1	Taxon-specific δ^{13} C analysis of chitinous invertebrate remains in sediments from		
2	Strandsjön, Sweden		
3			
4	M. van Hardenbroek ^{1,2} , A.F. Lotter ² , D. Bastviken ³ , T.J. Andersen ⁴ , O. Heiri ¹		
5			
6	¹ Institute of Plant Sciences and Oeschger Centre for Climate Change Research,		
7	University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland.		
8	² Palaeoecology, Department of Physical Geography, Laboratory of Palaeobotany and		
9	Palynology, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands.		
10	³ Department of Thematic Studies - Water and Environmental Studies, Linköping		
11	University, 58183 Linköping, Sweden.		
12	⁴ Department of Geography and Geology, University of Copenhagen, DK-1350		
13	Copenhagen K, Denmark.		
14			
15	Abstract		
16			
17	Taxon-specific stable carbon isotope (δ^{13} C) analysis of chitinous remains of		
18	invertebrates can provide valuable information about the carbon sources used by		
19	invertebrates living in specific habitats of lake ecosystems (for example, sediments,		
20	water column, or aquatic vegetation). This is complementary to $\delta^{13}C$ of sedimentary		
21	organic matter (SOM), which provides an integrated signal of organic matter		
22	produced in a lake and its catchment, and of diagenetic processes within sediments. In		
23	a sediment record from Strandsjön (Sweden) covering the past circa 140 years, we		
24	analyzed SOM geochemistry ($\delta^{13}C$, C:N _{atomic} , organic carbon content) and $\delta^{13}C$ of		
25	chitinous invertebrate remains in order to examine whether taxon-specific $\delta^{13}C$		

26	records could be developed for different invertebrate groups and whether these
27	analyses provide insights into past changes of organic carbon sources for lacustrine
28	invertebrates available in benthic and planktonic compartments of the lake.
29	Invertebrate taxa included benthic chironomids (Chironomus, Chironomini excluding
30	Chironomus, Tanytarsini, and Tanypodinae), filter-feeders on suspended particulate
31	organic matter (<i>Daphnia</i> , <i>Plumatella</i> and <i>Cristatella mucedo</i>), and Rhabdocoela. $\delta^{13}C$
32	of chironomid remains indicated periodic availability of ¹³ C-depleted carbon sources
33	in the benthic environment of the lake as δ^{13} C values of the different chironomid taxa
34	fluctuated simultaneously between -34.7 and -30.5‰ (VPDB). Daphnia and Bryozoa
35	showed parallel changes in their δ^{13} C values which did not coincide with variations in
36	δ^{13} C of chironomids, though, and a 2-3‰ decrease since circa AD 1960. The decrease
37	in $\delta^{13}C$ of <i>Daphnia</i> and Bryozoa could indicate a decrease in phytoplankton $\delta^{13}C$ as a
38	result of lower lake productivity, which is in accordance with historical information
39	about the lake that suggests a shift to less eutrophic conditions after AD 1960. In
40	contrast, Rhabdocoela cocoons were characterized by relatively high $\delta^{13}C$ values (-
41	30.4 to -28.2‰) that did not show a strong temporal trend, which could be related to
42	the predatory feeding mode and wide prey spectrum of this organism group. The
43	taxon-specific δ^{13} C analyses of invertebrate remains indicated that different carbon
44	sources were available for the benthic chironomid larvae than for the filter-feeding
45	Daphnia and bryozoans. Our results therefore demonstrate that taxon-specific analysis
46	of δ^{13} C of organic invertebrate remains can provide complementary information to
47	measurements on bulk SOM and that δ^{13} C of invertebrate remains may allow the
48	reconstruction of past changes in carbon sources and their $\delta^{13}C$ in different habitats of
49	lake ecosystems.

Keywords: invertebrates, chitinous remains, lake sediment, stable carbon isotopes, sedimentary organic matter

Introduction

The stable carbon isotope composition of bulk organic matter in lake sediments is determined by the δ^{13} C values of different organic matter sources available in a lake and its catchment and by the preservation of the various inputs (Meyers and Teranes 2001). Environmental changes can affect the isotopic composition of organic matter, the amount of organic matter produced within lakes, the relative contribution of autochtonous and allochthonous organic matter, and the transport processes leading to the incorporation of organic matter into lake sediments. Therefore, analysis of $\delta^{13}C$ of bulk sedimentary organic matter (SOM) can potentially provide information on past changes in climate, productivity, origin of organic matter, pollution, and in-lake carbon cycling (Leavitt et al. 2006; Leng et al. 2005; Meyers and Lallier-Vergès 1999). It is difficult, however, to reconstruct limnological changes in different compartments (benthic, pelagic) of a lake based on δ^{13} C of SOM only, since variations in δ^{13} C of SOM may reflect variations in the isotopic composition of organic matter within the lake as well as in the lake catchment. Even if the approach is applied to lakes in which SOM originates mainly from lacustrine sources, variations in bulk SOM isotopic composition may reflect changes in carbon sources and organic matter production in the littoral, pelagial or profundal of the lake and selective preservation/degradation of various compounds and tissues. A possible approach to circumvent this problem is to analyze organic remains of selected organisms

originating from the lake itself. If these remains can be identified and associated with organism groups with clear habitat preferences it may be possible to determine past changes in the carbon isotopic composition of aquatic organic matter in specific habitats and compartments of lakes.

Recently, a number of studies analyzed δ^{13} C of specific organic remains that preserve well in lake sediments. These include remains of primary producers such as Potamogeton (Herzschuh et al. 2010; Turney 1999) and traces of organic matter within diatom valves (Barker et al. 2013; Hurrell et al. 2011) as well as of secondary producers such as aquatic invertebrates (for example, cladocerans (Perga 2010) and chironomids (Wooller et al. 2008)). Chitinous invertebrate remains largely consist of proteins and chitin and are chemically robust and relatively resistant to microbial degradation, especially if buried under anoxic conditions (Verbruggen et al. 2010). They can be identified and associated with a particular group of organisms. Therefore, it can be ensured that the analysed remains originate from the lake itself. Since different invertebrate groups are characterized by different feeding modes (for example, filter-feeders, deposit-feeders, or predators) and habitat preferences (planktonic or benthic, littoral or profundal), taxon-specific analyses of invertebrate δ^{13} C can potentially provide insights on processes that are active in different compartments of lakes and affect the lacustrine food web and carbon cycle. Until very recently chitinous aquatic invertebrate remains have received little attention with regard to carbon isotope analysis. Perga (2010) demonstrated that strong correlations exist between δ^{13} C values of tissue of living cladocerans of the genus Bosmina and Daphnia and their chitinous carapaces. However, she concluded

that taphonomic processes should nevertheless be taken into account when

interpreting palaeoecological records as they can cause lower δ^{13} C (<1‰) of *Daphnia*

remains, because isotopically light C compounds seem to be selectively removed by microbial degradation (Perga 2011). Wooller et al. (2008) were the first to analyze δ^{13} C of head capsules of chironomid larvae in a sediment sequence from an Icelandic lake. They reported similar patterns in stable isotope values of chironomids and SOM. Van Hardenbroek et al. (2010a) studied the effects of various chemical pre-treatments commonly used in palaeoelimnological studies on carbon isotopic measurements on chitinous remains of chironomid larvae and showed that these treatments had little effect on the δ^{13} C of the larval head capsules. These authors also demonstrated that head capsule δ^{13} C is influenced by the chironomid diet and that methanogenic carbon can influence the carbon isotopic signature of chironomid exoskeletons. Heiri et al. (2012) and Frossard et al. (2013) demonstrated that δ^{13} C values of chironomid head capsules are closely related to the carbon isotopic composition of the larval body and the food ingested by chironomid larvae.

Only very few studies are available that examine $\delta^{13}C$ of remains of multiple invertebrate groups in the same sediment samples. Van Hardenbroek et al. (2012; 2013) examined the relationship of δ^{13} C values of remains of five chironomid taxa and the resting eggs (ephippia) of water fleas of the genus Daphnia with methane fluxes in 17 lakes in Sweden and Siberia and reported a statistically significant relationship between diffusive methane flux measured at the lake surface and δ^{13} C of the fossils of some chironomid groups and of *Daphnia* ephippia. Wooller et al. (2012) and van Hardenbroek et al. (2013) presented down-core records of chironomid and Daphnia δ^{13} C from thermokarst lakes in Alaska and Siberia, respectively, which suggest major changes in the carbon cycle and methane availability in these lakes. Similar results were obtained by Frossard et al. (2014) using sediment cores from Lake Annecy (France) in which they observed decreasing δ^{13} C values in remains of

pelagic Cladocera and three chironomid taxa during 1930s -1950s. Frossard et al.

(2014) suggest that eutrophication and increased hypoxia led to increased

incorporation of ¹³C-depleted carbon (microbially respired and/or methane-derived) into the invertebrate tissues, which explains the lower δ^{13} C values in the invertebrate remains.

Down-core studies examining δ^{13} C in the remains of multiple planktonic and benthic invertebrate groups such as cladocerans, bryozoans, chironomids, and other aquatic insects allows the taxon-specific records to be compared among indicator groups in order to detect variations in δ^{13} C common to several groups. Hence, past changes in the carbon cycle of lakes tracked by different invertebrates originating from the benthic or pelagic part of lakes could be separated from background variations in δ^{13} C common to all indicators. Here we present a first study presenting δ^{13} C records based not only on the chitinous remains of Chironomidae (non-biting midges) and Cladocera (water fleas), but also on the remains of Bryozoa (moss animals), and Rhabdocoela (Turbellaria: free-living flatworms). Analyses were made on sediments collected from Strandsjön, a small shallow lake in South-central Sweden (59°52'28" N and 17°10'5" E, 51 m a.s.l.). The catchment contains pine forests and pastures, some with dairy farming since the beginning of the 20th century that led to increased nutrient loading of the lake until the installation of waste water treatment in the 1960s (Brunberg and Blomqvist 1998) and TP values were lowered to 41.3 μ g L⁻¹ in 2008 (van Hardenbroek et al. 2012). The history of eutrophication and recovery of Strandsjön was expected to have affected δ^{13} C values of algae, as these are usually ¹³C-enriched when productivity is higher (Brenner et al. 1993; Hollander et al., 1993). We therefore expected productivity-related changes in algal δ^{13} C to be reflected in δ^{13} C of algivorous filter-feeders like *Daphnia* and Bryozoa and their remains. In

151	contrast, we expected that $\delta^{13}C$ values would be not (or less clearly) related to past
152	changes in productivity for predatory Rhabdocoela or for benthic chironomids, the
153	latter feeding on a mixture of detritus from the water column and decomposed SOM.
154	Strandsjön was selected because $\delta^{13}C$ of invertebrate remains in surface sediments
155	from this lake were previously analysed by van Hardenbroek et al. (2012), providing
156	additional information for the interpretation of invertebrate $\delta^{13}C$ in this down core
157	study.
158	
159	
160	Materials and Methods
161	
162	Sediment characteristics
163	
164	The coring location was chosen at 2.4 m water depth (Fig. 1). This location represents
165	the deepest part of a transect of surface sediment samples obtained from the lake in
166	which $\delta^{13}C$ of invertebrate remains were analyzed (van Hardenbroek et al. 2012). A
167	36 cm long core with undisturbed sediment-water interface was obtained using a
168	gravity corer (Uwitec, Austria) and sub-sampled in the field at 1 cm resolution. The
169	samples were stored in plastic bags and kept cool and dark until freeze-drying upon
170	arrival in the laboratory. Freeze-dried samples were used for dating by gamma
171	spectrometry using a Canberra low-background Ge-well detector. ²¹⁰ Pb was measured
172	via its gamma-peak at 46.5 keV, ²²⁶ Ra via the granddaughter ²¹⁴ Pb (peaks at 295 and
173	352 keV), and 137 Cs via its peak at 661 keV.
174	Samples for stable isotope analysis of SOM were rinsed in 2.5% HCl for 15
175	minutes to remove carbonates, rinsed three times with demineralized water,

176 centrifuged 4 min at 2000 rpm to remove excess water, and freeze-dried. C:N_{atomic} 177 ratios, as well as stable carbon isotopes of SOM were analyzed on a PDZ Europa 178 ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 IRMS. Two 179 laboratory standards (nylon and peach leaves) of known relation to international 180 standards for VPDB were used as references. Replicate sample measurements on 181 standards (n = 43) indicated an analytical error (2σ) of ±0.05‰ for δ^{13} C.

- δ^{13} C of invertebrate remains

Samples for δ^{13} C analysis of chitinous invertebrate remains were deflocculated in 10% KOH for 2 h at room temperature and sieved with 200- and 100-µm sieves (van Hardenbroek et al. 2010b). Rinsing with an alkaline solution has the added advantage of removing adsorbed fulvic and humic acids (Stevenson 1982). Sieve residues were soaked in 2.5% HCl for 15 minutes, rinsed three times and stored in demineralized water in the dark. Remains were identified under a dissecting microscope at 40–100x magnification following Wood and Okamura (2005) for Bryozoa statoblasts, Vanderkerkhove et al. (2004) for the resting stages of *Daphnia*, Brooks et al. (2007) for chironomid head capsules, and Luther (1955) and Frey (1964) for Rhabdocoela cocoons. Chironomid head capsules were separated into the categories Chironomus spp., Chironomini (excluding Chironomus), Tanytarsini, Orthocladiinae, and Tanypodinae. Furthermore, we differentiated ephippia of the cladoceran genus Daphnia, statoblasts of the Bryozoa taxa Plumatella and Cristatella mucedo, and the chitinous cocoons of Rhabdocoela. After identification, remains were transferred with forceps directly into pre-weighed ultraclean tin cups. Tin cups were dried on a hotplate at 50° C for 24 h after

201	which they were re-weighed and crimped for stable isotope analysis. The average
202	number of remains in a sample was 18 for Chironomus (average sample weight 23
203	μ g), 36 for other Chironomini (33 μ g), 45 for Tanytarsini (24 μ g), 22 for Tanypodinae
204	(22 μ g), 34 for <i>Daphnia</i> (40 μ g), 41 for <i>Plumatella</i> (18 μ g), 1.2 for <i>C. mucedo</i> (28 μ g),
205	and 40 for Rhabocoela (47 μg) (Supplementary Table S1). Control samples of water
206	from sieve residues were evaporated in tin cups and no carbon contamination was
207	detected. Samples of invertebrate remains were analyzed on a Fisons NA 1500 NCS
208	Elemental Analyzer coupled to a Thermo Electron Delta plus IRMS. A laboratory
209	standard (Naxos GQ graphite quartzite) of known relation to international standards
210	for VPDB was used as reference. Replicate sample measurements of the secondary
211	standard (n = 30) indicated an analytical error (2 σ) of ±0.05‰.
212	
213	Results
214	
215	Age-depth model
216	
217	High ¹³⁷ Cs-activity (>2000 Bq kg ⁻¹) was measured above 4.5 cm sediment depth in
218	the Strandsjön sediments pointing to a link to the 1986 Chernobyl accident for this
219	material. This suggests that the sediment at 4.5 cm depth was deposited in 1986 or
220	shortly thereafter. ¹³⁷ Cs-profiles measured in European lake sediments typically
221	feature a second maximum at AD 1963, coinciding with the peak in above-ground
222	nuclear bomb testing (Appleby 2001), but in Strandsjön this feature seems to be
223	hidden by slight downward penetration of the high ¹³⁷ Cs-activity in the top 4.5 cm.
224	We measured surface contents of unsupported ²¹⁰ Pb of approximately 220 Bq kg ⁻¹ .
225	²¹⁰ Pb contents decreased more or less exponentially down core with the exception of

1	226	two outliers at 17-18 and
1 2 3	227	$62 \text{ Bq m}^{-2} \text{ y}^{-1}$, which is si
4 5	228	y ⁻¹) presented by Appleby
6 7 8	229	modified constant rate of
9 10	230	cm fixed at 1986 as a refe
11 12 13	231	calculated on the basis of
14 15	232	accumulated mass above
16 17 18	233	below 31 cm, resulting in
19 20	234	1870 ± 15 years. Due to the
21 22 23	235	model less reliable in the
24 25	236	approximate ages for the
26 27	237	
28 29 30	238	Sediment geochemistry a
31 32	239	
33 34 35	240	SOM δ^{13} C decreased from
36 37	241	C:N _{atomic} ratio showed a s
38 39 40	242	The carbon content of the
41 42	243	of the record up to 15 cm
43 44 45	244	increased again to 12.6 w
46 47	245	δ^{13} C values of filt
48 49 50	246	column (Daphnia, C. mu
51 52	247	the record (approximately
53 54 55	248	between approximately 5
55 56 57	249	by approximately 1-1.5%
58 59	250	values (-35.1 to -31.2‰),
60 61 62		
63		
64 65		

6	two outliers at 17-18 and 25-26 cm depth. The calculated flux of unsupported ²¹⁰ Pb is
7	$62 \text{ Bq m}^{-2} \text{ y}^{-1}$, which is similar to the supply rate of Knud Sø in Denmark (58 Bq m ⁻²
8	y ⁻¹) presented by Appleby (2001). Age-depth modeling using ²¹⁰ Pb was based on a
9	modified constant rate of supply (CRS) model (Appleby and Oldfield 1978) with 4.5
0	cm fixed at 1986 as a reference point (Fig. 2). The inventory below 25 cm was
1	calculated on the basis of a regression of the content of unsupported ²¹⁰ Pb versus
2	accumulated mass above this level. The age-model was extrapolated for samples
3	below 31 cm, resulting in an estimated age for the lowermost sample of circa AD
4	1870 ±15 years. Due to the outliers in the 210 Pb measurements, we consider the CRS
5	model less reliable in the deeper section of the core, which is why we report
6	approximate ages for the record.
7	
8	Sediment geochemistry and $\delta^{13}C$ of invertebrate remains
9	
0	SOM δ^{13} C decreased from approximately -30 to -33‰ throughout the core and the
1	C:N _{atomic} ratio showed a small, gradual decrease from approximately 11 to 9 (Fig. 3).
2	The carbon content of the sediment was 15-20 % by weight (wt.%) in the lower part

of the record up to 15 cm depth, then decreased to 10.5 wt.% at 10 cm depth, andincreased again to 12.6 wt.% in the top 7 cm.

245 δ^{13} C values of filter-feeders that retrieve their food directly from the water 246 column (*Daphnia*, *C. mucedo*, and *Plumatella*) remained relatively stable for much of 247 the record (approximately 12-35 cm depth). Their δ^{13} C then decreased by 2-3‰ 248 between approximately 5 and 10 cm depth (AD ~1960 to 1980), and increased again 249 by approximately 1-1.5‰ in the uppermost sediments. *C. mucedo* had lowest δ^{13} C 250 values (-35.1 to -31.2‰), followed by *Daphnia* (-32.8 to -30.5‰) and *Plumatella* (-

251	33.1 to -28.0‰) (Fig. 4). δ^{13} C of larval remains of chironomids showed variations at a
252	higher frequency ranging between -34.6‰ and -30.3‰ (Fig. 3). Lowest values were
253	recorded by Chironomus (-34.6 to -30.8‰), followed by Tanytarsini (-33.9 to 30.3‰),
254	Tanypodinae (-33.8 to -31.0‰) and Chironomini other than Chironomus (-33.5 to -
255	30.5‰) (Fig. 4). For most chironomid groups, relative shifts in δ^{13} C values were very
256	similar with local minima in δ^{13} C recorded at 27.5, 20.5, 12.5, and 2.5-4.5 cm
257	sediment depth (AD ~1890, ~1920, ~1950, and ~1990, respectively). The exception
258	are the remains of Chironomus, which are present in the sediments between 0-19 cm
259	and at 32 cm depth, as they show higher amplitude variations than the other
260	chironomid groups. δ^{13} C of Rhabdocoela cocoons showed less temporal variability
261	and was around -29.5‰ (-30.2 to -28.4‰) throughout the core.
262	
263	Discussion
264	
264 265	Feeding ecology of invertebrate groups
264 265 266	Feeding ecology of invertebrate groups
264 265 266 267	Feeding ecology of invertebrate groups $\label{eq:Feeding}$ For the interpretation of $\delta^{13}C$ values it is important to understand the feeding ecology
264 265 266 267 268	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic
264 265 266 267 268 269	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged
264 265 266 267 268 269 270	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit-
264 265 266 267 268 269 270 271	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit- feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008;
264 265 266 267 268 269 270 271 271	Feeding ecology of invertebrate groups For the interpretation of δ ¹³ C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit- feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008; Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini
264 265 266 267 268 269 270 271 272 272	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit- feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008; Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini include a number of taxa that tend to burrow deeper into sediments, or in some cases
264 265 266 267 268 269 270 271 272 273 273	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit- feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008; Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini include a number of taxa that tend to burrow deeper into sediments, or in some cases into aquatic macrophytes. They feed predominantly as deposit-feeders, although some
264 265 266 267 268 269 270 271 272 273 274 275	Feeding ecology of invertebrate groups For the interpretation of δ^{13} C values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit- feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008; Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini include a number of taxa that tend to burrow deeper into sediments, or in some cases into aquatic macrophytes. They feed predominantly as deposit-feeders, although some groups also filter particles from water which is pumped through the tubes the larvae

1	276	live in (Merritt et al. 2008; Moller Pillot 2009
2 3	277	also access ¹³ C-depleted biomass of methane
4 5 6	278	The Tanypodinae are mainly free-living and
7 8	279	Tanypodinae are known to also feed on the s
9 10 11	280	2008; Vallenduuk and Moller Pillot 2007). B
12 13	281	attached to hard substrates and feed on suspe
14 15 16	282	water column, but they cannot move through
17 18	283	Okamura and Hatton-Ellis 1995; Wood and O
19 20	284	zooplankton feeding on fine particles in the v
21 22 23	285	$30 \ \mu m$ in diameter and include phytoplankton
24 25	286	can be assumed, therefore, that $\delta^{13}C$ of <i>Daph</i>
26 27 28	287	they filter from the water column. Rhabdoco
29 30	288	invertebrates and large ciliate protozoans as
31 32 33	289	Kolasa and Tyler 2010). In contrast to benthi
34 35	290	Daphnia and bryozoa, rhabdocoels are proba
36 37 29	291	δ^{13} C values of phytoplankton or SOM but ha
39 40	292	mixture of their prey organisms.
41 42	293	
43 44 45	294	Differences in δ^{13} C offsets between invertebr
46 47	295	
48 49 50	296	The carbon isotopic composition of invertebr
51 52	297	variability between the different invertebrate
53 54	298	of van Hardenbroek et al. (2013), the remain
55 56 57	299	relative to other chironomids and invertebrat
58 59	300	Chironomus head capsules were only slightly
60 61		
62		
63 64		
01		

76	live in (Merritt et al. 2008; Moller Pillot 2009; Moog 2002), a habitat where they can
77	also access ¹³ C-depleted biomass of methane oxidizing bacteria (Jones et al. 2008).
78	The Tanypodinae are mainly free-living and mobile predators. However, several
79	Tanypodinae are known to also feed on the substrate the larvae live on (Merritt et al.
80	2008; Vallenduuk and Moller Pillot 2007). Bryozoa live predominantly as colonies
81	attached to hard substrates and feed on suspended nanoplanktonic algae from the
82	water column, but they cannot move through the water themselves (Kaminski 1984;
83	Okamura and Hatton-Ellis 1995; Wood and Okamura 2005). Daphnia are mobile
84	zooplankton feeding on fine particles in the water column that range between 0.5 to
85	$30 \ \mu m$ in diameter and include phytoplankton and bacteria (Geller & Müller 1981). It
86	can be assumed, therefore, that δ^{13} C of <i>Daphnia</i> and Bryozoa reflects the material
87	they filter from the water column. Rhabdocoela are active predators feeding on small
88	invertebrates and large ciliate protozoans as well as scavengers (Jennings 1957;
89	Kolasa and Tyler 2010). In contrast to benthic chironomids and filter-feeding
90	Daphnia and bryozoa, rhabdocoels are probably less directly affected by changes in
91	δ^{13} C values of phytoplankton or SOM but have a δ^{13} C signature representing a
92	mixture of their prey organisms.
93	

rate taxa

rate remains in Strandsjön showed some groups (Fig. 4). Similar to the findings as of *Chironomus* were ¹³C-depleted e groups. In Strandsjön, δ^{13} C values of y more negative than the remains of other

301	Chironomidae and of Daphnia, which in turn tended to be only slightly more negative
302	than the $\delta^{13}C$ values recorded for bulk SOM. Interestingly, $\delta^{13}C$ values recorded for
303	statoblasts of the bryozoan C. mucedo were more negative than those of chironomid
304	remains in most samples, whereas the remains of <i>Plumatella</i> and of Rhabdocoela
305	cocoons were distinctly ¹³ C-enriched relatively to SOM and other invertebrates. We
306	found no published δ^{13} C values of sedimentary remains of <i>Plumatella</i> and
307	Rhabdocoela and only one published study with few δ^{13} C measurements on C.
308	mucedo from two sediment records in the United Kingdom (Turney, 1999). In this
309	study four δ^{13} C values of <i>C. mucedo</i> statoblasts from Llanilid are presented, ranging
310	between -29 and -25‰, approximately 0-1‰ lower than δ^{13} C values of SOM. For the
311	second site, Gransmoor, two δ^{13} C values of <i>C. mucedo</i> statoblasts are between -24
312	and -22‰ and approximately 0-4 ‰ higher than $\delta^{13}C$ of SOM. The variability in $\delta^{13}C$
313	offset between C. mucedo and SOM and the lack of stable isotope data clearly
314	indicates more work is required to understand which carbon sources are used by
315	Bryozoa and Rhabdocoela before their δ^{13} C values can be interpreted conclusively.
316	We expected that $\delta^{13}C$ offsets between taxa largely reflect differences in stable
317	isotope composition of their food sources. It must be considered, however, that
318	biochemical processes during the synthesis of the body tissues may also potentially
319	influence invertebrate $\delta^{13}C$ and can play a role when comparing remains from
320	different invertebrate taxa. Generally, the δ^{13} C of chitinous invertebrate structures is
321	closely related to the carbon isotopic composition of the soft tissue of the
322	invertebrates in question and of the food available for the animals (DeNiro and
323	Epstein 1978; Rau 1980). Schimmelmann (2011) reviewed the isotopic difference for
324	eight invertebrate-diet combinations and found that the isotopic difference between
325	diet and the corresponding D-glucosamine hydrochloride from invertebrate chitin

326	ranges between -2.2 and $+2.1\%$ (average $0.0 \pm 1.5\%$). His results suggest that taxa
327	can differ in the offset between $\delta^{13}C$ of ingested carbon and $\delta^{13}C$ of their chitinous
328	structures, but that this variation is mostly limited to approximately 0-2‰.
329	Experiments with laboratory cultures of Chironomus riparius indicated a ¹³ C-
330	depletion of 4 th instar larval head capsules compared with 4 th instar larval body tissue
331	of 1-2‰ (Heiri et al. 2012) and 0.9 ± 0.2 ‰ (Frossard et al. 2013). Perga (2011)
332	demonstrated that the offset in δ^{13} C between whole <i>Daphnia</i> and their ephippia
333	collected from the same water sample was within the standard deviation (0.2%) of
334	replicate δ^{13} C measurements. Similarly, Macko et al. (1989) reported a 0.4-0.8‰ 13 C-
335	depletion of carapaces compared to body tissue of three marine crustaceans. As
336	mentioned before, little to nothing has been published about the stable isotope
337	composition of Bryozoa and Rhabdocoela or their remains. The large difference in
338	δ^{13} C values of remains of Rhabdocoela and <i>Plumatella</i> , relative to other invertebrate
339	remains examined in our record suggests that these taxa were able to access different
340	carbon sources than the other analysed invertebrate taxa. This interpretation is
341	supported by the observation that an offset is apparent even between <i>Plumatella</i> and
342	C. mucedo, both bryozoans, for which a similar offset between food, body tissue and
343	statoblasts can be expected. In our record <i>Plumatella</i> had on average 3.5‰ higher
344	δ^{13} C than <i>C. mucedo</i> and it is possible that this was the result of a difference in the
345	δ^{13} C of the particles ingested by these two taxa when filtering the water. Kaminski
346	(1984) demonstrates that C. mucedo selects small seston (<7 μ m in diameter), which
347	can include bacteria, whereas Plumatella repens prefers slightly larger particles
348	(ranging from 5 to 17 μ m in diameter).
349	
350	Trends in δ^{13} C of invertebrate remains

1		
2	352	The four chironomid taxa showed similar variations in $\delta^{13}C$ with several minor
4 5	353	minima during the past circa 140 years (Fig. 3). All analyzed chironomid group
6 7 8	354	featured the same type of high frequency signal, which makes it very unlikely the
9 10	355	these variations in δ^{13} C are related to changes in taxonomic composition that
11 12 13	356	happened simultaneously within all four groups. Since Chironomus, other
14 15	357	Chironomini, and Tanytarsini are largely detritivores and partly grazers and filte
16 17 18	358	and Tanypodinae are predators, it can be expected that the $\delta^{13}C$ of the chironom
19 20	359	remains analyzed here mostly reflected the $\delta^{13}C$ of sedimentary detritus and
21 22 23	360	detritivorous prey animals. δ^{13} C of SOM in the Strandsjön record decreased gra
23 24 25	361	between 35 and 0 cm depth. However, chironomids did not show the same decr
26 27	362	trend in δ^{13} C that we observed for SOM. This suggests that the invertebrates we
28 29 30	363	selectively feeding on and digesting certain organic matter components in the
31 32	364	sediments. Wooller et al. (2008; 2012) observed trends in δ^{13} C of chironomid re-
33 34 35	365	that were roughly similar to the patterns observed for SOM. In these studies, ho
36 37	366	Wooller et al. (2008; 2012) did not include taxonomic information about the an
38 39 40	367	chironomids. This makes a direct comparison between their records and our stu
41 42	368	difficult, since not all chironomid tribes and subfamilies can be expected to hav
43 44 45	369	same δ^{13} C values (van Hardenbroek et al. 2012), at least not in every lake. In a
46 47	370	examining the sediments of a Siberian thermokarst lake, van Hardenbroek et al.
48 49	371	(2013) found that variations in δ^{13} C of <i>Chironomus</i> , Chironomini, Tanytarsini,
50 51 52	372	Daphnia did not follow the same pattern as δ^{13} C of SOM. Only the chironomid
53 54	373	subfamily Orthocladiinae was characterized by δ^{13} C values very similar to those
55 56 57	374	SOM. This could be explained by a preference of many Orthocladiinae for a die
58 59	375	periphyton or SOM, whereas the diet of Chironomus, Chironomini, Tanytarsini
60 61		
63		
64 65		
n n		

53	minima during the past circa 140 years (Fig. 3). All analyzed chironomid groups
54	featured the same type of high frequency signal, which makes it very unlikely that
55	these variations in $\delta^{13}C$ are related to changes in taxonomic composition that
56	happened simultaneously within all four groups. Since Chironomus, other
57	Chironomini, and Tanytarsini are largely detritivores and partly grazers and filterers,
58	and Tanypodinae are predators, it can be expected that the $\delta^{13}C$ of the chironomid
59	remains analyzed here mostly reflected the $\delta^{13}C$ of sedimentary detritus and
60	detritivorous prey animals. $\delta^{13}C$ of SOM in the Strandsjön record decreased gradually
61	between 35 and 0 cm depth. However, chironomids did not show the same decreasing
62	trend in δ^{13} C that we observed for SOM. This suggests that the invertebrates were
63	selectively feeding on and digesting certain organic matter components in the
64	sediments. Wooller et al. (2008; 2012) observed trends in δ^{13} C of chironomid remains
65	that were roughly similar to the patterns observed for SOM. In these studies, however,
66	Wooller et al. (2008; 2012) did not include taxonomic information about the analyzed
67	chironomids. This makes a direct comparison between their records and our study
68	difficult, since not all chironomid tribes and subfamilies can be expected to have the
69	same δ^{13} C values (van Hardenbroek et al. 2012), at least not in every lake. In a study
70	examining the sediments of a Siberian thermokarst lake, van Hardenbroek et al.
71	(2013) found that variations in δ^{13} C of <i>Chironomus</i> , Chironomini, Tanytarsini, and
72	<i>Daphnia</i> did not follow the same pattern as δ^{13} C of SOM. Only the chironomid
73	subfamily Orthocladiinae was characterized by $\delta^{13}C$ values very similar to those of
74	SOM. This could be explained by a preference of many Orthocladiinae for a diet of
75	periphyton or SOM, whereas the diet of Chironomus, Chironomini, Tanytarsini, and

Daphnia consisted, at least in part, of other components including planktonic algae and bacteria. Van Hardenbroek et al. (2012; 2013) suggested that varying proportions of methane oxidizing bacteria (MOB) in the diet of Chironomus, Chironomini, Tanytarsini, and *Daphnia* may be at least partly responsible for the difference in δ^{13} C between the different invertebrate groups examined in their study. Furthermore, Wooller et al. (2012) argued that methane-derived carbon led to lower δ^{13} C values of chironomids and Daphnia in sections of a sediment record from arctic Alaska. Frossard et al. (2014) also suggested that similar decreases of δ^{13} C values in two Chironomini taxa, Tanypodinae, and pelagic Cladocera in a sediment record Lake Annecy (France) were linked to increased availability of ¹³C-depleted methane-derived organic carbon. Chironomid larvae feeding partly on MOB can be strongly ¹³C-depleted, as shown for several species (Jones et al. 2008; Kiyashko et al. 2001; Zemskaya et al. 2012).

For Strandsjön, relatively high δ^{13} C values for all chironomid taxa and for Daphnia suggest that MOB may have been a less relevant food source than in the lakes studied by Wooller et al. (2012), van Hardenbroek et al. (2013), and Frossard et al. (2014). However, the finding that *Chironomus* and *C. mucedo* are relatively 13 C-depleted could be explained by a higher importance of MOB in their diet than in the food ingested by the other invertebrate groups. Larvae of several Chironomus species have been shown to incorporate carbon originating from methane into their body tissue (Jones et al. 2008). The feeding ecology of C. mucedo is still poorly studied, but as a filter-feeder that can ingest particles in the µm range (Kaminski 1984), the species may be able to feed on MOB in the water column. Similarly, the lower δ^{13} C values of chironomid remains of the groups Chironomini, Tanypodinae, and Tanytarsini around AD ~1890, ~1920, ~1950, and ~1990 could be explained by

401	increasing importance of MOB in their diet. However, variations in $\delta^{13}C$ of
402	chironomid remains in Strandsjön were muted and well within the range of values
403	expected for photosynthetically produced organic matter (France 1995a,b; Meyers and
404	Teranes 2001; Bade et al. 2006). Therefore, the observed changes in chironomid $\delta^{13}C$
405	might also be caused by changes in the relative contribution of isotopically different
406	carbon sources, including allochtonous inputs, remains of aquatic macrophytes,
407	benthic algae, and periphyton that all have higher $\delta^{13}C$ values than pelagic algae
408	(France 1995a,b; Meyers and Terranes 2001). Changes in allochtonous inputs are not
409	obvious from the low and relatively stable $C:N_{atomic}$ ratio of SOM that suggests
410	mainly autochthonous carbon sources. Benthic algae are not considered as an
411	important food source, as limited light penetration inhibits their growth in Strandsjön
412	with dissolved organic carbon concentrations as high as 20.8 mg L^{-1} (van
413	Hardenbroek et al. 2012). Finally, it is unlikely that chironomid δ^{13} C responded to
414	changes in concentration or $\delta^{13}C$ of dissolved inorganic carbon in the water column,
415	as this would lead to changes in $\delta^{13}C$ of pelagic algae as well, and would be expected
416	to be registered also in the δ^{13} C of the filter-feeding <i>Daphnia</i> and bryozoans.
417	Remaining explanations for the observed variations in chironomid δ^{13} C include
418	changes in the biomass of macrophytes, periphyton, or MOB in Strandsjön which may
419	have affected the importance of these carbon sources in the diet of chironomid larvae.
420	δ^{13} C values of the filter-feeding <i>Daphnia</i> , <i>C. mucedo</i> , and <i>Plumatella</i> were
421	relatively constant in the older part of the record compared with chironomids; the
422	filter-feeders had a decreasing trend in $\delta^{13}C$ in the youngest sediments, followed by a
423	slight reversal to higher values in the uppermost samples. This pattern could be
424	related to changes in δ^{13} C of phytoplankton in the water column, since planktonic
425	algae can be expected to be an important component of the diet of Daphnia and

1	426	Bryozo
1 2 3	427	product
4 5 6	428	often hi
7 8	429	et al. 19
9 10 11	430	showed
12 13	431	values i
14 15	432	for the
17 18	433	δ^{13} C va
19 20	434	during t
21 22 23	435	An incr
24 25	436	eutroph
26 27 28	437	installa
29 30	438	water q
31 32 33	439	Daphni
34 35	440	of the la
36 37	441	be cons
38 39 40	442	the wate
41 42	443	product
43 44 45	444	
46 47	445	Conclu
48 49 50	446	
51 52	447	Our res
53 54	448	of chitin
55 56 57	449	revealed
58 59	450	to past o
60 61 62		
63		
64 65		

426	Bryozoa. The observed variations would be easiest to explain by changes in lake
427	productivity, since δ^{13} C of phytoplankton in productive meso- to eutrophic lakes is
428	often higher than δ^{13} C of phytoplankton growing in oligotrophic conditions (Brenner
429	et al. 1999; Hollander et al. 1993). In Strandsjön, δ^{13} C of <i>Daphnia</i> and <i>C. mucedo</i>
430	showed a maximum at ~AD 1960. δ^{13} C of <i>Plumatella</i> also showed relatively high
431	values in this section of the record, although the maximum was not as pronounced as
432	for the other two taxa and <i>Plumatella</i> was already characterized by relatively high
433	δ^{13} C values in the period preceding ~AD 1960. A peak in lake nutrient concentration
434	during this period agrees with what is known of the trophic state history of the lake:
435	An increase in nutrient input as agriculture developed around the lake led to
436	eutrophication in the 20 th century, causing increasingly frequent algal blooms until the
437	installation of a water treatment plant for the inflow in AD 1960, after which the
438	water quality improved (Brunberg and Blomqvist 1998). In contrast to $\delta^{13}C$ of
439	Daphnia and bryozoans, δ^{13} C of SOM did not seem to track the eutrophication history
440	of the lake. This suggested that carbon isotopic analyses of invertebrate remains might
441	be considerably more sensitive to past changes in $\delta^{13}C$ of organic matter produced in
442	the water column than bulk SOM δ^{13} C, at least in relatively small, shallow, and
443	productive lakes such as Strandsjön.
444	
445	Conclusions

sults demonstrate that taxon-specific δ^{13} C records can be developed for a range nous invertebrate remains found in lake sediments. In Strandsjön, our approach d a differential response of δ^{13} C of benthic taxa (chironomids and rhabdocoels) environmental change compared with δ^{13} C of taxa feeding predominantly on

1	451	suspended particulate organic matter (Daphnia and bryozoans). Hence, our study									
2 3 4 5 6 7 8 9 10 11 12 13 14 15	452	indicates that $\delta^{13}C$ of chitinous invertebrate remains can provide valuable additional									
	453	information to δ^{13} C of SOM, as the latter cannot distinguish between carbon sources									
	454	in different compartments of a lake. Additional studies relating the stable isotope									
	455	composition of various aquatic invertebrates to the values of their fossilizing									
	456	structures, as well as studies establishing the link between $\delta^{13}C$ of the diet and $\delta^{13}C$ of									
	457	freshwater invertebrate tissues are urgently needed to improve the interpretation of									
16 17 18	458	invertebrate $\delta^{13}C$ in sediment records. More studies examining $\delta^{13}C$ of different									
19 20	459	organic invertebrate remains from the same sediment record are necessary to confirm									
21 22 23	460	that δ^{13} C of phytoplankton can be reliable traced by examining the carbon isotopic									
24 25	461	composition of the remains of filter-feeding Daphnia and Bryozoa, and that such									
26 27 28 29 30 31 32	462	analyses can reveal a different signal than the one captured by SOM or by the remains									
	463	of zoobenthos. The results from Strandsjön demonstrate the potential of taxon-									
	464	specific stable isotope analysis of invertebrate remains for environmental									
33 34 35	465	reconstructions and provide, to our knowledge, the taxonomically most diverse such									
36 37	466	study available to date. It is likely that similar taxon-specific records will soon be									
38 39 40	467	produced for stable isotopes of other elements (H, O, N, S) that can give further									
41 42	468	insights in the functioning of aquatic ecosystems over long time scales.									
43 44 45	469										
46 47	470	Acknowledgements									
48 49	471										
50 51 52	472	We thank Lotta Frisk Hagström and Kristina Eriksson for valuable historical									
53 54	473	information about the lake and Arndt Schimmelmann and three anonymous reviewers									
55 56 57	474	for their helpful suggestions to improve this manuscript. This research was supported									
58 59 60 61 62	475	by the Darwin Centre for Biogeosciences, the European Research Council (ERC)									
63 64 65											

1	476	Starting Grant project RECONMET (Project nr. 239858), and the Swedish Research
1 2 3	477	Council (Project no. VR 2006-3256).
4 5	478	
6 7 8	479	References
9	480	
L⊥ 2	481	Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: WM I ast
L 3	482	IP Smol (eds.) Tracking environmental change using lake sediments. Volume 1:
L4	483	Basin analysis coring and chronological techniques. Kluwer Academic Publishers
15	484	Dordrecht nn 171-203
L6 17	101	Dordroent, pp. 171 200
L8 L9	485 486	Appleby PG, Oldfield F (1978) The calculation of lead-210 dates assuming a constant rate of supply of unsupported ²¹⁰ Pb to the sediment. Catena 5: 1-8
20	100	
21	487	Bade D, Pace M, Cole J, Carpenter S (2006) Can algal photosynthetic inorganic
22	488	carbon isotope fractionation be predicted in lakes using existing models? Aquat Sci
24	489	68: 142-153
25		
26	490	Barker PA, Hurrell ER, Leng MJ, Verschuren D, Conley D, Plessens B (2013) The
27	491	changing carbon cycle of lakes revealed by the stable isotopes of diatom silica: a 25
28 29 20	492	ka record from Lake Challa, Kilimanjaro. Quaternary Sci Rev 66: 55-63
31	493	Brenner M, Whitmore TJ, Curtis JH, Hodell DA, Schelske CL (1999) Stable isotope
32	494	$(\delta^{13}C \text{ and } \delta^{15}N)$ signatures of sedimented organic matter as indicators of historic lake
33 34	495	trophic state. J Paleolimnol 22: 205-221
35	496	Brooks SJ, Langdon PG, Heiri O (2007) The identification and use of Palaearctic
30 37	497	Chironomidae larvae in palaeoecolgy, ORA Technical Guide No. 10. Ouaternary
38 39	498	Research Association, London
10	499	Brunberg A-K, Blomqvist P (1998) Vatten i Uppsala län 1997. Beskrivning,
11 12 12	500	utvärdering, åtgärdsförslag. Rapport nr 8/1998. Upplandsstiftelsen. [in Swedish]
±3 14	501	DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes
15	502	in animals. Geochim Cosmochim Acta 42: 495-506
16		
17	503	France RL (1995a) Carbon-13 enrichment in benthic compared to planktonic algae:
18 19 50	504	foodweb implications. Mar Ecol Prog Ser 124: 307-312
51	505	France RL (1995b) Stable isotopic survey of the role of macrophytes in the carbon
52 53	506	flow of aquatic foodwebs. Vegetatio 124: 67-72
54	507	Frey DG (1964) Remains of animals in Ouaternary lake and bog sediments and their
55 56	508	interpretation. Advances in Limnology 2: 1-114
58	509	Frossard V. Belle S. Verneaux Vr. Millet L. Magny M (2013) A study of the δ^{13} C
59	510	offset between chironomid larvae and their exuvial head capsules: implications for
50 51 52	511	palaeoecology. J Paleolimnol 50: 379-386
53		

	512	
1	512	
⊥ 2	513	Frossard V, Verneaux V, Millet L, Jenny J-P, Arnaud F, Magny M, Perga M-E (2014)
2	514	Reconstructing long-term changes (150 years) in the carbon cycle of a clear-water
4	515	lake based on the stable carbon isotope composition (δ^{13} C) of chironomid and
- 5	516	cladoceran subfossil remains. Freshwat Biol doi: 10.1111/fwb.12304
6	517	
3 7	510	Collar W. Müller H (1081) The filtration enversus of Cladecare: Filter mash sizes
8	510	Gener w, Muner H (1981) The initiation apparatus of Cladocera. Filter mesh-sizes
9	519	and their implications on food selectivity. Oecologia 49: 316-321
10		
11	520	Heiri O, Schilder J, van Hardenbroek M (2012) Stable isotopic analysis of fossil
12	521	chironomids as an approach to environmental reconstruction: State of development
13	522	and future challenges. Proceedings of the 18th International Symposium on
14	523	Chironomidae, Trondheim 4-6, August 2011, Fauna Norvegica 31: 7-18
15	010	
16	524	Herzschuh II Mischke S. Meyer H. Plessen B. Zhang C (2010) Lake nutrient
17	521	variability informed from alamental (C N S) and isotonia (\$12C \$15N) analyzes of
18	525	variability interfed from elemental (C, N, S) and isotopic (015C, 015N) analyses of
19	526	aquatic plant macrofossils. Quaternary Sci Rev 29: 2161-2172
∠U 21		
∠⊥ 22	527	Hollander DJ, McKenzie JA, Hsu KJ, Huc AY (1993) Application of an eutrophic
22	528	lake model to the origin of ancient organic-carbon-rich sediments. Global
2.4	529	Biogeochem Cycles 7: 157-179
25		
26	530	Hurrell FR Barker PA Leng MI Vane CH Wynn P Kendrick CP Verschuren D
27	521	Street Perrott EA (2011) Developing a methodology for earbon jectore analysis of
28	221	Succe-Ferrou FA (2011) Developing a methodology for carbon isotope analysis of
29	532	lacustrine diatoms. Rapid Commun Mass Sp 25: 1567-1574
30		
31	533	Jennings JB (1957) Studies on feeding, digestion, and food storage in free-living
32	534	flatworms (Platyhelminthes: Turbellaria). Biological Bulletin 112: 63-80
33		
34	535	Jones RI, Carter CE, Kelly A, Ward S, Kelly DJ, Grey J (2008) Widespread
35	536	contribution of methane-cycle bacteria to the diets of lake profundal chironomid
36	537	larvae Ecology 89: 857-864
3/	557	
38	520	Kaminski M (1084) Food composition of three bruczoan species (Pryozoa
39 40	530	Rammski W (1964) Food composition of unce of yozoan species (Diyozoa,
41	539	Phylactolaemata) in a mesotrophic lake. Polish Archive of Hydrobiology 31: 43-53
42		
43	540	Kiyashko SI, Narita T, Wada E (2001) Contribution of methanotrophs to freshwater
44	541	macroinvertebrates: evidence from stable isotope ratios. Aquat Microb Ecol 24: 203-
45	542	207
46		
47	543	Kolasa J. Tyler S (2010) Flatworms: Turbellaria and Nemertea. In: JH Thorp. AP
48	544	Covich (eds.) Ecology and classification of North American freshwater invertebrates
49		Academia Drass. London, np. 1/2 161
50	545	Academic Fless, London, pp. 145-101
51		
52	546	Leavitt PR, Brock CS, Ebel C, Patoine A (2006) Landscape-scale effects of urban
53	547	nitrogen on a chain of freshwater lakes in central North America. Limnol Oceanogr
54	548	51: 2262-2277
55		
57	549	Legendre P, Legendre L (1998) Numerical ecology. Developments in environmental
58	550	modelling 20. Elsevier Science, Amsterdam. 853 pp
59	551	,, ,, rr
60	001	
61		
62		
63		
64		

- Leng MJ, Lamb AL, Marshall JD, Wolfe BB, Jones MD, Holmes JA, Arrowsmith C (2005) Isotopes in lake sediments. In: MJ Leng (ed.), Isotopes in palaeoenvironmental research. Springer, Dordrecht, pp. 147-184 Luther A (1955) Die Dalyelliiden (Turbellaria Neorhabdocoela), eine monografie. von Tilgmann, Helsingfors, 337 pp б Macko SA, Helleur R, Hartley G, Jackman P (1990) Diagenesis of organic matter - a study using stable isotopes of individual carbohydrates. Org Geochem 16: 1129-1137 Merritt RW, Cummins KW, Berg MB (2008) An introduction to the Aquatic Insects of North America. Kendall and Hunt, Dubuque, 1158 pp Meyers PA, Lallier-Vergès E (1999) Lacustrine sedimentary organic matter records of Late Quaternary paleoclimates. J Paleolimnol 21: 345-372 Meyers PA, Teranes JL (2001) Sediment Organic Matter. In: WM Last, JP Smol (eds.), Tracking environmental change using lake sediments. Volume 2: Physical and Geochemical Techniques. Kluwer Academic Publishers, Dordrecht, pp. 239-269 Mihuc T, Toetz D (1994) Determination of Diets of Alpine Aquatic Insects Using Stable Isotopes and Gut Analysis. The American Midland Naturalist 131: 146-155 Moller Pillot HKM (2009) Chironomidae Larvae, Biology and ecology of the Chironomini. KNNV Publishing, Zeist, 272 pp Moog O (2002) Fauna Aquatica Austriaca. Wasserwirtshcaftskataster, Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Vienna Okamura B, Hatton-Ellis T (1995) Population biology of bryozoans: correlates of sessile, colonial life histories in freshwater habitats. Cell Mol Life Sci 51: 510-525 Perga M-E (2010) Potential of δ^{13} C and δ^{15} N of cladoceran subfossil exoskeletons for paleo-ecological studies. J Paleolimnol 44: 387-395 Perga M-E (2011) Taphonomic and early diagenetic effects on the C and N stable isotope composition of cladoceran remains: implications for paleoecological studies. J Paleolimnol 46: 203-213 Rau GH (1980) Carbon-13/Carbon-12 Variation in Subalpine Lake Aquatic Insects: Food Source Implications. Can J Fish Aquat Sci 37: 742-746 Schimmelmann A (2011) Carbon, Nitrogen and Oxygen Stable Isotope Ratios in Chitin. In: NS Gupta (ed.), Chitin. Springer Netherlands, pp. 81-103 Stevenson FJ (1982) Extraction, fractionation, and general chemical composition of soil organic matter. In: FJ Stevenson (ed.), Humus Chemistry. Genesis, Composition, Reactions, John Wiley and Sons, New York, pp. 26-54 Vallenduuk H, Moller Pillot HKM (2007) Chironomidae larvae, General introduction and Tanypodinae. KNNV Publishing, Zeist, 172 pp

- Vanderkerkhove J, Declerck S, Vanhove M, Brendonck L, Jeppesen E, Porcuna JM, De Meester L (2004) Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls. J Limnol 63: 75-84 Vander Zanden MJ, Rasmussen JB (1999) Primary consumer δ^{13} C and δ^{15} N and the trophic position of aquatic consumers. Ecology 80: 1395-1404 van Hardenbroek M, Heiri O, Grey J, Bodelier P, Verbruggen F, Lotter A (2010a) Fossil chironomid δ^{13} C as a proxy for past methanogenic contribution to benthic food webs in lakes? J Paleolimnol 43: 235-245 van Hardenbroek M, Heiri O, Lotter A (2010b) Efficiency of different mesh sizes for isolating fossil chironomids for stable isotope and radiocarbon analyses. J Paleolimnol 44: 721-729 van Hardenbroek M, Lotter AF, Bastviken D, Duc NT, Heiri O (2012) Relationship between δ^{13} C of chironomid remains and methane flux in Swedish lakes. Freshwat Biol 57: 166–177 van Hardenbroek M, Heiri O, Parmentier FJW, Bastviken D, Ilyashuk BP, Wiklund JA, Hall RI, Lotter AF (2013) Evidence for past variations in methane availability in a Siberian thermokarst lake based on δ^{13} C of chitinous invertebrate remains. Quaternary Sci Rev 66: 74-84 Verbruggen F, Heiri O, Reichart GJ, De Leeuw J, Nierop K, Lotter A (2010) Effects of chemical pretreatments on δ^{18} O measurements, chemical composition, and morphology of chironomid head capsules. J Paleolimnol 43: 857-872 Wood TS, Okamura B (2005) A new key to freshwater bryozoans of Britain, Ireland and continental Europe, with notes on their ecology. Freshwater Biological Association, London, 113 pp Wooller M, Pohlman J, Gaglioti B, Langdon P, Jones M, Walter Anthony K, Becker K, Hinrichs K-U, Elvert M (2012) Reconstruction of past methane availability in an Arctic Alaska wetland indicates climate influenced methane release during the past ~12,000 years. J Paleolimnol 48: 27-42 Wooller MJ, Wang Y, Axford Y (2008) A multiple stable isotope record of Late Quaternary limnological changes and chironomid paleoecology from northeastern Iceland. J Paleolimnol 40: 63-77 Zemskaya T, Sitnikova T, Kiyashko S, Kalmychkov G, Pogodaeva T, Mekhanikova I, Naumova T, Shubenkova O, Chernitsina S, Kotsar O, Chernyaev E, Khlystov O (2012) Faunal communities at sites of gas- and oil-bearing fluids in Lake Baikal. Geo-Mar Lett 32: 437-451

626 Figure captions

Fig. 1 Site location and bathymetric map of Strandsjön (redrawn after Brunberg and
Blomqvist 1998). Coring site is indicated by X, inlets and outlets are indicated by
arrows.

Fig. 2 Activity of ²¹⁰Pb (left) and ¹³⁷Cs (middle) and the age model (right) for the
sediment record from Strandsjön based on a constant rate of supply model for ²¹⁰Pb
with the 4.5 cm peak in ¹³⁷Cs activity as reference point for the 1986 Chernobyl
accident.

Fig. 3 δ^{13} C values of invertebrate remains and geochemistry of sedimentary organic 638 matter (δ^{13} C, organic carbon content, and C:N_{atomic} ratio) in the sediment record from 639 Strandsjön. Invertebrate taxa are indicated by open triangles (*Chironomus*), open 640 circles (Chironomini), open grey squares (Tanytarsini), open diamonds (Tanypodinae),

641 solid circles (*Daphnia*), solid triangles (*Cristatella mucedo*), solid diamonds

642 (*Plumatella*), and solid grey squares (Rhabdocoela).

Fig. 4 Boxplots with δ^{13} C values of the different invertebrate taxa in this study and of sedimentary organic matter (SOM).

Figure 1 Click here to download Figure: Fig 1 Location.eps



Figure 2 Click here to download Figure: Fig 2 Age depth v2.eps



Figure 4 Click here to download Figure: Fig 4 boxplot v2.eps





Supplementary data for van Hardenbroek et al "Taxon-specific 6¹³C analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden"

Supplementary table 1: δ^{13} C, number of remains, and weight (in microgram) for each invertebrate sample. δ^{13} C, δ^{15} N, $\delta^{C_{weighty}}$ And C:N_{atome} ratio for sedimentary organic matter (SOM)

	Chironomus		Chironomini		Tanytar	Tanytarsini		Tanypoo	Tanypodinae		Daphnic	Daphnia			Cristatella mucedo			Plumatella			Rhabdocoela								
Depth (cm)	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	δ ¹³ C	no.	mass	δ ¹³ C	no.	mass	δ ¹³ C	no.	mass	δ ¹³ C	no.	mass	δ ¹³ C	no.	mass	$\delta^{13}C$	no.	mass	δ ¹³ C	$\delta^{^{15}}N$	%C _{weight} %	6N _{weight} (C:N _{atomic}
0-2				-30.53	21	18	-30.3	39	17	-31.9	15	10	-31.45	13	15	-33.7	1.25	29	-31.27	30	17	-29.1	40	36	-32.8	3.4	12.6	1.4	10.6
2-3	-31.7	19	18	-32.2	41	29	-31.2	41	20	-31.9	19	9	-32.8	31	28				-32.1	40	21	-29.2	40	37	-32.8	3.4	12.3	1.3	10.7
4-5	-33.2	27	24	-32.1	41	23	-31.9	47	15	-32.4	23	15	-32.3	32	29	-34.2	1	22	-33.1	40	29	-29.2	40	33	-32.5	3.4	12.2	1.3	10.7
6-7	-33.0	31	36	-31.4	33	22	-31.8	45	22	-31.8	22	15	-31.7	36	42	-35.1	1.25	43	-30.7	40	24	-30.0	40	40	-31.4	3.5	11.1	1.2	11.0
8-9	-32.6	31	41	-31.4	38	40	-31.5	45	28				-31.4	41	36				-30.8	45	21	-29.2	40	47	-31.2	3.5	10.5	1.1	11.1
10-11	-31.7	12	26	-31.6	31	29	-32.2	45	28	-32.2	31	27	-30.5	40	37	-31.3	1.5	59	-29.1	40	23	-29.2	40	48	-31.0	3.3	10.9	1.1	11.5
12-13	-32.6	8	11	-33.4	44	39	-33.9	47	32	-33.8	19	22	-30.5	41	34	-32.6	1.25	21	-29.2	40	24	-30.2	40	56	-31.5	2.8	13.6	1.3	12.0
14-15	-34.6	12	19	-32.6	16	12	-33.5	47	23	-32.8	20	17	-30.9	40	29	-31.4	1.5	34	-28.2	40	18	-29.7	40	48	-31.4	2.6	15.3	1.5	12.2
16-17				-32.0	38	38	-32.5	35	16				-31.3	30	44				-29.6	40	16	-29.6	40	47					
18-19	-34.2	15	24	-33.2	38	36	-32.7	50	21	-32.9	25	21	-31.2	41	54				-28.9	45	18	-29.7	40	53	-30.8	2.4	15.9	1.5	12.4
20-21				-33.5	39	42	-32.1	45	25	-33.4	33	27	-31.6	31	49	-33.5	2	34	-28.9	35	15	-30.0	40	51	-30.6	2.7	15.0	1.4	13.4
22-23				-32.4	35	42	-32.5	46	27	-31.7	14	15	-32.1	32	48	-32.6	1.25	22	-29.5	40	15	-29.7	40	61	-30.6	2.7	15.8	1.5	12.0
24-25				-31.9	39	42	-32.3	45	23	-31.2	15	20	-31.9	29	40				-29.1	40	13	-29.5	40	53	-30.4	2.6	16.3	1.5	12.0
26-27				-31.8	40	49	-33.5	45	29	-32.0	19	27	-32.1	30	39	-33.1	1	20	-28.2	42	15	-29.3	40	53	-30.5	2.6	16.6	1.6	11.9
28-29				-32.7	38	30	-33.8	45	24	-32.6	26	33	-32.0	31	48				-29.4	40	17	-29.1	40	46	-30.5	2.4	18.9	1.9	10.4
30-32				-31.2	38	28	-32.3	45	21	-32.0	24	30	-32.0	31	42	-31.6	2	17	-28.6	40	13	-29.3	40	53	-30.1	2.4	20.0	1.9	11.7
32-33	-30.8	8	9	-31.1	31	32	-32.0	45	25	-31.0	18	26	-32.2	30	39	-31.2	0.5	10	-28.1	40	14	-29.0	40	44	-30.0	2.3	20.1	1.8	12.7
34-35				-31.3	37	23	-32.3	45	24	-31.5	25	32	-32.0	30	34	-34.1	1	21	-28.0	42	16	-28.4	40	38	-30.3	2.3	19.1	1.8	12.9

Below recommended weight of 20 microgram