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NOTE

Efficiency of different mesh sizes for isolating fossil chironomids for stable isotope and radiocarbon analyses

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Abstract We examined the effects of sieving with different mesh sizes on the efficiency of processing fossil chironomids from lake sediments for isotope analyses. Results obtained for three different sediments indicate that each of the studied sieve fractions (100–150, 150–200, 200–250, 250–300, >300 μm) contain a similar proportion of the overall mass of chironomid fossils in a sample. However, the sorting time needed to separate chironomids from other sieve residue is disproportionately large for smaller mesh sizes. Employing sieves with a 200-μm rather than the 100-μm mesh commonly used for standard palaeoecological analyses of fossil chironomids decreased processing time for a given mass of fossils by 30–58% in our study. For optimizing the efficiency of chironomid sample processing for stable isotope and radiocarbon analysis we therefore recommend a 200-μm mesh size sieve, although the sorting of all >100-μm fractions may be necessary in sediments with low chironomid abundances. Excluding certain small taxa from isotope analysis, may structurally bias isotope values of samples. Therefore, further studies on taxon-specific isotope analysis are required to quantify these effects.

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Introduction

Non-biting midges (Insecta: Diptera: Chironomidae) are sensitive indicators for a variety of environmental variables. The chitinous remains of chironomid larvae preserve well, are ubiquitous in lake sediments and have been used to reconstruct physical and chemical variables such as air or water temperature (Walker and Cwynar 2006; Brooks 2006; Heiri et al. 2007), total phosphorus (Brooks et al. 2001; Langdon et al. 2006), chlorophyll *a* (Brodersen and Lindegaard 1999), oxygen availability (Quinlan et al. 1998), or lake depth (Korhola et al. 2000).

The potential of fossil chironomids for isotope studies has first been shown for ^{14}C dating (Jones et al. 1993; Fallu et al. 2004). High-latitude or high-altitude sites are often devoid of terrestrial plant remains and bulk ^{14}C dates from lake sediments are often too old due to either contamination by allochtonous material or hard-water effects (Olsson 1991; Abbott and Stafford 1996). In such circumstances chironomids can be one of the few reliable sources of carbon available for dating. Recently, chironomid fossils have also been used in stable isotope studies, such as $\delta^{18}O$-based temperature reconstruction (Wooller et al. 2004, 2008) or the reconstruction of lake productivity using stable carbon and nitrogen isotopes (Wooller

et al. 2008). One of the major difficulties in all attempts to measure isotopes in chironomid fossils is to attain the required minimum sample mass for isotope analyses. The amount of chironomid material necessary for Accelerator Mass Spectrometry (AMS) ^{14}C dates was reported by Jones et al. (1993) as being 250–400 μg carbon (~800 head capsules), and Fallu et al. (2004) used between 180 and 370 μg chironomids (in their case equivalent to ~1,300–2,500 head capsules from unsieved sediment). The amount of larval chironomid head capsules necessary for an oxygen isotope measurement is approximately 100 μg (300–700 head capsules; Wooller et al. 2004) with a minimum of 50 μg (~120 head capsules) reported by Wang et al. (2008).

The most commonly used method to isolate head capsules from other sediment components is to wash the sediments through a 90–115-μm sieve and subsequently hand pick the remains under a dissecting microscope. The choice of the commonly used ~100-μm mesh size for sieving sediments is based on the observation by Walker and Paterson (1985) that most head capsules are larger than 100 μm in diameter and thus retained on a 100-μm sieve. Even the sorting of 50–100 head capsules per sample commonly used in palaeoecological analyses can take an analyst several hours for sediments with large amounts of obscuring debris. Therefore, the time needed for sorting and isolating chironomid remains is an important constraining factor for the number of samples that can be processed in chironomid-based isotope studies.

Missing certain small taxa due to sieving with a too wide a mesh and excluding them from numerical analyses may not only have considerable effects on the palaeoecological interpretation of the assemblage but can also significantly bias quantitative chironomid-based environmental reconstructions (Heiri and Lotter 2001; Quinlan and Smol 2001). For isotope analyses, however, mass is often more relevant than the number of individuals, at least when the isotopic composition of chironomids is expected to be similar within a lake basin and between species of different size. It may thus be beneficial to select a processing method that optimises the preparatory process, i.e., that yields the greatest mass of chironomid fossils in the shortest processing time.

A method to concentrate chitinous fossils from lake and stream deposits is the floatation of insect remains in a denser, apolar organic liquid. Using kerosene, a grade mineral oil, insects can be concentrated from any sediment material (Coope 1986). However, Rolland and Larocque (2007) recently demonstrated that this method yields reduced amounts of large head capsule types such as the fourth instars of *Chironomus* because these are often filled with sediment and are therefore heavier. This is unfortunate because large, heavily sclerotized head capsules provide a disproportionately large share of the chironomid sample mass available for isotope analyses. An additional drawback for carbon and hydrogen isotope analysis is the introduction of carbon and hydrogen from kerosene. Although it may be possible to remove the kerosene by chemically cleaning the samples, the additional time needed for such a cleaning step and the introduction of potential contaminants makes this approach unattractive for isotope studies.

Large head capsules yield several times more mass per specimen than small head capsules. Therefore, selectively isolating large head capsules from the sediments will concentrate a large proportion of the total chironomid mass available in a sample. This can be done in a standardized way by sieving with mesh sizes >100 μm which allows smaller head capsules and many other sedimentary particles to be washed through the sieve. Previous studies have examined the effect of different mesh sizes on chironomid sample processing. However, these studies mainly examined the effect of mesh size on the representativeness of chironomid assemblages identified and enumerated under the light microscope (Walker and Paterson 1985; Verschuren and Eggermont 2007) and no studies are available that document the effects of the mesh size used during sieving on the sorting time of chironomid samples. In this study we assess the effect of mesh size on fossil chironomid sample mass and processing time, with the aim of providing a recommendation for the most time-efficient mesh size to be used to concentrate fossil chironomids for stable isotope analyses and AMS radiocarbon dating.

Methods

Three types of lake sediments were selected that differ in age, water content, chironomid fossil concentration, and geographic setting (Table 1). Sediment sample A comes from an unnamed tundra pond in arctic Siberia, collected near the River Elon

and the town of Chokurdakh, Yakutia (van Huissteden et al. 2005). Sediment sample B was collected in subalpine Hinterburgsee, Switzerland (Heiri et al. 2003). Sediment sample C was collected from the former Slotseng lake basin, an archeological site in Denmark (Mortensen 2008). Further details on the different sediments are given in Table 1.

A known weight of freeze-dried sediment was rehydrated with 10% KOH for 2 h at room temperature and subsequently sieved with tap water through a set of nested sieves with mesh sizes of 300, 250, 200, 150, and 100 μm. The material in each size fraction was rinsed twice with demineralized water to eliminate residual KOH and carbonates in tap water. Head capsules were hand-picked from a Bogorov sorting tray using fine forceps by the same analyst (MvH) under a dissecting microscope at 16–100× magnification. The head capsules were placed on preweighted cover slips and dried on a hotplate at 50°C for 1 day before re-weighing the cover slips. The number of head capsules, their mass and the time necessary to sort the chironomid fossils were measured for each fraction separately and used to calculate sorting time per gram dry weight of fossil chironomids isolated from the fraction. In the following sections, data for the different size fractions are combined to calculate cumulative values. For example, the >150-μm fraction represents the combined data of the 150-, 200-, 250-, and 300-μm sieves and represents the fraction of a sample that would have been available for sorting if only a 150-μm mesh size sieve had been used for sample processing. Raw data for the individual sieve fractions can be found in Table 2.

Results and discussion

The number and mass of chironomid head capsules in each size fraction vary between the three analyzed sediment types. The total mass of the head capsules per gram dry weight of sediment is very similar in sediment samples A and C (1,317 and 1,539 μg, respectively), but only 43 μg in sediment sample B (Table 2). The highest concentration of head capsules was found in sediment sample C, which contains 980 head capsules per gram dry weight. This is twice as much as in sediment sample A and more than 15 times the concentration found in sediment sample B (Table 2). Furthermore, the average weight of a head capsule is higher in sediment sample A (4.1 μg) compared with sediment samples B and C (0.9 and 1.6 μg, respectively), indicating that the average mass of individual head capsules is site-specific.

Processing time for the cumulative sieve fractions decreased exponentially with increasing mesh size (Fig. 1). The 100- and 150-μm sieve fractions uniformly require 50–52 and 20–22% of overall picking time, respectively, in all three sediments (Table 2). This is disproportional to the mass these

Table 1 Main characteristics of the three lake sediment samples used in this study

Sediment	A	B	C
Site	Unnamed tundra pond, Russia	Hinterburgsee, Switzerland	Slotseng, Denmark
Latitude/longitude	70°49′46″N/147°29′13″E	46°43′5″N/8°4′2″E	55°19′48″N/9°16′17″E
Sediment age	Modern	Twentieth century	ca. 12,800–14,800 cal. years BP
Altitude (m a.s.l.)	50	1515	40
Water content (%)	98	69	50
Total number of head capsules >100 μm in sample	287	176	2258[a]
Dry weight of analyzed sample (g)	0.60	1.37	1.98
Number of head capsules per g dry weight	476	64	979
Total weight of head capsules >100 μm in sample (μg)	794	118	3550[a]

[a] Based on analysis of half of the 100 and 150 μm fraction. Fractions were subsampled following Heiri et al. (2003)

Table 2 Number of larval head capsules (HC), HC mass, and sorting time for 1.0 g dry weight of sediment samples A, B, and C

	Mesh size (μg)	Number of HC	Cumulative number of HC	Weight (μg)	Cumulative weight (μg)	Sorting time (min)	Cumulative sorting time (min)	Efficiency of sorting (μg/min)	Efficiency of sorting (HC/min)	HC weight (μg/HC)
Sediment A	300	40	40	332	332	83	83	4.00	0.48	8.33
	250	38	78	239	570	83	166	2.88	0.46	6.26
	200	166	244	262	833	191	357	1.37	0.87	1.58
	150	106	350	212	1045	282	638	0.75	0.38	2.00
	100	126	476	272	1317	647	1285	0.42	0.19	2.16
Average		95.2		263.3		257.0		1.9	0.5	4.1
Sediment B	300	4	4	9	9	11	11	0.80	0.40	2.00
	250	12	16	5	14	13	24	0.40	0.97	0.41
	200	8	24	4	18	16	40	0.27	0.49	0.55
	150	22	46	10	28	25	65	0.40	0.87	0.46
	100	17	63	15	43	71	136	0.21	0.24	0.85
Average		12.8		8.6		27.3		0.4	0.6	0.9
Sediment C	300	183	183	433	433	50	50	8.69	3.67	2.37
	250	134	317	190	623	65	115	2.93	2.05	1.43
	200	252	569	427	1050	56	171	7.69	4.53	1.70
	150	184	753	225	1275	113	284	2.00	1.63	1.23
	100	227	980	264	1539	308	592	0.86	0.74	1.16
Average		195.8		307.9		118.2		4.4	2.5	1.6

Values are calculated for the individual size fractions as well as cumulative for all fractions larger than a given mesh size. The three final columns show the efficiency of sorting fossil chironomids for the different fractions expressed as mass and number of HC per time, and the average mass per HC

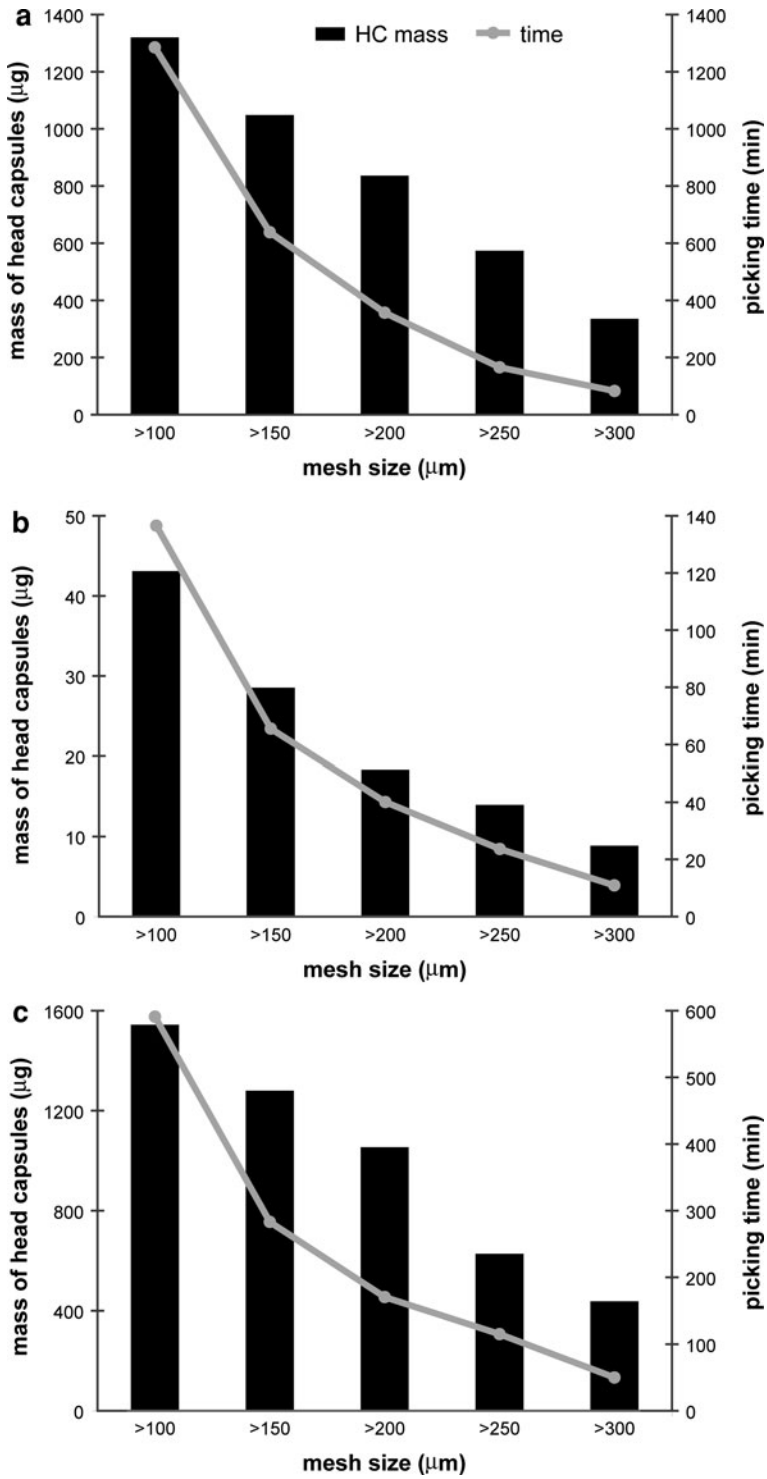

Fig. 1 Mass of chironomid head capsules (HC) isolated from 1.0 g of dry sediment sieved with a 100-, 150-, 200-, 250-, and 300-µg mesh size sieve (*black bars*) and the associated sorting time (*grey line*). Values are calculated based on cumulative data from Table 2; **a**–**c** refer to the sediment samples A–C

fractions yield (Fig. 1). Quantitatively, smaller head capsules dominate in sediment samples A–C, but their weight contribution to the combined weight of all size fractions varies between sediments (Table 2). In sediment sample A, each size fraction contains a similar mass of head capsules, in sediment sample B.

the small size fractions contain a larger mass than the large size fractions, and in sediment sample C, the small size fractions contain a smaller mass than the large size fractions. Table 2 also indicates that the sorting efficiency (fossil mass isolated per unit of time) generally increases with mesh size for each sediment type analyzed in this study. The only clear exception is the >250-µm fraction of sediment sample C, for which the sorting efficiency is lower than for the >200- and the >300-µm fractions. A possible explanation of this pattern is the relatively large number of light, weakly sclerotized Tanypodinae remains found in the 250-µm size fraction of sediment sample C. Our results indicate that the time necessary for sorting all chironomids in a sample of sieve residue is reduced by 50–52%, if a 150-µm mesh instead of a 100-µm mesh is used (Table 2). This reduction is very similar to the 50% reduction reported by Verschuren and Eggermont (2007) for African lake sediments.

Smaller mesh size sieves retain more debris particles that can obscure chironomid head capsules. Furthermore, the smaller head capsules are harder to see and handle than the large head capsules retained in large mesh sieves. This explains the exponential increase in picking time if smaller mesh size sieves are used. Larger mesh sizes have the advantage of saving time, but also the disadvantage of losing material that could be used for isotopic analyses. Therefore, a balance must be sought between the reduction of time and the mass loss associated with choosing coarser sieves for sample preparation. In order to find the optimal mesh size, we examined the relative decrease in sorting time and retained mass with increasing mesh sizes. The percentage of time and mass that is reduced by a given mesh size compared with the mesh that is 50 µm smaller is plotted in Fig. 2 for the tested mesh sizes of 150, 200, 250, and 300 µm. As long as the proportion of processing time saved by selecting a larger mesh size is larger than the relative amount of mass that is lost, it seems favorable to use the coarser mesh size. For sediment samples A and C, a mesh size of 200 µm seems optimal, since the proportion of material lost is less than the proportion of sorting time gained by choosing this coarser mesh size. The somewhat different pattern for sediment sample B suggests that the optimal mesh size for isolating chironomid remains from sediments depends on the size

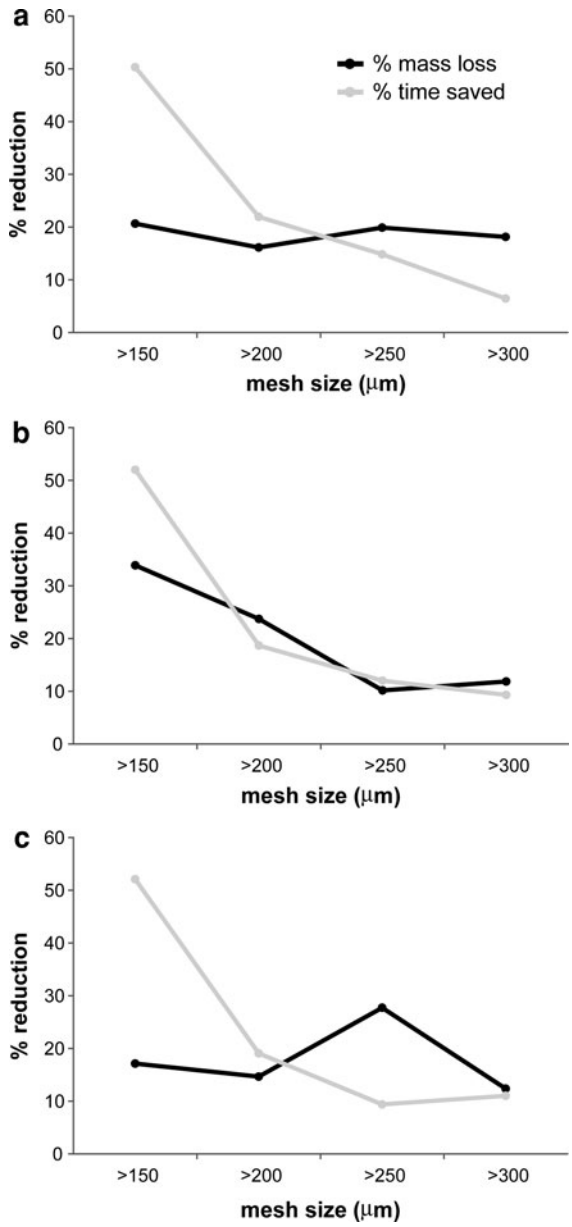

Fig. 2 Relative amount of time saved (*grey*) and head capsule mass lost (*black*) when using a mesh size of 150, 200, 250, and 300 µm for sample preparation relative to the sorting time and the head capsule mass retained when using a mesh size that is 50 µm smaller. Values are calculated based on cumulative weight and cumulative sorting time from Table 2; **a–c** refer to the sediment samples A–C

distribution and morphology (e.g., sclerotization) of head capsules in a given sediment type. However, overall choosing a 200-µm sieve increased the sorting efficiency for all three sediments we examined, and decreased picking time of the head capsules retained

in a sample by 71–72% compared to sorting through all material retained in a 100-μm sieve.

Our results have major implications for the potential of fossil chironomids in stable isotope and radiocarbon studies. Fallu et al. (2004) reported that 1,300–2,400 chironomid head capsules were necessary to obtain 180–370 μg fossils for radiocarbon dating using unsieved lake sediments. Based on the cumulative data provided in Table 2, the sorting of 180–370 μg of chironomid head capsules processed with a 100-μm sieve would have required 2.9–6.0, 9.5–19.6, and 1.2–2.4 h of sorting time for sediments samples A, B, and C, respectively, if head capsules of all sizes would have been picked (Table 3). With the use of a 200-μm sieve, the sorting time for the same mass of chironomid head capsules could be reduced to 1.3–2.6, 6.7–13.7, and 0.5–1.0 h, respectively, which is equivalent to a reduction of the sorting time by 56, 30, and 58% (Table 3). This shorter processing time would make it feasible to use chironomid head capsules for ^{14}C analysis at relatively high temporal resolution. Similarly, the isolation of 100 μg of chironomid remains recommended for $\delta^{18}O$ analysis by Wooller et al. (2004) would have taken us 1.6, 5.3, and 0.6 h for sediment samples A, B and C, respectively, if they were sieved with a 100-μm sieve, whereas processing time could have been reduced to 0.7, 3.7, and 0.27 h, respectively, to retrieve the same sample mass after sieving with a 200-μm sieve (Table 3).

Sample preparation with larger mesh sizes will require larger quantities of sediments to retrieve the same mass of head capsules. This is not necessarily problematic if the concentration of head capsules is high in the sediment record of interest, but it may decrease temporal resolution of palaeoenvironmental reconstructions if concentrations are low and adjacent samples have to be pooled. When preparing chironomid samples for isotopic studies, we therefore recommend to pre-screen sediment records to see if the concentrations of chironomids in the >200-μm fraction are sufficient before deciding on a certain mesh size. If chironomids are abundant, we recommend using 200-μm sieves to process samples rather than the 90–115-μm mesh commonly used for palaeolimnological studies as this may shorten sorting time by 30–58% (Table 3). However, we also recommend to retain the fractions <200 μm until the samples have been weighted, so that additional chironomids can be isolated from the 150–200- or 100–150-μm fractions if necessary to obtain the required minimum mass for analysis. In that case all samples should also include the 150–200- or 100–150-μm fractions to prevent size-dependent bias.

Our results indicate that sieving of chironomid samples with mesh sizes in the range of 150–200 μm can significantly reduce processing time compared with samples sieved with the standard mesh size of 90–115 μm. Selection of coarser sieve for chironomid sample preparation will therefore enhance the temporal resolution that can be achieved in studies of the isotopic composition of fossil chironomid assemblages. An important caveat, however, is that mesh size will potentially affect results if different chironomid size classes or taxa are characterized by different isotopic values. For example, stable carbon isotope values can be very variable in different chironomid taxa within a lake basin (Grey et al. 2004; van Hardenbroek et al. 2009), with strongly depleted values reported for some chironomids. A selective enrichment or elimination of head capsules of ^{13}C-depleted chironomids associated with choosing a

Table 3 Sorting time needed for isolating the minimum weight of 100 μg of fossil chironomids recommended for $\delta^{18}O$ analysis (Wooller et al. 2004) and 180–370 μg recommended for ^{14}C dating (Fallu et al. 2004) if samples are sieved with 100- or 200-μm mesh sieves

Chironomid mass (μg)	Time needed for sediment sample A (h)		Time needed for sediment sample B (h)		Time needed for sediment sample C (h)	
	100 μm	200 μm	100 μm	200 μm	100 μm	200 μm
100	1.6	0.7	5.3	3.7	0.6	0.3
180	2.9	1.3	9.5	6.6	1.2	0.5
370	6.0	2.6	19.6	13.6	2.4	1.0
Time reduction relative to 100 μm fraction	–	44%	–	70%	–	42%

Values are calculated using the cumulative data from Table 2

certain mesh size would therefore lead to biased isotopic measurements on fossil chironomid samples. In contrast, Fallu et al. (2004) assumed that for ^{14}C analyses the isotopic composition of chironomid fossils indiscriminately reflects isotope concentrations in the lake water. Similarly, Wooller et al. (2004) demonstrated that chironomid $\delta^{18}O$ is equilibrated with the $\delta^{18}O$ of lake waters in which larvae live, if lakes with short residence times are examined. In these situations it can be expected that mesh size will have a minor effect on isotopic measurements of fossil chironomid assemblages, although it remains to be demonstrated whether chironomid $\delta^{18}O$ is unaffected by the vital effects (e.g., habitat, instar effects, temperature fractionation), which have been described for inorganic remains of lacustrine invertebrates (Ito 2001).

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