoblasts, and 1.7‰ higher values than their food. At our field sites, we observed a strong positive correlation between median δ¹³C values of zooids and median δ¹³C 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,
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 $\delta D$ and $\delta^{18}O$ values of lacustrine invertebrates. The stable isotopic composition of H and O in aquatic invertebrate fossils reflects the $\delta D$ and $\delta^{18}O$ value of lake water at the time when these invertebrates were alive, and $\delta D$ and $\delta^{18}O$ values of invertebrate fossils can thus provide information about past climatic change. For example, $\delta^{18}O$ and $\delta D$ values of fossil remains of aquatic insects have been identified as proxies for reconstructing past variations in lake water $\delta^{18}O$ and $\delta D$ values (e.g. Wooller et al., 2004; Verbruggen et al., 2010; Van Hardenbroek et al.,...
2013b). However, $\delta^{18}$O and $\delta$D values of aquatic invertebrates are also influenced by
the $\delta^{18}$O and $\delta$D 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 $\delta$D 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 $\delta$D 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 $\delta$D 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 $\delta$D values of zooids relate to $\delta$D 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 $\delta$D values of zooids/statoblasts
reflect lake water δD values? Our study provides a basis for a future use of δ¹³C 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.
Mature statoblasts were identified with a dissection microscope (4-40x magnification) and opened to remove the mass of yolk granules and germinal tissue using lancet and forceps. Statoblasts were then treated with 10% KOH for 2 hours at room temperature to remove remaining attached soft tissue. This KOH treatment is commonly used in palaeolimnological studies of chitinous invertebrate remains and has been shown to have negligible effect on $\delta^{13}C$ values of chitinous sheaths and exoskeletons (van Hardenbroek et al., 2010; Schilder et al., 2015a). Samples were then rinsed with deionised water, freeze-dried, and transferred into silver cups for stable carbon and hydrogen isotope analysis.

Zooid tissue and matching statoblast samples from the field survey were measured on a high temperature elemental analyzer (ThermoFinnigan, Bremen, Germany) coupled to a mass spectrometer (Isoprime, Cheadle, UK). Pyrolysis temperature was set to 1450 °C. Since we attempted to measure stable isotope ratios for C and H simultaneously on small (60 to 160 µg) samples, the precision associated with the $\delta^{13}C$ and $\deltaD$ measurements is relatively low. Replicate measurements ($n = 37$) on a chitin standard (Sigma Aldrich, Zwijndrecht, The Netherlands) had a standard deviation of 1.1‰ for $\delta^{13}C$ and 3.1‰ for $\deltaD$. Replicate measurements ($n = 35$) of a cellulose standard (Merck, Darmstadt, Germany) had a standard deviation of 1.0‰ for $\delta^{13}C$ and 10.8‰ for $\deltaD$. Stable carbon isotopes are reported relative to VPDB and stable hydrogen isotopes relative to V-SMOW. $\deltaD$ values of bryozoan samples were corrected for exchangeable hydrogen using the method described by Filot et al. (2006):

In short, exchangeable hydrogen in the samples was equilibrated with standard water vapour of known isotopic composition. $\deltaD$ of bryozoan samples was calculated based on the measured $\deltaD$ after equilibration, the $\deltaD$ of the standard water vapour, and an
estimated percentage of 23.9% exchangeable H in the sample, assuming all samples have the same percentage of exchangeable H atoms.

Stable H and O isotopes of water samples from the field survey were analysed on a Finnigan MAT 250 mass spectrometer (Finnigan MAT, San Jose, CA) after equilibration of the water samples with a standard carbon dioxide using an equilibration device developed at the Physics Institute (University of Bern, Bern, Switzerland). Four small-volume samples were measured on a Picarro L1102-i analyser (Picarro Inc., Sunnyvale, CA) at the same laboratory. Standard deviations of measurements on water standards of known isotopic composition were better than 0.5‰ for δD and 0.1‰ for δ18O. For five sites only δ18O of lake water was measured. For these five lakes δD was estimated based on δ18O and the relationship between δ18O and δD observed for Swiss lakes: First, the difference (Δδ18O) between measured δ18O in lake water and estimated δ18O in precipitation (Bowen & Revenaugh, 2003; Bowen, 2014) was calculated for each of our sampling locations. Similarly, ΔδD was calculated as the difference between measured lake water δD and estimated δD of precipitation for those sites where we had measured lake water δD. A linear regression was then used to estimate ΔδD as a function of Δδ18O (n = 21, r = 0.99). This relationship was used to calculate ΔδD from Δδ18O for sites without lake water δD measurements and to estimate δD based on the δD of precipitation and ΔδD. Table 1 specifies the method used to derive δD for each water sample.

Laboratory study

Colonies of Lophopus crystallinus (Pallas, 1768) and Plumatella emarginata (Allman, 1844) were grown to evaluate the δ13C values of their zooids and statoblasts and how this relates to the δ13C of their diet, particulate organic matter (POM). A microcosm for
culturing bryozoans was established at constant 18 °C (± 1–2 °C) as in Hartikainen & Okamura (2012). The microcosm contained deionized water spiked periodically with natural pond water. The system comprised two 16-litre side tanks, housing the bryozoan colonies, connected to a 30-litre main tank containing 2 goldfish. A fluorescent light tube above the main tank (Tropic Sun 5500 K, ZooMed, Ekeren, Belgium) and fish excretions promoted algal and bacterial production and hence food for bryozoans. Water was continuously circulated between the main and side tanks via airlifts and U-tubes.

Bryozoa collected from three UK sites (Barton Blow Wells in Lincolnshire, the Norfolk Broads in Norfolk, and Padworth in Berkshire) were allowed to grow for at least 30 days in the laboratory. Zooids grown de novo in the laboratory were transparent, allowing the exclusion of sediment-covered zooids and statoblasts formed in the field. This allowed us to select laboratory-grown material, which had only incorporated carbon from the POM under the laboratory conditions. After 30 days, colonies were transferred to artificial pond water for 24 hours to allow gut evacuation.

Zooids and mature statoblasts were separated with forceps and a lancet under a stereomicroscope (4-40x magnification). Two types of statoblasts were collected from Plumatella colonies: sessoblasts, which remain attached inside colonies, and floatoblasts, which are released into the water. All zooid material per taxon was combined, freeze-dried, and homogenized. From this homogenized material, 3-4 replicate samples were weighed into tin capsules, and stored in a desiccator until stable isotope analysis. The same procedure was followed for statoblast material per taxon.

In addition, POM was collected at the start of the culture, at 14 days, and at 30 days to assess if δ¹³C values of POM changed during the study. POM was filtered onto
pre-combusted filters (Whatman GF/C), freeze-dried, weighed into tin capsules, and stored in a desiccator until stable isotope analysis.

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

Statistical analyses

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

**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\%\pm 4.4\% SD for *Plumatella*, 1.0\%\pm 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 ($\rho = 0.90$, $P < 0.0001$, $n = 16$).

$\delta$D values of zooids and statoblasts

The observed range of bryozoan $\delta$D values from our sites is nearly 100\% (Fig. 1b). $\delta$D 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 $\delta$D values between zooids and statoblasts ranged from -75 to +16 \% (Fig. 2b). Statoblast $\delta$D values appeared higher than $\delta$D values of zooids (Fig. 1b and 2b). The mean difference between median statoblast $\delta$D values and median zooid $\delta$D values per site was -44.8\% ± 15.5\% 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 zoooids ($\rho = 0.75, P = 0.0025, n = 9$; 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 zoid tissue in *Cristatella* than observed for the other bryozoan groups.

**δD values of bryozoans and lake water**

Sample pairs of zoooids 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 zoid δD values per site with lake water δD values based on Spearman correlation coefficients no systematic relationships between lake water and zoid 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 ($\rho = 0.56, P = 0.005, n = 24$) and when considering *Plumatella* ($\rho = 0.55, P = 0.027, n = 16$), 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 δ^{13}C values of zooids were in general very similar to δ^{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 *Plumatella* (Q = 8.14, P < 0.001). The mean 1.7‰ difference in δ^{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 δ^{3}C values*
The nearly 30%o-range of δ¹³C values observed for freshwater bryozoan tissues in this study is much larger than range of δ¹³C values previously reported by Turney (1999), Van Riel et al. (2006), and Van Hardenbroek et al. (2014). These earlier studies found δ¹³C values between -35 and -20%o that largely overlap with reported ranges for phytoplankton and POM (France, 1995; Vuorio et al., 2006). At 10 sites we found δ¹³C values that were lower than -35%o and at one site, Chli Moossee, values measured for zooids were as low as -48.2 and -47.2%o. Planktonic algae can in some situations be characterized by δ¹³C values lower than -35%o. For example, δ¹³C values of -41 to -37%o 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 δ¹³C values, however, are very unusual for eutrophic lakes like Chli Moossee, and an additional source of ¹³C-depleted carbon must have been available to bryozoans. Methane-derived carbon is strongly ¹³C-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 δ¹³C values as low as -70%o 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 δ¹³C values lower than -50%o 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
δ¹³C values of Bryozoa found at Aatalweiher, Sisselenweiher, Chli Moossee, Goliübweiher, Lobsigensee, and Piepertkolk (Table 3). However, more detailed measurements of δ¹³C values of bryozoans and POM, and of the abundance of MOB in POM in littoral habitats would be necessary to confirm this hypothesis.

δ¹³C offsets between POM, zooids, and statoblasts

Freshwater consumers are usually very similar in their δ¹³C values compared to their diet, with consumer δ¹³C 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 δ¹³C values of freshwater bryozoans reflect the δ¹³C 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 δ¹³C values of POM and cultured bryozoan zooids was statistically significant, it was small relative to the 30‰ range of δ¹³C 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 δ¹³C values of -31.1‰ and -28.8‰, respectively (van Riel et al., 2006). These values were substantially lower than δ¹³C 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 δ¹³C was only 0.9-3.3‰ higher than δ¹³C values of phytoplankton (-32‰) that they estimated based on the δ¹³C values of dissolved inorganic carbon. The δ¹³C 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.
study, suggesting that bryozoans were selectively feeding on phytoplankton, and that POM collected by van Riel et al. contained organic matter not assimilated by bryozoans.

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

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

Our laboratory study suggests that the $\delta^{13}C$ offset between food and zooids (1.7‰) does not vary greatly between colonies, at least for the two taxa investigated. In contrast, the offset between body tissue and fossilizing structure can vary between 0
and 1.2‰. Variations <1.2‰ in δ¹³C values of statoblasts from sediment samples could
therefore be the result of natural variability in the offset between zooids and
statoblasts. Variations >1.2‰ can thus be interpreted as a colony-independent signal
that has ecological or environmental significance. Certainly the large between-lake
variability in bryozoan δ¹³C values we observed in the 23 sites of our field survey
exceeds this 1.2‰ range. Other studies also indicated larger variability of δ¹³C values
in ecosystem studies and in down core records. For example, Vander Zanden &
Rasmussen (1999) report a range of 6‰ for δ¹³C values of primary consumers in
modern lake ecosystems, and Van Hardenbroek et al. (2014) report a range of 5‰ for
δ¹³C values of bryozoan statoblasts in a sediment record. The majority of this variation
in δ¹³C values can thus be interpreted in terms of changing carbon sources, or changing
δ¹³C values of these carbon sources.

Zooid and statoblast δ¹³C values under field conditions

We observed a strong correlation between the median δ¹³C values of zooids and
associated statoblasts at the different study sites (Fig. 3), which confirms that δ¹³C
values of statoblasts are systematically related to δ¹³C values of zooids. We observed a
clearly greater variability in offsets between zooids and statoblasts, however, in the
field survey than in the laboratory. In general, offsets in δ¹³C values between zooids
and statoblasts ranged between -3 and +4.5‰ (Fig. 2a), with average offsets of 1.0‰,
1.4‰, and -0.2‰ for Cristatella, Plumatella, and Pectinatella, respectively. However, in	hree cases statoblast δ¹³C was more than 10‰ lower than δ¹³C of zooids and the
opposite was observed in one instance. These four data points clearly fall outside the
regular range for offsets (Fig. 2a), and at two of these four extreme sites we also
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 $\delta D$ values**

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

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

In contrast to the $\delta^{13}C$ values, which were similar for zoids and statoblasts if median values were examined, we found that zoid $\delta D$ was substantially lower than statoblast $\delta D$ 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 $\delta D$ values for *Cristatella* obtained from 5 sites (i.e. Alte Aare, Piepertsolk, Schöhsee, Veenmeer in 2010, and Veenmeer in 2012), which are characterized by higher offsets between zoid and statoblast values and fall outside the scatter of other data points. These large offsets between $\delta D$ of zoids 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 $\delta D$ 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 zoids (mean 44%) compared to statoblasts (mean 30%) in our culture supports the idea of a higher lipid content in zoids. Further experiments with controlled $\delta D$ 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 $\delta D$ values between zoids and statoblasts, especially for *Cristatella*.

Relationship between $\delta D$ of lake water and Bryozoa

Lake water $\delta D$ values are more clearly related to the $\delta D$ values of statoblasts than to the $\delta D$ values of zoids (Fig. 5). One explanation for this may be that zoid 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 δ13C values. Offsets in δ13C 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 δ13C values from several colonies per sampling site, were strongly related with zooid δ13C 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 δ^{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).
References


Parasitology 139: 547-556.


Assessing the distribution of the freshwater bryozoan, Lophopus crystallinus.

Biological Conservation 135: 223-234.


Figure captions

**Fig. 1.** Boxplots of $\delta^{13}$C values (a) and $\delta$D 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 $\delta$D 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 $\delta$D values for each location.

**Fig. 4.** $\delta$D values of statoblasts plotted against $\delta$D 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 $\delta$D 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 δ^{13}C values of food (particulate organic matter, POM, grey circles) and δ^{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 δ^{13}C of POM in the culturing experiment.
Cristatella mucedo
Pectinatella magnifica
Plumatella spp.

Zooid $\delta D$ (‰ VSMOW)

Statoblast $\delta D$ (‰ VSMOW)

Median zooid $\delta D$ (‰ VSMOW)

Median statoblast $\delta D$ (‰ VSMOW)

- 1:1
- Cristatella mucedo
- Pectinatella magnifica
- Plumatella spp.
Figure 5

(a) Median zooid δD (‰ VSMOW) vs. δD lake water (‰ VSMOW) for 
Cristatella muceda, Pectinatella magnifica, and Plumatella spp.

(b) Median statoblast δD (‰ VSMOW) vs. δD lake water (‰ VSMOW) for 
Cristatella muceda, Pectinatella magnifica, and Plumatella spp.
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.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Coordinates</th>
<th>Date</th>
<th>Sampled substrate</th>
<th>Taxon</th>
<th>n_z</th>
<th>n_s</th>
<th>n_p</th>
<th>δD_water</th>
<th>δ¹⁸O_water</th>
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<tr>
<td>Aarbergerweiher, CH</td>
<td>47°32'20&quot;N / 7°17'4&quot;E</td>
<td>29-09-12</td>
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<td>0</td>
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<td>0</td>
<td>-63.0</td>
<td>-8.28</td>
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<td>10-09-11</td>
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<td>Plumatella</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>-64.6</td>
<td>-9.83</td>
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<td>47°6'10&quot;N / 8°38'7&quot;E</td>
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<td>breakwater 0.5m depth</td>
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<td>5</td>
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<td>-59.9</td>
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<td>10-09-11</td>
<td>breakwater 2.0m depth</td>
<td>Cristatella mucedo</td>
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<td>4</td>
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<td>-9.83</td>
</tr>
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<td>0</td>
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<td>-5.03</td>
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<td>3</td>
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<td>-5.03</td>
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<td>4</td>
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<td>-10.76</td>
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<td>rootlets</td>
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<td>-6.73</td>
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<td>submerged metal / vegetation</td>
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<td>2</td>
<td>2</td>
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<td>-6.31</td>
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<td>Plumatella</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-60.2</td>
<td>-8.26</td>
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<tr>
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<td>01-08-11</td>
<td>submerged branch</td>
<td>Plumatella</td>
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<td>0</td>
<td>-26.7</td>
<td>-3.63</td>
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<td>submerged branch</td>
<td>Plumatella</td>
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<td>1</td>
<td>1</td>
<td>-34.5</td>
<td>-4.69</td>
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<tr>
<td>Plusssee, D</td>
<td>54°10'58&quot;N / 10°26'47&quot;E</td>
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<td>6</td>
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<td>3</td>
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<td>old wooden board/rudder</td>
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<td>Plumatella</td>
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<td>0</td>
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<td>-5.32</td>
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<td></td>
<td></td>
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<td>Plumatella</td>
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<td>3</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
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</tbody>
</table>

**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 Δδ¹⁸O (lake water δ¹⁸O – estimated precipitation δ¹⁸O) and ΔδD (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.

<table>
<thead>
<tr>
<th></th>
<th>Cristatella colonies</th>
<th>Plumatella colonies</th>
<th>Pectinatella colonies</th>
<th>All Bryozoa colonies</th>
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<tr>
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<td>36</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>88</td>
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<td>Zooid samples</td>
<td>26</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>57</td>
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<td>Statoblasts samples</td>
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<td>8</td>
<td>2</td>
<td>1</td>
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<td>7</td>
<td>2</td>
<td>1</td>
<td>49</td>
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<td>Paired samples: water + zooid</td>
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<td>1</td>
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<td>26</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>72</td>
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</table>

**Table 3:** δ¹³C 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).

<table>
<thead>
<tr>
<th>Site</th>
<th>Taxon</th>
<th>mean zooid δ¹³C</th>
<th>SD</th>
<th>n</th>
<th>mean statoblast δ¹³C</th>
<th>SD</th>
<th>n</th>
<th>mean zooid δD</th>
<th>SD</th>
<th>n</th>
<th>mean statoblast δD</th>
<th>SD</th>
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<tbody>
<tr>
<td>Ågerisee 0.5m depth</td>
<td>C</td>
<td>-28.6</td>
<td>1.3</td>
<td>3</td>
<td>-31.6</td>
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<td>3</td>
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<td>3</td>
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<tr>
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<td>C</td>
<td>-29.1</td>
<td>0.2</td>
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<td>-31.3</td>
<td>0.9</td>
<td>5</td>
<td>-213.6</td>
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<td>5</td>
<td>-189.2</td>
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<tr>
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<td>-30.3</td>
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<td>Picardhofplas</td>
<td>C</td>
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