

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

M. van Hardenbroek  · M. Leuenberger ·
H. Hartikainen · B. Okamura · O. Heiri

Received: 15 November 2014 / Revised: 20 July 2015 / Accepted: 20 July 2015 / Published online: 29 July 2015
© Springer International Publishing Switzerland 2015

Abstract We explored the extent to which $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and 100‰ for δD values. $\delta^{13}\text{C}$ offsets between zooids and statoblasts generally ranged from -3 to $+4.5$ ‰, with larger offsets observed in four samples. However, a

laboratory study with *Plumatella emarginata* and *Lophopus crystallinus* demonstrated that, in controlled settings, zooids had only 0–1.2‰ higher $\delta^{13}\text{C}$ values than statoblasts, and 1.7‰ higher values than their food. At our field sites, we observed a strong positive correlation between median $\delta^{13}\text{C}$ values of zooids and median $\delta^{13}\text{C}$ 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.

Handling editor: M. Power

M. van Hardenbroek (✉) · O. Heiri
Institute of Plant Sciences and Oeschger Centre for
Climate Change Research, University of Bern,
Altenbergrain 21, 3013 Bern, Switzerland
e-mail: vanhardenbroek@soton.ac.uk

M. van Hardenbroek
Geography and Environment, University of Southampton,
Southampton SO17 1BJ, UK

M. Leuenberger
Physics Institute and Oeschger Centre for Climate Change
Research, University of Bern, Sidlerstrasse 5, 3012 Bern,
Switzerland

H. Hartikainen · B. Okamura
Department of Life Sciences, Natural History Museum,
Cromwell Road, London SW7 5BD, UK

H. Hartikainen
EAWAG, Department of Aquatic Ecology and ETH-
Zurich, Institute of Integrative Biology (IBZ),
Überlandstrasse 133, 8600 Dübendorf, Switzerland

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, which use ciliated tentacles to create feeding currents to capture suspended food particles, including phytoplankton and bacteria (Kaminski, 1984; Wood & 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 (Frey, 1964; Francis, 2001; Okamura et al., 2013).

In modern ecosystem studies, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) 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, $\delta^{13}\text{C}$ 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, $\delta^{13}\text{C}$ 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 $\delta^{18}\text{O}$ values of lacustrine invertebrates. The stable isotopic composition of H and O in aquatic invertebrate fossils reflects the δD and $\delta^{18}\text{O}$ value of lake water at the time when these invertebrates were alive, and δD and $\delta^{18}\text{O}$ values of invertebrate fossils can thus provide information about past climatic change. For example, $\delta^{18}\text{O}$ and δD values of fossil remains of aquatic insects have been identified as proxies for reconstructing past variations in lake water $\delta^{18}\text{O}$ and δD values (e.g. Wooller et al., 2004; Verbruggen et al., 2010; Van Hardenbroek et al., 2013b). However, $\delta^{18}\text{O}$ and δD values of aquatic invertebrates are also influenced by the $\delta^{18}\text{O}$ 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 $\delta^{13}\text{C}$ and δD values, as well as on the offsets between zooids and statoblasts under field conditions. We focused on stable carbon isotopes since invertebrate $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of bryozoan zooids and statoblasts under controlled conditions.

Our study first investigates the relationship between zooids and statoblasts regarding their $\delta^{13}\text{C}$ and δD values and secondly the relationship between bryozoans and their food/surrounding water. Specifically, we focus on the following questions: (1) How do $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of zooids/statoblasts related to $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 to 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 (Tables 1 and 3). Colonies were kept cool and dissected within 24 h 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 (4–40 × 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 h 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 $\delta^{13}\text{C}$ 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 1,450°C. Since we attempted to measure stable isotope ratios for C and H simultaneously on small (60–160 µg) samples, the precision associated with the $\delta^{13}\text{C}$ and δD 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 $\delta^{13}\text{C}$ and 3.1‰ for δD . Replicate measurements ($n = 35$) of a cellulose standard (Merck, Darmstadt, Germany) had a standard deviation of 1.0‰ for $\delta^{13}\text{C}$ and 10.8‰ for δD . Stable carbon isotopes are reported relative to VPDB and stable hydrogen isotopes relative to V-SMOW. δD 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. δD of bryozoan samples was calculated based on the measured δD after equilibration, the δD of the standard water vapour, and an estimated percentage of 23.9% exchangeable H in the sample, assuming that 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 $\delta^{18}\text{O}$. For five sites, only $\delta^{18}\text{O}$ of lake water was measured. For these five lakes, δD was estimated based on $\delta^{18}\text{O}$ and the relationship between $\delta^{18}\text{O}$ and δD was observed for Swiss lakes: first, the difference ($\Delta\delta^{18}\text{O}$) between measured $\delta^{18}\text{O}$ in lake water and estimated $\delta^{18}\text{O}$ in precipitation (Bowen & Revenaugh, 2003; Bowen, 2014) was calculated for each of our sampling locations. Similarly, $\Delta\delta\text{D}$ 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 $\Delta\delta\text{D}$ as a function of $\Delta\delta^{18}\text{O}$ ($n = 21$, $r = 0.99$). This relationship was used to calculate $\Delta\delta\text{D}$ from $\Delta\delta^{18}\text{O}$ for sites without lake water δD measurements and to estimate δD based on the δD of precipitation and $\Delta\delta\text{D}$. Table 1 specifies the method used to derive δD for each water sample.

Table 1 Location, date, and substrate of sampled Bryozoa

Lake	Coordinates	Date	Sampled substrate	Taxon	n_z	n_s	n_p	δD_{water}	$\delta^{18}O_{\text{water}}$
Aarbergerweiher, CH	47°3'20"N/7°17'4"E	29-09-12	Submerged vegetation	<i>Plumatella</i>	0	2	0	-63.0	-8.28
Aatalweiher, CH	47°14'23"N/8°57'9"E	10-09-11	Underside of stones	<i>Plumatella</i>	3	2	2	-64.6 ^a	-9.83
Ägerisee 0.5 m deep, CH	47°6'10"N/8°38'7"E	10-09-11	Breakwater 0.5 m depth	<i>Cristatella mucedo</i>	3	3	3	NA	NA
Ägerisee 2.0 m deep, CH	47°6'10"N/8°38'7"E	10-09-11	Breakwater 2.0 m depth	<i>Cristatella mucedo</i>	5	5	5	-56.9 ^a	-8.39
Alte Aare, CH	47°6'42"N/7°19'2"E	04-10-12	Submerged branch	<i>Cristatella mucedo</i>	4	4	4	-64.6 ^a	-9.83
Chli Golihübweiher, CH	47°4'33"N/7°22'31"E	04-10-12	Submerged wood of jetty	<i>Plumatella</i>	2	2	2	-38.3	-4.27
Chli Moossee, CH	47°1'36"N/7°28'12"E	04-10-12	Submerged branch	<i>Plumatella</i>	2	0	0	-66.4	-9.41
De Waay, NL	51°55'55"N/5°8'55"E	09-08-11	Submerged branch	<i>Plumatella geimermassardi</i>	3	3	3	-40.7	-4.90
Golihübweiher, CH	47°4'47"N/7°22'26"E	04-10-12	Submerged branch	<i>Plumatella</i>	4	3	3	-62.6	-9.00
Greifensee, CH	47°21'40"N/8°40'46"E	10-09-11	Submerged branch	<i>Plumatella</i>	5	4	4	-54.5 ^a	-8.15
Hinterburgsee, CH	46°43'5"N/8°41'E	16-09-11	Underside of old log	<i>Plumatella</i>	4	4	4	-72.8 ^a	-10.76
Holzsee, D	54°9'35"N/10°11'13"E	05-08-11	Metal poles of jetty	<i>Plumatella</i>	0	3	0	-41.3	-5.03
Inkwylersee, CH	47°11'50"N/7°39'39"E	15-09-12	Rootlets	<i>Plumatella</i>	0	2	0	-52.1	-6.73
Lobsigensee, CH	47°1'49"N/7°17'55"E	29-09-12	Submerged metal/vegetation	<i>Plumatella</i>	3	2	2	-49.4	-6.31
Moossee, CH	47°11'11"N/7°29'10"E	04-10-12	Submerged branch	<i>Plumatella</i>	2	2	2	-60.2	-8.26
Pieardhofplas, NL	53°11'16"N/6°32'23"E	01-08-11	Submerged branch	<i>Cristatella mucedo</i>	0	3	0	-26.7	-3.63
Pieperkolk, NL	52°38'43"N/6°3'56"E	07-08-11	Submerged branch	<i>Plumatella fruticosa</i>	1	1	1	-34.5	-4.69
		07-08-11	Submerged branch	<i>Cristatella mucedo</i>	4	4	4	-34.5	-4.69
		07-08-11	Submerged branch	<i>Pectinatella magnifica</i>	2	3	2	-34.5	-4.69
		05-08-12	Submerged branch	<i>Cristatella mucedo</i>	0	2	0	NA	NA
Plussee, D	54°10'58"N/ 10°26'47"E	06-08-11	Boat	<i>Plumatella</i>	0	6	0	-26.5	-2.10
			Boat	<i>Cristatella mucedo</i>	0	5	0	-26.5	-2.10
Steenbergen, NL	53°06'31"N/6°23'33"E	08-08-12	Submerged vegetation	<i>Plumatella</i>	0	2	0	-17.1	-1.50
Schöthsee, D	54°9'36"N/10°26'8"E	07-08-11	Submerged branch	<i>Cristatella mucedo</i>	3	3	3	-25.7	-2.72
Sempachersee, CH	47°9'48"N/8°8'42"E	15-09-12	Old wooden board/rudder	<i>Plumatella</i>	0	3	0	-49.5	-6.30
Siselenweiher, CH	47°1'38"N/7°12'16"E	29-09-12	Submerged branch	<i>Plumatella</i>	0	2	0	-44.7	-5.32
Veenmeer, NL	53°5'5"N/6°38'6"E	28-11-10	Submerged vegetation	<i>Cristatella mucedo</i>	4	2	2	-27.1	-3.33
		03-06-12	Submerged vegetation	<i>Cristatella mucedo</i>	3	3	3	NA	NA
			Total		57	80	49		

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

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

CH Switzerland, D Germany, NL The Netherlands

^a 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

	<i>Cristatella</i>		<i>Plumatella</i>		<i>Pectinatella</i>		All Bryozoa		Figure
	Colonies	Sites	Colonies	Sites	Colonies	Sites	Colonies	Sites	
Colonies sampled	36	8	49	17	3	1	88	23	Figs. 1, 2
Zooid samples	26	6	29	10	2	1	57	15	Figs. 1, 2
Statoblasts samples	34	8	43	16	3	1	80	22	Figs. 1, 2
Paired samples: zooid + statoblast	24	7	23	9	2	1	49	14	Figs. 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

'Paired samples' indicates 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

Laboratory study

Colonies of *Lophopus crystallinus* (Pallas, 1768) and *Plumatella emarginata* (Allman, 1844) were grown to evaluate the $\delta^{13}\text{C}$ values of their zooids and statoblasts and how this relates to the $\delta^{13}\text{C}$ of their diet, particulate organic matter (POM). A microcosm for culturing bryozoans was established at constant 18°C ($\pm 1 - 2^\circ\text{C}$) as in Hartikainen & Okamura (2012). The microcosm contained deionized water spiked periodically with natural pond water. The system comprised two 16-l side tanks, housing the bryozoan colonies, connected to a 30-l 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 h to allow gut evacuation. Zooids and mature statoblasts were separated with forceps and a lancet under a stereomicroscope (4–40× 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 materials per taxon were 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 $\delta^{13}\text{C}$ 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 analysed for $\delta^{13}\text{C}$ 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 $\pm 0.1\%$.

Statistical analyses

To examine the relationship between $\delta^{13}\text{C}$ 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

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

Site	Taxon	Mean zooid $\delta^{13}\text{C}$	SD	n	Mean statoblast $\delta^{13}\text{C}$	SD	n	mean zooid δD	SD	n	Mean statoblast δD	SD	n
Ägerisee 0.5 m depth	C	-28.6	1.3	3	-31.6	0.3	3	-216.4	2.3	3	-184.4	3.8	3
Ägerisee 2.0 m depth	C	-29.1	0.2	5	-31.3	0.9	5	-213.6	1.1	5	-189.2	7.8	5
Alte Aare	C	-30.3	0.8	4	-29.6	0.4	4	-190.4	3.0	4	-145.7	2.9	4
Picardhofplas	C				-37.4	1.3	3				-148.2	1.9	3
Piepertkolk '11	C	-39.5	0.5	4	-39.0	1.3	4	-220.3	1.0	4	-153.1	5.3	4
Piepertkolk '12	C				-36.5		2				-161.9		2
Plußsee	C				-32.7	1.6	5				-148.2	11.5	5
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
Inkwylensee	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			
Plußsee	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

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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of POM, zooids, and statoblast in the laboratory study using one-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

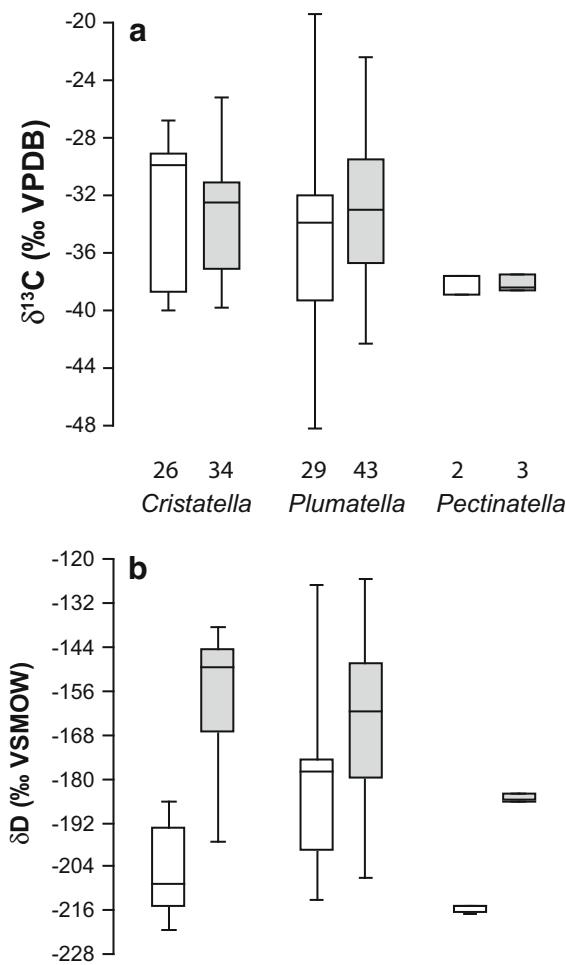


Fig. 1 Boxplots of $\delta^{13}\text{C}$ values (**a**) and δD values (**b**) of the three bryozoan genera investigated in this study. Values for zooids are shown in white and for statoblasts in grey. Numbers indicate how many data points constitute each boxplot

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).

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

A remarkably large range of $\delta^{13}\text{C}$ 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. $\delta^{13}\text{C}$ ranges of *Cristatella* were -40.0 to -26.8 ‰ for zooids and -39.8 to -25.2 ‰ for statoblasts. Offsets in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values and median statoblast $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values were not statistically significant for *Plumatella*, for *Cristatella*, or for all Bryozoa pooled (paired samples t-test).

Median $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 (Figs. 1b and 2b). The mean difference between median statoblast δD values and median zooid δD values per site was -44.8 ‰ \pm 15.5 ‰ SD for *Cristatella*, and -12.2 ‰ \pm 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

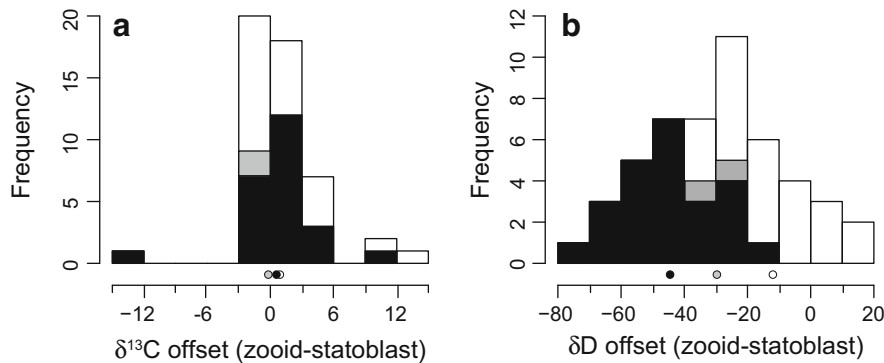


Fig. 2 Stacked histograms representing the offsets between $\delta^{13}\text{C}$ values (**a**) of zooids and statoblasts for *Cristatella* (black), *Pectinatella* (grey), and *Plumatella* (white). Average offsets

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

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 ($\rho = 0.75$, $P = 0.0025$, $n = 9$; Fig. 4), whereas the relationships between the 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's 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 ($\rho = 0.56$, $P = 0.005$, $n = 24$) 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 $\delta^{13}\text{C}$ values of zooids were in general very similar to $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values between POM and *Lophopus* statoblasts was significant (Tukey-HSD, $Q = 8.26$, $P < 0.001$), but no significant

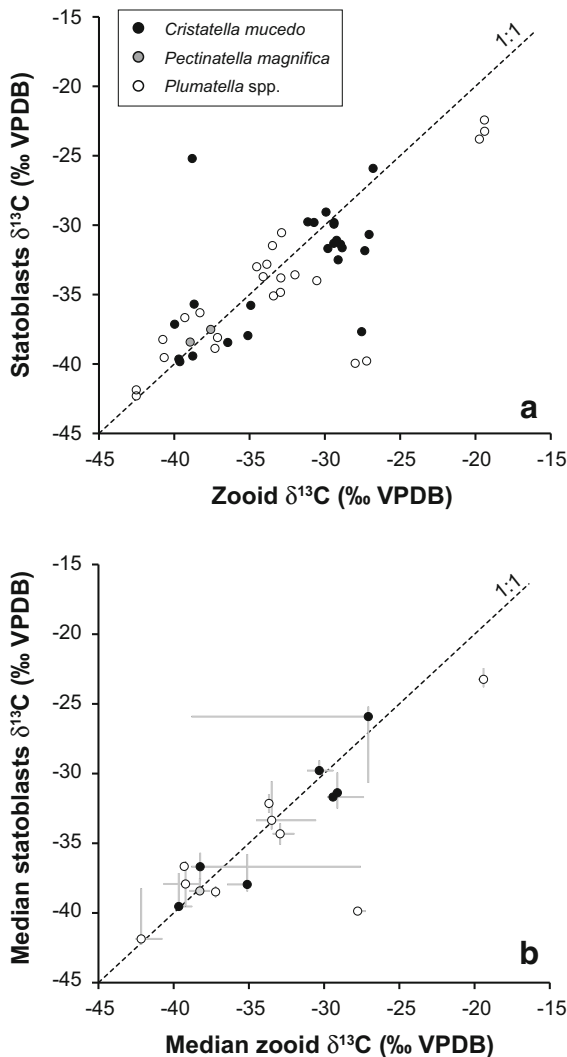


Fig. 3 $\delta^{13}\text{C}$ values of statoblasts plotted against $\delta^{13}\text{C}$ values of zooids for *Cristatella*, *Pectinatella*, and *Plumatella*. The dotted line indicates the 1:1 line. All data points are shown in (a); Median values for each sampling location are shown in (b) with grey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

difference was found between POM and *Plumatella* sessoblasts or floatoblasts (Tukey-HSD).

Discussion

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

The nearly 30‰ range of $\delta^{13}\text{C}$ values observed for freshwater bryozoan tissues in this study is much larger

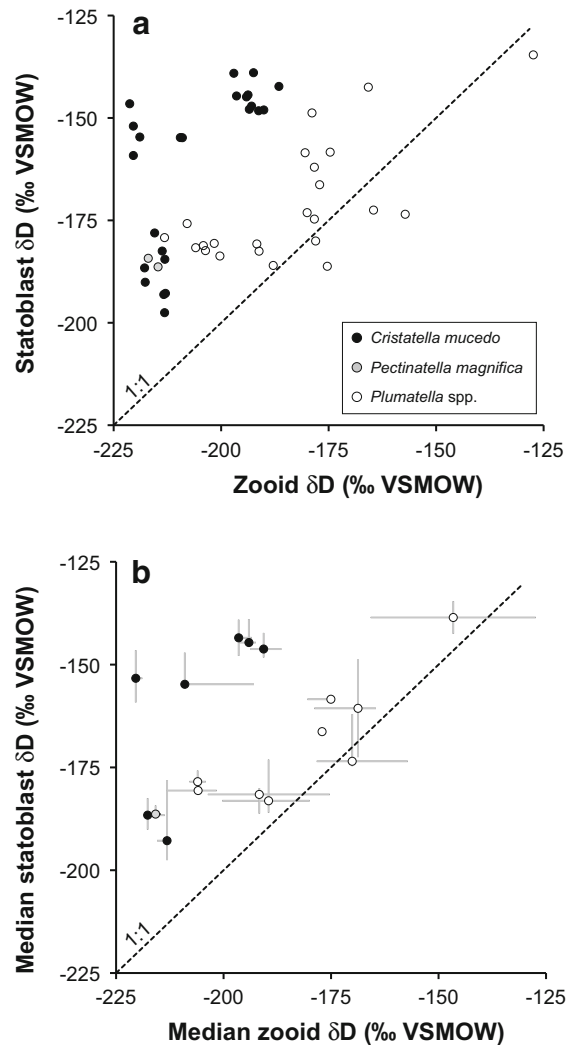


Fig. 4 δD values of statoblasts plotted against δD values of zooids for *Cristatella*, *Pectinatella*, and *Plumatella*. The dotted line indicates the 1:1 line. All data points are shown in (a); Median values for each sampling location are shown in (b) with grey lines representing the range of replicate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

than range of $\delta^{13}\text{C}$ values previously reported by Turney (1999), Van Riel et al. (2006), and Van Hardenbroek et al. (2014). These earlier studies found $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 characterised by $\delta^{13}\text{C}$ values lower than -35‰. For example, $\delta^{13}\text{C}$ values of -41 to

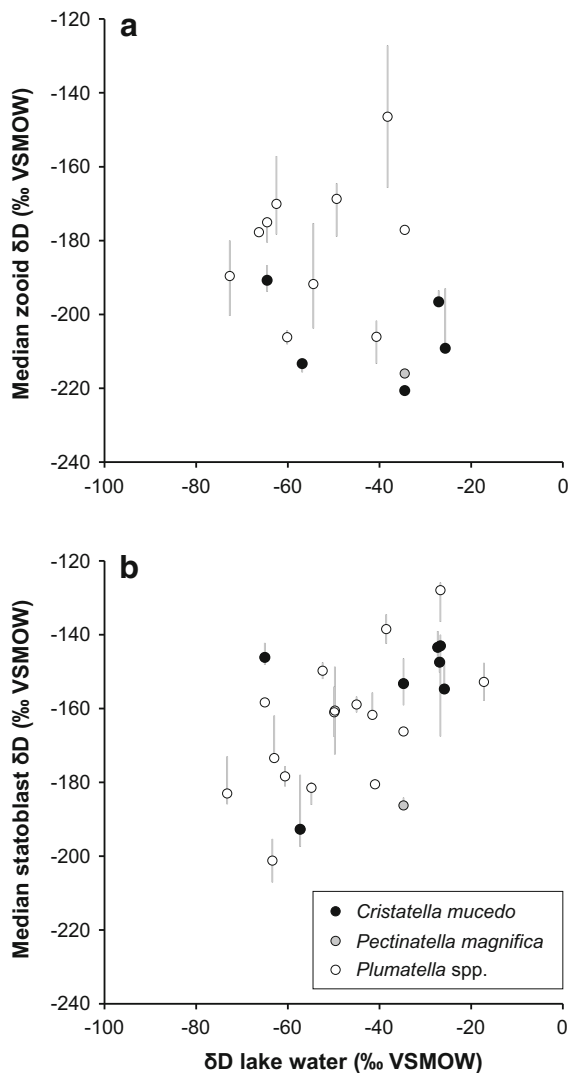


Fig. 5 Median δD values (with ranges indicated by grey lines) of *Cristatella*, *Pectinatella*, and *Plumatella* zoids (a) and statoblasts (b) plotted against δD of lake water. Grey lines represent the range of replicate δD values for each location

−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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values as low as −70‰ in some

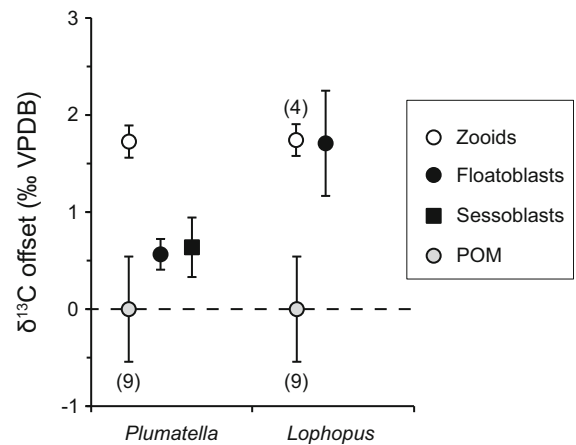


Fig. 6 Average offsets between $\delta^{13}\text{C}$ values of food (particulate organic matter, POM, grey circles) and $\delta^{13}\text{C}$ values of *Plumatella* and *Lophopus* zoids (open circles), floatoblasts (closed circles), and sessoblasts (closed squares) in the culturing experiment. Error 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 $\delta^{13}\text{C}$ of POM in the culturing experiment

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

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

Freshwater consumers are usually very similar in their $\delta^{13}\text{C}$ values compared to their diet, with consumer

$\delta^{13}\text{C}$ values on average 0–1.3‰ higher than those of their diet (DeNiro & Epstein, 1978; McCutchan et al., 2003; Peters et al., 2012). It has therefore been suggested that $\delta^{13}\text{C}$ values of freshwater bryozoans reflect the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of POM and cultured bryozoan zooids was statistically significant, it was small relative to the 30‰ range of $\delta^{13}\text{C}$ values observed for zooids in the field survey.

In a study on the River Rhine, colonies of *Plumatella repens* and *P. fungosa* were characterised by $\delta^{13}\text{C}$ values of -31.1‰ and -28.8‰ , respectively (Van Riel et al., 2006). These values were substantially lower than $\delta^{13}\text{C}$ 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* $\delta^{13}\text{C}$ was only 0.9–3.3‰ higher than $\delta^{13}\text{C}$ values of phytoplankton (-32‰) that they estimated based on the $\delta^{13}\text{C}$ values of dissolved inorganic carbon. The $\delta^{13}\text{C}$ 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 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 characterised by $\delta^{13}\text{C}$ values 1.7–3.1‰ higher than their food (Power et al., 2003). In another study with *Daphnia pulex* (Forbes, 1893), this difference was $0.5 \pm 0.3\text{‰}$ (Schilder et al., 2015a). In our laboratory study, the 1.7‰ higher $\delta^{13}\text{C}$ values of zooids of *Plumatella* and *Lophopus* compared with the $\delta^{13}\text{C}$ values of their food are of similar magnitude, suggesting that zooid $\delta^{13}\text{C}$ values provide a direct indication of the $\delta^{13}\text{C}$ values of bryozoan diet.

In our laboratory study, we found very small offsets between $\delta^{13}\text{C}$ values of bryozoan zooids and statoblasts, based on a diet with constant $\delta^{13}\text{C}$ values. We observed no significant offset between $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of the ephippia of *Daphnia* from Lake Geneva were indistinguishable ($\pm 0.1\text{‰}$) from $\delta^{13}\text{C}$ values of whole body tissue. Similarly, a culturing experiment by Schilder et al. (2015a) indicated that $\delta^{13}\text{C}$ values of *Daphnia* ephippia were on average $0.2 \pm 0.4\text{‰}$ higher than whole body tissue. Head capsules of 4th instar *Chironomus riparius* (Meigen 1804) larvae were on average $1.2 \pm 0.9\text{‰}$ and $0.9 \pm 0.2\text{‰}$ 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 $\delta^{13}\text{C}$ 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 $<1.2\text{‰}$ in $\delta^{13}\text{C}$ values of statoblasts from sediment samples could therefore be the result of natural variability in the offset between zooids and statoblasts. Variations $>1.2\text{‰}$ can thus be interpreted as a colony-independent signal that has ecological or environmental significance. Certainly the large between-lake variability in bryozoan $\delta^{13}\text{C}$ values we observed in the 23 sites of our field survey exceeds this 1.2‰ range. Other studies also indicated larger variability of $\delta^{13}\text{C}$ values in ecosystem studies and in down core records. For example, Vander Zanden & Rasmussen (1999) report a range of 6‰ for $\delta^{13}\text{C}$ values of primary consumers in modern lake ecosystems, and Van Hardenbroek et al. (2014) report a range of 5‰ for $\delta^{13}\text{C}$ values of bryozoan statoblasts in a sediment record. The majority of this variation in $\delta^{13}\text{C}$ values can thus be interpreted in terms of changing carbon sources, or changing $\delta^{13}\text{C}$ values of these carbon sources.

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

We observed a strong correlation between the median $\delta^{13}\text{C}$ values of zooids and associated statoblasts at the different study sites (Fig. 3), which confirms that $\delta^{13}\text{C}$ values of statoblasts are systematically related to $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values between zooids and statoblasts ranged between -3 and $+4.5\text{‰}$ (Fig. 2a), with average offsets of 1.0, 1.4, and -0.2‰ for *Cristatella*, *Plumatella*, and *Pectinatella*, respectively. However, in three cases, statoblast $\delta^{13}\text{C}$ was more than 10‰ lower than $\delta^{13}\text{C}$ 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 – 3.6‰ . This suggests that the availability of food sources that differ $>10\text{‰}$ can be a very localized phenomenon in time and space, possibly occurring in particular microhabitats. Differences in $\delta^{13}\text{C}$ values of similar magnitude (10 – 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 (Figs. 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 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 characterised 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 ($<7\ \mu\text{m}$ in diameter), which can include bacteria, whereas *Plumatella repens* prefers slightly larger particles (ranging from 5 to $17\ \mu\text{m}$ 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 $20\ \text{m}$ (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* zooids and *Cristatella* tissues.

δD offsets between zooids and statoblasts

In contrast to the $\delta^{13}\text{C}$ 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 characterised 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 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.

Acknowledgments 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

- Agasild, H., P. Zingel, L. Tuvikene, A. Tuvikene, H. Timm, T. Feldmann, J. Salujõe, K. Toming, R. I. Jones & T. Nõges, 2014. Biogenic methane contributes to the food web of a large, shallow lake. *Freshwater Biology* 59: 272–285.
- Belle, S., C. Parent, V. Frossard, V. R. Verneaux, L. Millet, P.-M. Chronopoulou, P. Sabatier & M. Magny, 2014. Temporal changes in the contribution of methane-oxidizing bacteria to the biomass of chironomid larvae determined using stable carbon isotopes and ancient DNA. *Journal of Paleolimnology* 52: 215–228.
- Bowen, G. J., 2014. The Online Isotopes in Precipitation Calculator, version 2.2. <http://www.waterisotopes.org>.
- Bowen, G. J. & J. Revenaugh, 2003. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research* 39: 1299.
- Deines, P., M. J. Wooller & J. Grey, 2009. Unraveling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. *Freshwater Biology* 54: 2243–2251.
- DeNiro, M. J. & S. Epstein, 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica and Cosmochimica Acta* 42: 495–506.
- Filot, M. S., M. Leuenberger, A. Pazdur & T. Boettger, 2006. Rapid online equilibration method to determine the D/H ratios of non-exchangeable hydrogen in cellulose. *Rapid Communications in Mass Spectrometry* 20: 3337–3344.
- France, R. L., 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography* 40: 1310–1313.
- Francis, D., 2001. Bryozoan Statoblasts. In Smol, J., H. J. Birks & W. Last (eds), *Tracking Environmental Change Using Lake Sediments. Developments in Paleoenvironmental Research*, Vol. 4. Springer, Dordrecht: 105–123.
- Frey, D. G., 1964. Remains of animals in Quaternary lake and bog sediments and their interpretation. *Archiv für Hydrobiologie Supplement* 2: 1–114.
- Frossard, V., S. Belle, V. Verneaux, L. Millet & M. Magny, 2013. A study of the $\delta^{13}\text{C}$ offset between chironomid larvae and their exuvial head capsules: implications for palaeoecology. *Journal of Paleolimnology* 50: 379–386.
- Frossard, V., V. Verneaux, L. Millet, J.-P. Jenny, F. Arnaud, M. Magny & M.-E. Perga, 2014. Reconstructing long-term changes (150 years) in the carbon cycle of a clear-water lake based on the stable carbon isotope composition ($\delta^{13}\text{C}$) of chironomid and cladoceran subfossil remains. *Freshwater Biology* 59: 789–802.
- Gat, J. R., 1995. Stable Isotopes of Fresh and Saline Lakes. In Lerman, A., D. M. Imboden & J. R. Gat (eds), *Physics and Chemistry of Lakes*. Springer, Berlin: 139–195.
- Grey, J., S. Waldron & R. Hutchinson, 2004a. The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: a pilot study in Esthwaite Water, UK. *Hydrobiologia* 524: 253–262.
- Grey, J., A. Kelly, S. Ward, N. Sommerwerk & R. I. Jones, 2004b. Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. *Freshwater Biology* 49: 681–689.
- Hammer, Ø., D. A. T. Harper, & P. D. Ryan, 2001. PAST: paleontological statistics software package for education and data analysis. *Palaentologia Electronica* 4: 9 pp.
- Hartikainen, H. & B. Okamura, 2012. Castrating parasites and colonial hosts. *Parasitology* 139: 547–556.
- Heiri, O., J. Schilder & M. van Hardenbroek, 2012. Stable isotopic analysis of fossil chironomids as an approach to environmental reconstruction: state of development and future challenges. *Fauna Norvegica* 31: 7–18.
- Hill, S. L. L., C. D. Sayer, P. M. Hammond, V. K. Rimmer, T. A. Davidson, D. J. Hoare, A. Burgess & B. Okamura, 2007. Are rare species rare or just overlooked? Assessing the distribution of the freshwater bryozoan, *Lophopus crystallinus*. *Biological Conservation* 135: 223–234.
- Hobson, K. A., L. Atwell & L. I. Wassenaar, 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences of the United States of America* 96: 8003–8006.
- Jones, R. I. & J. Grey, 2011. Biogenic methane in freshwater food webs. *Freshwater Biology* 56: 213–229.
- Jones, R. I., J. Grey, D. Sleep & L. Arvola, 1999. Stable isotope analysis of zooplankton carbon nutrition in humic lakes. *Oikos* 86: 97–104.
- Kaminski, M., 1984. Food composition of three bryozoan species (Bryozoa, Phylactolaemata) in a mesotrophic lake. *Polish Archive of Hydrobiology* 31: 45–53.
- Kankaala, P., S. Taipale, L. Li & R. Jones, 2010. Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquatic Ecology* 44: 781–795.
- Karlsson, J., M. Berggren, J. Ask, P. Byström, A. Jonsson, H. Laudon & M. Jansson, 2012. Terrestrial organic matter support of lake food webs: evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnology and Oceanography* 57: 1042–1048.
- Lacourt, A., 1968. A monograph of the freshwater Bryozoa-Phylactolaemata. *Zoologische Verhandlungen* 93: 1–155.
- McCutchan, J. H., W. M. Lewis, C. Kendall & C. C. McGrath, 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–390.
- Okamura, B., K. Ayres, J. Salgado, T. Davidson, R. Shaw, T. Stephens, D. Hoare & C. Sayer, 2013. Shallow lake sediments provide evidence for metapopulation dynamics: a pilot study. *Aquatic Ecology* 47: 163–176.
- Perga, M.-E., 2011. Taphonomic and early diagenetic effects on the C and N stable isotope composition of cladoceran remains: implications for paleoecological studies. *Journal of Paleolimnology* 46: 203–213.
- Peters, L., C. Faust & W. Traunspurger, 2012. Changes in community composition, carbon and nitrogen stable isotope signatures and feeding strategy in epilithic aquatic nematodes along a depth gradient. *Aquatic Ecology* 46: 371–384.
- Post, D. M., 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83: 703–718.
- Power, M., K. R. R. A. Guiguer & D. R. Barton, 2003. Effects of temperature on isotopic enrichment in *Daphnia magna*:

- implications for aquatic food-web studies. *Rapid Communications in Mass Spectrometry* 17: 1619–1625.
- R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Richelle, E., Z. Moureau & G. Van de Vyver, 1994. Bacterial feeding by the freshwater bryozoan *Plumatella fungosa* (Pallas, 1768). *Hydrobiologia* 291: 193–199.
- Schilder, J., C. Tellenbach, M. Möst, P. Spaak, M. van Hardenbroek, M. J. Wooller & O. Heiri, 2015a. Experimental assessment of environmental influences on the stable isotopic composition of *Daphnia pulex* and their ephippia. *Biogeosciences* 12: 3819–3830.
- Schilder, J., D. Bastviken, M. van Hardenbroek, M. Leuenberger, P. Rinta, T. Stötter & O. Heiri, 2015b. The stable carbon isotopic composition of *Daphnia* ephippia in small, temperate lakes reflects in-lake methane availability. *Limnology and Oceanography* 60: 1064–1075.
- Schimmelmann, A., A. L. Sessions & M. Mastalerz, 2006. Hydrogen isotopic (D/H) composition of organic matter during diagenesis and thermal maturation. *Annual Review of Earth and Planetary Sciences* 34: 501–533.
- Schürch, M., R. Kozel, U. Schotterer & J.-P. Tripet, 2003. Observation of isotopes in the water cycle – the Swiss National Network (NISOT). *Environmental Geology* 45: 1–11.
- Solomon, C., J. Cole, R. Doucett, M. Pace, N. Preston, L. Smith & B. Weidel, 2009. The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* 161: 313–324.
- Soto, D. X., L. I. Wassenaar & K. A. Hobson, 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Functional Ecology* 27: 535–543.
- Taipale, S., P. Kankaala & R. I. Jones, 2007. Contributions of different organic carbon sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: results from mesocosm DI^{13}C -additions. *Ecosystems* 10: 757–772.
- Turney, C. S. M., 1999. Lacustrine bulk organic $\delta^{13}\text{C}$ in the British Isles during the last glacial-Holocene transition (14–9 ka C-14 BP). *Arctic Antarctic and Alpine Research* 31: 71–81.
- Vander Zanden, M. J. & J. B. Rasmussen, 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80: 1395–1404.
- Van Hardenbroek, M., O. Heiri, J. Grey, P. Bodelier, F. Verbruggen & A. F. Lotter, 2010. Fossil chironomid $\delta^{13}\text{C}$ as a proxy for past methanogenic contribution to benthic food webs in lakes? *Journal of Paleolimnology* 43: 235–245.
- Van Hardenbroek, M., O. Heiri, F. J. W. Parmentier, D. Bastviken, B. P. Ilyashuk, J. A. Wiklund, R. I. Hall & A. F. Lotter, 2013a. Evidence for past variations in methane availability in a Siberian thermokarst lake based on $\delta^{13}\text{C}$ of chitinous invertebrate remains. *Quaternary Science Reviews* 66: 74–84.
- Van Hardenbroek, M., D. R. Gröcke, P. E. Sauer & S. A. Elias, 2013b. North American transect of stable hydrogen and oxygen isotopes in water beetles from a museum collection. *Journal of Paleolimnology* 48: 461–470.
- Van Hardenbroek, M., A. F. Lotter, D. Bastviken, T. J. Andersen & O. Heiri, 2014. Taxon-specific $\delta^{13}\text{C}$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden. *Journal of Paleolimnology* 52: 95–105.
- Van Riel, M. C., G. Velde, S. Rajagopal, S. Marguillier, F. Dehaers & A. B. Vaate, 2006. Trophic Relationships in the Rhine Food Web During Invasion and After Establishment of the Ponto-Caspian Invader *Dikerogammarus Villosus*. In Leuven, R. S. E. W., A. M. J. Ragas, A. J. M. Smits & G. Velde (eds), *Living Rivers: Trends and Challenges in Science and Management*, Developments in Hydrobiology. Springer, Dordrecht: 39–58.
- Vuorio, K., M. Meili & J. Sarvala, 2006. Taxon-specific variation in the stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of lake phytoplankton. *Freshwater Biology* 51: 807–822.
- Wang, Y., D. O'Brien, J. Jenson, D. Francis & M. Wooller, 2009. The influence of diet and water on the stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. *Oecologia* 160: 225–233.
- Wassenaar, L. I. & K. A. Hobson, 2000. Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. *Environmental Science & Technology* 34: 2354–2360.
- Whiticar, M. J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* 161: 291–314.
- Wood, T. S. & B. Okamura, 2005. *A New Key to Freshwater Bryozoans of Britain, Ireland and Continental Europe, With Notes on Their Ecology*. Freshwater Biological Association, London.
- Wooller, M. J., D. Francis, M. L. Fogel, G. H. Miller, I. R. Walker & A. P. Wolfe, 2004. Quantitative paleotemperature estimates from $\delta^{18}\text{O}$ of chironomid head capsules preserved in arctic lake sediments. *Journal of Paleolimnology* 31: 267–274.
- Wooller, M. J., Y. Wang & Y. Axford, 2008. A multiple stable isotope record of Late Quaternary limnological changes and chironomid paleoecology from northeastern Iceland. *Journal of Paleolimnology* 40: 63–77.
- Wooller, M., J. Pohlman, B. Gaglioti, P. Langdon, M. Jones, K. Walter Anthony, K. Becker, K.-U. Hinrichs & M. Elvert, 2012. Reconstruction of past methane availability in an Arctic Alaska wetland indicates climate influenced methane release during the past ~ 12,000 years. *Journal of Paleolimnology* 48: 27–42.