

Effect of removing small (<150 µm) chironomids on inferring temperature in cold lakes

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Abstract Sieving samples for chironomid analysis with a 150 µm mesh was shown to greatly reduce sample preparation time, and use of only larger specimens did not affect chironomid-inferred salinities in African lakes. Here, we tested if this method is suitable for temperature reconstruction in colder lakes at higher latitudes. Removal of specimens <150 µm in two training sets, one from Canada and one from Sweden, had little impact on the performance statistics of the calibration models. Chironomid abundance, however, decreased greatly because more than half of the head capsules in assemblages were <150 µm. This

had major impacts on the temperature reconstructions. Inferences were on average 2°C warmer with the modified models (all specimens >150 µm) than those obtained with the full model (all specimens >100 µm). General patterns of temperature change were also altered. For Lake 7 on Southampton Island, Canada, a cooling trend was reconstructed with the full Canadian model while the modified Canadian model yielded a warming trend. When only specimens >150 µm were used, two to three times more wet sediment was needed to obtain a sufficient number of head capsules. These results indicate that, in cold lakes (mean July/August air temperature $\leq 11^{\circ}\text{C}$), large proportions of head capsules are <150 µm, and sieving the samples in a 150 µm mesh leads to altered temperature reconstructions.

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Introduction

Chironomid analysis has been used successfully to reconstruct temperature on short (last 100 years) and longer (The Holocene, the Last Glacial) temporal scales in Europe and North America (Brooks 2006; Walker and Cwynar 2006). Although the method is effective, sample preparation is time consuming,

limiting the number of samples that can be analyzed, thus sometimes constraining high-resolution or multi-core studies. Alternate methods to decrease the time of sample preparation include kerosene flotation of the head capsules (Rolland and Larocque 2007) and adding exotic markers to quantify the absolute concentration of head capsules (Velle and Larocque 2008). Recently, Verschuren and Eggermont (2007) demonstrated that sieving lake samples with a 150 µm mesh instead of a 100 µm mesh, as is standard practice, contributed to a 50% time reduction for chironomid sample preparation. More importantly, their study showed that using only larger specimens (>150 µm) did not significantly alter the performance statistics of the salinity transfer function or the salinity reconstruction.

Verschuren and Eggermont (2007) tested the effect of increasing the mesh size in warm African lakes. According to our experience from higher latitude, colder lakes, many chironomid head capsules are small and/or broken in halves. Thus, in the present study we try to (a) evaluate if colder lakes have smaller specimens than warmer lakes, (b) test the effect of removing smaller specimens from the training set, as if we had sieved the sample in a 150 µm mesh and (c) compare the temperature reconstructions obtained from transfer functions using all specimens (>100 µm) with those using only specimens >150 µm. These questions were addressed for different sites in Canada and in Sweden. The effect of removing specimens <150 µm on the percentages of

fossil assemblages was also assessed in one high elevation lake in Switzerland and two lakes in Norway.

Methods

Chironomid analysis

For the transfer function and the fossil sample analyses, samples were sieved with a 100 µm mesh, following methods described in Larocque and Hall (2004), Larocque et al. (2006) and Rolland et al. (2008). While Verschuren and Eggermont (2007) used both a 100 and 150 µm mesh to test their hypothesis, we measured the head capsule size (i.e. the longest segment of the occipital) and removed all head capsules <150 µm under the assumption that these sub-fossils normally would be lost by using a 150 µm mesh.

To explore the potential of using chironomid assemblages containing only head capsules >150 µm in down-core studies of high latitude/altitude lakes, we measured the fraction of head capsules <150 µm in two lakes in Sweden (Lake Njulla, Lake 850), one lake in Switzerland (Lake Silvaplana), two lakes in Norway (Brurskardstjønn, and Vestre Øykjamyrkjøn) and two lakes in Canada (see Table 1 for lake details). For details on lake coring, sieving procedure, and the species assemblages from the Norwegian lakes, see Velle et al. (2005), for the Swedish lakes see Larocque and Hall (2004), for the Swiss lake see Blass et al.

Table 1 Characteristics of the studied lakes

Sites	Latitude	Longitude	Elevation (m a.s.l.)	Mean July air T (°C)	Maximum depth (m)
Sweden					
Lake Njulla	68°22'	18°42'	999	8.1	4
Lake 850	68°18'	19°07'	850	9.1	7.5
Norway					
Brurskardstjønn	61°25'	8°40'	1,309	8.7	12
Vestre Øykjamyrkjøn	59°32'	6°06'	594	11	7.4
Switzerland					
Lake Silvaplana	46°26'	9°47'	1,791	10.1	77
Canada					
Lake 4	65°05'	83°47'	100	9.3	36.5
Lake 7	65°12'	83°47'	155	9	19

(2007), Bigler et al. (2007), Larocque et al. (2009) and for the Canadian lakes see Rolland et al. (2009).

Training sets

A Canadian chironomid-temperature inference model, modified from Larocque et al. (2006), and a Swedish chironomid-temperature inference model (Larocque et al. 2001), were used to test the hypotheses. The Canadian model reconstructs mean August air temperature for lakes between the Abitibi region in Quebec, Canada and Ellesmere Island in the Canadian arctic, covering a temperature gradient of ca. 20°C. The r^2 of this model is 0.85, the root-mean-square error of prediction (RMSEP) is 1.12°C, and the maximum bias is 3.0°C. Two lakes are used for temperature reconstruction. These lakes [Lake 7, Rolland et al. (2008) and Lake 4, Rolland et al. (2009)] are located on Southampton Island and have a mean August air temperature of 7.3°C (average 1995–2000). The Swedish training set ($r^2 = 0.63$, RMSEP = 1.13°C, max bias = 2.1°C) is used to reconstruct the temperature in two lakes: Lake Njulla (Bigler et al. 2003) and Lake 850 (Larocque and Bigler 2004).

New transfer functions were created by removing all head capsules <150 µm from the training sets. These new transfer functions are referred to in the following text as the “Modified Canadian (or Swedish) model.” The existing transfer functions, which include all head capsules >100 µm are referred to as the “Full Canadian (or Swedish) model.” The statistics (r^2 , RMSEP, maximum bias) are compared between the modified and the full models to determine the effect of removing the smaller head capsules. In addition, we measured the fraction of head capsules >150 µm.

Results and discussion

Our first objective was to evaluate whether cold lakes have smaller chironomid head capsules than warmer lakes. In the Canadian training set, there are 25 lakes with a mean July air temperature colder than 11°C. When organizing the training set according to head capsule size, 18 of the cold lakes have modified assemblages (all specimens >150 µm) that include <60% of the full assemblage (all specimens >100 µm; Fig. 1a). In warmer lakes, most of the modified

samples still have at least 80% of the full assemblages. In the Swedish training set, 48 out of 100 lakes have mean July air temperature below 11°C. In 29 of these lakes (60%), the modified chironomid assemblages include <60% of the full assemblage. Although the relationship between lake temperature and head capsule size is not straightforward ($r^2 = 0.32$ and 0.31; $P \leq 0.001$), the results indicate that colder lakes tend to have smaller chironomid head capsules than warmer lakes.

The relationship between average head capsule size and mean July/August air temperature (Fig. 1b) shows similar patterns. All of the 25 cold lakes in the Canadian training set have head capsules with average sizes <200 µm while most of the warmer lakes have average head capsule sizes >200 µm. In the Swedish training set, 30 of the 48 cold lakes (63%) have average sizes smaller than 180 µm. Here, the relationship between average head capsule size and mean July/August air temperature was significant ($r^2 = 0.56$ and 0.53; $P \leq 0.001$). These results indicate that cold lakes have smaller specimens than warmer lakes, and that removing specimens <150 µm, as would be done if the samples were sieved on a 150 µm mesh, would significantly alter the chironomid assemblages.

In ectotherms, there is a relationship between temperature and size. Individuals in colder environments tend to grow more slowly, but are larger as adults (Ray 1960). For chironomids, temperature is a driving variable for growth rates (Sweeney 1984) and for short-term and seasonal abundance patterns (Hodkinson et al. 1996). Sizes of chironomids grown in experimental conditions tend to follow the temperature-size rule (McKie and Cranston 2005). However, species often have a narrow temperature tolerance and there are few studies comparing the average size of specimens in warm-water assemblages to specimens in cold-water assemblages. Brundin (1958) observed that cold-water chironomids such as Orthocladiinae and Diamesinae generally consist of small specimens, however, it should be noted that the size range in both sub-families is large. Twenty-seven of the 79 taxa found in the Canadian training set had average sizes <150 µm (Table 2; Fig. 1c), mainly consisting of Orthocladiinae and Diamesinae, and 16 (59%) of those had temperature optima lower than 11°C. In the Swedish training set, 25 of the 48 taxa had sizes <150 µm and of those, 14 (58%) had temperature optima lower than 11°C. These results suggest that

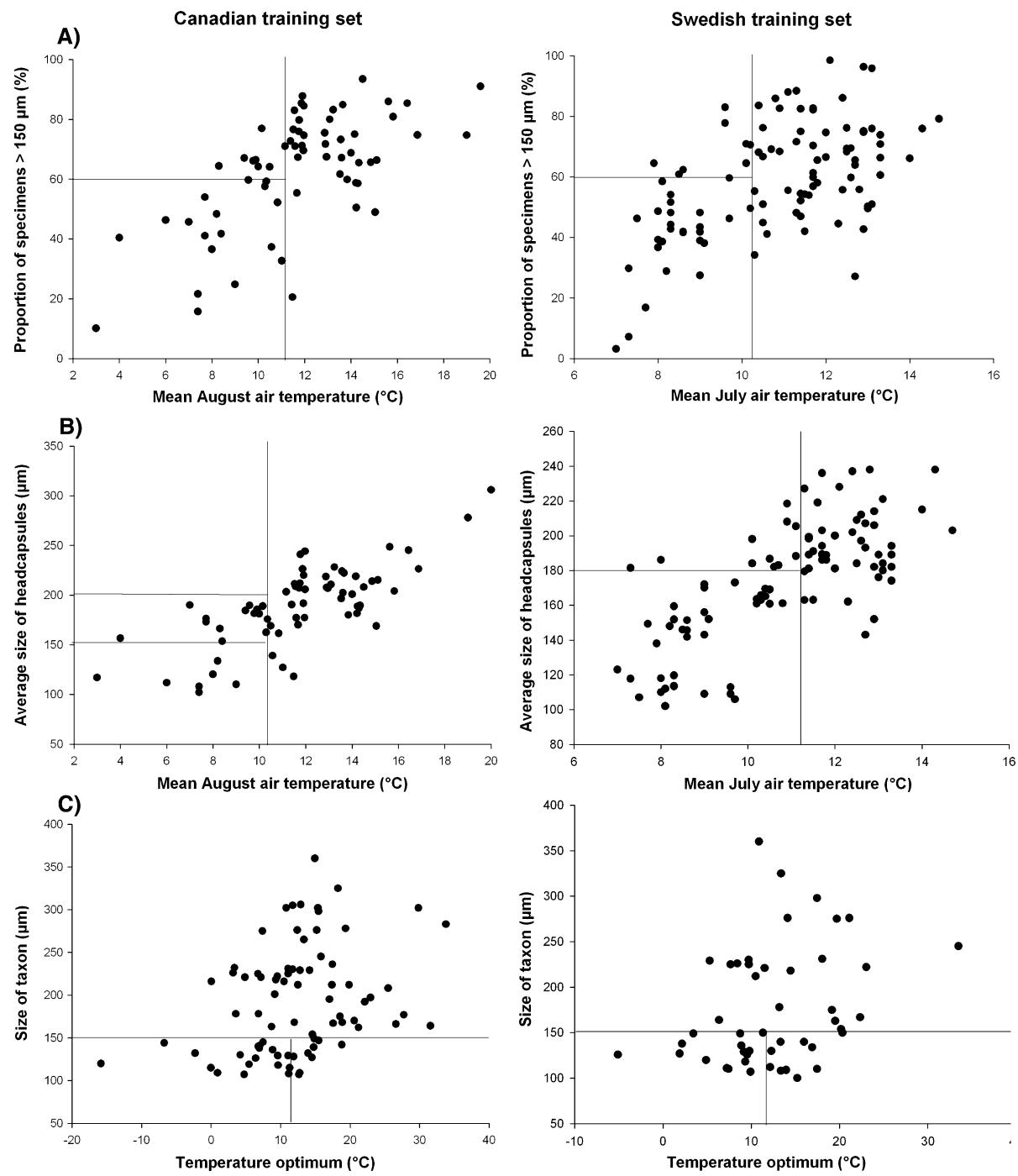


Fig 1 Graphs on the *left* relate to the Canadian training set and graphs on the *right* relate to the Swedish training set. **a** Percentages of the chironomid assemblages in the surface samples after removing specimens $<150 \mu\text{m}$ in relation to the mean August air temperature of the lakes. Lines indicate limits at 11°C (the warmest lake reconstructed in this study) and 60% of the specimens remaining. **b** Relationship between the average size of head capsules in each sample and the mean August air temperature ($^{\circ}\text{C}$). The vertical line is the temperature limit at 11°C (the warmest lake reconstructed in this study). **c** Relationship between the temperature optima and the average size of each taxon recorded in the training sets. The vertical line indicates the limit at 150 μm (specimens $<150 \mu\text{m}$ will be removed in the “Modified” model) and the horizontal line indicates the limit at 11°C (the warmest lake reconstructed in this study)

mean August air temperature ($^{\circ}\text{C}$). The vertical line is the temperature limit at 11°C (the warmest lake reconstructed in this study). The vertical line indicates the limit at 150 μm (specimens $<150 \mu\text{m}$ will be removed in the “Modified” model) and the horizontal line indicates the limit at 11°C (the warmest lake reconstructed in this study)

Table 2 Average size (in μm) and standard deviation (\pm) of taxa in the Swedish (Swed TF) and in the North-America (NA TF) training sets

Taxa	Swed TF	NA TF
Orthocladiinae		
<i>Abiskomyia</i>	138 \pm 12	142 \pm 12
<i>Brillia</i>		106 \pm 11
<i>Chaetocladius</i>		129 \pm 9
<i>Corynoneura scutellata</i>	110 \pm 9	121 \pm 7
<i>Cricotopus</i>	118 \pm 7	115 \pm 12
<i>Cricotopus/Orthocladius</i>	110 \pm 13	
<i>Eukiefferiella</i>		119 \pm 9
<i>Orthocladius</i>	120 \pm 12	132 \pm 8
<i>Georthocladius</i>		108 \pm 13
<i>Heterotanytarsus</i>	175 \pm 13	182 \pm 8
<i>Heterotriassocladus undiff.</i>	111 \pm 8	
<i>Heterotriassocladus grimshawi-group</i>	100 \pm 13	116 \pm 11
<i>H. maeri-group</i>	108 \pm 6	112 \pm 15
<i>H. marcidus-group</i>	109 \pm 11	115 \pm 20
<i>H. subpilosus-group</i>	110 \pm 10	115 \pm 13
<i>Hydrobaenus</i>	134 \pm 9	141 \pm 6
<i>Nanocladius</i>	140 \pm 10	132 \pm 12
<i>Oliveridia</i>	136 \pm 8	143 \pm 10
<i>Mesocricotopus</i>		128 \pm 5
<i>Paracricotopus</i>		130 \pm 10
<i>Paracladius</i>		115 \pm 7
<i>Parakiefferiella</i>	126 \pm 10	116 \pm 11
<i>Paralimnophyes</i>		141 \pm 8
<i>Parasmittia</i>		112 \pm 9
<i>Paraphaenocladius</i>		127 \pm 11
<i>Psectrocladius undiff.</i>	140 \pm 8	
<i>Allopsectrocaldius</i>	130 \pm 6	122 \pm 6
<i>Psectrocladius septentrionalis-group</i>	130 \pm 11	128 \pm 7
<i>P. sordidellus-group</i>	129 \pm 12	131 \pm 8
<i>Pseudoorthocladius</i>		130 \pm 11
<i>Smittia</i>		119 \pm 13
<i>Thienemanniella</i>		120 \pm 12
<i>Zalutschia zalutschicola</i>	112 \pm 8	117 \pm 4
<i>Zalutschia lingulata pauca</i>	127 \pm 12	119 \pm 12
Chironominae		
<i>Chironomus anthracinus-group</i>	275 \pm 15	281 \pm 9
<i>Chironomus plumosus-group</i>	276 \pm 12	278 \pm 21
<i>Cladopelma</i>	222 \pm 17	231 \pm 11
<i>Criptochironomus</i>		162 \pm 8
<i>Cryptotendipes</i>		197 \pm 13

Table 2 continued

Taxa	Swed TF	NA TF
<i>Dicrotendipes</i>	276 \pm 21	264 \pm 14
<i>Einfeldia</i>	245 \pm 18	256 \pm 11
<i>Endochironomus</i>		265 \pm 26
<i>Endochironomus tendens-type</i>		278 \pm 30
<i>Glyptotendipes</i>		236 \pm 12
<i>Microtendipes</i>	231 \pm 21	226 \pm 31
<i>Paracladopelma</i>		212 \pm 21
<i>Parachironomus</i>		178 \pm 11
<i>Phaenopsectra</i>		216 \pm 7
<i>Polypedilum</i>	298 \pm 12	276 \pm 11
<i>Sergentia</i>	226 \pm 14	235 \pm 12
<i>Stempellinella</i>		212 \pm 16
<i>Stictochironomus</i>		232 \pm 11
<i>Cladotanytarsus</i>	163 \pm 21	143 \pm 8
<i>Constempellina brevicosta</i>	154 \pm 12	142 \pm 11
<i>Corynocera oliveri-type</i>	225 \pm 16	183 \pm 7
<i>Corynocera ambigua</i>	164 \pm 30	209 \pm 13
<i>Micropsectra undiff</i>	229 \pm 31	
<i>Micropsectra radialis-type</i>	149 \pm 12	161 \pm 12
<i>Micropsectra insignilobus-type</i>	178 \pm 13	163 \pm 17
<i>Pagastiella orophila</i>	167 \pm 11	
<i>Paratanytarsus</i>	225 \pm 16	217 \pm 22
<i>Tanytarsus undiff</i>	230 \pm 15	238 \pm 16
<i>Tanytarsus pallidicornis-type</i>	218 \pm 12	193 \pm 11
<i>Tanytarsus sp. B</i>	212 \pm 8	197 \pm 6
<i>Tanytarsus sp. C</i>	150 \pm 7	161 \pm 15
<i>Tanytarsus lugens-type</i>	221 \pm 11	198 \pm 21
Diamesinae/Prodiamesinae		
<i>Protanypus</i>	149 \pm 8	151 \pm 11
<i>Pseudodiamesa</i>	150 \pm 7	148 \pm 9
<i>Diamesa</i>	126 \pm 10	131 \pm 12
Tanypodinae		
<i>Ablabesmyia</i>		306 \pm 16
<i>Pentaneurini</i>	325 \pm 21	341 \pm 18
<i>Procladius</i>	360 \pm 17	364 \pm 25
<i>Telopelopia</i>		305 \pm 31
<i>Zavrelymia</i>		302 \pm 21

The taxa in bold are mostly found in halves. The numbers in bold are $<150 \mu\text{m}$

removing specimens $<150 \mu\text{m}$ could lead to warmer inferences than those obtained when all specimens are considered, especially if the smaller specimens are dominant in the fossil assemblages. Table 2 also suggests that removing all head capsules $<150 \mu\text{m}$

could lead to removing complete taxa because many of the Orthocladiinae have average sizes (with standard deviations) $<150\text{ }\mu\text{m}$. Those small sizes are often due

Table 3 Statistics of models

	Full model	Modified model
Canada		
r^2	0.85	0.82
RMSEP	1.12°C	1.23°C
Maximum Bias	3.0°C	2.59°C
Sweden		
r^2	0.63	0.65
RMSEP	1.13°C	1.11°C
Maximum Bias	2.1°C	2.03°C

to the fact that the head capsules of the Orthocladiinae are found only in halves, and those halves are generally $<150\text{ }\mu\text{m}$. In Lac 4 and 7 on Southampton Island, the chironomid assemblages are composed, on average, of 75–82% of Orthocladiinae of small sizes. Removal of many of these specimens should have an influence on the temperature reconstruction.

Model performance

Our second objective was to determine the effect of removing smaller specimens from the training sets. Similar to the results obtained by Verschuren and Eggermont (2007), removing all specimens $<150\text{ }\mu\text{m}$

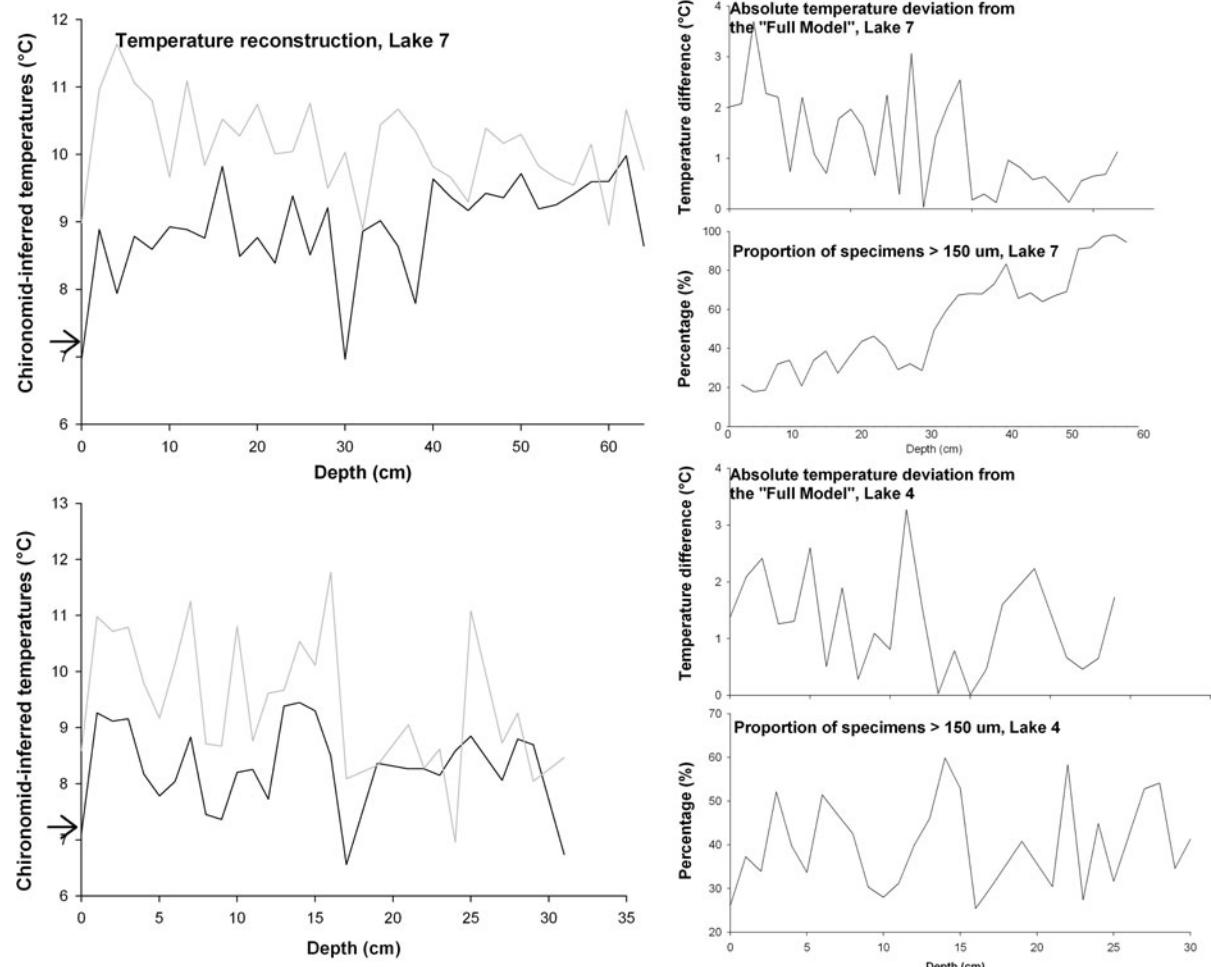


Fig 2 Left panels: temperature reconstructions of lakes located on Southampton Island, Canada, using the full model (black line) and the modified model (gray line) in Lake 7 (upper graph) and Lake 4 (lower graph). The arrows indicate

the climate normals (1961–1990). Right panels: temperature differences between the modified and the full model, and percentages of specimens $>150\text{ }\mu\text{m}$ in each sample of lake 7 (first two graphs) and lake 4 (last two graphs)

does not affect the numerical performance of the transfer functions considerably, and the r^2 is even slightly improved with the modified Swedish model (Table 3). Surprisingly, it seems that sieving the samples on a 150 μm mesh instead of the standard 100 μm mesh would not alter the statistics of the inference model. It is clear that weighted averaging techniques, and especially weighted average partial least squares employed to construct most chironomid-inference models, are robust (Birks 1995, 1998). However, good performance statistics for the modern inference model do not necessarily ensure reliable reconstructions of past change (Velle et al. 2005), as shown in the subsequent section.

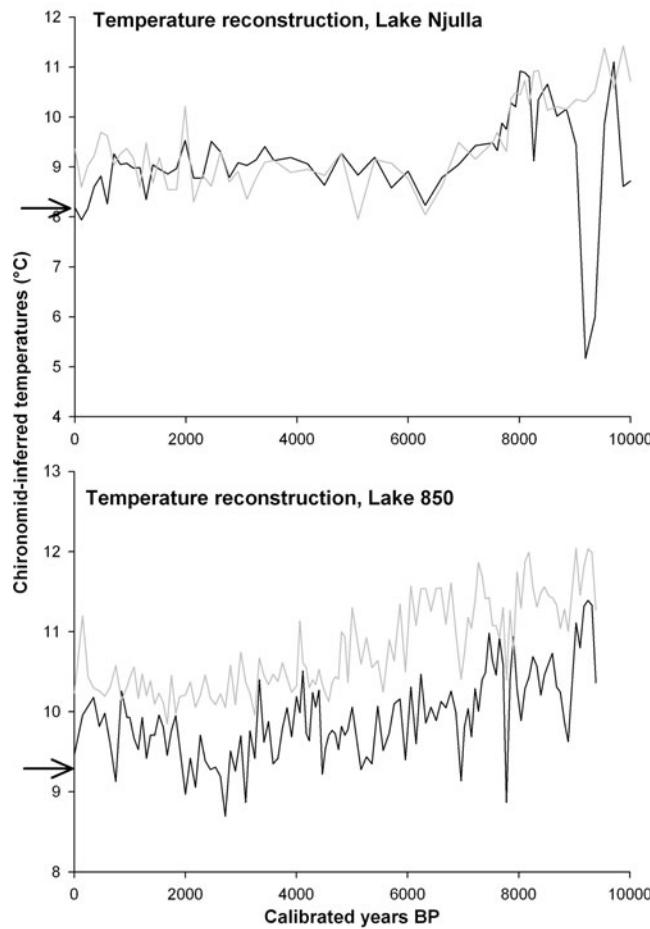
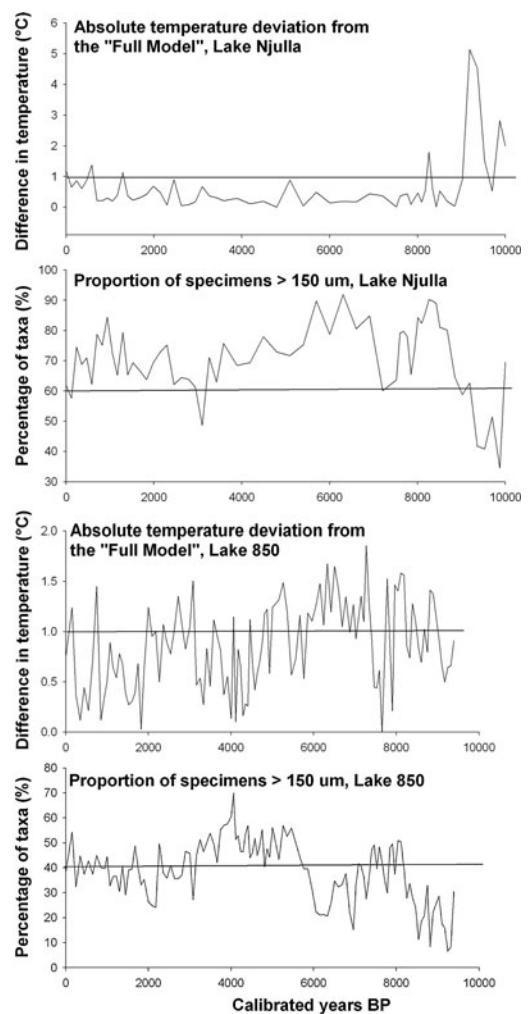


Fig 3 Left panel: Temperature reconstructions of lakes located in the Abisko region (Sweden), using the full model (black line) and the modified model (gray line) in Lake Njulla (upper graph) and Lake 850 (lower graph). The arrows indicate the start of the modified model period around 1000–2000 years BP. Right panel: temperature differences between the modified and the full model, and percentages of specimens $>150 \mu\text{m}$ in each sample of Lake Njulla (first two graphs) and Lake 850 (last two graphs)

Down-core temperature inferences

Although the performance statistics of the inference model are not significantly altered by removing specimens $<150 \mu\text{m}$, the general pattern of down-core temperature reconstructions is changed (Figs. 2, 3). In Lake 7, the full Canadian model reconstructs a temperature decrease through time while the modified Canadian model reconstructs a small warming (Fig. 2). The inferences obtained by the modified Canadian model are up to 4°C warmer than inferences obtained with the full Canadian model, and the percentages of specimens remaining after removing all taxa $>150 \mu\text{m}$ can be as low as 20%. In addition,



climate normals (1961–1990). Right panel: temperature differences between the modified and the full model, and percentages of specimens $>150 \mu\text{m}$ in each sample of Lake Njulla (first two graphs) and Lake 850 (last two graphs)

the inferred temperature at the top of the core obtained by using the full Canadian model corresponds to the mean August air temperature at this lake (7.3°C) while the modified model yields an inference 1.7°C warmer than the measured temperature. In Lake 4, the general pattern of temperature change obtained with both models is similar, but the modified Canadian model yields temperature inferences about $1.5\text{--}2^{\circ}\text{C}$ warmer than the inferences obtained with the full Canadian model (Fig. 2). Most modified assemblages contain $\leq 50\%$ the number of specimens in the full assemblages. Again, for Lake 4, the full model yields a better inference for modern temperature (7.3°C) than the modified model (1.4°C warmer than the climate normal).

The reconstructions for the two Swedish lakes are also affected by removing head capsules $<150 \mu\text{m}$, but to a lesser extent than reconstructions for the Canadian lakes (Fig. 3). The general temperature patterns are similar with the two models (i.e. a decrease of temperature through time). At Lake Njulla, most of

the samples still had at least 60% of the individuals in full assemblages, and the differences between the inferences obtained with models were smaller (less than the RMSEP), except at the beginning of the record when small specimens dominated the assemblages. At Lake 850, the percentages of specimens remaining after sieving are lower than at Lake Njulla, and these might be associated with the warmer inferences obtained with the modified Swedish model. For both records, the full Swedish model provided the best estimates for today's temperature (8.1°C for Lake Njulla, 0.3°C warmer than the climate normal for Lake 850), while the modified model provided estimates at least 1°C warmer than the climate normal.

The results obtained from the four lakes suggest that for down-core temperature inferences there may be a size-dependent bias when small specimens are removed because many of these smaller specimens have cold-temperature optima (Fig. 1c). In general, warmer inferences are obtained with the modified models and the general pattern may also change.

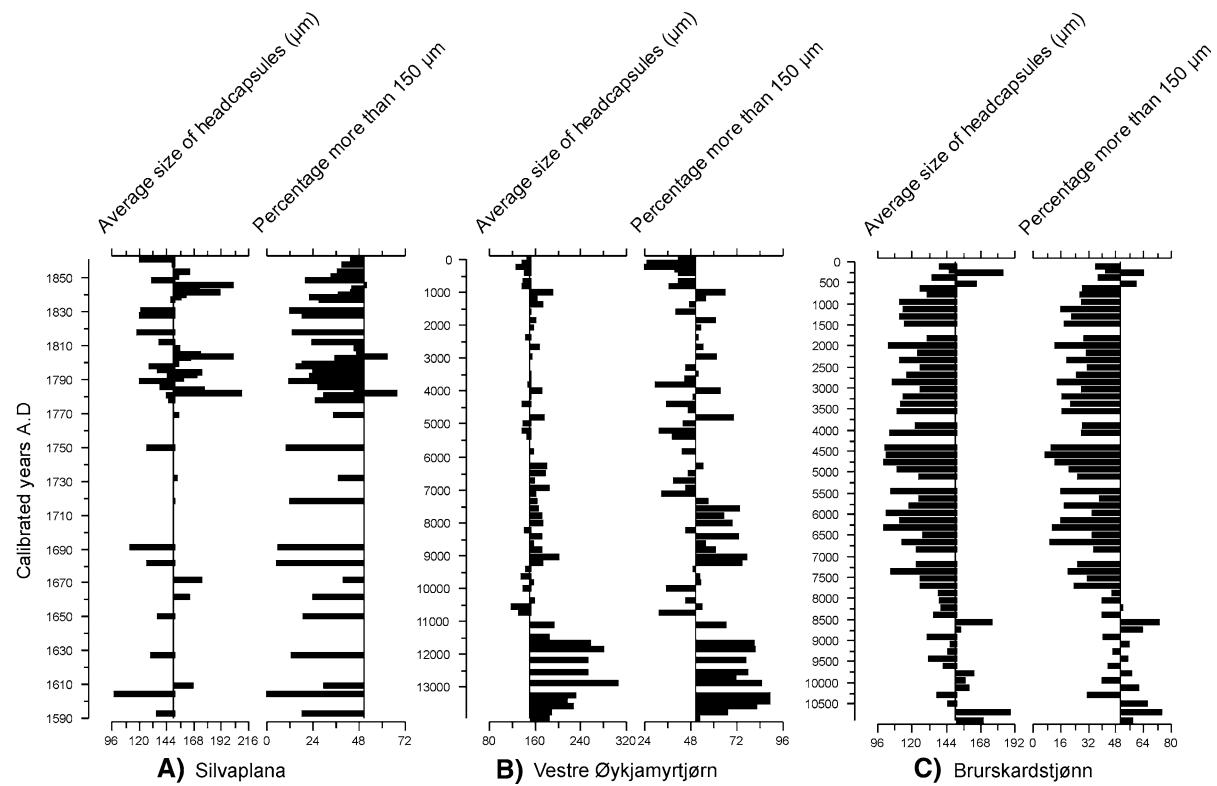


Fig 4 Average size of head capsules in each fossil sample and percentages of specimens $>150 \mu\text{m}$ in cores of **a** Lake Silvaplana, Switzerland, **b** Lake Vestre Øykjamyrkjøn, Norway and **c** in Lake Brurskardstjønn, Norway

Thus, sieving the sediment samples through a 150 µm mesh is not recommended when analyzing cold lakes.

Proportion of head capsules >150 µm and “time-saving” consideration

One advantage of sieving samples through a 150 µm mesh is, according to Verschuren and Eggermont (2007), that this method is “time-saving.” But this should be the case only if (a) the assemblages

obtained are representative of the whole samples, and (b) the amount of sediment needed to obtain at least 50–75 head capsules (number recommended for temperature reconstruction; Heiri and Lotter 2001; Larocque 2001; Velle and Larocque 2008) does not greatly exceed the amount generally used. In cold lakes of Switzerland and Norway, there is a close relationship between average head capsule size in a sample and the proportion of head capsules >150 µm (Fig. 4; Table 4). In all cores, a substantial proportion of the head capsules have sizes <150 µm and in

Table 4 Number of samples in each lake, number of these samples with average size of head capsules (hc) <150 µm and number of these samples with assemblages lower than 50% when only head capsules >150 µm are considered

Lake	Number of samples	Number of samples (and %) with average hc <150 µm	Samples (and %) with <50% of specimens >150 µm
Silvaplana	46	21 (45%)	42 (94%)
Brurskardstjønn	61	52 (84%)	49 (80%)
Vestre Øykjamyrkjørn	66	23 (34%)	28 (42%)

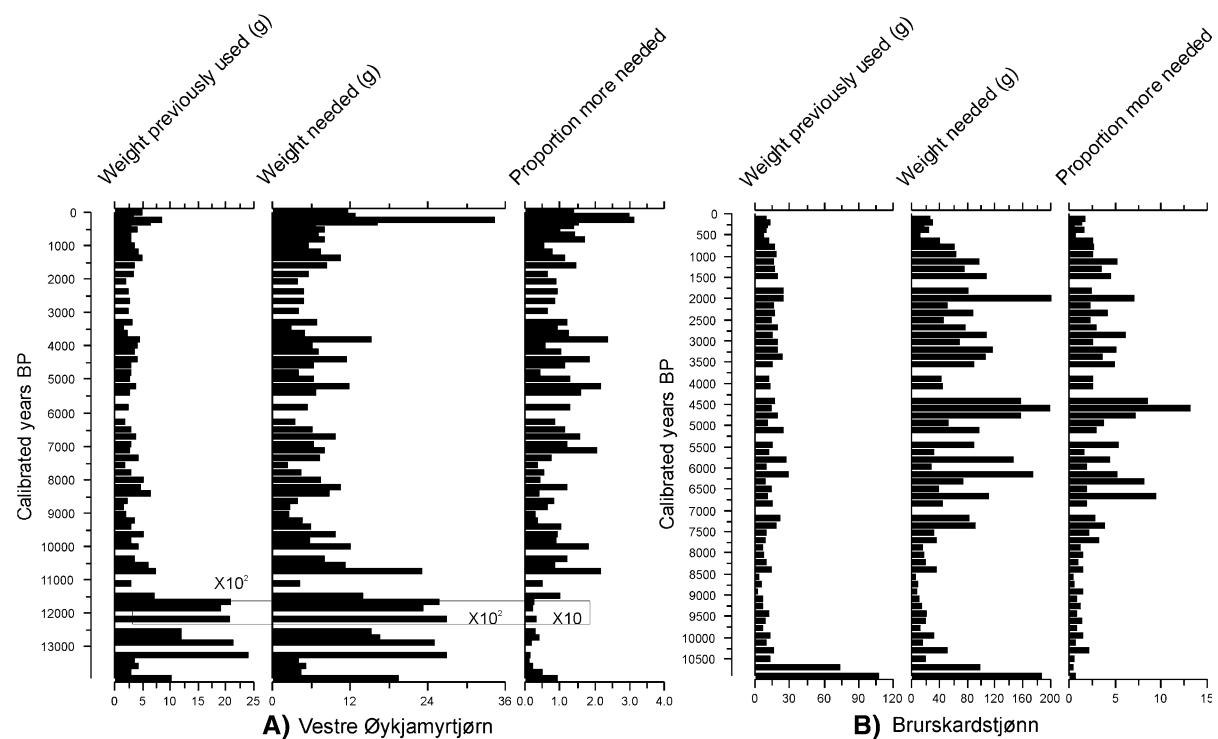


Fig. 5 Weight of wet sediment needed to find 75 head capsules >100 µm, weight needed if only specimens >150 µm are considered and the proportion more sediment needed when only specimens >150 µm are considered in **a** Lake Vestre Øykjamyrkjørn and **b** Lake Brurskardstjønn, Norway. Note that in (a), data for three samples must be multiplied by 100 (i.e. the

weights needed are 2,696, 1,528 and 1,661 g) for the weight of sediment used and the weight needed, and by 10 for the proportion more needed. The *baselines* are the average. These averages do not take into account the three samples with extreme data in (a)

Brorskardstjønn as much as 84% of the samples have average head capsule sizes $<150 \mu\text{m}$ (Table 4). These results violate condition a. By removing the fraction $<150 \mu\text{m}$, we obtain a chironomid assemblage that represents <50% of the total assemblage. Furthermore, the temperature inferences obtained from these samples would probably be over-estimated because the smaller head capsules (often with colder temperature optima, Fig. 1c) are removed.

Figure 5 indicates that if samples had been sieved on a 150 μm mesh, a much greater mass of sediment would be needed to obtain the 75 head capsules necessary for temperature reconstruction. Even without considering the three extreme data points in Vestre Øykjamyrkjørn, 9 g of sediment would be needed on average in this core. This represents twice the sediment used when all specimens $>100 \mu\text{m}$ are considered. In Brorskardstjønn, the difference is even more striking: 64 g would be needed on average to obtain 75 head capsules, representing three times the amount needed when all specimens $>100 \mu\text{m}$ are included. These results indicate that, at least for the sites we have examined, sieving samples from cold lakes on a 150 μm mesh would require use of a large amount of sediment. Such an amount of sediment may or may not be available when multi-proxy analyses are performed on a core. Furthermore, the amount of sediment needed to obtain a sufficient number of fossils suggests that the processing time may increase.

Conclusion

In African lakes, Verschuren and Eggermont (2007) found that salinity patterns inferred using chironomid assemblages were similar whether they were sieved in a 100 or 150 μm mesh. This method considerably reduced the time for chironomid sub-sampling. Although this method works for inferring salinity in warm lakes, it would be highly ineffective for inferring temperature in cold lakes because (a) the assemblages are composed of smaller chironomid head capsules, (b) most of these smaller head capsules have colder optima, (c) removing specimens $<150 \mu\text{m}$ might considerably alter temperature reconstructions, and (d) as much as 93% of the head capsules in a sample may be $<150 \mu\text{m}$. Furthermore, by removing specimens $<150 \mu\text{m}$, complete taxa

might be discarded because 27 of the 79 taxa considered here had average sizes $<150 \mu\text{m}$. To save time when preparing samples from cold lakes, alternate methods should be used, such as flotation (Rolland and Larocque 2007) or the microsphere technique (Velle and Larocque 2008).

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