

1 **Taxon-specific $\delta^{13}\text{C}$ analysis of chitinous invertebrate remains in sediments from**
2 **Strandsjön, Sweden**

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7 4 M. van Hardenbroek^{1,2}, A.F. Lotter², D. Bastviken³, T.J. Andersen⁴, O. Heiri¹
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12 6 ¹ Institute of Plant Sciences and Oeschger Centre for Climate Change Research,
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14 7 University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland.
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17 8 ² Palaeoecology, Department of Physical Geography, Laboratory of Palaeobotany and
18

19 9 Palynology, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands.
20

21 10 ³ Department of Thematic Studies - Water and Environmental Studies, Linköping
22

23 11 University, 58183 Linköping, Sweden.
24
25

26 12 ⁴ Department of Geography and Geology, University of Copenhagen, DK-1350
27

28 13 Copenhagen K, Denmark.
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34 15 **Abstract**
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39 17 Taxon-specific stable carbon isotope ($\delta^{13}\text{C}$) analysis of chitinous remains of
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41 18 invertebrates can provide valuable information about the carbon sources used by
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43 19 invertebrates living in specific habitats of lake ecosystems (for example, sediments,
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45 20 water column, or aquatic vegetation). This is complementary to $\delta^{13}\text{C}$ of sedimentary
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47 21 organic matter (SOM), which provides an integrated signal of organic matter
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49 22 produced in a lake and its catchment, and of diagenetic processes within sediments. In
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52 23 a sediment record from Strandsjön (Sweden) covering the past circa 140 years, we
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54 24 analyzed SOM geochemistry ($\delta^{13}\text{C}$, $\text{C:N}_{\text{atomic}}$, organic carbon content) and $\delta^{13}\text{C}$ of
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57 25 chitinous invertebrate remains in order to examine whether taxon-specific $\delta^{13}\text{C}$
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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 (*Daphnia*, *Plumatella* and *Cristatella mucedo*), and Rhabdocoela. $\delta^{13}\text{C}$
32 of chironomid remains indicated periodic availability of ^{13}C -depleted carbon sources
33 in the benthic environment of the lake as $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values which did not coincide with variations in
36 $\delta^{13}\text{C}$ of chironomids, though, and a 2-3‰ decrease since circa AD 1960. The decrease
37 in $\delta^{13}\text{C}$ of *Daphnia* and Bryozoa could indicate a decrease in phytoplankton $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of organic invertebrate remains can provide complementary information to
47 measurements on bulk SOM and that $\delta^{13}\text{C}$ of invertebrate remains may allow the
48 reconstruction of past changes in carbon sources and their $\delta^{13}\text{C}$ in different habitats of
49 lake ecosystems.

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

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55 **Introduction**

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57 The stable carbon isotope composition of bulk organic matter in lake sediments is
58 determined by the $\delta^{13}\text{C}$ values of different organic matter sources available in a lake
59 and its catchment and by the preservation of the various inputs (Meyers and Teranes
60 2001). Environmental changes can affect the isotopic composition of organic matter,
61 the amount of organic matter produced within lakes, the relative contribution of
62 autochthonous and allochthonous organic matter, and the transport processes leading to
63 the incorporation of organic matter into lake sediments. Therefore, analysis of $\delta^{13}\text{C}$ of
64 bulk sedimentary organic matter (SOM) can potentially provide information on past
65 changes in climate, productivity, origin of organic matter, pollution, and in-lake
66 carbon cycling (Leavitt et al. 2006; Leng et al. 2005; Meyers and Lallier-Vergès
67 1999). It is difficult, however, to reconstruct limnological changes in different
68 compartments (benthic, pelagic) of a lake based on $\delta^{13}\text{C}$ of SOM only, since
69 variations in $\delta^{13}\text{C}$ of SOM may reflect variations in the isotopic composition of
70 organic matter within the lake as well as in the lake catchment. Even if the approach
71 is applied to lakes in which SOM originates mainly from lacustrine sources, variations
72 in bulk SOM isotopic composition may reflect changes in carbon sources and organic
73 matter production in the littoral, pelagial or profundal of the lake and selective
74 preservation/degradation of various compounds and tissues. A possible approach to
75 circumvent this problem is to analyze organic remains of selected organisms

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

80 Recently, a number of studies analyzed $\delta^{13}\text{C}$ of specific organic remains that
81 preserve well in lake sediments. These include remains of primary producers such as
82 *Potamogeton* (Herzschuh et al. 2010; Turney 1999) and traces of organic matter
83 within diatom valves (Barker et al. 2013; Hurrell et al. 2011) as well as of secondary
84 producers such as aquatic invertebrates (for example, cladocerans (Perga 2010) and
85 chironomids (Wooller et al. 2008)). Chitinous invertebrate remains largely consist of
86 proteins and chitin and are chemically robust and relatively resistant to microbial
87 degradation, especially if buried under anoxic conditions (Verbruggen et al. 2010).
88 They can be identified and associated with a particular group of organisms. Therefore,
89 it can be ensured that the analysed remains originate from the lake itself. Since
90 different invertebrate groups are characterized by different feeding modes (for
91 example, filter-feeders, deposit-feeders, or predators) and habitat preferences
92 (planktonic or benthic, littoral or profundal), taxon-specific analyses of invertebrate
93 $\delta^{13}\text{C}$ can potentially provide insights on processes that are active in different
94 compartments of lakes and affect the lacustrine food web and carbon cycle.

95 Until very recently chitinous aquatic invertebrate remains have received little
96 attention with regard to carbon isotope analysis. Perga (2010) demonstrated that
97 strong correlations exist between $\delta^{13}\text{C}$ values of tissue of living cladocerans of the
98 genus *Bosmina* and *Daphnia* and their chitinous carapaces. However, she concluded
99 that taphonomic processes should nevertheless be taken into account when
100 interpreting palaeoecological records as they can cause lower $\delta^{13}\text{C}$ (<1‰) of *Daphnia*

101 remains, because isotopically light C compounds seem to be selectively removed by
102 microbial degradation (Perga 2011). Wooller et al. (2008) were the first to analyze
103 $\delta^{13}\text{C}$ of head capsules of chironomid larvae in a sediment sequence from an Icelandic
104 lake. They reported similar patterns in stable isotope values of chironomids and SOM.
105 Van Hardenbroek et al. (2010a) studied the effects of various chemical pre-treatments
106 commonly used in palaeolimnological studies on carbon isotopic measurements on
107 chitinous remains of chironomid larvae and showed that these treatments had little
108 effect on the $\delta^{13}\text{C}$ of the larval head capsules. These authors also demonstrated that
109 head capsule $\delta^{13}\text{C}$ is influenced by the chironomid diet and that methanogenic carbon
110 can influence the carbon isotopic signature of chironomid exoskeletons. Heiri et al.
111 (2012) and Frossard et al. (2013) demonstrated that $\delta^{13}\text{C}$ values of chironomid head
112 capsules are closely related to the carbon isotopic composition of the larval body and
113 the food ingested by chironomid larvae.

114 Only very few studies are available that examine $\delta^{13}\text{C}$ of remains of multiple
115 invertebrate groups in the same sediment samples. Van Hardenbroek et al. (2012;
116 2013) examined the relationship of $\delta^{13}\text{C}$ values of remains of five chironomid taxa
117 and the resting eggs (ephippia) of water fleas of the genus *Daphnia* with methane
118 fluxes in 17 lakes in Sweden and Siberia and reported a statistically significant
119 relationship between diffusive methane flux measured at the lake surface and $\delta^{13}\text{C}$ of
120 the fossils of some chironomid groups and of *Daphnia* ephippia. Wooller et al. (2012)
121 and van Hardenbroek et al. (2013) presented down-core records of chironomid and
122 *Daphnia* $\delta^{13}\text{C}$ from thermokarst lakes in Alaska and Siberia, respectively, which
123 suggest major changes in the carbon cycle and methane availability in these lakes.
124 Similar results were obtained by Frossard et al. (2014) using sediment cores from
125 Lake Annecy (France) in which they observed decreasing $\delta^{13}\text{C}$ values in remains of

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126 pelagic Cladocera and three chironomid taxa during 1930s -1950s. Frossard et al.
127 (2014) suggest that eutrophication and increased hypoxia led to increased
128 incorporation of ^{13}C -depleted carbon (microbially respired and/or methane-derived)
129 into the invertebrate tissues, which explains the lower $\delta^{13}\text{C}$ values in the invertebrate
130 remains.

131 Down-core studies examining $\delta^{13}\text{C}$ in the remains of multiple planktonic and
132 benthic invertebrate groups such as cladocerans, bryozoans, chironomids, and other
133 aquatic insects allows the taxon-specific records to be compared among indicator
134 groups in order to detect variations in $\delta^{13}\text{C}$ common to several groups. Hence, past
135 changes in the carbon cycle of lakes tracked by different invertebrates originating
136 from the benthic or pelagic part of lakes could be separated from background
137 variations in $\delta^{13}\text{C}$ common to all indicators. Here we present a first study presenting
138 $\delta^{13}\text{C}$ records based not only on the chitinous remains of Chironomidae (non-biting
139 midges) and Cladocera (water fleas), but also on the remains of Bryozoa (moss
140 animals), and Rhabdocoela (Turbellaria: free-living flatworms). Analyses were made
141 on sediments collected from Strandsjön, a small shallow lake in South-central Sweden
142 ($59^{\circ}52'28''$ N and $17^{\circ}10'5''$ E, 51 m a.s.l.). The catchment contains pine forests and
143 pastures, some with dairy farming since the beginning of the 20th century that led to
144 increased nutrient loading of the lake until the installation of waste water treatment in
145 the 1960s (Brunberg and Blomqvist 1998) and TP values were lowered to $41.3 \mu\text{g L}^{-1}$
146 in 2008 (van Hardenbroek et al. 2012). The history of eutrophication and recovery of
147 Strandsjön was expected to have affected $\delta^{13}\text{C}$ values of algae, as these are usually
148 ^{13}C -enriched when productivity is higher (Brenner et al. 1993; Hollander et al., 1993).
149 We therefore expected productivity-related changes in algal $\delta^{13}\text{C}$ to be reflected in
150 $\delta^{13}\text{C}$ of algivorous filter-feeders like *Daphnia* and Bryozoa and their remains. In

151 contrast, we expected that $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ in this down core
157 study.

160 **Materials and Methods**

162 Sediment characteristics

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 $\delta^{13}\text{C}$ 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 δ¹³C.
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183 δ¹³C of invertebrate remains
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185 Samples for δ¹³C analysis of chitinous invertebrate remains were deflocculated in
186 10% KOH for 2 h at room temperature and sieved with 200- and 100-μm sieves (van
187 Hardenbroek et al. 2010b). Rinsing with an alkaline solution has the added advantage
188 of removing adsorbed fulvic and humic acids (Stevenson 1982). Sieve residues were
189 soaked in 2.5% HCl for 15 minutes, rinsed three times and stored in demineralized
190 water in the dark. Remains were identified under a dissecting microscope at 40–100x
191 magnification following Wood and Okamura (2005) for Bryozoa statoblasts,
192 Vanderkerkhove et al. (2004) for the resting stages of *Daphnia*, Brooks et al. (2007)
193 for chironomid head capsules, and Luther (1955) and Frey (1964) for Rhabdocoela
194 cocoons. Chironomid head capsules were separated into the categories *Chironomus*
195 spp., Chironomini (excluding *Chironomus*), Tanytarsini, Orthoclaadiinae, and
196 Tanypodinae. Furthermore, we differentiated ephippia of the cladoceran genus
197 *Daphnia*, statoblasts of the Bryozoa taxa *Plumatella* and *Cristatella mucedo*, and the
198 chitinous cocoons of Rhabdocoela.

199 After identification, remains were transferred with forceps directly into pre-
200 weighed ultraclean tin cups. Tin cups were dried on a hotplate at 50°C for 24 h after

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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 μg), 36 for other Chironomini (33 μg), 45 for Tanytarsini (24 μg), 22 for Tanypodinae
204 (22 μg), 34 for *Daphnia* (40 μg), 41 for *Plumatella* (18 μg), 1.2 for *C. mucedo* (28 μg),
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 (2σ) of $\pm 0.05\%$.

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213 **Results**

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215 Age-depth model

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217 High ^{137}Cs -activity ($>2000 \text{ Bq kg}^{-1}$) 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

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226 two outliers at 17-18 and 25-26 cm depth. The calculated flux of unsupported ^{210}Pb is
227 $62 \text{ Bq m}^{-2} \text{ y}^{-1}$, which is similar to the supply rate of Knud Sø in Denmark (58 Bq m^{-2}
228 y^{-1}) presented by Appleby (2001). Age-depth modeling using ^{210}Pb was based on a
229 modified constant rate of supply (CRS) model (Appleby and Oldfield 1978) with 4.5
230 cm fixed at 1986 as a reference point (Fig. 2). The inventory below 25 cm was
231 calculated on the basis of a regression of the content of unsupported ^{210}Pb versus
232 accumulated mass above this level. The age-model was extrapolated for samples
233 below 31 cm, resulting in an estimated age for the lowermost sample of circa AD
234 1870 ± 15 years. Due to the outliers in the ^{210}Pb measurements, we consider the CRS
235 model less reliable in the deeper section of the core, which is why we report
236 approximate ages for the record.

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238 Sediment geochemistry and $\delta^{13}\text{C}$ of invertebrate remains

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240 SOM $\delta^{13}\text{C}$ decreased from approximately -30 to -33‰ throughout the core and the
241 C:N_{atomic} ratio showed a small, gradual decrease from approximately 11 to 9 (Fig. 3).
242 The carbon content of the sediment was 15-20 % by weight (wt.%) in the lower part
243 of the record up to 15 cm depth, then decreased to 10.5 wt.% at 10 cm depth, and
244 increased again to 12.6 wt.% in the top 7 cm.

245 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$
250 values (-35.1 to -31.2‰), followed by *Daphnia* (-32.8 to -30.5‰) and *Plumatella* (-

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251 33.1 to -28.0‰) (Fig. 4). $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values were very
256 similar with local minima in $\delta^{13}\text{C}$ 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. $\delta^{13}\text{C}$ of Rhabdocoela cocoons showed less temporal variability
261 and was around -29.5‰ (-30.2 to -28.4‰) throughout the core.

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263 **Discussion**

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265 Feeding ecology of invertebrate groups

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267 For the interpretation of $\delta^{13}\text{C}$ values it is important to understand the feeding ecology
268 and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic
269 and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged
270 wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit-
271 feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008;
272 Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini
273 include a number of taxa that tend to burrow deeper into sediments, or in some cases
274 into aquatic macrophytes. They feed predominantly as deposit-feeders, although some
275 groups also filter particles from water which is pumped through the tubes the larvae

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276 live in (Merritt et al. 2008; Moller Pillot 2009; Moog 2002), a habitat where they can
277 also access ^{13}C -depleted biomass of methane oxidizing bacteria (Jones et al. 2008).
278 The Tanypodinae are mainly free-living and mobile predators. However, several
279 Tanypodinae are known to also feed on the substrate the larvae live on (Merritt et al.
280 2008; Vallenduuk and Moller Pillot 2007). Bryozoa live predominantly as colonies
281 attached to hard substrates and feed on suspended nanoplanktonic algae from the
282 water column, but they cannot move through the water themselves (Kaminski 1984;
283 Okamura and Hatton-Ellis 1995; Wood and Okamura 2005). *Daphnia* are mobile
284 zooplankton feeding on fine particles in the water column that range between 0.5 to
285 30 μm in diameter and include phytoplankton and bacteria (Geller & Müller 1981). It
286 can be assumed, therefore, that $\delta^{13}\text{C}$ of *Daphnia* and Bryozoa reflects the material
287 they filter from the water column. Rhabdozoela are active predators feeding on small
288 invertebrates and large ciliate protozoans as well as scavengers (Jennings 1957;
289 Kolasa and Tyler 2010). In contrast to benthic chironomids and filter-feeding
290 *Daphnia* and bryozoa, rhabdozoels are probably less directly affected by changes in
291 $\delta^{13}\text{C}$ values of phytoplankton or SOM but have a $\delta^{13}\text{C}$ signature representing a
292 mixture of their prey organisms.

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294 Differences in $\delta^{13}\text{C}$ offsets between invertebrate taxa

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296 The carbon isotopic composition of invertebrate remains in Strandsjön showed some
297 variability between the different invertebrate groups (Fig. 4). Similar to the findings
298 of van Hardenbroek et al. (2013), the remains of *Chironomus* were ^{13}C -depleted
299 relative to other chironomids and invertebrate groups. In Strandsjön, $\delta^{13}\text{C}$ values of
300 *Chironomus* head capsules were only slightly more negative than the remains of other

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301 Chironomidae and of *Daphnia*, which in turn tended to be only slightly more negative
302 than the $\delta^{13}\text{C}$ values recorded for bulk SOM. Interestingly, $\delta^{13}\text{C}$ 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 *Plumatella* and of Rhabdozoela
305 cocoons were distinctly ^{13}C -enriched relatively to SOM and other invertebrates. We
306 found no published $\delta^{13}\text{C}$ values of sedimentary remains of *Plumatella* and
307 Rhabdozoela and only one published study with few $\delta^{13}\text{C}$ measurements on *C.*
308 *mucedo* from two sediment records in the United Kingdom (Turney, 1999). In this
309 study four $\delta^{13}\text{C}$ values of *C. mucedo* statoblasts from Llanilid are presented, ranging
310 between -29 and -25‰, approximately 0-1‰ lower than $\delta^{13}\text{C}$ values of SOM. For the
311 second site, Gransmoor, two $\delta^{13}\text{C}$ values of *C. mucedo* statoblasts are between -24
312 and -22‰ and approximately 0-4 ‰ higher than $\delta^{13}\text{C}$ of SOM. The variability in $\delta^{13}\text{C}$
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 Rhabdozoela before their $\delta^{13}\text{C}$ values can be interpreted conclusively.

316 We expected that $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and can play a role when comparing remains from
320 different invertebrate taxa. Generally, the $\delta^{13}\text{C}$ 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

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326 ranges between -2.2 and $+2.1\text{‰}$ (average $0.0 \pm 1.5\text{‰}$). His results suggest that taxa
327 can differ in the offset between $\delta^{13}\text{C}$ of ingested carbon and $\delta^{13}\text{C}$ 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 4th instar larval head capsules compared with 4th instar larval body tissue
331 of 1-2‰ (Heiri et al. 2012) and $0.9 \pm 0.2\text{‰}$ (Frossard et al. 2013). Perga (2011)
332 demonstrated that the offset in $\delta^{13}\text{C}$ between whole *Daphnia* and their ephippia
333 collected from the same water sample was within the standard deviation (0.2‰) of
334 replicate $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of remains of Rhabdocoela and *Plumatella*, 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 *Plumatella* and
342 *C. mucedo*, both bryozoans, for which a similar offset between food, body tissue and
343 statoblasts can be expected. In our record *Plumatella* had on average 3.5‰ higher
344 $\delta^{13}\text{C}$ than *C. mucedo* and it is possible that this was the result of a difference in the
345 $\delta^{13}\text{C}$ of the particles ingested by these two taxa when filtering the water. Kaminski
346 (1984) demonstrates that *C. mucedo* selects small seston ($<7 \mu\text{m}$ in diameter), which
347 can include bacteria, whereas *Plumatella repens* prefers slightly larger particles
348 (ranging from 5 to 17 μm in diameter).
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350 Trends in $\delta^{13}\text{C}$ of invertebrate remains

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2 352 The four chironomid taxa showed similar variations in $\delta^{13}\text{C}$ with several minor
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5 353 minima during the past circa 140 years (Fig. 3). All analyzed chironomid groups
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7 354 featured the same type of high frequency signal, which makes it very unlikely that
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9 355 these variations in $\delta^{13}\text{C}$ are related to changes in taxonomic composition that
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11 356 happened simultaneously within all four groups. Since *Chironomus*, other
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13 357 Chironomini, and Tanytarsini are largely detritivores and partly grazers and filterers,
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15 358 and Tanypodinae are predators, it can be expected that the $\delta^{13}\text{C}$ of the chironomid
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17 359 remains analyzed here mostly reflected the $\delta^{13}\text{C}$ of sedimentary detritus and
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19 360 detritivorous prey animals. $\delta^{13}\text{C}$ of SOM in the Strandsjön record decreased gradually
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21 361 between 35 and 0 cm depth. However, chironomids did not show the same decreasing
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23 362 trend in $\delta^{13}\text{C}$ that we observed for SOM. This suggests that the invertebrates were
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25 363 selectively feeding on and digesting certain organic matter components in the
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27 364 sediments. Wooller et al. (2008; 2012) observed trends in $\delta^{13}\text{C}$ of chironomid remains
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29 365 that were roughly similar to the patterns observed for SOM. In these studies, however,
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31 366 Wooller et al. (2008; 2012) did not include taxonomic information about the analyzed
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33 367 chironomids. This makes a direct comparison between their records and our study
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35 368 difficult, since not all chironomid tribes and subfamilies can be expected to have the
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37 369 same $\delta^{13}\text{C}$ values (van Hardenbroek et al. 2012), at least not in every lake. In a study
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39 370 examining the sediments of a Siberian thermokarst lake, van Hardenbroek et al.
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41 371 (2013) found that variations in $\delta^{13}\text{C}$ of *Chironomus*, Chironomini, Tanytarsini, and
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43 372 *Daphnia* did not follow the same pattern as $\delta^{13}\text{C}$ of SOM. Only the chironomid
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45 373 subfamily Orthocladiinae was characterized by $\delta^{13}\text{C}$ values very similar to those of
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47 374 SOM. This could be explained by a preference of many Orthocladiinae for a diet of
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49 375 periphyton or SOM, whereas the diet of *Chironomus*, Chironomini, Tanytarsini, and
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376 *Daphnia* consisted, at least in part, of other components including planktonic algae
377 and bacteria. Van Hardenbroek et al. (2012; 2013) suggested that varying proportions
378 of methane oxidizing bacteria (MOB) in the diet of *Chironomus*, Chironomini,
379 Tanytarsini, and *Daphnia* may be at least partly responsible for the difference in $\delta^{13}\text{C}$
380 between the different invertebrate groups examined in their study. Furthermore,
381 Wooller et al. (2012) argued that methane-derived carbon led to lower $\delta^{13}\text{C}$ values of
382 chironomids and *Daphnia* in sections of a sediment record from arctic Alaska.
383 Frossard et al. (2014) also suggested that similar decreases of $\delta^{13}\text{C}$ values in two
384 Chironomini taxa, Tanypodinae, and pelagic Cladocera in a sediment record Lake
385 Annecy (France) were linked to increased availability of ^{13}C -depleted methane-
386 derived organic carbon. Chironomid larvae feeding partly on MOB can be strongly
387 ^{13}C -depleted, as shown for several species (Jones et al. 2008; Kiyashko et al. 2001;
388 Zenskaya et al. 2012).

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

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401 increasing importance of MOB in their diet. However, variations in $\delta^{13}\text{C}$ 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 Terranes 2001; Bade et al. 2006). Therefore, the observed changes in chironomid $\delta^{13}\text{C}$
405 might also be caused by changes in the relative contribution of isotopically different
406 carbon sources, including allochthonous inputs, remains of aquatic macrophytes,
407 benthic algae, and periphyton that all have higher $\delta^{13}\text{C}$ values than pelagic algae
408 (France 1995a,b; Meyers and Terranes 2001). Changes in allochthonous 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 $\delta^{13}\text{C}$ responded to
414 changes in concentration or $\delta^{13}\text{C}$ of dissolved inorganic carbon in the water column,
415 as this would lead to changes in $\delta^{13}\text{C}$ of pelagic algae as well, and would be expected
416 to be registered also in the $\delta^{13}\text{C}$ of the filter-feeding *Daphnia* and bryozoans.
417 Remaining explanations for the observed variations in chironomid $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of the filter-feeding *Daphnia*, *C. mucedo*, and *Plumatella* were
421 relatively constant in the older part of the record compared with chironomids; the
422 filter-feeders had a decreasing trend in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of phytoplankton in the water column, since planktonic
425 algae can be expected to be an important component of the diet of *Daphnia* and

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426 Bryozoa. The observed variations would be easiest to explain by changes in lake
427 productivity, since $\delta^{13}\text{C}$ of phytoplankton in productive meso- to eutrophic lakes is
428 often higher than $\delta^{13}\text{C}$ of phytoplankton growing in oligotrophic conditions (Brenner
429 et al. 1999; Hollander et al. 1993). In Strandsjön, $\delta^{13}\text{C}$ of *Daphnia* and *C. mucedo*
430 showed a maximum at ~AD 1960. $\delta^{13}\text{C}$ of *Plumatella* 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 *Plumatella* was already characterized by relatively high
433 $\delta^{13}\text{C}$ 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 20th 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 $\delta^{13}\text{C}$ of
439 *Daphnia* and bryozoans, $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of organic matter produced in
442 the water column than bulk SOM $\delta^{13}\text{C}$, at least in relatively small, shallow, and
443 productive lakes such as Strandsjön.

444 445 **Conclusions**

446
447 Our results demonstrate that taxon-specific $\delta^{13}\text{C}$ records can be developed for a range
448 of chitinous invertebrate remains found in lake sediments. In Strandsjön, our approach
449 revealed a differential response of $\delta^{13}\text{C}$ of benthic taxa (chironomids and rhabdocoels)
450 to past environmental change compared with $\delta^{13}\text{C}$ of taxa feeding predominantly on

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451 suspended particulate organic matter (*Daphnia* and bryozoans). Hence, our study
452 indicates that $\delta^{13}\text{C}$ of chitinous invertebrate remains can provide valuable additional
453 information to $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of the diet and $\delta^{13}\text{C}$ of
457 freshwater invertebrate tissues are urgently needed to improve the interpretation of
458 invertebrate $\delta^{13}\text{C}$ in sediment records. More studies examining $\delta^{13}\text{C}$ of different
459 organic invertebrate remains from the same sediment record are necessary to confirm
460 that $\delta^{13}\text{C}$ of phytoplankton can be reliably traced by examining the carbon isotopic
461 composition of the remains of filter-feeding *Daphnia* and Bryozoa, and that such
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
465 reconstructions and provide, to our knowledge, the taxonomically most diverse such
466 study available to date. It is likely that similar taxon-specific records will soon be
467 produced for stable isotopes of other elements (H, O, N, S) that can give further
468 insights in the functioning of aquatic ecosystems over long time scales.

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626 **Figure captions**

627

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

631

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

636

637 **Fig. 3** $\delta^{13}\text{C}$ values of invertebrate remains and geochemistry of sedimentary organic
638 matter ($\delta^{13}\text{C}$, organic carbon content, and $\text{C:N}_{\text{atomic}}$ 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).

643

644 **Fig. 4** Boxplots with $\delta^{13}\text{C}$ values of the different invertebrate taxa in this study and of
645 sedimentary organic matter (SOM).

646

Figure 1
[Click here to download Figure: Fig 1 Location.eps](#)

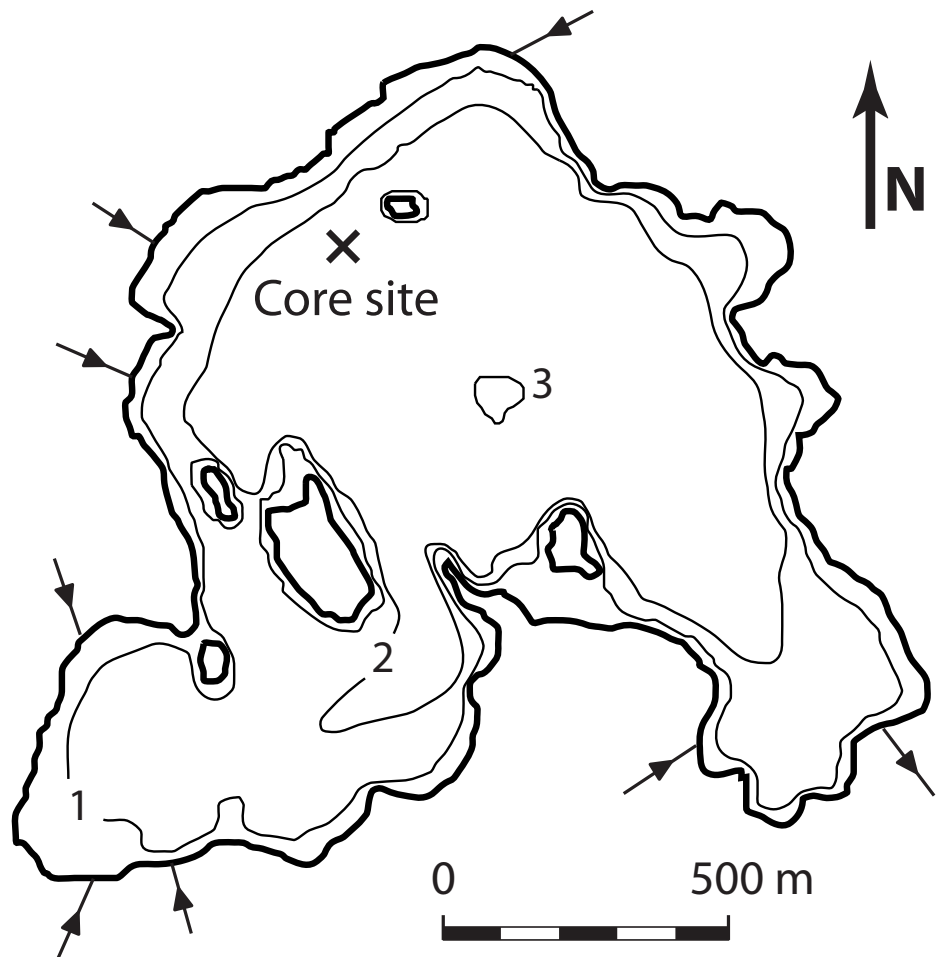


Figure 2
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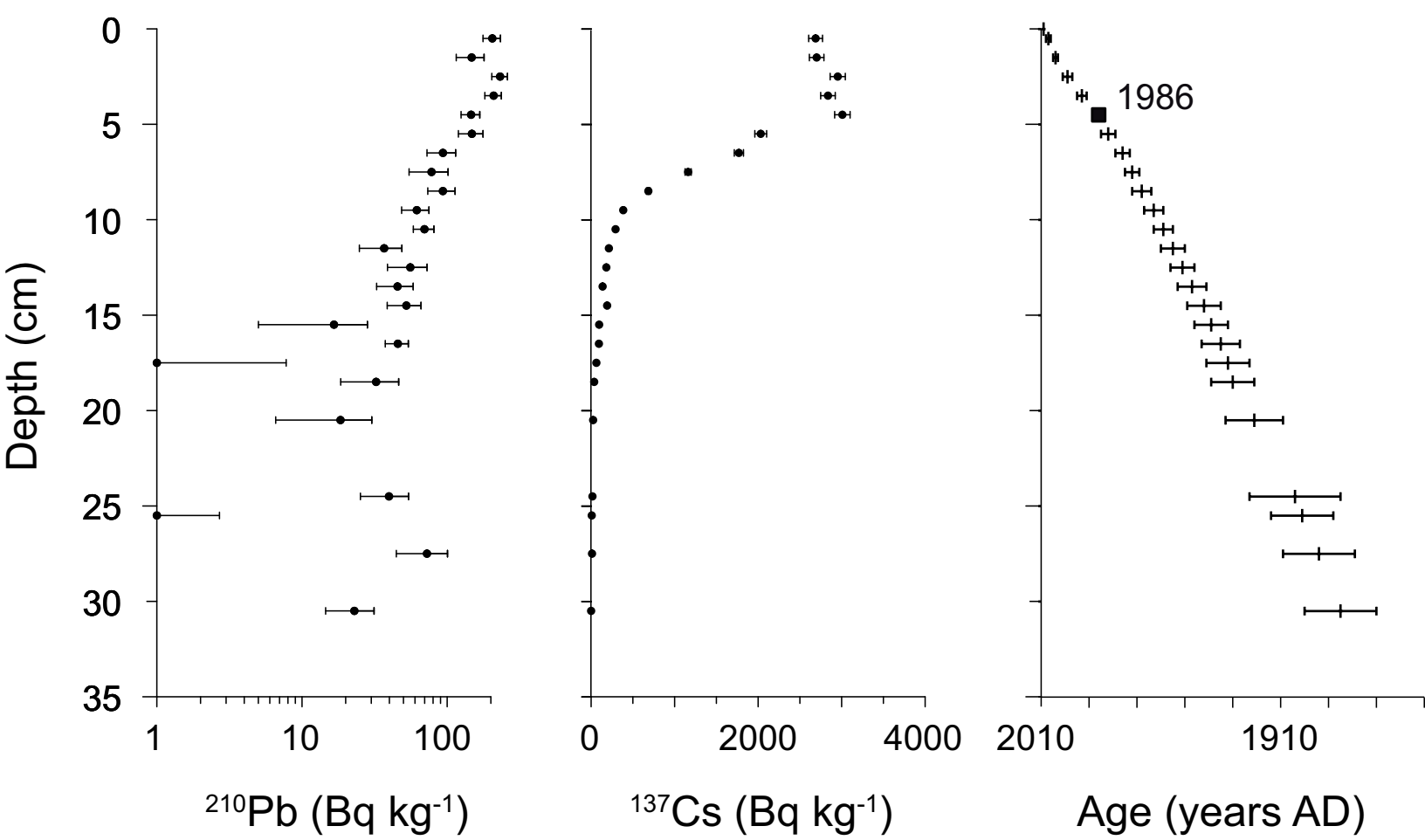


Figure 4

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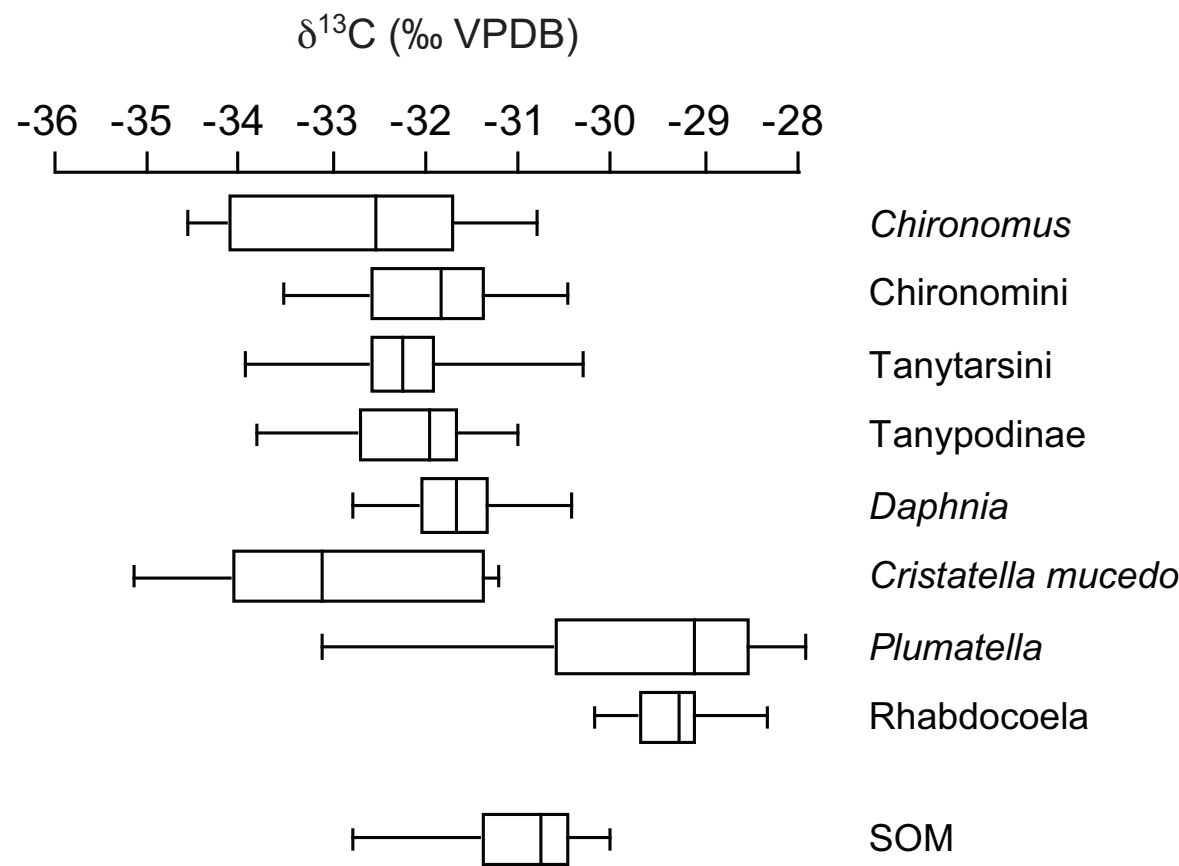
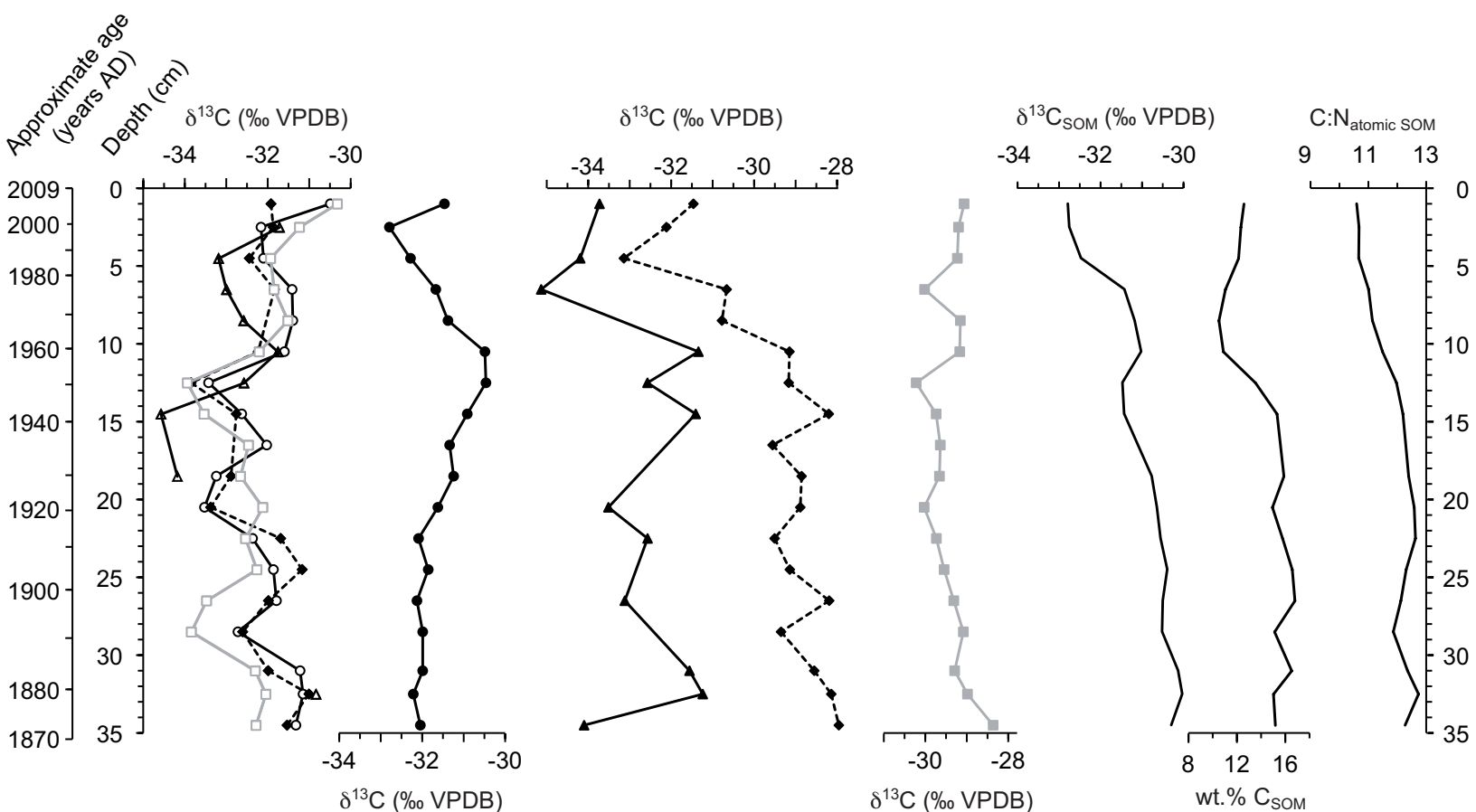


Figure 3

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- ▲— *Chironomus*
- *Chironomini*
- - ◆ - - *Tanypodinae*
- *Tanytarsini*
- *Daphnia*
- ▲— *Cristatella mucedo*
- - ◆ - - *Plumatella*
- *Rhabdoceola*
- *SOM*

Supplementary data for van Hardenbroek et al "Taxon-specific $\delta^{13}\text{C}$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden"

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

Depth (cm)	<i>Chironomus</i>			Chironomini			Tanytarsini			Tanypodinae			<i>Daphnia</i>			<i>Cristatella mucedo</i>			<i>Plumatella</i>			Rhabdoceola			SOM				
	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	no.	mass	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	%C _{weight}	%N _{weight}	C:N _{atomic}
0-2	-31.7	19	18	-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