

# Stable oxygen isotopes in fossil chironomids

development of a new proxy for past climate change

Frederike Verbruggen





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**LPP Foundation 2010**

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**ISBN 978-90-393-5286-1**  
**NSG Publication No. 20100225**  
**LPP Contribution Series 30**  
**Cover design: Leonard Bik**  
**Printed by GVO Drukkers en Vormgevers | Ponsen & Looijen**

# **Stable oxygen isotopes in fossil chironomids**

*development of a new proxy for past climate change*

# **Stabiele zuurstofisotopen in fossiele dansmuggen**

*ontwikkeling van een nieuwe proxy voor klimaatsverandering in het verleden*

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht  
op gezag van de rector magnificus, prof. dr. J.C. Stoof,  
ingevolge het besluit van het college voor promoties  
in het openbaar te verdedigen  
op donderdag 25 februari 2010 des middags te 2.30 uur

door

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geboren op 29 juli 1980 te Eindhoven

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This research was financially supported by the Darwin Center for Biogeosciences and the Laboratory of Palaeobotany and Palynology.

depende, de que depende  
de según como se mire, todo depende

*Jarabedepalo*



I was just guessing at numbers and figures  
pulling the puzzles apart  
Questions of science, science and progress  
do not speak as loud as my heart

*Coldplay*  
*'The scientist'*



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# General Introduction and Synopsis



# General introduction

Climate is changing rapidly. The Intergovernmental Panel on Climate Change (IPCC) recently published a comprehensive report stating that the current climate warming is unequivocal (IPCC, 2007). It assessed the possible causes and magnitude of future climate warming as well as the expected consequences. Many ecosystems are likely to be affected by changing environmental conditions (e.g. Parmesan *et al.*, 1999; Walther *et al.*, 2002). In order to obtain a better insight into naturally and anthropogenically forced climate variability and possible impacts of future environmental change on ecosystems we need to understand past climate variations and their effects on the biosphere. A better understanding of past and future climate change requires development of proxies that have the potential to provide long-term palaeoclimate records as instrumental measurements of climatic variables have often started after the onset of anthropogenically-induced warming and, hence, are often too short to assess natural variability or long-term trends. Therefore, natural archives need to be consulted when reconstructing environments older than ~100 years. In this thesis, a proxy for inferring past climate change is developed based on the stable oxygen isotopic composition of chironomids and is eventually applied to sediments of late glacial and early Holocene age.

## Chironomids and climate

Lakes are known to record environmental changes in their sediments. The sediment record of many lakes in Europe reaches back to the end of the last glacial period, approximately 16,000 years ago. Fossil remains from a wide variety of aquatic organisms have been used to reconstruct past environmental conditions from lake sediments (e.g. Smol, 2008). Chironomids, or non-biting midges (Dutch: 'dansmuggen'), are an example of an organism group that received increasing attention in palaeolimnology (Brooks and Birks, 2001; Brooks, 2006). Adult chironomids spend their lives in the air, where they mate. Subsequently, females deposit eggs in aquatic environments in which the larvae hatch. Chironomid larvae go through four larval stages before pupation and emergence. Chironomid larvae are ubiquitous and abundant in freshwater lakes. Their head capsules preserve well in sediments and remain identifiable. Many chironomid taxa are stenotopic and respond quickly to changes in their environments due to their relatively short generation time and high dispersal rates (e.g. Brooks *et al.*, 2007).

Many studies have empirically linked modern chironomid distributions to environmental conditions. Such modern training or calibration sets, are available for different regions, such as Iceland (Langdon *et al.*, 2008; Holmes *et al.*, 2009), Finland (Olander *et al.*, 1999; Luoto, 2009a), Sweden (Larocque *et al.*, 2001), the UK (Brooks *et al.*, 2001), Switzerland (Lotter *et al.*, 1997, 1998; Heiri *et al.*, 2003; Bigler *et al.*, 2006), Africa (Eggermont *et al.*, 2009), the Tibetan Plateau (Zhang *et al.*, 2007), New Zealand (Rees *et al.*, 2008), California (Porinchi *et al.*, 2002), Alaska (Kurek *et al.*, 2009), Beringia (Barley *et al.*, 2006), and Canada (Walker *et al.*, 1991; Quinlan *et al.*, 1998; Larocque *et al.*, 2006; Porinchi *et al.*, 2009). In each region, the distribution of chironomids is influenced by a set of environmental

variables. Although the effect of individual parameters may vary per region, temperature is considered the most important parameter in driving broad-scale chironomid distributions (Brooks, 2006), by exerting either a direct influence on chironomid assemblages by altering lake water temperatures, or indirectly through hypolimnetic oxygen and nutrient availability. Sediment organic matter and dissolved organic carbon content, pH, and conductivity are usually driven by changes in the lake's catchment and can influence chironomid distributions on a more local scale (Pinder, 1995).

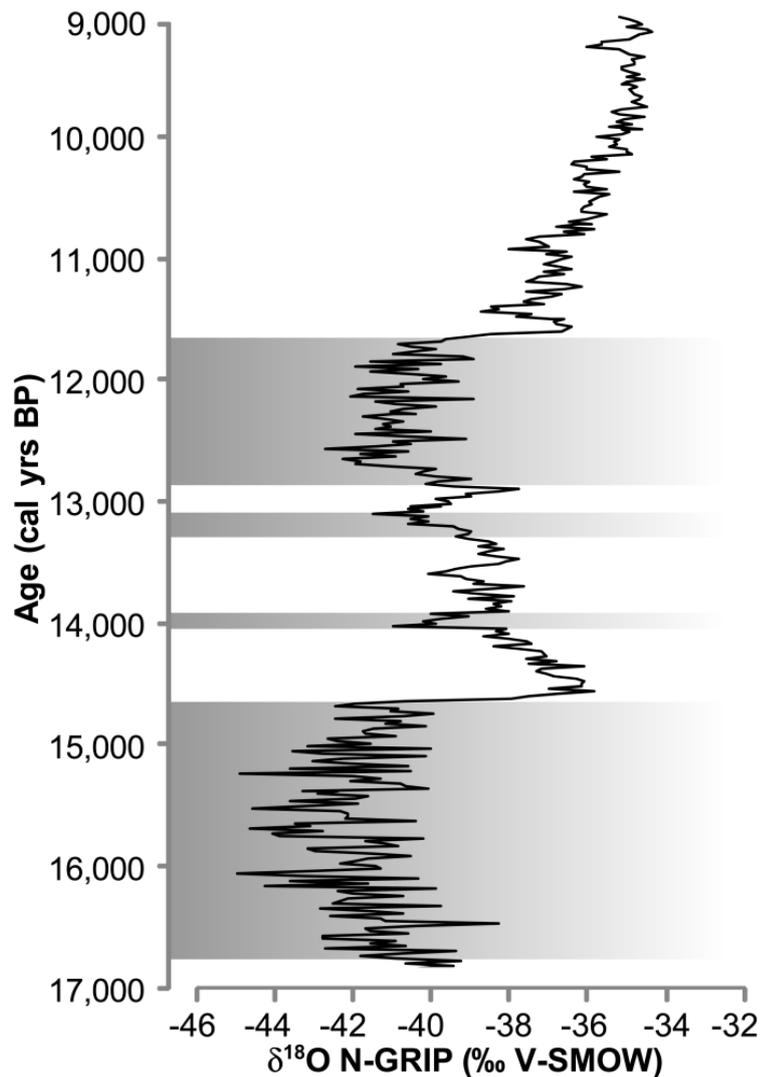
Once robust chironomid-environment relationships have been established and evaluated, this information can be used to reconstruct parameters of ecological relevance in and around lakes from fossil chironomid assemblages preserved in lake sediments via transfer functions. Available quantitative, chironomid-based reconstructions include records of summer lake water and air temperature (e.g. Heiri and Lotter, 2005; Heiri *et al.*, 2007), trophic conditions and oxygen levels (e.g. Brodersen and Quinlan, 2006), salinity (Eggermont *et al.*, 2008), acidity (Brodin and Gransberg, 1993), chlorophyll  $\alpha$  concentrations (Brodersen and Lindegaard, 1999), organic matter content (Nyman *et al.*, 2005), and water depth (Korhola *et al.*, 2000; Luoto, 2009b). Particularly, temperature reconstructions based on fossil chironomid assemblages have shown to work well in many regions (Lotter *et al.*, 1999; Brooks, 2006; Walker and Cwynar, 2006).

## Stable oxygen isotopes and climate

Temperature in Europe has shown large fluctuations since the Last Glacial Maximum, about 20,000 calibrated years before present (cal yrs BP; 'present' being AD 1950). From that period onward, alternations of relatively colder (stadials) and warmer (interstadials) periods are observed. This thesis focuses on the period from 17,000 to 9,000 cal yrs BP, which is characterized by two stadials and one interstadial. The latter comprises two smaller-scaled climatic reversals. These climatic reversals have been inferred by fluctuations in stable oxygen isotope ( $\delta^{18}\text{O}$ ) records obtained from the well-dated Greenland ice cores (Johnsen *et al.*, 2001; Fig. 1). Furthermore, similar oscillations are also observed in continental stable oxygen isotope records in Europe (e.g. Eicher and Siegenthaler, 1976; Lotter *et al.*, 1992) and allow  $\delta^{18}\text{O}$  records to be used as a means of correlating and dating continental archives.

The stable oxygen isotopic composition in continental archives, such as ice cores, speleothems, and lake sediments is mostly dependent on the stable oxygen isotopic composition of precipitation, the most important source of oxygen for these archives. The ratio of heavy ( $^{18}\text{O}$ ) and light ( $^{16}\text{O}$ ) stable oxygen isotopes in precipitation, expressed in per mille (‰) relative to a standard (e.g. Vienna-Standard Mean Ocean Water, V-SMOW; Coplen, 1996), is controlled by several parameters. Regular monitoring of precipitation since the 1960s (IAEA/WMO, 2006) has shown that temperature is often the most important factor determining  $\delta^{18}\text{O}$  in precipitation in mid and high latitudes (Dansgaard, 1964; Siegenthaler and Oeschger, 1980; Rozanski *et al.*, 1993). As air masses cool along their way from low latitudes toward the poles, they become progressively depleted in  $^{18}\text{O}$  due to preferential condensation of this isotope, leaving more of the lighter  $^{16}\text{O}$  in air mass vapor (Gat, 1996). Precipitation  $\delta^{18}\text{O}$  is also influenced by other factors, such as the amount effect (locations with more precipitation will have depleted precipitation  $\delta^{18}\text{O}$ , as the heavy isotope  $^{18}\text{O}$

preferentially rains out, see Dansgaard, 1964), the altitude effect (Poage and Chamberlain, 2001; Siegenthaler and Oeschger, 1980), and the continental effect (or distance from coast, see Alley and Cuffey, 2001). Although the major part of water in precipitation originates from oceanic evaporation at low to mid latitudes (Rozanski *et al.*, 1993), the exact water source in air masses and the path these air masses follow over the continents also play a role in altering precipitation  $\delta^{18}\text{O}$  (Alley and Cuffey, 2001). In general, however, precipitation will display a lower  $\delta^{18}\text{O}$  value towards colder regions, regions further from the sea, or towards higher elevations (Fricke and O'Neil, 1999).



**Figure 1** Subdivision of period 17,000-9,000 cal yrs BP based on the stable oxygen isotope ( $\delta^{18}\text{O}$ ) record from a Greenland ice core (N-GRIP). Periods with relatively low  $\delta^{18}\text{O}$  values (indicated by grey areas) are characterized by relatively low temperatures, and vice versa

## Chironomids and stable oxygen isotopes

Precipitation is the major source of stable oxygen isotopes in lake water. Lake water  $\delta^{18}\text{O}$  is therefore dependent on the  $\delta^{18}\text{O}$  of precipitation (Gonfiantini, 1986) but can be altered by changes in the local hydrology. For example, increased evaporation causes an enrichment of lake water  $\delta^{18}\text{O}$  as the lighter isotope ( $^{16}\text{O}$ ) preferentially evaporates (Gonfiantini, 1986). Input of isotopically light ice and snow melt water can also affect lake water  $\delta^{18}\text{O}$  (Bottomley *et al.*, 1986).

Oxygen is incorporated into the internal or external skeleton of most aquatic organisms. The isotopic composition of these structures depends on the oxygen isotope composition of lake water (Wang *et al.*, 2009). Exoskeleton parts of some aquatic organisms preserve well as fossils in lake sediments and can therefore potentially be used to infer past lake water  $\delta^{18}\text{O}$ , and hence, past climate change. Remains of several aquatic organism groups, such as ostracods or molluscs (von Grafenstein *et al.*, 1999, Schwalb, 2003) and diatoms (Barker *et al.*, 2001; Leng and Barker, 2006) have been used to obtain  $\delta^{18}\text{O}$  records, in addition to the often used  $\delta^{18}\text{O}$  of bulk carbonate in sediments. However, ostracod remains are not always present in lake sediments and extensive sediment purification is required to obtain diatom-based  $\delta^{18}\text{O}$  records since small amounts of contamination can have a large impact on stable oxygen isotope values (Leng and Barker, 2006). Furthermore, aquatic cellulose can be used to infer past lake water  $\delta^{18}\text{O}$  (Wolfe *et al.*, 2007). The interpretation of  $\delta^{18}\text{O}$  in sedimentary cellulose, however, may be complicated by the presence of terrestrial cellulose (Sauer *et al.*, 2001).

Chitin (Poly-*N*-acetyl-D-glucosamine) is structurally very similar to cellulose and preserves well in sediments under suitable conditions (Muzzarelli, 1977). As chironomid head capsules are chitinous they have the potential to be used to generate  $\delta^{18}\text{O}$  records. This approach was first explored by Wooller *et al.* (2004, 2008), who found a relationship between  $\delta^{18}\text{O}$  in chironomid remains and precipitation. A protocol regarding sample pretreatment, storage, and measurement of  $\delta^{18}\text{O}$  in fossil chironomids was established by Wang *et al.* (2008). Furthermore, Wang *et al.* (2009) demonstrated in a culturing experiment that almost 70% of the oxygen in chironomid head capsules is derived from ambient water and that the stable oxygen isotopic composition of chironomids is therefore largely dependent on the  $\delta^{18}\text{O}$  of the water they are living in. However, as the methodology regarding chironomid  $\delta^{18}\text{O}$  analysis is still under development, many aspects associated with the use and interpretation of this new climate proxy still remain to be evaluated.

## Research aim

The main aim of this thesis was to further develop stable oxygen isotopes in chironomids as a proxy for past changes in lake water  $\delta^{18}\text{O}$  and, therefore, for past climate change. As chironomids have previously been used to successfully reconstruct past temperatures based on modern species-environment relationships, this approach would provide an opportunity to use fossil remains of one organism group for two independent climate reconstructions. One reconstruction would then be based on the identification of chironomid fossils in lake sediments and the interpretation of changes in the taxonomic composition of fossil chironomid assemblages, the other on the signature of past lake water  $\delta^{18}\text{O}$  preserved in the chironomid exoskeleton. The use of chironomids to obtain  $\delta^{18}\text{O}$  records would eventually allow stable oxygen isotope records to be obtained from lakes where  $\delta^{18}\text{O}$  records based on other organism groups are difficult to develop, e.g. lakes on acid bedrock or lakes which are characterized by a significant inwash of terrestrial silica or cellulose.

## Summary

In a first step to achieve this aim, the effect of chemical pretreatments with acids and alkali, chemicals that are commonly applied in palaeoecology, on  $\delta^{18}\text{O}$ , chemical composition, and morphology of chironomid head capsules was explored. In a second step,  $\delta^{18}\text{O}$  analysis was performed on subfossil chironomid head capsules and ephippia from cladocerans isolated from surface sediments of thirty deep and stratified lakes along a transect ranging from northern Sweden to southern Italy. The results indicated that chironomid  $\delta^{18}\text{O}$  was strongly and positively related to lake water and precipitation  $\delta^{18}\text{O}$ . In a third step, stable oxygen isotopes in fossil chironomids from late glacial and early Holocene sediments from Rotsee (Switzerland) were compared to bulk carbonate  $\delta^{18}\text{O}$  from the same sediment core to examine whether chironomid  $\delta^{18}\text{O}$  can track centennial- to millennial-scale changes in past lake water  $\delta^{18}\text{O}$ . Carbonate particles adhering to chironomid remains resulted in a masking of the signal, leading to a poor correlation between chironomid and bulk carbonate  $\delta^{18}\text{O}$ . Therefore, a pretreatment to remove contaminating carbonates in a pH-neutral environment was applied to the head capsules. Eventually, a reliable late glacial and early Holocene chironomid  $\delta^{18}\text{O}$  record was obtained that agreed well with the one obtained from bulk carbonates, which allowed for inferences of past lake water  $\delta^{18}\text{O}$  and climatic variability. Chironomid analysis of lake surface sediments furthermore provided an opportunity to study the relation between chironomid assemblage composition and environmental parameters such as temperature, nutrient concentration, and oxygen availability in deep stratified lakes on a continental scale. Similarly, the examined down-core record allowed testing of different hypotheses about the extent that forcing factors in and around the investigated lake affected the composition of the late glacial chironomid assemblages.

# Synopsis

Prior to the research presented in this thesis, only limited information was available about the effects of chemical pretreatments on the stable oxygen isotopic composition of chironomid head capsules. **Chapter 1** shows that sample pretreatment with alkali, acids, and sodium chlorite can have an effect on the chemical composition of chironomid exoskeletons, as assessed by Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), the stable oxygen isotopic composition of chironomid remains, as measured by High Temperature Conversion-Elemental Analyzer/Isotope Ratio Mass Spectrometry (TC-EA/IRMS), and the morphology of head capsule cuticles as examined by Scanning Electron Microscopy (SEM). Gas chromatograms of pyrolyzates showed that hot alkali and sodium chlorite selectively removed proteins from cuticles, which resulted in relatively enriched  $\delta^{18}\text{O}$  values during analysis. SEM images showed that layers of chitin fibers were protruding from head capsules pretreated with alkali. Acids on the other hand, caused a depletion of chironomid  $\delta^{18}\text{O}$ . This is thought to have been caused by acid-induced oxygen exchange between chironomid remains and water and/or de-N-acetylation of chitin to chitosan, which is soluble in water. The selective removal of chitin by acids was also apparent in the gas chromatograms in which mainly protein-derived compounds were detected after acid treatment. A proteinaceous matrix without protruding chitin rods was apparent in SEM images of acid-treated head capsules. Based on these results the use of hot and strong alkali and acid treatments prior to chironomid  $\delta^{18}\text{O}$  analysis is discouraged.

Modern training sets have been used to establish empirical relationships between chironomid assemblages and their environment for different regions in Europe. In many of these datasets temperature was found to be an important variable in explaining chironomid distributions. Deep and stratified lakes however, have largely been neglected in these surveys. In addition, most calibration sets have focused on relatively small geographical regions and did not include continental-scale transects. In **Chapter 2**, profundal chironomid assemblages in surface sediments from deep lakes along a transect ranging from northern Sweden to southern Italy were examined to assess the relationship between chironomid assemblages and July air temperature, summer surface water temperature, total phosphorus concentration, hypolimnetic oxygen concentration, conductivity, pH, lake area, maximum lake depth, Secchi depth, catchment size, latitude, and organic matter content of the sediment. Canonical correspondence analyses (CCA) showed that chironomid assemblages were significantly related to latitude, temperature, nutrient content and oxygen availability in deep lakes. These variables co-varied in the dataset and were either positively or negatively correlated to the first axis of a detrended correspondence analysis (DCA) of the chironomid data. This suggested a direct (food and oxygen) and indirect (temperature) influence of climatic and trophic conditions on profundal chironomid assemblages. Despite the co-variation, a statistically significant relationship between temperature and chironomid assemblages was evident in the dataset, even when the effects of nutrient content and oxygen availability were statistically partialled out. This suggested that indirect effects via lake trophic state are not the only way that temperature affects chironomids populations in these deep, stratified lake systems. As trophic state is shown to explain a significant amount of

variance in the chironomid data a reconstruction of past total phosphorus (TP) concentrations was made using the Benthic Quality Index (BQI). The BQI relies on indicator scores for selected sensitive chironomid taxa. BQI values calculated for the study lakes showed a linear relationship with log-transformed TP. Application of this relationship to fossil chironomid assemblages from Lake Päijänne (Finland) produced a TP reconstruction that agreed well with measured TP values from AD 1970-1990. This demonstrated the potential of the BQI for providing quantitative reconstructions of past changes in the trophic state of deep, stratified lakes.

In addition to studying the relationships between subfossil chironomid assemblages from deep lakes and their environment, as discussed in Chapter 2, a study was carried out on the same lakes to relate the stable oxygen isotopic composition of aquatic invertebrate remains to the  $\delta^{18}\text{O}$  of the water the animals originally lived in. Understanding modern relationships between lake water and aquatic invertebrate  $\delta^{18}\text{O}$  is essential for the interpretation of palaeoclimate records based on stable oxygen isotopes in these fossils. In **Chapter 3**, the  $\delta^{18}\text{O}$  composition of head capsules of chironomids as well as resting eggs (ephippia) of planktonic cladocerans (water fleas) in the surface sediments of the studied lakes have been related to the  $\delta^{18}\text{O}$  composition of lake water and regional precipitation. A robust and statistically significant linear relationship was observed between precipitation  $\delta^{18}\text{O}$  and mean annual air temperature at the studied lakes. Precipitation  $\delta^{18}\text{O}$  was also positively correlated to lake water  $\delta^{18}\text{O}$ , although this relationship was less strong. The stable oxygen isotopic composition of chironomid and cladoceran remains showed a strong correlation with lake water  $\delta^{18}\text{O}$  and a less strong correlation with mean annual air temperature. Although slopes of regressions between aquatic invertebrate and lake water  $\delta^{18}\text{O}$  were similar for both groups, a systematic offset was seen between chironomid and cladoceran  $\delta^{18}\text{O}$  throughout the transect. This was attributed to differences in ecology, metabolism, and/or behavior of chironomids and cladocerans. This study showed that  $\delta^{18}\text{O}$  can be reliably measured on chironomid head capsules and ephippia from cladocerans, that oxygen isotopes in these remains reflect  $\delta^{18}\text{O}$  of the lake water, and that chironomid or cladoceran-based  $\delta^{18}\text{O}$  records have the potential to be used to infer past lake water  $\delta^{18}\text{O}$  from lake sediments, and hence, to reconstruct past climatic change.

The remaining two chapters focus on the lake sediments covering the late glacial and early Holocene period (17,000-9,000 calibrated years BP). In **Chapter 4** a multi-proxy analysis including chironomids, pollen, and plant macrofossils was carried out on littoral sediments from Rotsee, which was complemented by analyses of organic matter and carbonate content, and stable oxygen isotope in bulk carbonates. An age model was established based on high-resolution wiggle-match dating supported by tephrochronology. The aim of this study was to explore leads and lags of biotic response to distinct changes in the late glacial climate, as well as to assess the importance of external and lake-internal forcing factors for changes in the littoral chironomid communities. DCA was used to infer timing and magnitude of past compositional changes in the biotic data. Boundaries of biotic assemblage zones coincided with shifts in  $\delta^{18}\text{O}$  at the transitions to the interstadial and to the Younger Dryas cold phase, which suggested that climatic changes influenced biotic communities. Cold-indicating chironomids however were not abundant in the littoral zone of Rotsee during the Younger

Dryas and early Holocene, probably due to a lake level lowering, as suggested by the macrophyte communities. Furthermore, the effects of climate, catchment and lake processes on littoral chironomid assemblages were explored by (partial) CCAs using one or several independent proxies as predictors or explanatory variables for these forcings. In order of explanatory power, catchment and lake processes, and climate were found to be statistically significant in explaining the variation in the chironomid data, even when the effects of long-term trends were partialled out. Because of the location of the coring site in the shallow, littoral zone of Rotsee and the influence of lake level fluctuations, lake-internal dynamics and catchment-related processes seem to be important drivers that determine changes in the late glacial aquatic ecosystem of Rotsee. This may be the reason why catchment-related as well as lake-internal processes explained a larger amount of the total variance in the littoral chironomid communities than climate.

Finally, the applicability of stable oxygen isotope analysis on fossil chironomids was tested in **Chapter 5**. This was done for sediments deposited in Rotsee during the late glacial and early Holocene, which is a period when climatic shifts are distinct and well documented in scientific literature. High concentrations of head capsules in littoral Rotsee sediments ensured the presence of sufficient material for  $\delta^{18}\text{O}$  analysis. The aim of this study was to test whether the chironomid  $\delta^{18}\text{O}$  record is in agreement with  $\delta^{18}\text{O}$  record measured on bulk carbonates. Prior to  $\delta^{18}\text{O}$  analysis, chironomids were pretreated using standard palaeoecological methods (treatment with weak KOH solution, sieving, and ultrasonic treatment). The presence of lake marls in the Rotsee sediment may result in contamination of chironomid head capsules and lead to uncontrolled fractionation during measurement of chironomid  $\delta^{18}\text{O}$ . A second chironomid  $\delta^{18}\text{O}$  record was therefore obtained with head capsules that had been exposed to a pretreatment to remove carbonate particles. An initial test confirmed that the use of acids prior to chironomid  $\delta^{18}\text{O}$  analysis may induce oxygen exchange between chironomid remains and water as suggested in Chapter 1. Therefore, carbonate particles were eliminated using a buffered ammonium chloride ( $\text{NH}_4\text{Cl}$ ) solution in a circum-neutral pH environment. The record obtained using standard palaeoecological pretreatments resulted in a poor correlation between chironomid and bulk carbonate  $\delta^{18}\text{O}$ , whereas the one obtained from chironomids pretreated with  $\text{NH}_4\text{Cl}$  showed an excellent agreement with bulk carbonate  $\delta^{18}\text{O}$ , in which colder and warmer periods were reflected by relatively depleted and enriched  $\delta^{18}\text{O}$  values, respectively. Differences in amplitude of variations in the chironomid and carbonate  $\delta^{18}\text{O}$  records are attributed to differential effects of temperature on oxygen isotope fractionation during the formation of bulk carbonates and chironomid cuticles or seasonal changes of lake water  $\delta^{18}\text{O}$  within Rotsee, potentially affecting  $\delta^{18}\text{O}$  of carbonates and chironomids to a different extent. The good agreement between the chironomid and carbonate-based  $\delta^{18}\text{O}$  records suggested that this approach is useful in various sedimentary settings. Chironomid  $\delta^{18}\text{O}$  can be an alternative in lakes where carbonates are absent and in lakes with a high input of terrestrial material. Ultimately, it can be used in carbonate-rich lakes provided that residual carbonate particles are adequately removed using an  $\text{NH}_4\text{Cl}$  solution prior to chironomid  $\delta^{18}\text{O}$  analysis. If oxygen isotope fractionation in chironomid head capsules is independent of temperature this would allow chironomid  $\delta^{18}\text{O}$  to be used in combination with carbonate  $\delta^{18}\text{O}$  to reconstruct water temperature in lakes.  $\delta^{18}\text{O}$  of carbonate reflects past lake water  $\delta^{18}\text{O}$  modified by a temperature-dependent fractionation, whereas chironomid

$\delta^{18}\text{O}$  would then allow an estimate of past lake water  $\delta^{18}\text{O}$  unaffected by temperature. Since the relationship between lake water  $\delta^{18}\text{O}$ , carbonate  $\delta^{18}\text{O}$ , and temperature is well studied and follows a straightforward relationship this would allow inferring water temperature during formation of lacustrine carbonate.

## Conclusions

This thesis shows that stable oxygen isotopes can be reliably measured on fresh, subfossil and fossil chironomid head capsules. The following conclusions regarding chironomid  $\delta^{18}\text{O}$  analysis can be drawn:

1) Chemical pretreatments should be avoided when possible, as they may affect the  $\delta^{18}\text{O}$  signature chironomids. However, when working with lake marl deposits a solution of ammonium chloride adequately removes residual carbonates that may obscure the original  $\delta^{18}\text{O}$  signal stored in chironomids. This treatment does not have a noticeable effect on chironomid  $\delta^{18}\text{O}$ .

2) In deep European lakes  $\delta^{18}\text{O}$  of chironomid remains is strongly correlated with lake water  $\delta^{18}\text{O}$ . The strong correlation of precipitation  $\delta^{18}\text{O}$  with, on the one hand, lake water  $\delta^{18}\text{O}$  and, on the other hand, mean annual air temperature, suggests that chironomid  $\delta^{18}\text{O}$  has the potential to be used to infer past lake water  $\delta^{18}\text{O}$ , and indirectly, past climatic change.

3) A  $\delta^{18}\text{O}$  record was successfully obtained from fossil chironomids of late glacial and early Holocene age. Relative changes in the  $\delta^{18}\text{O}$  record analyzed on fossil chironomids agree well with variations in bulk carbonate  $\delta^{18}\text{O}$ , indicating that climatic changes have influenced the stable oxygen isotopic composition of chironomids in the past. This indicates that chironomid remains can be used to produce reliable  $\delta^{18}\text{O}$  records, even in lakes where no carbonates are produced or in lakes with a high input of terrestrial material.

4) Many aspects regarding this new proxy for lake water  $\delta^{18}\text{O}$  remain to be studied. For example no or limited information is available about the effects of temperature on  $\delta^{18}\text{O}$  of the chironomid exoskeleton during the formation of the cuticle, the effects of seasonal changes of lake water  $\delta^{18}\text{O}$  on the stable oxygen isotope composition of chironomid remains, and the within-lake variability of fossil chironomid  $\delta^{18}\text{O}$ . However, this thesis documents the potential of this approach for producing high-quality reconstructions of temperature and hydrology in lakes and shows the first evidence that chironomid  $\delta^{18}\text{O}$  provides reconstructions of the oxygen isotope composition of lake water that are in agreement with records based on other sediment components.

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# Chapter 1



# Effects of chemical pretreatments on $\delta^{18}\text{O}$ measurements, chemical composition, and morphology of chironomid head capsules

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*Published in Journal of Paleolimnology (2009)*

## Abstract

Stable oxygen isotope measurements on fossil chironomid head capsules from lake sediments show that these chitinous remains can be used to reconstruct past lake water  $\delta^{18}\text{O}$  and, indirectly, past climate change. We examined the impact of chemical pretreatment procedures on the chemical and stable oxygen isotope composition, and morphology of chironomid cuticles. Use of alkali, acids, and sodium chlorite alters the chemical composition and the morphological structure of chironomid cuticles by selective removal of chitin or proteins. Gas chromatograms of pyrolyzates show that  $\text{NaClO}_2$  causes deproteination, whereas the combined use of  $\text{HCl}$  and  $\text{HF}$  results in partial chitin removal. Head capsules pretreated with  $\text{KOH}$  contained both chitin- and protein-derived moieties, although the concentration of protein was reduced, especially after  $\text{KOH}$  treatment at high concentration (28%) and temperature ( $100^\circ\text{C}$ ). Scanning Electron Microscopy confirmed that a proteinaceous matrix is still present in modern and fossil head capsules after  $\text{KOH}$  treatment. This matrix, however, is largely absent in head capsules pretreated with  $\text{NaClO}_2$ . A change in the proportion of chitin and proteins in our samples was associated with differences in chironomid  $\delta^{18}\text{O}$  values. Our results suggest that deproteination results in a relative increase of chironomid  $\delta^{18}\text{O}$ , whereas removal of chitin leads to decreased  $\delta^{18}\text{O}$  values. We therefore discourage the use of acids or prolonged ( $\geq 1$  h) exposure to hot alkali ( $70^\circ\text{C}$ ) prior to chironomid  $\delta^{18}\text{O}$  analysis. Chitin purification by sodium chlorite causes significant weight loss, which may preclude down-core chironomid  $\delta^{18}\text{O}$  measurements. Caution and standardization are required when pretreating samples for chironomid  $\delta^{18}\text{O}$  analysis to ensure reliable, comparable, and reproducible results.

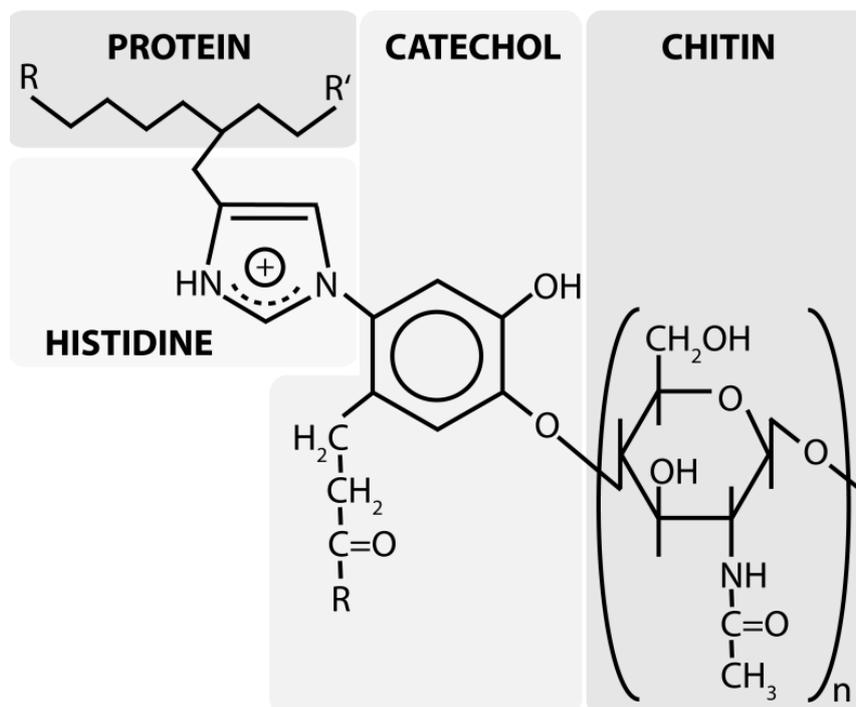
# Introduction

Uncertainties about impacts of future environmental change, including global warming, have led to growing public concern. Understanding past climate variations is important for assessing the potential impacts of future climatic changes. Lakes preserve a record of environmental change in their sediments, which can be used to infer climate changes in the pre-instrumental period (Smol, 2008). A number of biotic and abiotic variables in lake sediments provide valuable information on past environmental changes (Battarbee, 2000; von Gunten *et al.*, 2008; Bjune *et al.*, 2009; Moore *et al.*, 2001; Loso, 2009). Although some of these proxy variables have been applied successfully to reconstruct past climate shifts, considerable effort is required to develop accurate temperature indicators. Temperature proxies are most reliable if applied within a multi-proxy framework (Lotter, 2003; Birks and Birks, 2006). Application of multiple temperature indicators, however, is limited to certain regions, lakes, or sediment types, and although a few methods can be used universally (Blaga *et al.*, 2009), they often apply only to the warm season.

Stable oxygen isotope analysis of lacustrine carbonates, such as shells of molluscs or ostracods, is a common approach for reconstructing past lake water isotopic composition and for obtaining information on past temperature changes (von Grafenstein *et al.*, 1999; Schwalb, 2003; Leng and Marshall, 2004). Furthermore,  $\delta^{18}\text{O}$  in bulk carbonate is an excellent tool for correlating regional lake sediment records during phases of distinct climatic changes (Lotter *et al.*, 1992). Many lake sediments, especially those from silicate bedrock regions, do not contain carbonates. In such cases, isotopic measurements on other autochthonous substances preserved in lake sediments provide an alternative approach for reconstructing past lake water  $\delta^{18}\text{O}$ . For instance, several methods have been developed to extract, e.g. algal cellulose from sediments for  $\delta^{18}\text{O}$  analysis (Wolfe *et al.*, 2001; Wissel *et al.*, 2008). Chitin, a component of exoskeletons of many aquatic invertebrates, is another common biomacromolecule in lake sediments. Chitin (Poly-*N*-acetyl-D-glucosamine; Fig. 1) is a biopolymer that preserves well under suitable conditions (Muzzarelli, 1977), such as environments characterized by high sedimentation rates (van Waveren, 1994), high productivity, and reducing conditions in bottom waters (Stankiewicz *et al.*, 1997a). Chitin in insect cuticles is thought to be cross-linked with proteins through quinonoid derivatives of catecholamines (Schaefer *et al.*, 1987). Besides chitin and proteins, lipids and pigments constitute a minor part of insect cuticles (Richards, 1978).

Chironomids (non-biting midges; Diptera: Chironomidae) are insects whose larvae are abundant in lakes. Their exoskeletons contain chitin. Chironomid larvae live in benthic freshwater environments and undergo several molts (ecdysis) during which the head capsule is shed (Hopkins and Kramer, 1992). Both head capsules released during ecdysis and those from deceased specimens are usually well preserved in lake sediments. Past environments have been inferred from changes in the taxonomic composition of fossil chironomid assemblages, using information on the ecology and distribution of different taxa (Brooks, 2006; Walker and Cwynar, 2006; Heiri *et al.*, 2007; Engels *et al.*, 2008).

Wooller *et al.* (2004, 2008) and Wang *et al.* (2008) demonstrated the use of chitinous chironomid head capsules to generate  $\delta^{18}\text{O}$  records, which have great potential for palaeotemperature reconstruction. The effects of chemical pretreatment procedures on



**Figure 1** Chemical structure of poly-N-Acetyl-D-Glucosamine (chitin) linked to proteins in insect cuticles through catecholamines and histidine moieties (modified after Schaefer *et al.*, 1987)

chironomid  $\delta^{18}\text{O}$ , however, still must be fully evaluated. Detailed information on changes in the chemical composition and  $\delta^{18}\text{O}$  of chironomid head capsules associated with different chemical pretreatments will allow further development of this method and will reduce uncertainties associated with  $\delta^{18}\text{O}$  analysis.

In the 1980s and 1990s the  $\delta^{18}\text{O}$  composition of purified arthropod chitin was analyzed in several studies (Schimmelmann and DeNiro, 1985, 1986a, 1986b; Schimmelmann *et al.*, 1986) and the chemical composition of chitinous insect cuticles was characterized (van der Kaaden *et al.*, 1984; Schaefer *et al.*, 1987; Kramer *et al.*, 1995; Stankiewicz *et al.*, 1996, 1997a, 1997b, 1997c, 1998; Briggs *et al.*, 1998). Schimmelmann and DeNiro (1986a) recommend purifying chitin in modern and fossil cuticles to D-glucosamine hydrochloride (GlcN.HCl; Schimmelmann and DeNiro, 1986b). However, D-glucosamine preparation and extraction is labor-intensive and may cause contamination by introduction of non-authigenic oxygen. Moreover, purification leads to weight loss and may eventually yield a sample too small to be measured (Hodgins *et al.*, 2001). Wooller *et al.* (2004) completed a study of how various chemicals influence chironomid  $\delta^{18}\text{O}$ , but did not detect any effect, and a protocol was proposed for preparing chironomid remains for  $\delta^{18}\text{O}$  measurement, using alkali and acids (Wang *et al.*, 2008). We studied the effects of several commonly used chemicals (e.g. KOH, HF, and HCl) on the chemical composition and  $\delta^{18}\text{O}$  of chironomid head capsules. Because chitin is similar to cellulose, differing only in the presence of N-acetyl groups in chitin, a method developed to process and purify cellulose samples prior to  $\delta^{18}\text{O}$  analysis (Leavitt and Danzer, 1993) was also tested. We used Scanning Electron Microscopy (SEM) to detect morphological changes in head capsule cuticles.

In this study, we addressed the following questions: a) what effects do the various pretreatments have on the relative abundances of chitin and proteins in chironomid head capsules, and on the morphology of their cuticles?, and b) what are the implications of changes in chemical composition for chironomid  $\delta^{18}\text{O}$  analysis? Sorting chironomid head capsules from lake sediments under a dissecting microscope is very labor intensive. We thus determined the minimum chironomid sample weight that can be used to produce a reliable isotopic measurement and we discuss the suitability of the different chemical pretreatment methods in the context of the minimum weight necessary for  $\delta^{18}\text{O}$  analysis.

## Materials and methods

Chironomid (*Chironomus riparius*) larvae, commonly used as fish food, were obtained from a commercial source (Discusfarm Marsilea, Lelystad, The Netherlands). To guarantee preservation, frozen larvae were obtained. We assume that the freezing process did not affect the chemical and stable oxygen isotopic composition, or morphology of the head capsules. A purified chitin standard (Sigma-Aldrich, C9752, St. Louis, MO, USA), derived from marine crab shells, was compared to the chemical composition of chironomid head capsules. Pretreatment chemicals were added to the head capsules in a 100-ml glass beaker containing a stirring magnet, unless indicated otherwise. Fossil chironomid head capsules from the sediments of Rotsee, Switzerland, which date to ~16,000 cal yrs BP (Lotter and Zbinden, 1989), were used for comparison with fresh head capsules.

### Pretreatments

Palaeoecological analyses often involve chemical pretreatment of sediments. KOH, HCl, and HF are commonly used. They alter the sample pH and may induce changes in chemical composition of organic materials. We tested the effects of these chemicals on the morphology, as well as the chemical and stable oxygen isotopic composition of chironomid head capsules. We also evaluated a method that is used to purify cellulose prior to  $\delta^{18}\text{O}$  analysis.

#### *Demineralized water pretreatment*

A batch of chironomid larvae was treated with demineralized water for 24 h at room temperature (~20 °C) to represent chemically untreated chironomid head capsules. Larval head capsules were subsequently removed from the bodies under a stereo microscope using forceps. Digestive tracts and muscle tissue were carefully detached from the head capsules to avoid contamination by organic material in the larval gut. Head capsules containing sandy detritus were eliminated from further analysis.

### *Alkaline pretreatments*

Potassium hydroxide (KOH) is often used to deflocculate sediment organic material prior to sieving and is used in the standard preparation of samples for microscopic identification of fossil chironomids (Walker, 2001; Brooks *et al.*, 2007). This alkaline treatment causes deproteination of insect cuticles (Schimmelmann *et al.*, 1986; Einbu and Vårum, 2008). Because this process accelerates with increasing temperature, the effect of different temperatures and exposure times on head capsule composition was examined. Larvae were soaked in 10% KOH for 2 h at room temperature (~20°C) or for 1 h at 70°C. An additional batch of larvae was soaked in 28% KOH for 24 h at 100°C. After exposure to KOH, larvae were rinsed 10 times with demineralized water and head capsules were removed manually under a dissecting microscope.

### *Acid pretreatment*

Hydrochloric acid (HCl) and hydrofluoric acid (HF) are commonly used to remove carbonates and other minerals (e.g. silicates), respectively. Acids may induce exchange of OH groups (Roberts and Urey, 1939). This exchange may lead to  $\delta^{18}\text{O}$  values that do not reflect the original signal in the head capsules. We analyzed chironomid larvae that were exposed to 30% HCl, and subsequently to 40% HF, for 1 and 2 h at room temperature, respectively, in a plastic pot on a shaker apparatus. After acid treatment, larvae were washed thoroughly with demineralized water. Head capsules were subsequently removed from larval bodies as described above.

### *Sodium chlorite pretreatment*

Head capsules were pretreated using a common method to purify cellulose for  $\delta^{18}\text{O}$  analysis. This method, modified from the “Jayme-Wise” technique (Green, 1963), is described in detail by Leavitt and Danzer (1993) and will be referred to as the LD method. The method involves transfer of head capsules to glass fiber filters, followed by Accelerated Solvent Extraction (ASE) using a mixture of dichloromethane and methanol (9:1) to remove waxes, oils, and resins. This first step is followed by boiling in deionized water for 6 h to remove inorganic salts and low molecular weight polysaccharides. Next, several additions of sodium chlorite and glacial acetic acid are used to purify the chitin. Finally, samples are washed multiple times with demineralized water and oven dried over night at 60°C.

### **Curie-point Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)**

Flash pyrolysis allows macromolecular organic substances to be characterized by their low molecular weight pyrolysis products, after rapid heating in an inert environment. The chemical composition of head capsules and chitin can be assessed by examining mass spectrometric

fragment ions that characterize components reflected as peaks in the chromatograms. Samples were pressed onto a flattened ferromagnetic wire and pyrolyzed at 610°C for 10 sec in a continuous He flow using a Curie Point Pyrolyzer (FOM) coupled directly to a Thermo Finnigan Trace GC Ultra gas chromatograph, interfaced to a Thermo Finnigan Trace DSQ mass spectrometer. Compounds were separated using a silica capillary column with an inner diameter of 0.32 mm coated with a 0.4- $\mu\text{m}$  film (Varian, CP-Sil-5CB). The oven was programmed from 40°C (constant for 5 min) to 230°C at a rate of 3°C/min and further increased to 300°C at a rate of 20°C/min, maintaining this endpoint temperature for 10 min. The MS was operated in full-scan mode ( $m/z$  50-800, 2.5 scans/sec, 70eV electron energy, 250°C source temperature). Compounds were identified by their retention times and mass spectra using literature data (van der Kaaden *et al.*, 1984; Boon and de Leeuw, 1987; Chiavari and Galletti, 1992; Stankiewicz *et al.*, 1996; Stankiewicz *et al.*, 1997a, 1997b; Stankiewicz *et al.*, 1998; Bierstedt *et al.*, 1998; Flannery *et al.*, 2001)

### Stable oxygen isotope analysis (TC-EA/IRMS)

A high-temperature conversion elemental analyzer (TC-EA; Thermo Finnigan) coupled to an isotope ratio mass spectrometer (IRMS; Thermo Finnigan Delta<sup>plus</sup>) was used to determine ratios of stable oxygen isotopes of organic substances in relatively small amounts (50-100  $\mu\text{g}$  range; Kornexl *et al.*, 1999). Both head capsules and a chitin standard (Sigma-Aldrich) were used to assess the minimum weight needed for  $\delta^{18}\text{O}$  analysis. All samples were analyzed using 4- x 3.2-mm silver cups (Elemental Microanalysis Ltd, batch number 128843). Two blanks were measured at the start of every run. Standardization was achieved using a cellulose standard (IAEA-C3), benzoic acid standard (HEKAtech, batch number 33822501), and two international potassium nitrate standards (IAEA-NO-3 en USGS-32).  $\delta^{18}\text{O}$  data are reported in per mille (‰) relative to the V-SMOW (Vienna Standard Mean Ocean Water) standard (Coplen, 1996). Two outliers were detected using Grubbs' test and were removed from the data set. Contamination probably explains these outliers. Only measurements that passed strict data quality control criteria for minimum voltage (>950 mV) and stable backgrounds were used for data interpretation.

### Scanning Electron Microscopy (SEM)

Morphology of the cuticles of chironomid head capsules subjected to the various pretreatments was studied by Scanning Electron Microscopy (SEM). Head capsules were cut into smaller fragments and rinsed in demineralized water before mounting. Fragments were mounted onto negative film attached to a stub with Araldite in such a way that the inner structures of the cuticles (i.e. perpendicular to the surface) were displayed. Fragments were coated with 12 nm platinum using a sputter coater (Cressington 208hr), and examined under a SEM (Philips XL30S FEG) at 65,000x magnification.

# Results

## Pyrolysis-GC/MS

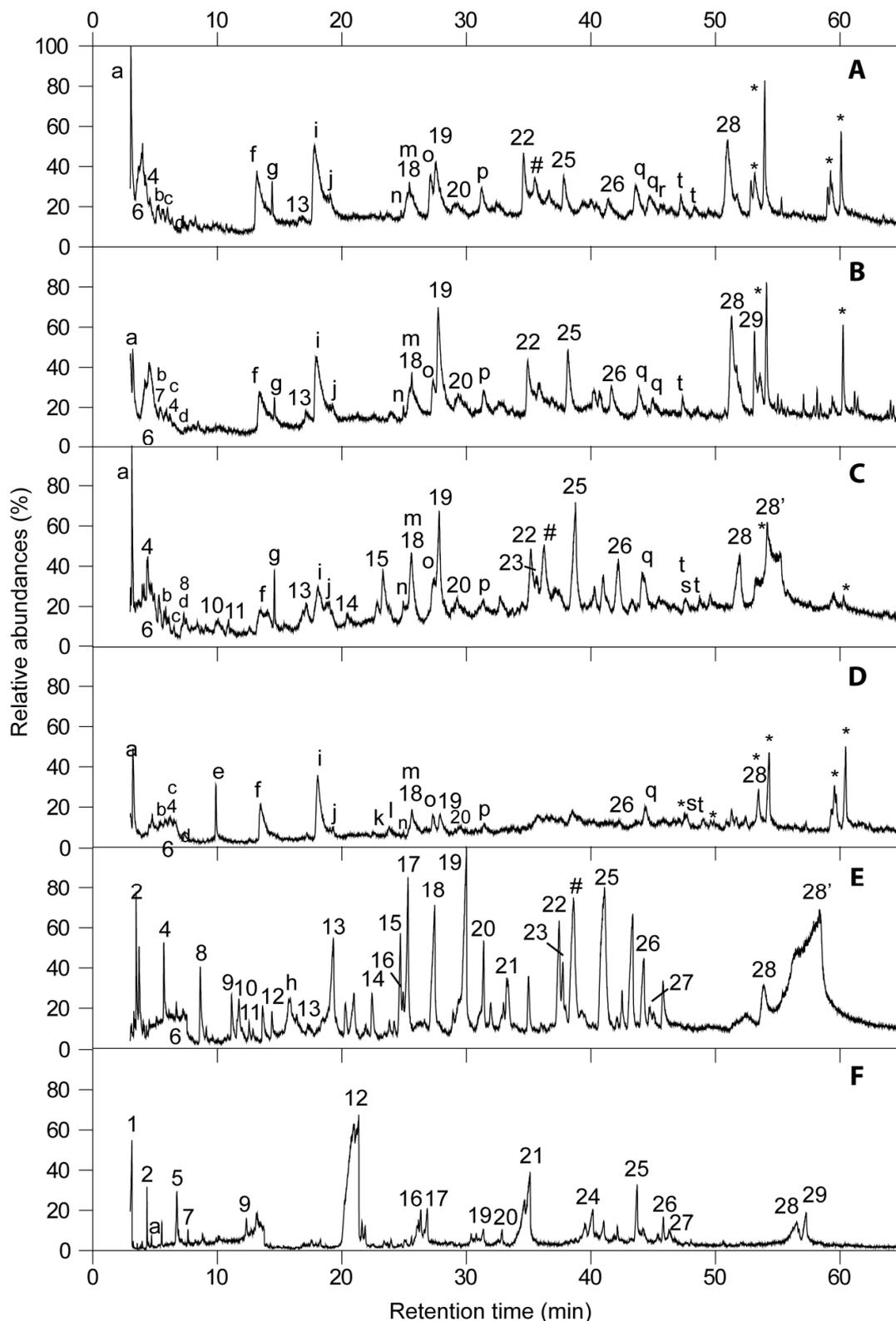
### *Pretreatment effects*

The impact of the various pretreatments on the chemical composition of chironomid head capsules was deduced from the chromatograms of the pyrolyzates in Fig. 2. Pyrolysis products are reflected by peaks in these chromatograms and are identified based on their characteristic mass spectra (Table 1). All compounds are derived from either chitin or proteins. 1,6-Anhydro-2-acetamido-2-deoxyglucose (28) is the most abundant pyrolysis product and shows up as a high and broad peak in most chromatograms.

The chromatograms indicate that the proportion of chitin and protein in chironomid head capsules varies with pretreatment. Many chitin-derived compounds are present in head capsules that were treated with NaClO<sub>2</sub>, as well as in the crab chitin standard. However, some of these components, such as acetylpyridone (17) and N-hydroxyphenylacetamide (21) are not observed in the chromatograms of the head capsules treated with demineralized water or KOH. Even fewer chitin-derived compounds are seen in the acid-treated samples. On the other hand, only a fraction of the protein-associated peaks that are recognized in the chromatograms of head capsules pretreated with demineralized water, alkali, or acids are present in the chromatograms of head capsules that underwent the LD pretreatment and of purified crab chitin.

Although Py-GC/MS cannot quantify absolute concentrations, differences in the areas under various peaks allow estimation of relative abundances of compounds in head capsules. A chitin:protein ratio was calculated to infer quantitative changes in relative abundances of chitin to proteins. This ratio was calculated by dividing the sum of areas of compounds 28 and 28', i.e. dominant chitin-derived compounds, by the sum of areas of compounds i and j, i.e. dominant protein-derived components. A ratio of 1 indicates equal abundances of chitin and protein-derived pyrolysis products. The lowest ratio was found in the head capsules pretreated with acids, followed by head capsules treated with demineralized water, 2 h KOH 10% at 20°C, and 1 h KOH 10% at 70°C (Table 2). Head capsules pretreated with the LD method consist almost entirely of chitin.

The LD-pretreated head capsules resemble purified crab chitin in that both contain few protein-derived pyrolysis products. Differences among the chromatograms from head capsules pretreated with KOH, acids, or demineralized water, are relatively small, indicating a similar mixture of chitin and proteins. Head capsules pretreated with HCl and HF, however, contain relatively low abundances of pyrolysis products indicative of chitin, and the highest amount of pyrolysis products derived from proteins. The presence of many acetylated pyrolysis products suggests that chitin in crab chitin and chironomid head capsules is not entirely deacetylated to chitosan.



**Figure 2** Total ion current traces obtained by Curie-point pyrolysis-gas chromatography mass spectrometric analysis of chironomid head capsules pretreated 24 h in demineralized water at 20°C (a), 2 h in KOH 10% at 20°C (b), 1 h in KOH 10% at 70°C (c), 1 h in HCl 30% and 2 h in HF 40% at 20°C (d) and in sodium chlorite (Leavitt and Danzer (1993) method; e), as well as of crab chitin standard (Sigma-Aldrich; f). Numbers indicate peaks characteristic of chitin and letters mark peaks indicative of proteins

**Table 1** List of major pyrolysis products characteristic for pretreated chironomid head capsule and crab chitin pyrolyzates. Numbers refer to components derived from chitin (C), letters to those from proteins (P). Fragment ions in bold indicate base peaks and masses underlined indicate molecular ions (M<sup>+</sup>). M/z values after base peak values are in descending order of relative abundance. Phe=Phenylalanine; Hpro=Hydroxyproline; Tyr=Tyrosine; Pro=Proline; Trp=Tryptophan; Ala=Alanine; Leu=Leucine; Lys=Lysine; Gly=Glycine; Val=Valine; Arg=Arginine. C<sub>1</sub>=methyl; C<sub>2</sub>=dimethyl or ethyl.

Peak	MS characteristics	Compound Name	Origin	Pretreatment						Fossil
				demi water	KOH 20°C	KOH 70°C	HCl/HF	LD method	crab chitin	
1	<b>60</b>	Acetic acid	C							x
2	<b>79</b> 52	Pyridine	C					x		x
3	<b>84</b> 54 55	Furanone	C							x
4	<b>93</b> 66	C <sub>1</sub> -Pyridine	C	x	x	x	x	x		x
5	<b>96</b> 95	Furfural	C						x	
6	<b>59</b>	Acetamide	C	x	x	x	x	x	x	x
7	<b>109</b> 81 53	C <sub>1</sub> -Pyridone	C		x				x	x
8	<b>111</b> 70 83 53	3-Hydroxy-2-pyridone	C			x		x		
9	<b>107</b> <b>79</b> 52 51 78	2-Pyridine-carboxaldehyde	C					x	x	
10	<b>113</b> <b>71</b>	Acetylpyrroline	C			x		x		
11	<b>125</b> 110 84 54 82	Amine derivative	C			x		x		
12	<b>126</b> <b>68</b> 96 97 98	Levoglucosenone	C			x		x	x	
13	<b>127</b> <b>85</b> 56	N-Acetyl-N-ethenylacetamide	C	x	x	x		x		
14	<b>123</b> <b>80</b> 81	Acetyldihydropyridine	C			x		x		
15	<b>137</b> <b>109</b> 95 81 68	Acetylpyridone	C			x		x		x
16	<b>151</b> <b>108</b> 109 65 79 80 78	2-Pyridinemethanol acetate	C					x	x	
17	<b>137</b> <b>109</b> 95 81 68	Acetylpyridone	C					x	x	
18	<b>125</b> <b>83</b> 54 55	3-Acetamidofuran	C	x	x	x	x	x		x
19	<b>139</b> <b>97</b> 69	3-Acetamido-5-methylfuran	C	x	x	x	x	x	x	x
20	<b>153</b> <b>111</b> 82 83	3-Acetamido-4-pyrone	C	x	x	x	x	x	x	x
21	<b>151</b> <b>109</b> 80 81	N-Hydroxyphenylacetamide	C					x	x	x
22	<b>185</b> <b>84</b> 83 55 56	Oxazoline structure	C	x	x	x		x		x
23	<b>169</b> <b>82</b> 127 80	?	C			x		x		x
24	<b>165</b> <b>125</b> 55 84	Trihydro-2-acetamido-2-deoxyglucose	C						x	
25	<b>185</b> <b>84</b> 83 55 56	Oxazoline structure	C	x	x	x		x		x
26	<b>185</b> <b>69</b> 97 81 111	Dianhydro-2-acetamido-2-deoxyglucose	C	x	x	x	x	x	x	x
27	<b>167</b> <b>110</b> 125 96 83 54 68	3-Acetamido-5-acetylfuran	C					x	x	
28	<b>204</b> <b>59</b> 101 114 72	1,6-Anhydro-2-acetamido-2-deoxyglucose	C	x	x	x	x	x	x	x

Table 1 Continued.

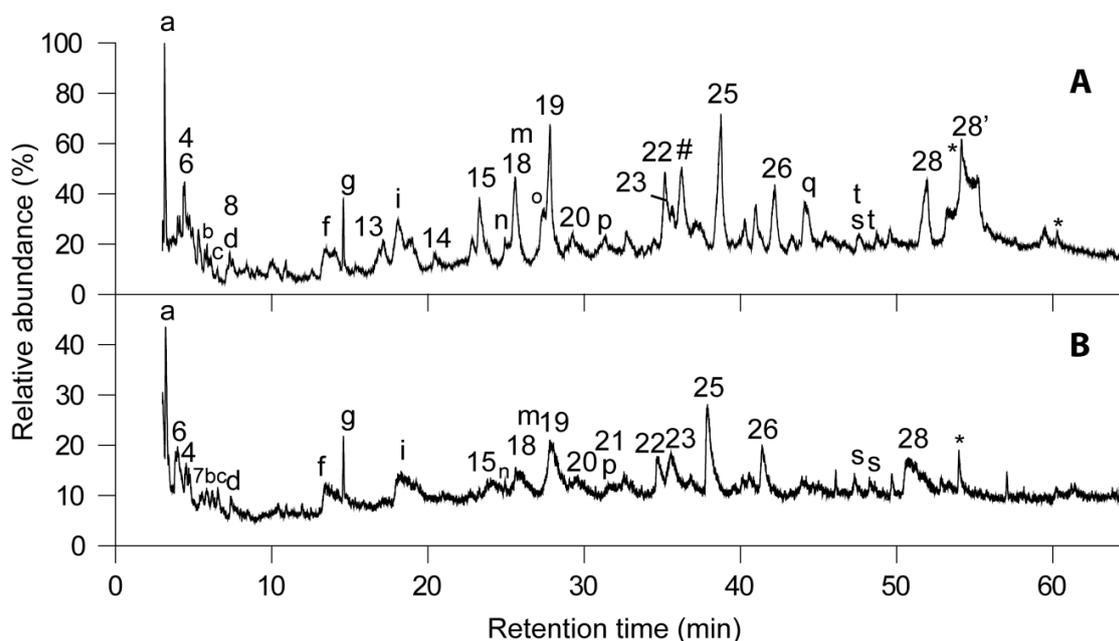
Peak	MS characteristics	Compound Name	Origin	Pretreatment						Fossil
				demi water	KOH 20°C	KOH 70°C	HCl/HF	LD method	crab chitin	
28'	<u>204 59</u> 101 114 72	isomer of compound 28	C			x		x		
29	<u>204 101</u> 59 98 139	3,4-di-O-Acetyl-1:6-anhydro-2-acetamido-2-deoxy-D-altrosan	C		x				x	
a	<u>92 91</u> 65	Toluene	P (Phe)	x	x	x	x		x	
b	<u>81 80</u> 53	C <sub>1</sub> -Pyrroles	P (Hpro)	x	x	x	x		x	
c	<u>106 91</u>	Ethylbenzene	P (Phe)	x	x	x	x		x	
d	<u>104</u> 78 51	Styrene	P (Phe)	x	x	x	x		x	
e	<u>99 69</u> 56 87	2-Propenoic acid, 2-methyl-, 2-methylpropyl ester	P (Phe)				x			
f	<u>94</u> 66	Phenol	P (Tyr)	x	x	x	x		x	
g	<u>136 68</u> 67 93 79 53 107 121	Limonene	P (Phe)	x	x	x			x	
h	<u>95</u> 94 66	Ethylpyrrole	P (Pro)					x		
i	<u>108 107</u> 77	C <sub>1</sub> -Phenol	P (Tyr)	x	x	x	x		x	
j	<u>153 69</u> 56 110 84	3-(2-Methylpropenyl)-5-(2-methylbutyl)-3,4-dihydro-2H-pyrrole-2,4-dione	P (Val-Leu)	x	x	x	x			
k	<u>122 107</u>	C <sub>2</sub> -Phenol	P (Tyr)				x			
l	<u>131 91</u> 65	Propylcyanobenzene	P (Phe)				x			
m	<u>120</u> 91 65	Vinylphenol	P (Tyr)	x	x	x	x		x	
n	<u>181 69</u> 84 56 166	3-(2-Methylpropenyl)-5-(2-methylpropyl)-3,4-dihydro-2H-pyrrole-2,4-dione	P (Val-Val)	x	x	x	x		x	
o	<u>117</u> 90 89	Indole	P (Trp)	x	x	x	x			
p	<u>131 130</u> 77	C <sub>1</sub> -indole	P (Trp)	x	x	x	x		x	
q	<u>168 70</u> 97 125	2,5-Diketopiperazine derivative	P (Pro-Ala)	x	x	x	x			
r	<u>154 83</u> 111 70 98	Pyrrolidinopiperazine derivative	P (Pro-Gly, Pro-Lys)	x						
s	<u>154 70</u> 72 125 55	2,5-Diketopiperazine derivative	P (Pro-Val, Pro-Arg)			x	x		x	
t	<u>154 70</u> 125 55	2,5-Diketopiperazine derivative	P (Pro-Arg)	x	x	x	x			
#	<u>154 83</u> 96 71 55	?	?	x		x		x		
*	<u>60 73</u>	Fatty acids				x				

**Table 2** Chitin:protein ratios of pretreated head capsules, as well as fossil head capsules, and crab chitin standard as calculated by dividing the sum of the peak areas of compounds 28 and 28' by the sum of the peak areas of compounds i and j.

Pretreatment	Chitin:protein ratio
24h demineralized water	0.77
2h KOH10% 20°C	0.95
1h KOH10% 70°C	2.37
LD method	no protein detected
1h HCl30% 2h HF 40%	0.19
Fossil chironomids	1.08
Crab chitin	no protein detected

### *Fossil head capsules*

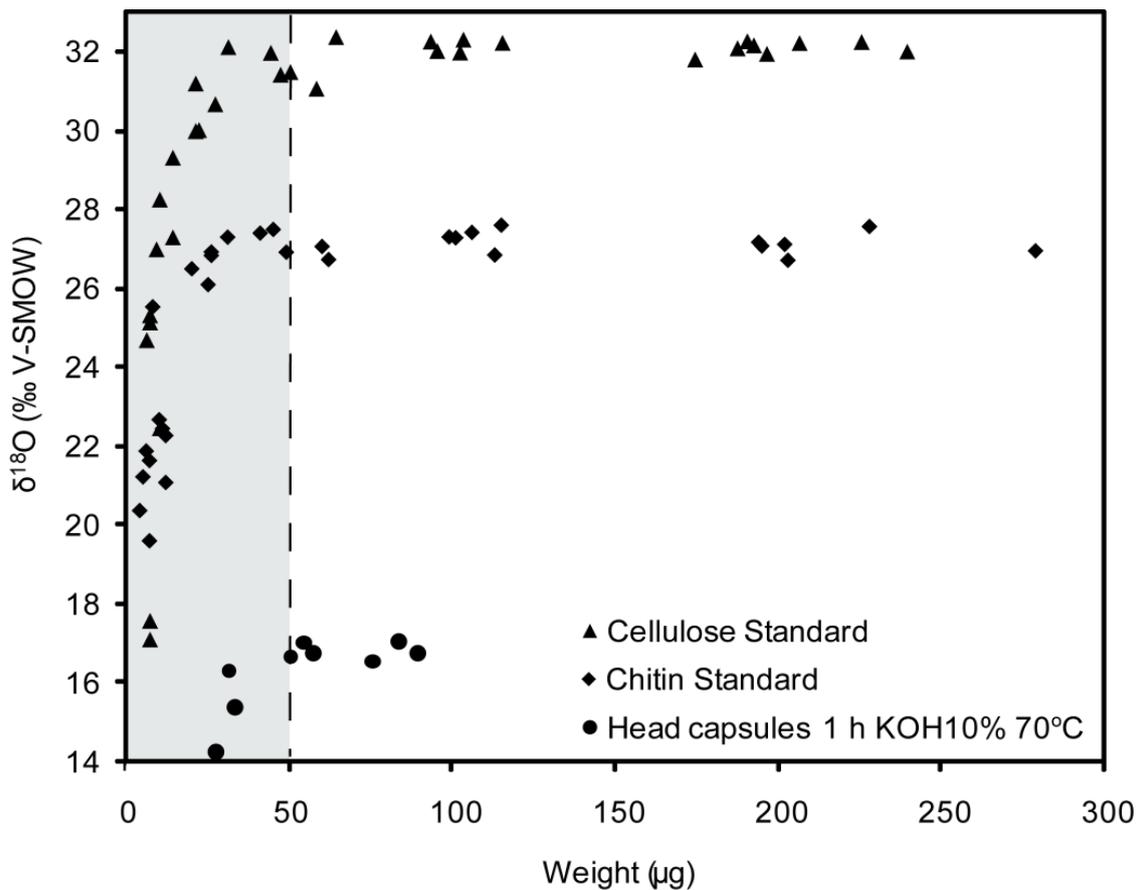
Fossil head capsules from late glacial sediment of Rotsee, Switzerland, were also analyzed using Py-GC/MS, and their composition was compared with pretreated head capsules. These ~16,000-year-old fossil head capsules contain both abundant chitin- and protein-derived compounds (Fig. 3b). Pyrolysis products are most similar to those in head capsules pretreated with 10% KOH for 1 h at 70°C, indicating a similar chemical composition.



**Figure 3** Total ion current traces of the Curie-point pyrolysis-gas chromatography mass spectrometric analysis of modern chironomid head capsules pretreated with 10% KOH for 1 h at 70°C (a) and fossil chironomid head capsules isolated from Rotsee sediments of late glacial age (~16,000 cal yrs BP) (b). Numbers and letters refer to peaks derived from chitin and proteins, respectively (note the different scales of y-axes)

$\delta^{18}\text{O}$  analysis*Minimum weight assessment*

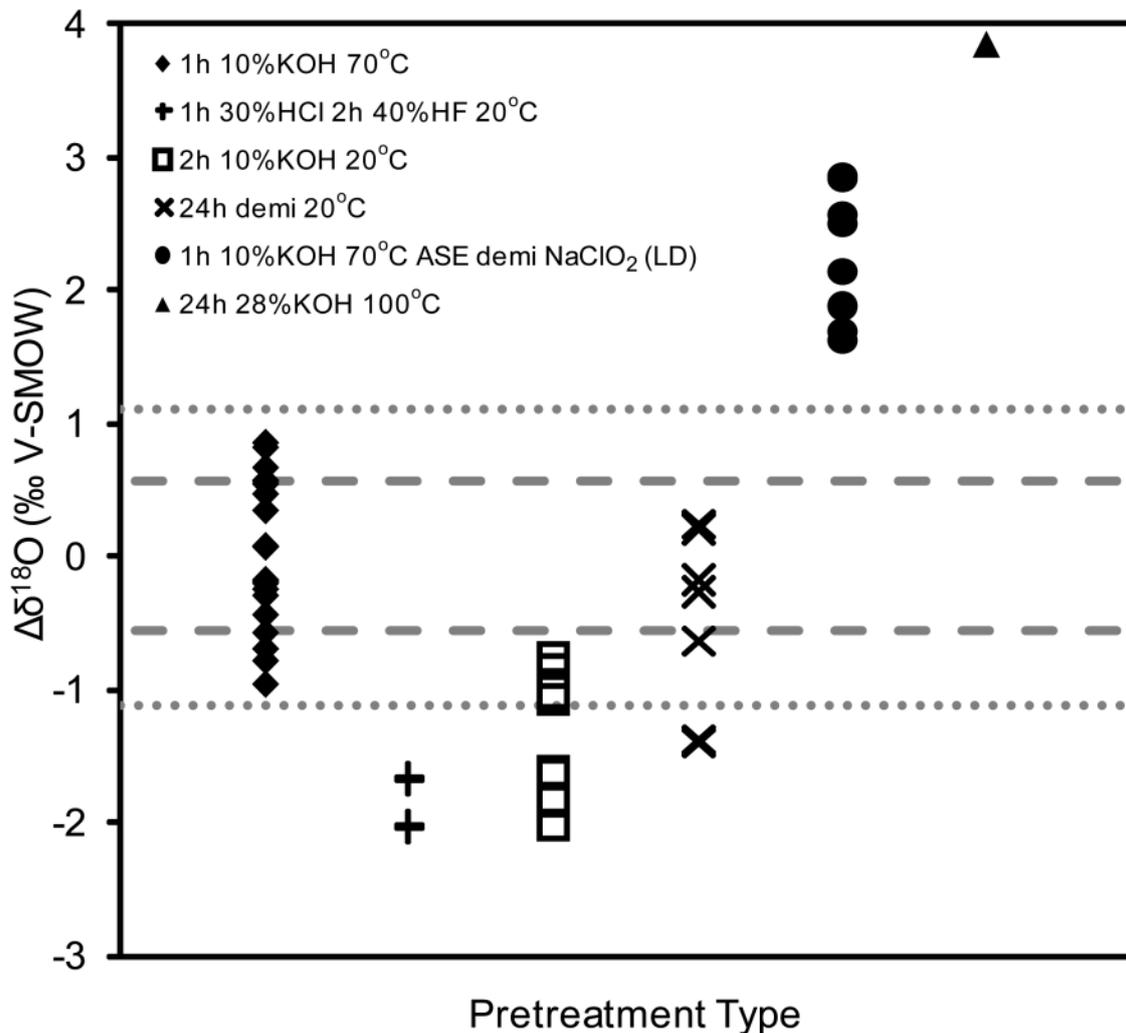
For chironomid head capsules, as well as for both the chitin and cellulose standards, the minimum sample size necessary for  $\delta^{18}\text{O}$  analysis was determined by analyzing samples of weights ranging from 4 to 250  $\mu\text{g}$  (Fig. 4). Samples display stable  $\delta^{18}\text{O}$  values when weights are  $>50 \mu\text{g}$ . This limit corresponds to a voltage of 950 mV and was adopted as the minimum weight for chironomid  $\delta^{18}\text{O}$  analysis using our equipment.



**Figure 4**  $\delta^{18}\text{O}$  values of cellulose (triangles) and chitin (diamonds) standards and chironomid head capsules (circles) plotted against sample weight. The shaded area shows weights  $< 50 \mu\text{g}$ , which produced unreliable results

*Pretreatment impact*

We evaluated effects of pH-neutral, alkaline, and acidic pretreatments, as well as the LD pretreatment on  $\delta^{18}\text{O}$  values of chironomid head capsules (Fig. 5). Head capsules exposed to 10% KOH for 1 h at 70°C were taken as a matrix-matched reference and used for inter-calibration of  $\delta^{18}\text{O}$  values. This pretreatment was chosen as a reference because it facilitates



**Figure 5** Effects of various pretreatments involving alkali (diamonds, squares, triangles), acids (crosses), demineralized water (x-symbols) and sodium chlorite (circles) on the stable oxygen isotopic composition of chironomid head capsules (1 h 10% KOH 70°C measurements are used as reference). Y axis shows differences in  $\delta^{18}\text{O}$  values with respect to reference. Dashed and dotted lines delimit the  $\pm 1$  and 2 standard deviation range of the reference, respectively

removal of head capsules from larval bodies, eliminates soft body tissue, and therefore mimics the fossilization process. The inter-calibration is done to compensate for possible drift in  $\delta^{18}\text{O}$  values for every run, and changes with respect to this reference are shown in Fig. 5. Dashed and dotted lines represent the 1- and 2- $\sigma$  range of reference  $\delta^{18}\text{O}$  values, respectively. Different pretreatments produced statistically significant differences in  $\delta^{18}\text{O}$  values in all cases (two-tailed  $t$ -test,  $\alpha=0.05$ ), except for the groups pretreated with acids and KOH 10% for 1 h at 20°C ( $P=0.11$ ; Table 3). The higher the temperature applied during KOH pretreatment, the higher the  $\delta^{18}\text{O}$  offset, leading to increased chironomid  $\delta^{18}\text{O}$  (Fig. 5). The use of acids, however, removes isotopically heavier components and/or causes isotope exchange between oxygen in cuticles and the water in which the acid is dissolved, which results in relatively depleted  $\delta^{18}\text{O}$  values for head capsules. The LD method results in

relatively  $^{18}\text{O}$ -enriched head capsules. This enrichment is not an artifact of adherence of glass fiber filters to the head capsules during the LD pretreatment, as filters were measured separately for  $\delta^{18}\text{O}$  and found to contain too little oxygen to interfere with chironomid  $\delta^{18}\text{O}$  measurements.

**Table 3** P-values obtained by a statistical comparison of  $\delta^{18}\text{O}$  of chironomid head capsules pretreated with alkali, acids, demineralized water, and sodium chlorite (LD method) by two-tailed *t*-tests. The asterisk marks the two pretreatments that produce chironomid  $\delta^{18}\text{O}$  values that are not statistically different ( $\alpha=0.05$ ).

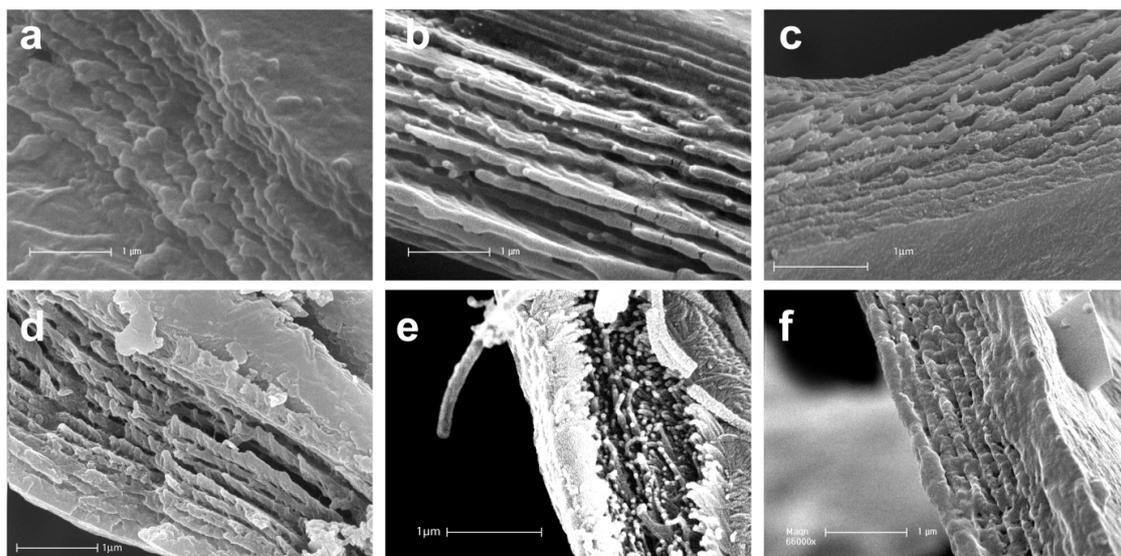
Pretreatment	24h demi water	2h KOH10% 20°C	1h KOH10% 70°C	24h KOH28% 100°C	LD method	1h HCl30% 2h HF 40%
24h demi water		0.02	0.03	0.002	<0.0001	0.007
2h KOH10% 20°C			<0.0001	<0.0001	<0.0001	0.11*
1h KOH10% 70°C				<0.0001	<0.0001	<0.0001
24h KOH28% 100°C					0.02	0.0002
LD method						<0.0001
1h HCl30% 2h HF 40%						

## Head capsule morphology

We detected differences in head capsule morphology for the different treatments by examining fractured edges of chironomid head capsules under a SEM (Fig. 6). Insect cuticles consist mainly of multiple overlapping layers of chitin fibers, or rods, embedded in a protein matrix (Nation, 2002). This is seen clearly in cuticles of head capsules pretreated with demineralized water (Fig. 6a), whereas this matrix is slightly reduced in fossil head capsules (Fig. 6f). The matrix is, to a lesser extent, apparent in head capsules pretreated with KOH (Figs. 6b and 6c), where the ‘plywood’ structure that is typical for insect exoskeletons (Nation, 2002) can clearly be distinguished. The protein matrix is clearly seen in head capsules pretreated with acids (Fig. 6d). However, it seems to be absent in cuticles pretreated with the LD method (Fig. 6e). In the latter case, long fibers protrude from the fractured cuticle edges. SEM analyses thus indicate that cuticles are morphologically altered by pretreatment.

## Discussion

We analyzed the chemical composition of chironomid head capsules and showed that chitin moieties are present in head capsule cuticles, regardless of pretreatment and age in this case. Often the most dominant pyrolysis product of chitin was 1,6-anhydro-2-acetamido-2-deoxyglucose (28), which was present in every pyrolyzate (Figs. 2 and 3). It is the primary pyrolysis product of the intact monomer, or building block of chitin, and is produced by



**Figure 6** SEM images of fractured edges of chironomid head capsule cuticles pretreated with demineralized water for 24 h at 20°C (a), KOH for 2 h at 20°C (b), KOH for 1 h at 70°C (c), HCl 1 h and HF 2 h (d), sodium chlorite (Leavitt and Danzer (1993) method; e), as well as of a fossil chironomid head capsule from the sediment of Rotsee (f)

depolymerization of chitin, followed by dehydration (van der Kaaden *et al.*, 1984). Both pyrolysis results and SEM images show that pretreatments have an effect on the relative amount of chitin and proteins present in chironomid head capsules, which in turn affects cuticle  $\delta^{18}\text{O}$  values. Relative abundances of chitin and proteins in head capsules pretreated with either demineralized water or KOH are similar (Figs. 2a, b, c), although the chitin:protein ratio is higher in the hot alkali pretreatment (Table 2). The presence of pyrolysis products of proteinaceous origin indicates that amino acids such as tyrosine (characterized by pyrolysis products such as phenols), tryptophan (indoles), phenylalanine (toluene), and proline (pyrroles) are, to some extent, resistant to these pretreatments. This implies that deproteination caused by base hydrolysis did not occur extensively during either the hot or cold alkali pretreatment. This is in agreement with Brine and Austin (1981), who exposed horseshoe crab shells to 1M NaOH for 6 h at 50°C and found proteins after treatment. The fact that little deproteination occurs is also evident from the SEM images (Figs. 6a, b, c), in which sheets of chitin fibers are seen embedded in a proteinaceous matrix in the typical ‘plywood’ structure of the insect exocuticle (Giraud-Guille and Bouligand, 1986; Nation, 2002). Enriched  $\delta^{18}\text{O}$  values after KOH pretreatment at higher temperatures suggest that temperature enhances the reaction between KOH and head capsules, whereas the length of treatment was less important in our experiments. It also implies that material removed using KOH has a lighter  $\delta^{18}\text{O}$  value than the remaining material.

Acid pretreatment leads to a substantial decrease in the amount of head capsule chitin, as seen in the low chitin:protein ratio (0.19; Table 2). Only a few chitin-derived compounds, such as anhydrosugars, remain in the pyrolyzates at low relative abundances. Acids are known to catalyze the loss of N-acetyl groups (de-N-acetylation; Einbu and Vårum, 2007). De-N-acetylation is therefore thought to be the mechanism behind this chitin decrease. If de-N-acetylation occurs extensively, chitin is partly converted to chitosan, which is soluble in acidic aqueous solutions (Pariser and Lombardi, 1980). If most chitosan is

dissolved, only a small amount of chitin remains relative to proteins. This is reflected in the reduced relative abundances of chitin-derived compounds in the chromatogram of acid-treated head capsules (Fig. 2d) and the relatively low abundance of chitin fibers observed in the SEM image (Fig. 6d). Furthermore, acids induce exchange of oxygen between chitin and water. Because the  $\delta^{18}\text{O}$  of head capsules ( $\sim 16\text{‰}$ ) is heavier than that of the water used ( $\sim -7\text{‰}$ ), head capsules soaked in acids end up isotopically lighter due to oxygen exchange (Fig. 5).

Head capsules pretreated with the LD method contain many chitin-derived compounds and only a few compounds associated with proteins (Fig. 2e). The chitin:protein ratio, which is calculated using protein pyrolysis products i and j, cannot be determined due to the absence of these compounds. Substantial deproteination is attributed to the use of  $\text{NaClO}_2$ . The resultant morphological change is visible in the SEM image (Fig. 6e), in which chitin rods are seen protruding from a fractured edge of an acid-treated head capsule cuticle that lacks the proteinaceous matrix.

The pyrolyzate of purified crab chitin standard contains only one minor pyrolysis product derived from protein moieties (Fig. 5f), and therefore consists predominantly of chitin, which confirms the findings by Stankiewicz *et al.* (1996). Our results show that chemical pretreatment can cause selective removal of chitin and/or protein moieties.

The chemical stability of chitin and persistence of proteins is demonstrated by their abundance in fossil chironomids of late glacial age (Fig. 3b). Traces of chitin have even been found in 25-Ma-old insect remains from lacustrine deposits of the Enspel Formation, Germany (Stankiewicz *et al.*, 1997a; Gupta *et al.*, 2007). Although chitin can be degraded both aerobically (Boyer, 1994; Reguera and Leschine, 2001) and anaerobically (Sturz and Robinson, 1986), the depositional environment is thought to play a prominent role in the molecular preservation of both chitin and proteins (Stankiewicz *et al.*, 1997a, 1998; Briggs *et al.*, 1998; Gupta *et al.*, 2007). Lakes may provide a suitable environment to preserve these biopolymers on long timescales. Although proteins are more susceptible to degradation than chitin (Baas *et al.*, 1995; Briggs *et al.*, 1998), their pyrolysis products are still present in the late glacial chironomids as well as in the Miocene beetles studied by Stankiewicz *et al.*, (1997a). Fossil chironomids analyzed here mostly resemble the head capsules pretreated with demineralized water or KOH, with respect to their chemical composition and morphology (Figs. 3 and 6). Distributions of pyrolysis products and the chitin:protein ratio in fossil head capsules correspond most closely to those of head capsules pretreated with KOH (Tables 1 and 2, Fig. 3).

Relative amounts of chitin in extant insect cuticles range between 20 and 50% (Hackman, 1974; Muzzarelli, 1977; Andersen, 1979; Kramer *et al.*, 1995; Bierstedt *et al.*, 1998). The proportion of chitin in insects from Pleistocene sediments is usually between 10 and 30% (Miller *et al.*, 1993), but can approach 40% even in Pliocene deposits (Bierstedt *et al.*, 1998). Although pyrolysis does not allow absolute quantification of the amount of chitin, the estimated chitin:protein ratio of  $\sim 1$  in fossil chironomid head capsules (Table 2) indicates that the chitin proportion in the sample is in agreement with the literature.

## Pretreatment effects on chironomid $\delta^{18}\text{O}$

Different pretreatments have different effects on the stable oxygen isotopic composition of chironomid head capsules (Fig. 5). Progressive deproteinization, as seen in chromatograms and SEM images of the head capsules pretreated with the LD method, as well as in head capsules exposed to prolonged hot alkali pretreatment (28% KOH for 24 h at 100 °C), causes enrichment of  $\delta^{18}\text{O}$  values. On the other hand, head capsules that experience removal of chitin relative to proteins, such as those processed by acids, display depleted  $\delta^{18}\text{O}$  values. Two main processes may contribute to these shifts in isotope values. First, oxygen isotope exchange induced by the use of acids alters the  $\delta^{18}\text{O}$  of head capsules toward the  $\delta^{18}\text{O}$  value of the water in which the acid is dissolved, thus leading to a depletion in the  $\delta^{18}\text{O}$  value of the head capsule in our study. Second, fractionation processes that occur when the chironomid exoskeleton is formed affect protein and chitin components of head capsules differently. Proteins and chitin are formed via different biochemical pathways (Nation 2002), which may explain differences in oxygen isotope fractionation. Our experiments suggest that pretreatments that cause deproteinization may produce head capsules with up to 5-6‰ heavier  $\delta^{18}\text{O}$  values (Fig. 5). Acid pretreatment, on the other hand, results in selective preservation of proteins as opposed to chitin, but also induces oxygen isotope exchange, the combination of which leads to significant oxygen isotopic depletion (2‰) in head capsules. Currently, we cannot assess the relative contribution of oxygen isotope exchange versus compound-specific fractionation, to this oxygen isotope shift. Nevertheless,  $\delta^{18}\text{O}$  enrichment of head capsules that have undergone progressive deproteinization in a pH-neutral or alkaline environment, where no oxygen isotope exchange has taken place, suggests that chitin is isotopically heavy with respect to proteins, and that selective chitin removal in the acid treatment is an important factor causing changes in  $\delta^{18}\text{O}$ .

Wooller *et al.* (2004, 2008) demonstrated that chironomid  $\delta^{18}\text{O}$  is in equilibrium with lake water  $\delta^{18}\text{O}$  and therefore has the potential to be used as a palaeotemperature proxy. Wang *et al.* (2008) recently proposed a protocol for chironomid pretreatment prior to  $\delta^{18}\text{O}$  analysis, including treatments with 10% HCl for 24 h and 5% KOH for 15-20 min at 60-70 °C. The advantage of their two-step approach is that chironomid samples, once prepared, can be stored before final transfer to silver cups for TC-EA/IRMS analysis. Wang *et al.* (2008) used weak to moderate alkali (KOH) and acid (HCl) treatments. We demonstrated that prolonged exposure to hot alkali, and even more so, use of acids, alters the chemical composition and stable oxygen isotopic composition of head capsules. When applied in palaeolimnological studies, down-core shifts in chironomid  $\delta^{18}\text{O}$  are still observed, even if the values are affected by a systematic bias. Reconstructions of the past oxygen isotopic composition of lake water, however, may be obscured by the biases caused by such chemicals. This problem may be minimized by performing alkali and acid pretreatments under closely controlled conditions, that is, by using consistent sample exposure times and acid/alkali concentrations, and using water with the same  $\delta^{18}\text{O}$  composition. Nevertheless, because acid treatment has been shown to promote exchange of oxygen atoms, at least in organic material derived from algal biomass (Wedeking and Hayes, 1983), we strongly discourage the use of acids.

Proteins are thought to have a different stable oxygen isotopic composition than chitin, and their  $\delta^{18}\text{O}$  value is also affected by their amino acid composition. A possible preparation procedure might be to isolate the chitin component of the head capsules before

analysis. The LD method seems a suitable method for chitin purification. This processing step, however, leads to major weight loss in samples, >45% in our experiments. Hodgins *et al.* (2001) applied labor-intensive preparation approaches to purify chitin for dating purposes and found similar yields after deproteinization of insect chitin. Such a weight loss may preclude using chironomid  $\delta^{18}\text{O}$  down-core, because chironomid concentrations are often low. Even in lakes with very high head capsule concentrations, production of high-resolution records may be difficult given the long time required to sort sufficient chironomid remains from the sediments. The use of chemicals similar to those investigated in this study apparently does not alter the  $\delta^{13}\text{C}$  of chironomid head capsules (van Hardenbroek *et al.*, 2009).

## Conclusions

We assessed the effect of chemical pretreatments that are commonly used in palaeolimnology, on the chemical composition,  $\delta^{18}\text{O}$ , and morphology of chironomid head capsules. The chemical composition and morphology of late glacial chironomid fossils from Rotsee are very similar to head capsules of extant chironomid larvae that were exposed briefly to alkali, or exposed for longer duration to demineralized water. This suggests that extensive protein and chitin degradation did not occur during the fossilization process. Presence of the chitin monomer in pyrolyzates of all studied samples confirms the chemical stability of chitin, regardless of sample age or chemical pretreatment.

Whereas cold alkali pretreatments have little effect on chironomid remains, prolonged ( $\geq 1\text{h}$ ) exposure to hot alkali, acids, or  $\text{NaClO}_2$  (LD method) alters their chemical and stable oxygen isotope composition by oxygen isotope exchange, and/or removal of proteins or chitin, two main components of chironomid head capsules. Selective removal of these components introduces a bias in the  $\delta^{18}\text{O}$  signal in head capsules, which is thought to reflect past lake water  $\delta^{18}\text{O}$ , and, hence, past temperatures. We, therefore, strongly discourage the use of acids or prolonged exposure to hot alkali in preparing chironomids for  $\delta^{18}\text{O}$  analysis. Purification of chitin from head capsules in a pH-neutral or alkaline environment does not alter the  $\delta^{18}\text{O}$  signal in the purified chitin, and probably yields the most reproducible chironomid  $\delta^{18}\text{O}$  records. The purification process, however, causes significant sample weight loss, thereby precluding the use of this approach for developing high-resolution, down-core records. Because different pretreatments alter the  $\delta^{18}\text{O}$  of chironomid remains in different ways, it will be necessary to separately calibrate the relationship between  $\delta^{18}\text{O}$  of chironomids treated with acids, hot alkali, or less aggressive pretreatment methods, and lake water  $\delta^{18}\text{O}$  values. Given the differential effects of various preparation techniques on the chemical and oxygen isotope signature of chironomid head capsules, care must be taken when comparing chironomid  $\delta^{18}\text{O}$  records that used different pretreatment methods.

# Acknowledgements

We thank Arnold van Dijk and Gijs Nobbe for laboratory assistance. Jan van Tongeren helped with Scanning Electron Microscopy. Maarten van Hardenbroek provided fruitful discussions. We thank Matthew J. Wooller, an anonymous reviewer, and an associate editor of the Journal of Paleolimnology for valuable comments on an earlier version of the manuscript. This is publication number DW-2009-5001 of the Darwin Center for Biogeosciences, Utrecht, The Netherlands ([www.darwincenter.nl](http://www.darwincenter.nl)), which financially supported this study. This is Netherlands Research School of Sedimentary Geology publication no. 20090701.

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## Chapter 2



# Subfossil chironomid assemblages in deep, stratified European lakes: relation with temperature, trophic state and oxygen

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*To be submitted for publication in Freshwater Biology*

## Abstract

The distribution of subfossil remains of chironomid larvae in 28 large, deep, and stratified lakes in Europe was examined in surface sediments along a latitudinal transect ranging from northern Sweden to southern Italy. The relationship between chironomid assemblages and mean July air temperature, summer surface water temperature, total phosphorus concentration, hypolimnetic oxygen concentration, conductivity, pH, lake area, maximum lake depth, Secchi depth, catchment size, latitude, and organic matter content of the sediment was examined. Canonical correspondence analysis (CCA) showed that latitude, summer surface water and July air temperature, as well as nutrient and hypolimnetic oxygen availability were statistically significant ( $P < 0.05$ ) explanatory variables for the chironomid distribution, explaining between 11 and 14% of the variance in the chironomid assemblage data. Due to the spatial scale covered by our study many environmental variables were co-varying. Latitude, temperature, and nutrient concentration and oxygen availability were positively or negatively correlated with the first axis of a detrended correspondence analysis (DCA) of chironomid assemblages, suggesting that climatic and trophic conditions influenced profundal chironomid assemblages either in a direct (food and oxygen) or indirect (temperature) way. Parameters related to local environmental conditions, lake morphology, and bedrock geology, such as organic matter content of the sediment, maximum lake depth, Secchi depth, pH, and conductivity, were not significant in explaining the distribution of chironomid assemblages in our study lakes. The strong relationship between chironomid

assemblages and summer temperature in our dataset may be related to the co-variation of temperature with parameters known to affect chironomid assemblages in deep stratified lakes, such as nutrient and oxygen availability. However, summer temperature explained a statistically significant proportion of the variance in the chironomid assemblages even when effects of oxygen and food availability were partialled out. This suggests that summer temperature has an effect on chironomid assemblages in deep lakes that is not related to its co-variation with trophic state. The potential of chironomid assemblages in deep stratified lakes for quantitative reconstruction of past nutrient conditions was examined by calculating the Benthic Quality Index (BQI) based on profundal chironomids and comparing BQI values with observed total phosphorus (TP) concentrations. In our study lakes the BQI was linearly related with log-transformed TP. Applying this relationship to fossil chironomid assemblages from Lake Päijänne (Finland) produced a TP reconstruction which was in agreement with measured TP during the period AD 1970-1990, demonstrating that this approach can provide quantitative estimates of past nutrient concentrations in deep, stratified lakes.

## Introduction

Studies on the composition and distribution of biotic communities in lake systems in relation to environmental conditions provide insights into factors affecting the occurrence and abundance of aquatic organisms such as cladocerans (e.g. Korhola, 1999; Bigler *et al.*, 2006), diatoms (e.g. Cameron *et al.*, 1999; Fallu *et al.*, 2002), chrysophyte cysts (e.g. Pla and Catalan, 2005), chaoborids (e.g. Luoto, 2009a) and chironomids (e.g. Walker *et al.*, 1991; Lotter *et al.*, 1997, 1998). In addition, such studies form the basis for palaeolimnological reconstructions of past climate (e.g. Lotter *et al.*, 2000; Heiri *et al.*, 2003) and water quality parameters such as nutrient concentrations (e.g. Brooks *et al.*, 2001; Lotter *et al.*, 1998) or pH (e.g. Curtis *et al.*, 2009). Chironomid larvae are abundant and ubiquitous in lakes (Armitage *et al.*, 1995) and their head capsules preserve well in sediments. Fossil remains of chironomid larvae can usually be identified to generic, species-group, or species level (e.g. Rieradevall and Brooks, 2001; Brooks *et al.*, 2007). A number of studies have been conducted that link the distribution of chironomid assemblages to environmental conditions along environmental gradients (e.g. Larocque *et al.*, 2006; Langdon *et al.*, 2008; Rees *et al.*, 2008; Luoto, 2009b; Porinchi *et al.*, 2009; Eggermont *et al.*, 2009). In many of these studies a strong relationship between the taxonomic composition of chironomid assemblages and summer lake water and air temperature has been described (e.g. Heiri and Lotter, 2005; Brooks, 2006; Walker and Cwynar, 2006). However, other factors, such as trophic conditions and oxygen levels (e.g. Brodersen and Quinlan, 2006), salinity (Eggermont *et al.*, 2008), acidity (Brodin and Gransberg, 1993), chlorophyll  $\alpha$  concentrations (Brodersen and Lindegaard, 1999), organic matter content (Nyman *et al.*, 2005), and water depth (Korhola *et al.*, 2000; Luoto, 2009a) have also been reported to influence assemblage composition. Many of these surveys have focused on comparatively small and shallow lakes, and most are restricted to a particular geographical region (Larocque *et al.*, 2006).

Due to the sensitivity of many chironomid species to their environment, chironomids have been used as indicators of limnological conditions in lakes since the 1920s

(Thienemann, 1921; Gams, 1927). The strong relationship between environmental parameters such as temperature, nutrient concentrations, oxygen, and salinity and the distribution of chironomid assemblages has led to the development of empirical chironomid-based inference models (transfer functions), which have been used to provide quantitative estimates of past environmental changes. Available chironomid-based inference models and quantitative palaeoenvironmental reconstructions have mainly been developed from lakes which are relatively small (surface area 0.003-6.94 km<sup>2</sup>, average 0.41 km<sup>2</sup>) and shallow (maximum water depth 0.09-98 m, average 7.3 m) (data from Lotter *et al.*, 1997, 1998; Heiri, 2001; Larocque *et al.*, 2006; Langdon *et al.*, 2008; Rees *et al.*, 2008; Luoto, 2009b; Porinchu *et al.*, 2009; Eggermont *et al.*, 2009). For temperature reconstructions in particular, large and deep lakes have so far been neglected since these lakes are thermally stratified during most of the growing season. It can be expected that chironomid larvae living at the bottom of such lakes will be exposed to temperatures close to 4°C throughout the whole annual cycle and under a range of climatic conditions. However, it has been suggested that the observed relationship between chironomid assemblages and temperature may be the consequence of the indirect effects of temperature on limnological parameters rather than due to a direct influence on development and growth of chironomid larvae (e.g. Hann *et al.*, 1992; Brodersen and Quinlan, 2006). If this is the case, a relationship between surface water or air temperatures and chironomids may also be apparent in deep lakes.

Chironomids have long been used as indicators of trophic conditions and nutrient concentrations in deep, stratified lakes (Brundin, 1956; Saether, 1979; Wiederholm, 1980). However, fossil chironomids have rarely been used to reconstruct past trophic changes in these lakes, although the few available case studies (e.g. Meriläinen and Hamina, 1993; Itkonen *et al.*, 1999; Meriläinen *et al.*, 2000, 2003) indicate considerable potential for using fossil chironomid records in deep lakes for inferring past nutrient availability. This implies that chironomid-based reconstructions of lake trophic conditions can provide an assessment of baseline nutrient conditions, as previously was done using diatoms (e.g. Lotter, 1998, 2001; Kirilova *et al.*, 2009). Assessment of such reference conditions is essential for effective ecosystem management and restoration (Bennion and Battarbee, 2007; Smol, 2008).

Here, we present the first study that explores the distribution of subfossil chironomid assemblages in the surface sediments of large, deep, and stratified lakes along a continental-scale north-south transect across Europe. The 28 study lakes were selected to cover a wide range of environmental conditions. We aim to determine whether a relationship exists between the distribution of fossil chironomid assemblages in lake surface sediments and physical and chemical parameters, such as lake surface water or air temperature, nutrient availability, and oxygen concentrations. Furthermore, we want to assess whether an index-based approach, the Benthic Quality Index (BQI) (Wiederholm, 1980), which has been used to determine lake trophic state based on surveys of the modern chironomid fauna, can reliably infer trophic conditions from fossil chironomids. Based on these investigations we will provide an assessment of the potential of fossil chironomids in deep and stratified lakes as quantitative indicators for climatic and limnological change.

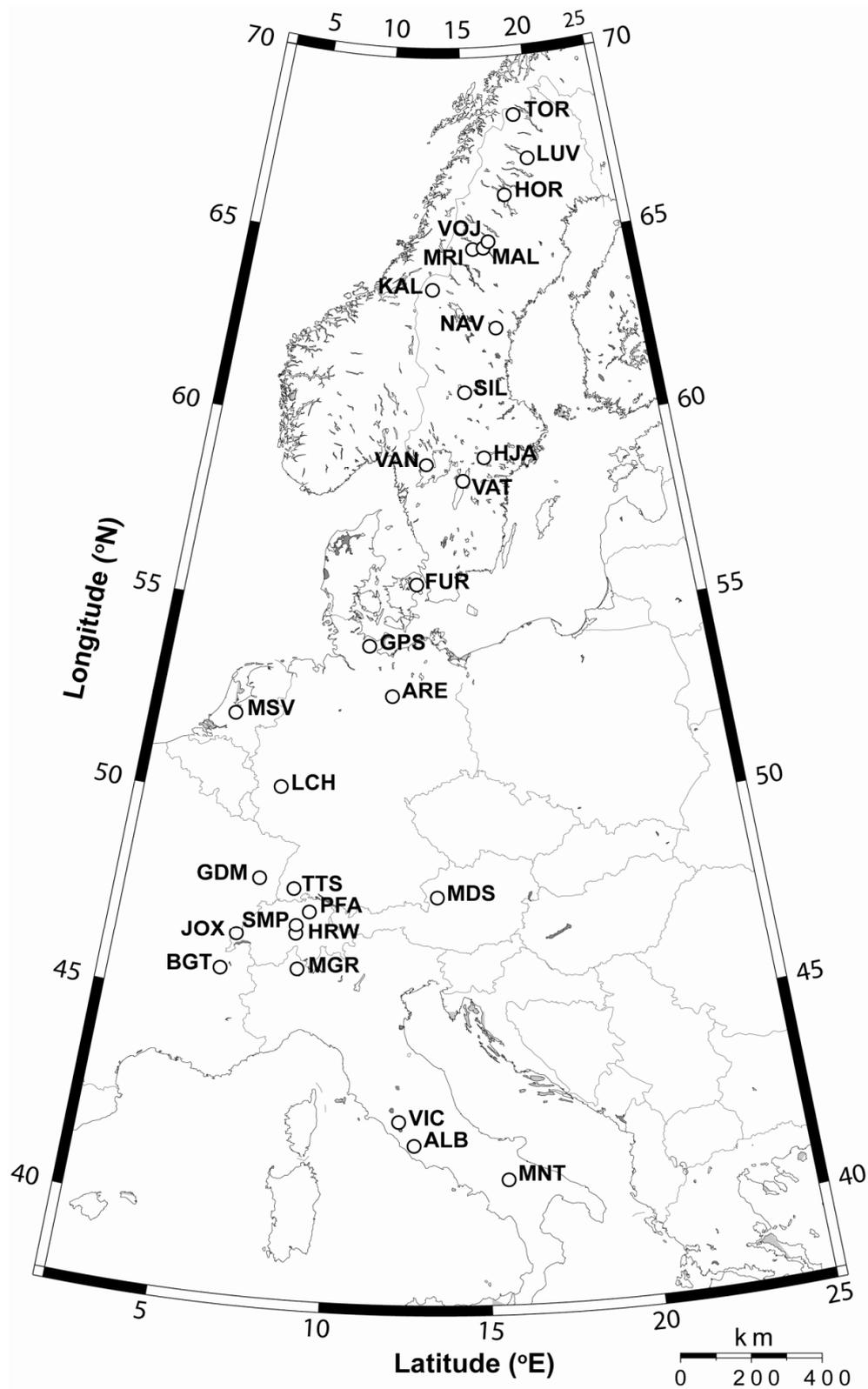
# Materials and methods

## Fieldwork

During two campaigns in January and July 2006 surface sediments were obtained from 28 large, deep, and stratified lakes along a north-south transect in Sweden, Denmark, Germany, the Netherlands, Austria, Switzerland, France, and Italy (Fig. 1). Physical and climatological characteristics of the lakes are listed in Table 1. Surface sediments were taken near the deepest part of the lake basin (LUV, MRI, NAV, HJA, VAN, FUR, GPS, ARE, MSV, LCH, GDM, TTS, MDS, PFA, SMP, JOX, BGT, VIC, MNT), unless the maximum water depth exceeded 100 m, in which case the sediments were taken closer to the shore (TOR, HOR, VOJ, MAL, KAL, SIL, VAT, HRW, MGR, ALB). Most sediment cores were taken at water depths well below the thermocline during summer stratification (20-25 m). In one lake (MRI) sampling took place in the lower littoral to sublittoral of the lake (6 m). Sediment cores were obtained using a gravity corer (Uwitec, Austria). The sediments were extruded in contiguous 1 cm slices in the field and samples were freeze dried.

## Chironomid analysis

Freeze-dried surface sediments were treated with 10% KOH for 2 h at room temperature to deflocculate organic material and then washed through a 100 µm mesh sieve following Walker (2001) and Brooks *et al.* (2007) (no acid treatment). Chironomid head capsules were sorted from the sieve residue in a Bogorov tray under a stereo microscope (magnification 40x) using fine forceps and permanently mounted onto microscope slides using Euparal® as a mounting medium. For lakes TOR, LUV, HOR, MRI, NAV, HJA, FUR, GPS, ARE, GDM and JOX the 0-1 cm sediment interval yielded sufficient head capsules. For VOJ, VAN, VAT, MSV, LCH, TTS, and HRW 0-2 cm was used, whereas for MAL, KAL and SIL 0-3 cm was needed. Finally, the top 5 cm of sediment were needed for lakes MDS, PFA, SMP, MGR, BGT, VIC, ALB, and MNT, as for these lakes chironomid concentrations were very low. Due to these low head capsule concentrations, the number of chironomid head capsules extracted from sediment was as low as 40 in some instances (KAL, VIC, ALB). HRW only yielded 22 head capsules. All other lakes yielded more than 45 head capsules, which is recommended for quantitative analyses (Heiri and Lotter, 2001). Chironomids were identified under a compound microscope (magnification 400x) following identification keys by Wiederholm (1983), Schmid (1993), Rieradevall and Brooks (2001), and Brooks *et al.* (2007). Half head capsules were counted as halves but counts were expressed as the minimum number of whole specimens per sample before transformation to relative abundances per lake. Chironomid distributions were graphically displayed using TGView (Grimm, 1991-2004).



**Figure 1** Location of the 28 studied lakes along a north-south transect in Europe. For abbreviations of the lakes see Table 1

**Table 1** Physical, morphological, and climatological characteristics of the studied lakes. (LOI550: Loss-on-ignition at 550 °C; MJAT: mean July air temperature; MAAT: mean annual air temperature; SSWT: summer surface water temperature, MAP: mean annual precipitation; N/A: not available; DBGZ: Datenbank Gewässerzustand, Bundesamt für Umwelt, Switzerland; EEA: European Environmental Agency; SLU: Sveriges Lantbruksuniversitet). a) annual; bm) bimonthly; bw) biweekly; m) monthly; s) seasonal; sm) summer; ty) twice per year; w) weekly

Lake name	Physical and climatological data													Environmental data			Source	
	Code	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	Open water area (km <sup>2</sup> )	Volume (km <sup>3</sup> )	Maximum depth (m)	Average depth (m)	Sampling depth (m)	Catchment area (km <sup>2</sup> )	Retention Time (years)	LOI550 (%)	MJAT (°C)	MAAT (°C)	SSWT (°C)	MAP (mm)		Sampling frequency
Lago di Albano	ALB	41.75	12.67	293	6	0.5	175	77	53	3.7	47	21.1	22.5	18.3	24.1	868	2004;1997 (a); 2001;2005 (m)	Messineo <i>et al.</i> , 2009; Cioni <i>et al.</i> , 2003; Bruno, pers. comm.
Lago di Vico	VIC	42.31	12.17	507	12.1	0.3	49	22	48	41	17	31.2	20.7	13.2	22.7	1316	2000 (s)	Scialanca, pers. comm.
Lac du Bourget	BGT	45.74	5.86	231	42	3.5	145	80	144	560	8	13.8	20.4	10.3	22.9	1333	2006 (w)	Paolini, pers. comm.
Lago Maggiore	MGR	45.90	8.52	193	213	37.1	370	177	22	6599	4	5.4	21.9	12.1	23.5	1703	2004-2005 (s)	C.N.R.-I.S.E. Sede di Verbania. 2005; 2006
Lac de Joux	JOX	46.63	6.26	1004	9	0.2	38	21	12	211	N/A	12.5	15.3	6.6	20	1770	1998-2006 (m)	DBGZ, Lods-Crozet, pers. comm.
Vierwaldstättersee	HRW	46.99	8.34	433	114	11.8	214	104	20	2124	3.4	19.0	18.0	8.9	21	1157	2003-2005 (m)	DBGZ
Sempachersee	SMP	47.16	8.14	504	14.4	0.6	87	44	76	62.6	16.9	14.8	17.6	9.2	22.3	1114	1995-2005 (m)	DBGZ
Präffikersee	PFA	47.35	8.78	537	3.3	0.06	36	19	32	40	2.1	17.1	17.6	8.4	22.3	1162	1995-2005 (m)	DBGZ
Mondsee	MDS	47.82	13.38	481	14.2	0.5	68	36	48	247.2	1.7	11.6	17.8	9.7	20.9	1545	2003-2006 (m)	Dokullil, pers. comm.
Titisee	TTS	47.89	8.15	846	1.1	0.02	39	20	37	24.2	22.5	34.9	14.5	5.6	N/A	1329	1989 (a)	Rosknecht, 1992; Nixdorf <i>et al.</i> , 2004
Lac de Gerardmer	GDM	48.07	6.85	660	1.2	0.02	38	13	32	N/A	2.1	26.0	17.9	8.8	N/A	581	1976 (ty)	Lafont <i>et al.</i> , 1991
Laachersee	LCH	50.41	7.27	275	3.3	0.1	52	31	50	12.2	21	56.2	17.1	8.9	20.1	703	2000-2003 (m)	Landesamt fuer Wasserwirtschaft, Germany
Maarsveense Plassen A	MSV	52.14	5.09	1	0.7	0.09	31	12	24	N/A	N/A	27.8	17.4	9.8	20.8	793	1976-1985 (s)	Swain <i>et al.</i> , 1987; Lingeman <i>et al.</i> , 1987
Arendsee	ARE	52.89	11.49	23	5.2	0.2	49	29	43	29.8	114	25.9	17.3	8.5	N/A	566	2006 (s)	Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen ; Walpersdorf <i>et al.</i> , 2004; EEA

Table 1 Continued.

Lake name	Physical and climatological data											Environmental data				Source		
	Code	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	Open water area (km <sup>2</sup> )	Volume (km <sup>3</sup> )	Maximum depth (m)	Average depth (m)	Sampling depth (m)	Catchment area (km <sup>2</sup> )	Retention Time (years)	LOI550 (%)	MJAT (°C)	MAAT (°C)	SSWT (°C)		MAP (mm)	Sampling frequency
Grosser Plönersee	GPS	54.16	10.42	21	30	0.4	58	12	41	382	3	25.4	16.5	8.4	19.3	654	1998-2006 (bw)	Landesamt für Natur und Umwelt des Landes Schleswig-Holstein, EEA
Furesø	FUR	55.80	12.42	80	7.4	0.1	38	17	36	79	10.6	23.3	16.4	8.0	19	613	1989-2002; 2005 (a); 1997(m)	EEA; De Vicente <i>et al.</i> , 2008; Københavns Amt, 2001
Vättern	VAT	58.52	14.62	89	189	77.6	128	39	61	4447	55.9	12.2	15.8	6.5	17.1	468	1998-2003 (m)	Institutionen för vatten och miljö; SLU
Vänern	VAN	58.96	12.79	44	565	153	106	27	61	411809	7.7	16.4	6.4	17.5	517	1998-2006 (m)	Institutionen för vatten och miljö; SLU	
Hjälmarén	HJA	59.15	15.71	22	483	3	22	6	10	3575	3.5	16.2	16.5	5.9	18.6	614	1990-1995; 2003 (m)	Institutionen för vatten och miljö; SLU
Siljan	SIL	60.89	14.80	161	292	8.1	134	27	83	11894	N/A	16.9	15.8	5.8	17.9	647	1990-2005 (m)	Institutionen för vatten och miljö; SLU
Navarn	NAV	62.59	16.69	280	9.6	0.1	52	10	49	37	N/A	12.5	12.9	2.7	18.4	598	1998-2006 (m)	Institutionen för vatten och miljö; SLU; EEA
Kallsjön	KAL	63.66	13.02	380	158	6.1	134	40	73	N/A	N/A	36.9	13.4	2.5	N/A	662	-	-
Mellan Rissjön	MRI	64.75	15.52	443	1.6	0.02	29	11	6	82.9	N/A	29.0	12.9	-0.1	14.5	682	1998-2006 (m)	Institutionen för vatten och miljö; SLU
Malgomaj	MAL	64.77	16.19	341	101	3.1	148	117	78	N/A	N/A	26.8	13.4	0.4	15.8	682	2007 (sm)	Fisk o Vattenvård i Norrland AB, 2007
Vojmsjön	VOJ	64.94	16.49	420	70	3.1	145	39	71	2253	N/A	13.7	12.9	-0.1	15.9	682	2007 (sm)	Fisk o Vattenvård i Norrland AB, 2007
Hornavan	HRW	66.18	17.76	425	283	11.2	228	45	32	N/A	N/A	14.1	12.3	-1.3	15.1	598	2006 (sm; m)	Institutionen för vatten och miljö; SLU
Stora Lulevatten	LUV	67.13	19.53	365	155	1.3	29	9	21	9793	N/A	22.0	12.6	-1.8	N/A	522	-	-
Torneträsk	TOR	68.36	18.85	341	332	17.1	168	51	29	3300	N/A	6.1	12.0	-0.5	8.2	304	2004-2005 (m)	Länsstyrelsen i Norrbottens län, Sweden

## Environmental parameters

Single spot measurements of water temperatures and chemistry provide a very incomplete picture of local limnological conditions (e.g. Livingstone and Lotter, 1998; Bunbury and Gajewski, 2008). Therefore, we relied on time-series and multiple measurements of environmental parameters at the study lakes instead, which were available from local monitoring programs, governmental agencies, and literature. Data on mean July and mean annual air temperatures (MJAT and MAAT, respectively) were retrieved as Climatic Normals (1961-1990 period) from databases of national meteorological institutes. Site-specific air temperatures were inferred by averaging measurements from stations close to study sites and were corrected for altitude (standard lapse rate of  $-0.6^{\circ}\text{C}/100\text{ m}$  altitude; Tabony, 1985; Rolland, 2002). Information on the physical and chemical characteristics of the lakes was obtained from online databases (Institutionen för vatten och miljö, <http://www.ma.slu.se/>), national and regional water protection agencies, and via personal communication with scientists involved in local lake monitoring programs (Table 1). Water temperature profiles were available for many lakes and allowed calculations of summer surface water temperatures (SSWT) to compare with air temperatures. These data also allowed assessment of the timing of summer stratification and spring circulation. Oxygen concentrations in the bottom waters during summer stratification ( $\text{O}_{2\text{bwss}}$ ) were used as a measure for oxygen availability. Total phosphorus (TP) concentration was used as an estimate of nutrient availability and was calculated as depth-weighted mean based on measurements obtained during the mixing period in spring. The same calculation was done for lake water conductivity, alkalinity and pH. Loss-on-ignition at  $550^{\circ}\text{C}$  (LOI550) was carried out on surface sediments according to Heiri *et al.* (2001) to estimate the amount of organic matter in the sediment. Since many of the available parameters co-varied along the transect and, therefore, do not provide additional information on the species-environment relationships, we included only a selection of variables in numerical analyses of the chironomid data. This resulted in the exclusion of MAAT (co-varying with MJAT), average depth (co-varying with maximum depth), volume (co-varying with area), and alkalinity (co-varying with conductivity). The following parameters were available for the majority of lakes and were included in the environmental data set: MJAT, SSWT, TP,  $\text{O}_{2\text{bwss}}$ , conductivity, pH, lake area, maximum lake depth, Secchi depth, catchment size, latitude, and LOI550. Environmental parameters that showed skewed distributions were log-transformed (TP, area, maximum depth, catchment size).

## Multivariate statistics

Multivariate analyses were carried out using CANOCO 4.52 (ter Braak and Šmilauer, 1998; ter Braak, 2003). Chironomid percentages were square-root transformed and rare species were downweighted. Detrended correspondence analysis (DCA; Hill and Gauch, 1980) on chironomid data showed a gradient length of DCA axis 1 of 2.9 standard deviation units, indicating that ordination methods based on unimodal species-response models were appropriate for analysis (ter Braak and Prentice, 1988; Birks, 1995). Environmental variables were not available for every parameter for all lakes and different environmental parameters

were missing for different study sites. Therefore, it was not possible to assess the relation between chironomid assemblages and the combined dataset of environmental parameters in a single direct gradient analysis run. Instead, we used indirect gradient analysis (DCA; detrended by segments) to explore major faunistic patterns in the 28 chironomid assemblages. In a second step, the correlation between environmental parameters and DCA axes 1 and 2 was examined to determine which parameters were closely related to the dominant ordination axes. The strength of the relationship between individual parameters and the chironomid assemblages was assessed by calculating a series of individual canonical correspondence analyses (CCA) on the assemblage data with a sole constraining environmental variable (Lotter *et al.*, 1997). Statistical significance of these relationships was assessed by Monte Carlo permutation tests with 9999 unrestricted permutations. Tests for significance were subjected to a Bonferroni correction ( $\alpha=0.05/k=12$ ; Legendre and Legendre, 1998). Unconstrained cluster analysis on chironomid assemblage data was calculated using Ward's method and Euclidean distances were used as the distance metric using the program PAST (Hammer *et al.*, 2001). To avoid over-fitting a similarity of >50% was chosen as threshold for accepting clusters.

## Results

### Relative abundances chironomids

Concentrations of the head capsules of chironomid larvae showed large variations in the different studied lake sediments, ranging from 3.4 (KAL) to 266 (NAV) head capsules per gram sediment (dry weight). The average concentration was 60 head capsules per gram of freeze dried surface sediment. In total, 106 different taxa were identified in the 28 samples. The most abundant taxa were *Heterotrissocladius subpilosus*-type, *Cladotanytarsus*, *Cricotopus*, *Tanytarsus lugens*-type, *Procladius*, *Psectrocladius sordidellus*-type, *Micropsectra radialis*-type, and *Dicrotendipes* (Fig. 2). *Heterotrissocladius subpilosus*-type showed the highest maximum abundance in the dataset with 53% in VOJ (Sweden). Many taxa were restricted to specific regions of the transect. For example, most morphotypes of *Heterotrissocladius* (with the exception of *H. grimshawi*-type) occurred exclusively in Sweden. Similarly, taxa such as *Abiskomyia*, *Parakiefferriella nigra*-type, *Protanypus*, *Paracladius*, *Constempellina*, *Psectrocladius septentrionalis*-type, and *Mesocricotopus* were restricted to northern lakes, while others such as *Paratanytarsus penicillatus*-type, *Paratendipes nudisquama*-type, *Micropsectra insignilobus*-type, and *Sergentia coracina*-type were more common in southern lakes. Other taxa such as *Procladius*, *Cladotanytarsus*, *Cricotopus*, *Microtendipes pedellus*-type, *Dicrotendipes*, *Psectrocladius sordidellus*-type, and *Polypedilum* showed a wider distribution and occurred in more than 85% of the deep lakes.



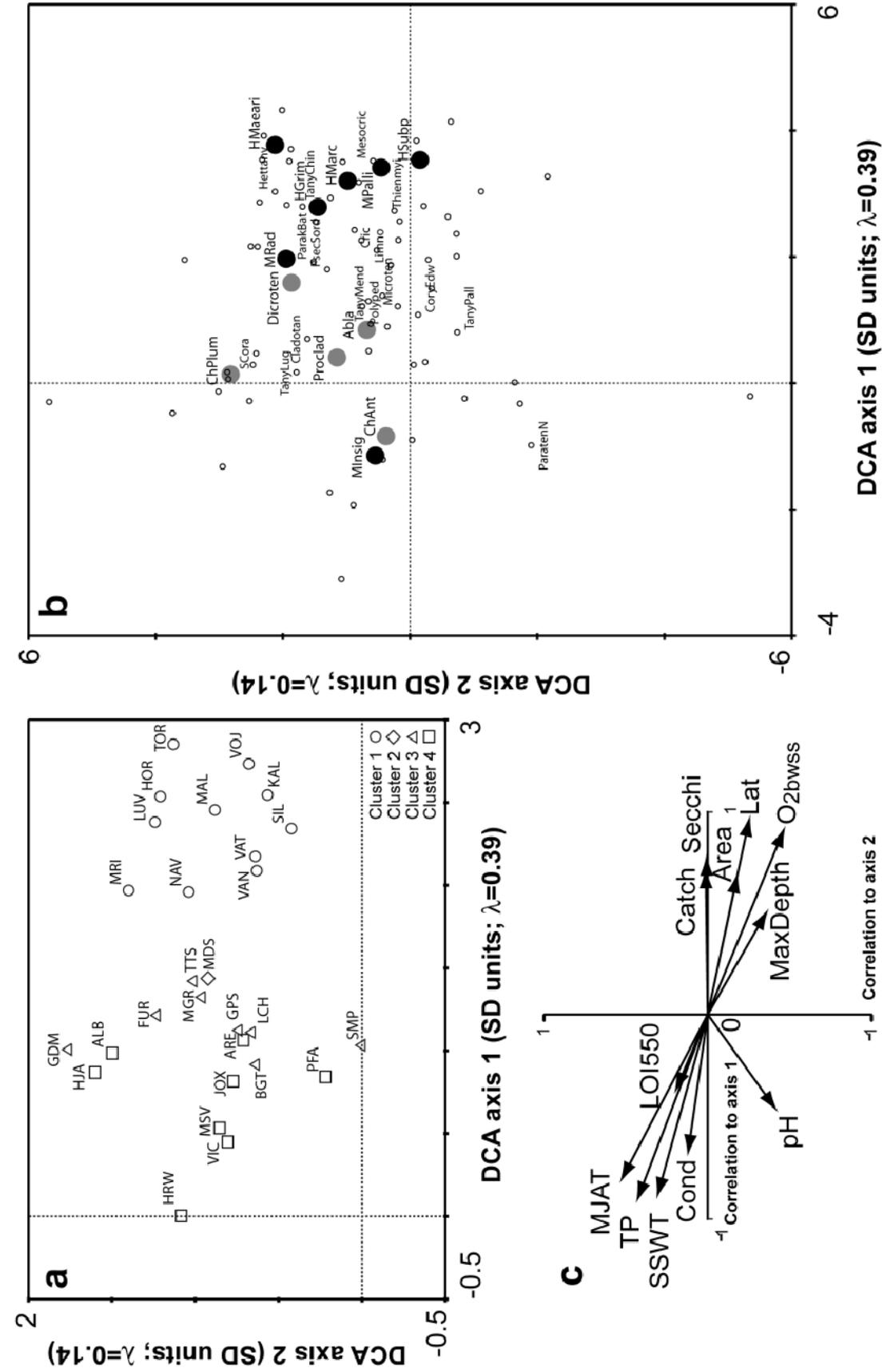
## Gradient analyses

### *Lakes*

DCA was used to explore major faunistic trends and similarities in the assemblage data (Fig. 3). In the scatter plots the sites are arranged in several clusters along DCA axis 1 ( $\lambda=0.39$ , 17.1% of variance explained) and axis 2 ( $\lambda=0.14$ , 5.9% of variance explained) (Fig. 3a). The first cluster consists of 11 lakes (indicated by circles). These sites display relatively high DCA axis 1 scores (TOR, VOJ, KAL, HOR, MAL, LUV, SIL, VAT, VAN, NAV and MRI). Most of these lakes are situated in the northern section of the transect and are apparently dominated by very similar chironomid assemblages. Cluster 2 (Fig. 3a, diamonds) consists of a single site, MDS, which displays an intermediate score for both DCA axes. The third cluster contains eight lakes from the southern section of the transect (TTS, MGR, FUR, GPS, LCH, SMP, BGT and GDM; Fig. 3a, triangles), and displays intermediate DCA axis 1 scores that are lower than for MDS and comprise a large range of DCA axis 2 scores. Finally, the fourth cluster (Fig. 3a, squares) is characterized by the lowest scores on DCA axis 1 and also consists of seven lakes from the southern region of the transect (ARE, ALB, PFA, JOX, MSV, VIC, HRW), as well as one from Sweden (HJA). Similar to cluster 3, lakes in cluster 4 also show a wide range of DCA axis 2 scores.

### *Taxa*

Fig. 3b shows the scores of common chironomid taxa on DCA axes 1 and 2. Taxa such as *Abiskomyia*, *Pseudodiamesa*, *Rheocricotopus*, *Parakiefferiella nigra*-type, all *Heterotrissocladius* morphotypes, *Mesocricotopus*, *Paracladius*, *Parachaeotocladius*, and *Protanypus* display high DCA axis 1 scores (Fig. 3b) and occur predominantly in lakes that form cluster 1. *Micropsectra radialis*-type, *Dicrotendipes*, *Limnophyes*, *Psectrocladius sordidellus*-type, *Cricotopus*-type, many *Tanytarsus* morphotypes, and *Ablabesmyia* are examples of taxa characterized by intermediate values on DCA axis 1. The chironomid assemblage in MDS (cluster 2) consists for 50% of *Micropsectra radialis*-type. Finally, low scores for the first DCA axis are apparent for taxa such as *Macropelopia*, *Paratendipes nudisquama*-type, *Micropsectra insignilobus*-type, *Chironomus*, *Cladopelma laccophila*-type, and *Endochironomus albipennis*-type. Lakes in clusters 3 and 4 mainly consist of species displaying intermediate to relatively high DCA axis 1 scores. Taxa with relatively high DCA axis 1 scores, such as *Heterotrissocladius grimshawi*-type, *Tanytarsus chinyensis*-type, and *Parakiefferiella bathophila*-type are more abundant in lakes forming cluster 3 relative to cluster 4, which accounts for the higher scores of lakes in that cluster. Lakes in cluster 4, on the other hand, display higher abundances for *Chironomus plumosus*-type, *Micropsectra insignilobus*-type, *Macropelopia*, and *Cladopelma laccophila*-type, which all have relatively low scores on DCA axis 1.



**Figure 3 (previous page)** Site (a) and species scores (b) of a detrended correspondence analysis (DCA) based on chironomid assemblage data. Enlarged grey and black symbols in (b) indicate species that are tolerant to low oxygen conditions or indicative for high oxygen levels, respectively (based on Brodersen *et al.*, 2004). (c) Correlation coefficients ( $r$ ) of environmental parameters (arrows) with DCA axes 1 and 2. Lake abbreviations are as listed in Table 1. Species abbreviations are listed for taxa that occur >2 times and have a total abundance >30% throughout the data set. Abla *Ablabesmyia*; ChAnt *Chironomus anthracinus*-type; ChPlum *Chironomus plumosus*-type; Cladotan *Cladotanytarsus mancus*-type; CoryEdw *Corynoneura edwardsi*-type; Cric *Cricotopus*-type; Dicroten *Dicrotendipes*-type; Hettany *Heterotanytarsus*; HGrim *Heterotrissocladius grimshawi*-type; HMaeari *Heterotrissocladius maearei*-type; HMarc *Heterotrissocladius marcidus*-type; HSubp *Heterotrissocladius subpilosus*-type; Limno *Limnophyes*; Mesocric *Mesocricotopus*; Microten *Microtendipes pedellus*-type; MInsig *Micropsectra insignilobus*-type; MPalli *Micropsectra pallidula*-type; MRad *Micropsectra radialis*-type; ParakBat *Parakiefferiella bathophila*-type; ParatenN *Paratendipes nudisquama*-type; Polyped *Polypedilum*; Proclad *Procladius*; PsecSord *Psectrocladius sordidellus*-type; SCora *Sergentia coracina*-type; TanyChin *Tanytarsus chinyensis*-type; TanyLug *Tanytarsus lugens*-type; TanyMend *Tanytarsus mendax*-type; TanyPall *Tanytarsus pallidicomis*-type; Thienmyi *Thienemannimyia*

### *Species-environment relationship*

All variables, except for pH and LOI550 show a strong positive or negative correlation with DCA axis 1 (Fig. 3c). Latitude and hypolimnetic oxygen concentrations during summer stratification show the strongest positive correlations with the first DCA axis, whereas Secchi depth, catchment size, lake area, and maximum depth show weaker correlations to this axis. TP concentrations, and both water and air temperatures are strongly negatively correlated with axis 1. Conductivity is also negatively correlated with the first DCA axis, however the correlation is less strong. Correlations with DCA axis 2 are less pronounced. pH and oxygen concentrations during the summer stratification are negatively correlated with axis 2, whereas temperature during the summer months and TP show the strongest positive correlation with this axis.

### *Statistical significance of environmental variables*

The statistical significance of relationships between these environmental variables and chironomid assemblages was assessed by Monte Carlo permutation tests in a series of CCAs with a single constraining environmental variable (Table 2). Latitude and parameters closely related to latitude, such as temperature (MJAT, SSWT), oxygen availability ( $O_{2bwss}$ ), and conductivity explained the largest proportion of variance in the chironomid assemblage data (14-9%; Table 2). Moreover, TP, which in our data set is significantly correlated with temperature ( $P < 0.05$ ) explains a large proportion of the variance in the chironomid data (13%). In addition, pH, lake area, Secchi depth, maximum depth, and catchment area explained noticeable proportions of variance (7-8%). Organic matter content of the sediments (LOI550) only showed a weak relationship with the chironomid assemblages. After applying a Bonferroni adjustment the following environmental variables were statistically significant ( $P < 0.05$ ) in order of explanatory power: latitude (14% of variance explained), SSWT (13.7%), TP concentration (12.6%),  $O_{2bwss}$  (11.4%), and MJAT (11.2%).

**Table 2** Summary of different runs of canonical correspondence analyses (CCA) performed on chironomid assemblage data and a single explanatory environmental variable. For each variable the number of lakes with this parameter available, percentage variance explained,  $\lambda_1/\lambda_2$  and P-values are indicated. Parameters marked with an asterisk are significant ( $\alpha=0.05$ ;  $k=12$ ) after a Bonferroni correction.

Analysis	Abbreviation	Unit	Number of lakes	% variance explained	$\lambda_1/\lambda_2$	P-value	Range	Median	SD
Latitude	LAT	°N	28	14	1.59	0.0001*	41.7-68.4	53.5	8.4
Summer surface water temperature	SSWT	°C	23	13.7	1.3	0.0001*	8.2-24.1	19.3	3.6
Total phosphorus	TP	µg/l	26	12.6	1.13	0.0001*	1.5-180	12	46.2
Main July air temperature	MJAT	°C	28	11.2	1.09	0.0001*	12-22.5	16.5	2.9
O <sub>2</sub> in bottom water during summer stratification	O <sub>2, bWSS</sub>	mg/l	21	11.4	0.93	0.0008*	0.0-12.6	3.3	4.4
Conductivity	COND	µS/cm	23	9.2	0.79	0.0027	1.3-550	146	183
pH	PH		25	8.4	0.62	0.0029	6.16-8.7	7.6	0.6
Area	AREA	km <sup>2</sup>	28	7.6	0.56	0.0031	0.7-5648	22.2	1100
Secchi depth	SECCHI	m	22	8.3	0.59	0.0087	2.2-14.9	4.7	4
Maximum depth	MAX DEPTH	m	28	6.6	0.43	0.0099	22-370	63.2	80.6
Catchment area	CATCH	km <sup>2</sup>	23	7.5	0.55	0.0136	3.7-41180	247.2	8800
Loss-on-ignition at 550 °C	LOI550	%	28	3.9	0.24	0.3571	5.4-56.2	18.1	10.9

# Discussion

A range of studies is available that describe the distribution of subfossil chironomid assemblages in different regions of Europe. For example, for northern Fennoscandia Olander *et al.* (1999), Larocque *et al.* (2001), and Nyman *et al.* (2005) examined the distribution of chironomids in lakes and demonstrated a strong relationship between chironomid assemblages and temperature (measured as air or water temperature), as well as organic matter content of the sediment. Additionally, in the former two studies chironomid assemblages also showed a strong and statistically significant relationship with maximum lake depth, whereas in the latter study chironomid distributions were significantly related to total organic carbon concentration and pH of the lake water. Similarly, Luoto (2009b) determined that the distribution of chironomid assemblages in lakes in Finland is strongly related to MJAT, although their relation with conductivity, sampling depth, and dissolved oxygen was also statistically significant. Langdon *et al.* (2008) related chironomid distributions to environmental parameters in northwestern Iceland and found that chironomid assemblages were significantly related to substrate (measured as total carbon content) and MJAT. Chlorophyll  $\alpha$  and Secchi depth were found to be strong explanatory variables for the distribution of chironomid assemblages in lakes in Denmark (Brodersen and Lindegaard, 1999), where temperature was not included in the analysis. In the United Kingdom, chironomid assemblages were strongly related to TP, hypolimnetic dissolved oxygen, maximum lake depth, and Secchi depth (Brooks *et al.*, 2001). In lakes in the Swiss Alps, however, dissolved organic carbon, air and water temperatures, maximum depth, organic matter content of the sediments, and conductivity were found to be the parameters which best explained the distribution of lake-living chironomids (Bigler *et al.*, 2006). Finally, Lotter *et al.* (1997, 1998) demonstrated that MJAT and trophic state explain a large proportion of variance in chironomid distributions in Swiss lakes. These studies indicate that summer temperature (measured either in water or air) seems to be the dominant or one of the dominant environmental parameters explaining chironomid distributions in Europe. However, all of the above-mentioned studies focused on comparatively shallow lakes and on restricted geographical regions. Moreover, they did not always include all parameters that are generally considered to exert a major influence on chironomid distributions. For example, few studies are available which include oxygen concentrations at the lake bottom (e.g. Brooks *et al.*, 2001; Luoto, 2009b). Our study includes lakes that have different physical characteristics, such as lake area, depth, and catchment area. However, they all share that they are deep and thermally stratified during summer. Direct gradient analyses revealed a statistically significant relationship between chironomid assemblage composition and latitude, SSWT, MJAT, TP, and hypolimnetic oxygen concentrations during summer stratification (Table 2).

## Latitude, temperature, and geology

In our dataset latitude, SSWT, MJAT, TP,  $O_{2\text{bwss}}$ , and conductivity are all closely and statistically significantly correlated with each other ( $r^2 = 0.21-0.82$ ;  $P < 0.05$ ). Many studies have demonstrated a strong relationship between air or water temperature, and correlated parameters, with chironomid assemblages (e.g. Walker *et al.*, 1997; Heiri *et al.*, 2003; Lotter *et al.*, 1997, Brooks and Birks, 2001; Larocque *et al.*, 2001; Luoto, 2009b; Eggermont *et al.*, 2009). However, considerable controversy has developed over the question whether a causal link exists between temperature and the taxonomic composition of chironomid assemblages in lakes (Walker and Mathewes 1987a, 1987b; Warner and Hann, 1987; Walker *et al.*, 1991, 1992; Hann *et al.*, 1992). Recent research demonstrated that this relationship between temperature and chironomid assemblages is apparent in temperate to subarctic regions in both the northern and southern hemisphere (e.g. Larocque *et al.*, 2001; Porinchu *et al.*, 2002, 2009; Heiri *et al.*, 2003; Dieffenbacher-Krall *et al.*, 2007) and can also be seen in low latitude lakes (Eggermont *et al.*, 2009). This strongly suggests that, at the spatial scales examined, water temperature is a key ecological factor affecting the distribution of chironomid assemblages. Our study is the first that examines the distribution of subfossil chironomids in a dataset which consists exclusively of deep lakes (Table 1). In most of these lakes, hypolimnetic water temperatures will be close to 4°C during the entire year. Both summer surface water and air temperatures are, nevertheless, strong and statistically significant explanatory variables for the distribution of chironomid assemblages in our study lakes. Taphonomic processes may partly explain this apparent relationship between chironomids and temperature. Sediment samples taken in the deepest part of lake basins may contain head capsules from both the hypolimnion and the shallower parts of lakes (Frey, 1988; Schmäh, 1993; Heiri, 2004). Generally, it can be expected that most chironomid head capsules in a sample will originate from the vicinity of the coring site (Iovino, 1975). However, the situation may be different in lakes where the abundance of chironomid larvae in deep-water habitats is low, e.g. in lakes with permanently anoxic bottom waters. In profundal sediments of such lakes a considerable proportion of chironomids in subfossil assemblages may be redeposited from shallower parts of the lake basin where chironomids may be subject to the direct influence of elevated temperatures during summer. However, in our data the shift in chironomid assemblages with respect to air and lake surface temperatures is also apparent in taxa typically restricted to profundal environments. For example, profundal taxa such as *Heterotrissocladius subpilosus*-type, *Heterotrissocladius maeaeri*-type, *Paracladius*, *Micropsectra radialis*-type, *Micropsectra insignilobus*-type, *Sergentia coracina*-type, *Chironomus anthracinus*-type, and *Chironomus plumosus*-type are all restricted to specific regions of the latitudinal gradient (Fig. 2). This indicates that redeposited chironomid remains from the littoral cannot be the only explanation for the strong relationship between chironomid assemblages and temperature.

In our dataset, a number of limnological variables important for the survival of chironomid larvae, such as nutrient and oxygen concentration of the lake water, are closely correlated with temperature. Lakes situated in regions of cool climatic conditions are commonly less rich in nutrients than lakes in warmer regions (Brodersen and Anderson, 2002). In our dataset warmer lakes are also located in lower latitude regions with a higher human population density. Many of these lakes have been affected by anthropogenic nutrient

enrichment which may have reinforced the natural relationship between temperature and nutrient availability in the set of lakes sampled for this study. Nutrient availability determines the productivity of lakes and therefore also affects their susceptibility to hypoxia. Furthermore, in seasonally stratified lakes summer stratification can be expected to be more stable under warmer climatic conditions thereby also promoting hypoxic conditions during the summer months. Hence, it is not surprising that oxygen concentrations during summer were negatively correlated with summer temperature in our dataset.

The close correlation of summer air temperature and water temperature with other parameters makes it difficult to disentangle the relationship between chironomid assemblages and temperature from their relation with lake nutrient status and productivity. Temperature influences emergence, growth and development, flight, reproduction, and dispersal of adult chironomids and can also affect egg and larval development and pupation, especially in the shallower, littoral regions of lakes (e.g. Walker and Mathewes, 1989; Rossaro, 1991; Walker *et al.*, 1991). Temperature indirectly influences chironomid distributions via changes in oxygen conditions, length of ice-cover period, thermal stratification, and food quantity and quality (e.g. Walker and Mathewes, 1989; Rossaro, 1991; Walker *et al.*, 1991). Brooks and Birks (2001) argue that air temperature influences the winged adult stage and water temperatures has an impact on chironomid distributions by influencing the larval stage. In our dataset MJAT and SSWT are significantly correlated ( $r = 0.87$ ), which is in agreement with earlier studies (Livingstone and Lotter 1998; Livingstone *et al.*, 1999), which examined the relation between surface water and air temperatures in lakes in the Alpine region. In our study the relationship between chironomid assemblages and temperature is best explained by surface water temperature (13.7% of variance explained), as opposed to air temperature (11.2%). The opposite is seen in other surveys (e.g. Bigler *et al.*, 2006), which is attributed to the poor approximation of monthly water temperature values by the single spot measurements commonly used to estimate summer surface water temperature. We used variance partitioning (Lepš and Šmilauer, 2003) to assess whether water temperature can explain an independent part of the variance in chironomid assemblages that is not related to nutrient and oxygen availability (Table 3). Our results indicate that both MJAT and SSWT explain a statistically significant proportion of the variance in the chironomid assemblage data if the effects of  $O_{2bwss}$  and TP are partialled out. This suggests that even though lake nutrient status and oxygen availability may explain a large part of the relationship between chironomids and temperature, MJAT and SSWT have an effect on chironomid assemblages in deep lakes that is not related to the effects of climate on lake trophic status.

Conductivity is strongly dependent on the geology of the catchment. Water from lakes in catchments containing carbonate-rich bedrock generally display high conductivity values, as opposed to softwater lakes in catchments dominated by siliceous bedrock. The northern part of our transect mainly consists of siliceous bedrock and low conductivities are therefore measured in lakes in Sweden. Due to the distribution of bedrock geology in Europe, conductivity is therefore correlated with latitude in our dataset, and hence, also with temperature.

**Table 3** Summary of Canonical Correspondence Analyses (CCAs) performed on chironomid assemblage data and a single explanatory environmental variable with one or two co-variables. For each analysis the number of lakes with the environmental and co-variable data available, percentage variance explained, and P-values are indicated.

Variable	Co-variable(s)	Number of lakes	% variance explained	P-value
MJAT	TP	26	6.8	0.0051
MJAT	O <sub>2</sub> bwss	21	7.7	0.0266
MJAT	TP and O <sub>2</sub> bwss	21	8.1	0.0327
SSWT	TP	23	8.4	0.0014
SSWT	O <sub>2</sub> bwss	19	10.2	0.0044
SSWT	TP and O <sub>2</sub> bwss	19	10	0.013

### Nutrient and oxygen availability

Profundal chironomid communities have been extensively used to classify lakes with regard to their trophic conditions (e.g. Saether, 1979; Wiederholm, 1980). Chironomid distributions in lakes in the UK are strongly related to TP (Brooks *et al.*, 2001). Lindegaard (1995) and Wiederholm (1980) stated that chironomid communities in stratified lakes are influenced by trophic conditions through an interaction between food and oxygen availability. Brodersen and Quinlan (2006) indicated that lake temperature and productivity are closely linked. This concurs with the correlation between temperature and the availability of nutrients and oxygen in the deep and stratified lakes studied here. Oxygen measurements are rarely incorporated in surveys examining the relationship between subfossil chironomid assemblages and their environment. A number of available studies, however, have shown that dissolved oxygen in the hypolimnion can be an important factor affecting chironomid distributions (Brooks *et al.*, 2001; Quinlan *et al.*, 2003; Luoto, 2009b). Hypolimnetic oxygen concentrations are influenced by physical, chemical, and biological variables, such as lake volume and depth, water temperature, length and strength of thermal stratification period, trophic state, and input of material from the surrounding catchment (Brodersen and Quinlan, 2006). In this survey, oxygen concentrations were assessed as hypolimnetic means during summer, which is the period when oxygen stress likely becomes most acute and is most likely to affect benthic profundal biota. Hypolimnetic oxygen levels close to 0 mg O<sub>2</sub>/l occur in many of the study lakes. Some chironomid taxa are able to tolerate low oxygen levels due to high levels of haemoglobin in their haemolymph (e.g. Int Panis *et al.*, 1996) or can adapt to anoxia by switching to anaerobic metabolism (Brodersen *et al.*, 2004). In our study chironomids known

to be tolerant to low oxygen levels (e.g. *Chironomus*, *Dicrotendipes*, *Ablabesmyia*, and *Procladius*; Fig. 3b) were predominantly found in lower latitude lakes that are characterized by higher nutrient levels and, in general, lower oxygen availability. In contrast, taxa sensitive to hypoxia were predominantly found in more northerly, cool lakes characterized by lower nutrient concentrations and higher hypolimnetic oxygen content. This suggests that oxygenation of the deep-water environments is a major factor affecting chironomid assemblages in the examined lakes.

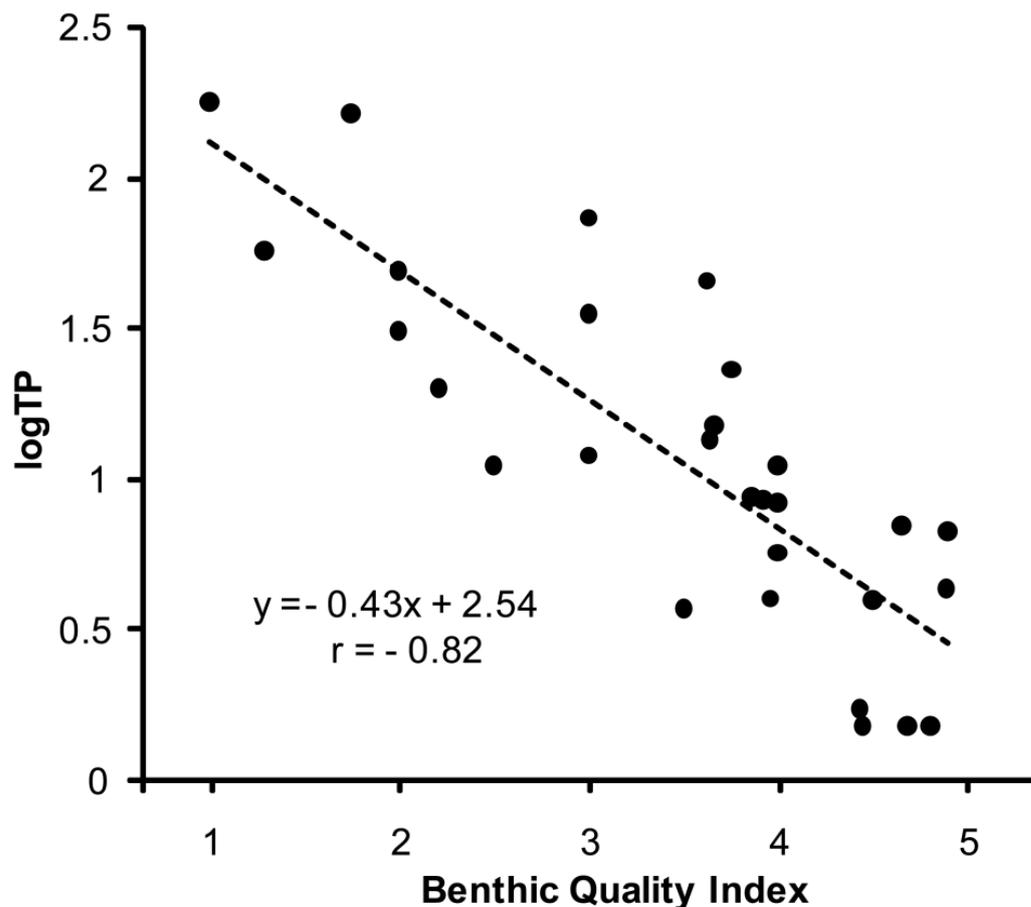
### Other factors

The distribution of subfossil chironomid assemblages is related to lake depth in a number of available studies (e.g. Olander *et al.*, 1999; Larocque *et al.*, 2001; Brooks *et al.*, 2001; Bigler *et al.*, 2006). Walker *et al.* (1991) suggested that lake depth affects chironomid assemblages by influencing water temperature. In our study, however, lake depth does not seem to play a major role. Lake depth is not significantly correlated with either MJAT or SSWT ( $P < 0.05$ ) and explains only a comparatively minor part of the variance in the chironomid assemblages. Water depth ranges from 22 to 370 m in the examined lakes. However, these lakes are comparatively deep, stratified systems and chironomid taxa indicative for profundal environments can be found in all of them. It seems, therefore, that beyond a certain depth range, the importance of water depth for the development and survival of chironomid larvae may be less important than in shallower stratified and unstratified lakes. Water transparency (as Secchi depth), sediment organic matter content, and pH have also been put forward as factors that may influence the distribution of chironomid larvae (e.g. Brodersen and Lindegaard, 1999; Olander *et al.*, 1999; Brooks *et al.*, 2001; Larocque *et al.*, 2001; Nyman *et al.*, 2005; Bigler *et al.*, 2006; Langdon *et al.*, 2008). However, none of these parameters was identified as a significant explanatory variable in our dataset, even though they cover a comparatively large gradient of environmental conditions. This suggests that these parameters are either not as important for chironomid assemblages in deep, stratified lakes, or that their explanatory power in shallower lake systems is largely due to their co-variation with parameters such as lake trophic state or climate.

### Benthic Quality Index as an indicator for trophic state

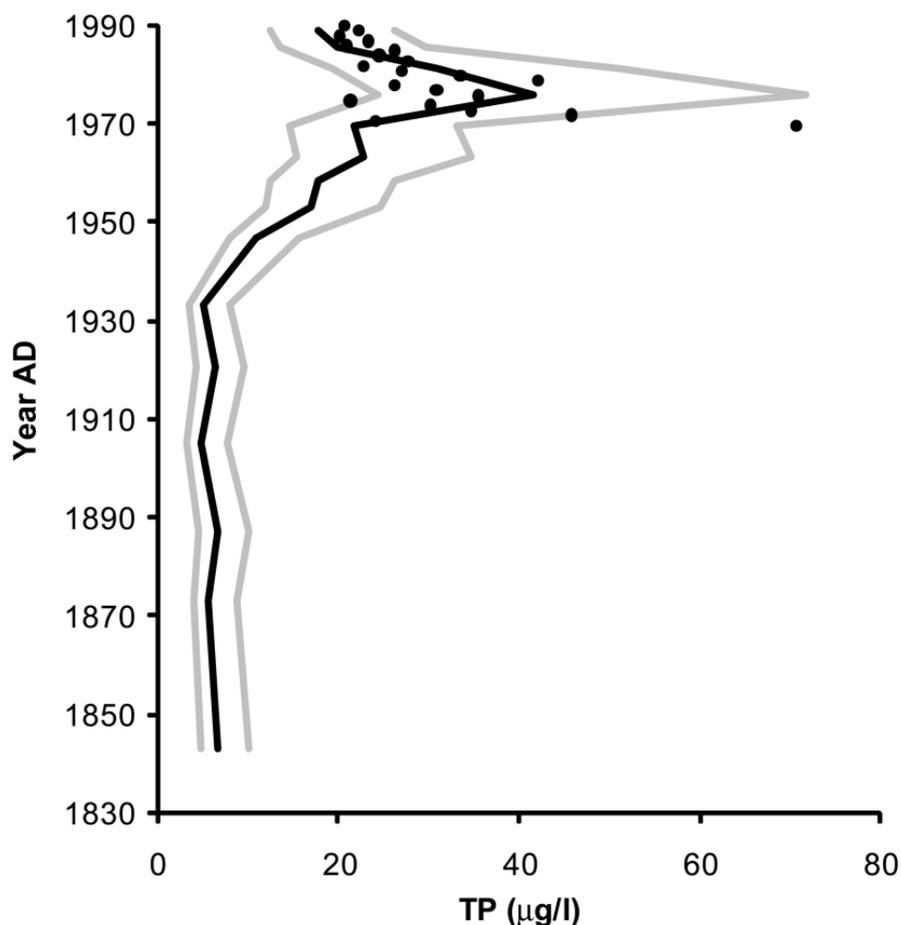
Trophic state has a strong effect on chironomid larvae in lakes (e.g. Brundin, 1949, 1956; Saether, 1979). However, fossil chironomids are less frequently used for inferring past changes in nutrient availability and lake productivity than other palaeolimnological proxies such as diatoms (e.g. Lotter, 1998, 2001; Kirilova *et al.*, 2009). Some quantitative reconstructions of TP and oxygen based on fossil chironomid assemblages are available, mainly from unstratified or shallow, stratified lake ecosystems (e.g. Brooks *et al.*, 2001; Quinlan and Smol, 2001; Langdon *et al.*, 2006). In deep, stratified lakes, classification schemes and biomonitoring approaches developed for profundal chironomid assemblages have been applied to sediment sequences. For example, Benthic Quality Index (BQI) values

Wiederholm, 1980) calculated from subfossil assemblages in deep stratified Finnish lakes have been used to infer past changes in lake trophic state (e.g. Itkonen *et al.*, 1999; Meriläinen *et al.*, 2003). The BQI has successfully tracked past phases of organic pollution in the studied lake systems and allowed a detailed assessment of the first occurrence of nutrient enrichment and of the pre-impact state of the lakes' chironomid fauna (e.g. Meriläinen and Hamina, 1993; Meriläinen *et al.*, 2003). However, the BQI, which relies on indicator scores for selected sensitive chironomid species, has been developed for assessments using the modern chironomid fauna of lakes rather than subfossil assemblages. The approach remains to be validated on subfossil chironomids and presently allows at the most semi-quantitative statements of past changes in lake nutrient conditions. Our dataset provides information on the occurrence and abundance of chironomid remains in the surface sediments of deep lakes covering a large trophic gradient. It therefore allows an evaluation of the BQI by comparing index values against the observed nutrient concentrations in lakes. For this comparison we have supplemented our dataset with chironomid assemblages from 5 deep lakes in the Alpine area that were sampled in previous studies of subfossil assemblages in surface sediments (Arnensee, maximum depth 47.2 m; Lej da Silvaplauna, 77 m; Lej da Champfèr, 36 m; Silsersee, 71 m; Lej da San Murezzan, 44 m; Heiri, 2001; Bigler *et al.*, 2006; Verbruggen, unpublished). BQI values based on subfossil chironomid assemblages ranged from 1 to 4.9 (Fig. 4) and, hence, covered almost the entire range of possible BQI values from 1 (strongly



**Figure 4** Relationship of the Benthic Quality Index with log-transformed TP (logTP) of the studied lakes

eutrophic) to 5 (ultraoligotrophic) (Lindegaard, 1995). Saether (1979) and Wiederholm (1980) related chironomid classification schemes and the BQI to lake TP concentrations divided by mean lake depth. The rationale behind this approach was that deeper lakes are more susceptible to anoxia and therefore the effects of nutrient enrichment on chironomid larvae can be expected to be more severe in shallow, stratified lakes than in deeper ones. The BQI calculated based on lake surface sediments from our study lakes is negatively correlated with both TP ( $r = -0.71$ ) and TP divided by mean depth ( $r = -0.68$ ), although the former relationship is stronger. TP after log-transformation shows an even stronger linear relationship with the BQI ( $r = -0.82$ ), which can be expressed as a simple linear regression (slope  $-0.43$ , constant  $2.54$ ; Fig. 4). The residuals of this regression amount to a root mean square error of  $0.31$  logTP units, indicating that in deep, stratified European lakes TP can be predicted fairly well based on the BQI calculated from fossil chironomids. To examine whether this relationship holds for down-core records we applied the linear regression to reconstructed BQI from Lake Päijänne, Finland (Meriläinen and Hamina, 1993). Calculated BQI values in this record vary between 2 and 4.5 for sediments deposited between AD 1840 and 1990. As the water quality in this lake has been regularly monitored since the late 1960s TP values predicted based on the linear relationship between BQI and log-transformed TP can



**Figure 5** Total phosphorus (TP) reconstruction of Lake Päijänne for the period AD 1840-1990 (black curve) based on the relationship between the Benthic Quality Index and fossil chironomid assemblages presented in Fig. 4. Grey curves show 95% confidence intervals. Black circles represent measured TP in the lake for AD 1970-1990 (Water quality data bank, Finnish Environment Institute)

be directly compared to instrumental measurements. Reconstructed values suggest that Lake Päijänne was oligotrophic before ca. AD 1930 (Fig. 5) and that the lake progressed through a rapid eutrophication from the 1940s to the 1960s, leading to TP concentrations in the meso- to eutrophic range in the 1970s. Inferred TP values are in good agreement with measured TP in Lake Päijänne between AD 1970-1990. The results suggest that this approach of reconstructing past TP has considerable potential for reconstructing trophic change in deep, stratified lakes, especially in combination with other, independent palaeolimnological proxies for inferring lake water TP, such as fossil diatom assemblages. Since the BQI has been defined exclusively for deep, stratified lakes, it is expected that this approach will not be applicable in shallow lake systems where deep-water chironomid assemblages are supplemented by chironomid remains originating from the littoral region. In such lakes, TP reconstruction based on multivariate transfer function techniques (see, e.g. Lotter *et al.*, 1998; Brooks *et al.*, 2001) may be more promising.

## Conclusions

On local and regional scales, site-specific variables, such as organic matter content and pH have a strong effect on aquatic invertebrate communities (Pinder, 1995; Johnson and Goedkoop, 2002). On larger (continental to global) scales, chironomid distributions are often influenced by large-scale climatic patterns, such as temperature variations. We have shown that at the continental scale chironomid assemblages in deep, stratified lakes in Europe are strongly related with water surface and air temperatures. In the examined dataset temperature is correlated with parameters reflecting lake nutrient content and trophic state, such as TP and oxygen concentrations. However, a statistically significant relationship between temperature and chironomid assemblages exists in our dataset which is independent of the lake water nutrient content and oxygen availability. This suggests that indirect effects via lake trophic state are not the only way that temperature affects chironomid populations in these deep, stratified lake systems. Indirect effects of temperature via limnological parameters not examined in this study (e.g. dissolved organic carbon content) or direct effects of temperature on adults during the emergence period may be responsible for this relation. However, considering the strong correlation between surface water temperature and trophic state in deep stratified lakes in Europe and the indirect nature of the effects of temperature on chironomid larvae it remains to be determined whether fossil chironomid assemblages can be used to produce chironomid-temperature transfer functions for these systems. Trophic conditions are a major determinant of chironomid assemblages in deep lakes, an observation confirmed by the strong relationship between chironomids and TP in our data. A comparison of BQI values calculated from subfossil chironomid assemblages with lake water TP suggests that the BQI can be used to approximate past TP in deep, stratified lakes, as is further supported by the good agreement of a down-core TP reconstruction based on the BQI with historical records of measured TP in Lake Päijänne. However, this approach clearly needs to be validated on additional down-core records before it can be widely applied for quantifying past nutrient dynamics from lake sediments.

# Acknowledgements

Liesbeth Vissers, Cornelia Blaga and Koos Swart are thanked for assistance in the field. We are indebted to the many contributors of environmental data, in particular Gertrud Bernhart-Dietrich (Landesamt für Wasserwirtschaft, Germany), Milena Bruno (National Institute of Health, Italy), Brigitte Lods-Crozet (Laboratoire du Service des Eaux, Sols et Assainissement, Switzerland), Martin Dokulil (Institut für Limnologie, Mondsee), Letizia Garibaldi (Università degli Studi di Milano Bicocca), Lisa Lundstedt (Länsstyrelsen i Norrbottens län, Sweden), Detlef Möbes (Landesbetrieb für Hochwasserschutz und Wasserwirtschaft (LHW) Sachsen-Anhalt, Germany), Gérard Paolini (Cellule Technique de l'Aquarium du Bourget), Gudrun Plambeck (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein, Germany), Fabrizio Scialanca (Tuscia University). Environmental data has furthermore been provided by the Bundesamt für Umwelt, Switzerland, European Environmental Agency, and Sveriges Lantbruksuniversitet, Sweden. Jarmo Meriläinen is cordially thanked for providing data on recent TP concentrations from Lake Päijänne. We thank Walter Finsinger for linguistic help and providing us with important contacts. This study was financially supported by the Darwin Center for Biogeosciences (Utrecht, the Netherlands; [www.darwincenter.nl](http://www.darwincenter.nl)) and a Schure-Beijerinck-Popping Grant to AFL.

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## Chapter 3



# Stable oxygen isotopes in subfossil aquatic invertebrates (chironomids, cladocerans) from deep European lakes

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*To be submitted to Limnology and Oceanography*

## Abstract

Understanding modern relationships between lake water and aquatic invertebrate  $\delta^{18}\text{O}$  is essential for the interpretation of palaeoclimate records based on stable oxygen isotopes in these fossils. We analyzed the stable oxygen isotope composition ( $\delta^{18}\text{O}$ ) of lake water and invertebrate remains, such as the head capsules of chironomid larvae and the resting eggs (ephippia) of planktonic cladocerans in surface sediments from 31 large, deep, and stratified lakes along a latitudinal transect through Europe.  $\delta^{18}\text{O}$  was measured on both lake water and remains from aquatic invertebrates and compared to modeled  $\delta^{18}\text{O}$  in precipitation. A strong linear relationship between mean annual air temperature and  $\delta^{18}\text{O}$  of precipitation along the transect ( $r=0.97$ ) was observed, whereas the relationship between precipitation  $\delta^{18}\text{O}$  and lake water  $\delta^{18}\text{O}$  was less pronounced ( $r=0.80$ ). A strong positive correlation was observed between  $\delta^{18}\text{O}$  of aquatic invertebrates and lake water ( $r=0.95$  and  $0.94$  for chironomids and cladocerans, respectively). Although slopes of linear regressions between lake water  $\delta^{18}\text{O}$  and  $\delta^{18}\text{O}$  of both aquatic invertebrate groups are similar, there was a systematic offset between the absolute  $\delta^{18}\text{O}$  values of chironomids and cladocerans, with chironomid samples generally characterized by a 2‰ heavier oxygen isotopic composition. We attribute this to differences in ecology, metabolism, and/or behavior between benthic chironomid larvae and planktonic cladocerans. Although many aspects regarding  $\delta^{18}\text{O}$  of aquatic invertebrates still remain to be evaluated, we demonstrate that chironomid and cladoceran  $\delta^{18}\text{O}$  records have the potential to be used to infer past lake water  $\delta^{18}\text{O}$  and, indirectly, past climatic changes from lake sediments.

# Introduction

Recently, Wooller *et al.* (2004, 2008) demonstrated the potential of fossil remains of chironomid larvae (non-biting midges) as a proxy for reconstructing the past oxygen isotopic composition of lake water, and indirectly, past variations in temperatures and/or humidity. Larval head capsules of chironomids preserve well in lake sediments for thousands to ten thousands of years (e.g. Heiri *et al.*, 2007; Engels *et al.*, 2008). The chironomid exoskeleton mainly consists of chitin and proteins (Verbruggen *et al.*, 2009), biomacromolecules that contain a significant amount of oxygen. The stable oxygen isotope composition of chironomid head capsules, expressed as the ratio of  $^{18}\text{O}$  to  $^{16}\text{O}$  ( $\delta^{18}\text{O}$ ) relative to a standard, can therefore provide information on the past  $\delta^{18}\text{O}$  of the lake water in which the larvae were living (Wooller *et al.*, 2004). However, several aspects regarding this new approach for palaeoclimatic reconstruction still need to be investigated. The methodology for sorting and sieving chironomid remains has been established (Wang *et al.*, 2008; van Hardenbroek *et al.*, 2009a) and the effects of chemical pretreatments on fossil chironomids prior to stable oxygen isotope analysis have been examined (Wooller *et al.*, 2004; Verbruggen *et al.*, 2009). Lake water  $\delta^{18}\text{O}$  largely depends on the  $\delta^{18}\text{O}$  of precipitation and can be altered by evaporation, which is generally a function of local humidity and temperature (Gonfiantini, 1986; Darling *et al.*, 2005). Wooller *et al.* (2004) showed that chironomid and lake water  $\delta^{18}\text{O}$  are in equilibrium within the analytical errors of  $\delta^{18}\text{O}$  analysis. Based on culturing experiments with chironomid larvae Wang *et al.* (2009) found that approximately 70% of oxygen in chironomid head capsule tissue was derived from ambient water.

Since the 1960s knowledge on stable isotopic compositions of precipitation has been expanded significantly by the establishment of the Global Network for Isotopes in Precipitation (GNIP) by the World Meteorological Organization (WMO) and International Atomic Energy Agency (IAEA). Monthly monitoring at more than 800 meteorological stations worldwide has provided insights on temporal and spatial variations of  $\delta^{18}\text{O}$  in precipitation (IAEA/WMO, 2006). These measurements demonstrated that the oxygen isotopic composition of precipitation is mainly dependent on temperature, with  $\delta^{18}\text{O}$  becoming progressively lighter towards the poles where cooler temperatures prevail (Dansgaard, 1964; Siegenthaler and Oeschger, 1980; Rozanski *et al.*, 1993). Other factors influencing  $\delta^{18}\text{O}$  in precipitation are altitude (Siegenthaler and Oeschger, 1980; Poage and Chamberlain, 2001), continentality (Alley and Cuffey, 2001) and the moisture source area (Alley and Cuffey, 2001). Especially at low latitudes the so-called amount effect, which leads to depleted  $\delta^{18}\text{O}$  at locations with enhanced precipitation (Dansgaard, 1964), plays an important role as well. Overall, precipitation will display lower  $\delta^{18}\text{O}$  values towards colder regions, more continental regions, or towards higher elevations (Fricke and O'Neil, 1999).

Stable oxygen isotopes are widely used in palaeoclimatic reconstructions (e.g. Johnsen *et al.*, 1995; von Grafenstein *et al.*, 1999; Leng and Marshall, 2004; McCarroll and Loader, 2004; Leng and Barker, 2006). In lake sediments they are generally measured on carbonate shells of molluscs or ostracods (e.g. Holmes *et al.*, 1997; von Grafenstein *et al.*, 2000), or on bulk carbonates (e.g. Siegenthaler and Eicher, 1986). However, even in hardwater lakes carbonates may sometimes not be present in lake sediments. In some settings other sediment components produced within lake ecosystems, such as aquatic

cellulose (Wolfe *et al.*, 2007) or biogenic silica (Leng and Marshall, 2004) have been analyzed to obtain stable oxygen isotope records. Chitinous remains of aquatic invertebrates, e.g. chironomid head capsules or cladoceran exoskeletons, are another potential material for stable isotope analysis (e.g. Wooller *et al.*, 2004; van Hardenbroek *et al.*, 2009b; Perga, 2009; Griffiths *et al.*, 2009), since they are well preserved as fossils and are almost ubiquitously present in lake sediments. However, little attention has so far been given to the relationship between  $\delta^{18}\text{O}$  in invertebrate remains and in lake water and regional precipitation. Wooller *et al.* (2004) compared  $\delta^{18}\text{O}$  in precipitation and subfossil chironomids from four lakes in eastern North America. Their results suggested that there is an offset of 28.5‰ between  $\delta^{18}\text{O}$  in chironomid head capsules and in precipitation, a value comparable to the offset found for aquatic mosses in lakes (Sauer *et al.*, 2001). However, if  $\delta^{18}\text{O}$  in fossil remains of aquatic invertebrates is to be used as a reliable proxy for past lake water  $\delta^{18}\text{O}$ , additional studies examining this relationship for a larger number of sites and for different aquatic invertebrate groups are needed.

Here, we present data on  $\delta^{18}\text{O}$  measurements of both lake water and subfossil aquatic invertebrate remains, such as chironomid head capsules and resting eggs (ephippia) of cladocerans. These remains have been isolated from surface sediments of 27 mainly deep and stratified lakes along an extensive latitudinal transect through Europe. The transect spans a large temperature gradient and will allow assessing the relationship of  $\delta^{18}\text{O}$  of subfossil invertebrates with lake water and with precipitation. Once established, this relationship will form the basis for the future use of aquatic invertebrate records for reconstructing past changes in lake water  $\delta^{18}\text{O}$ , and hence climate.

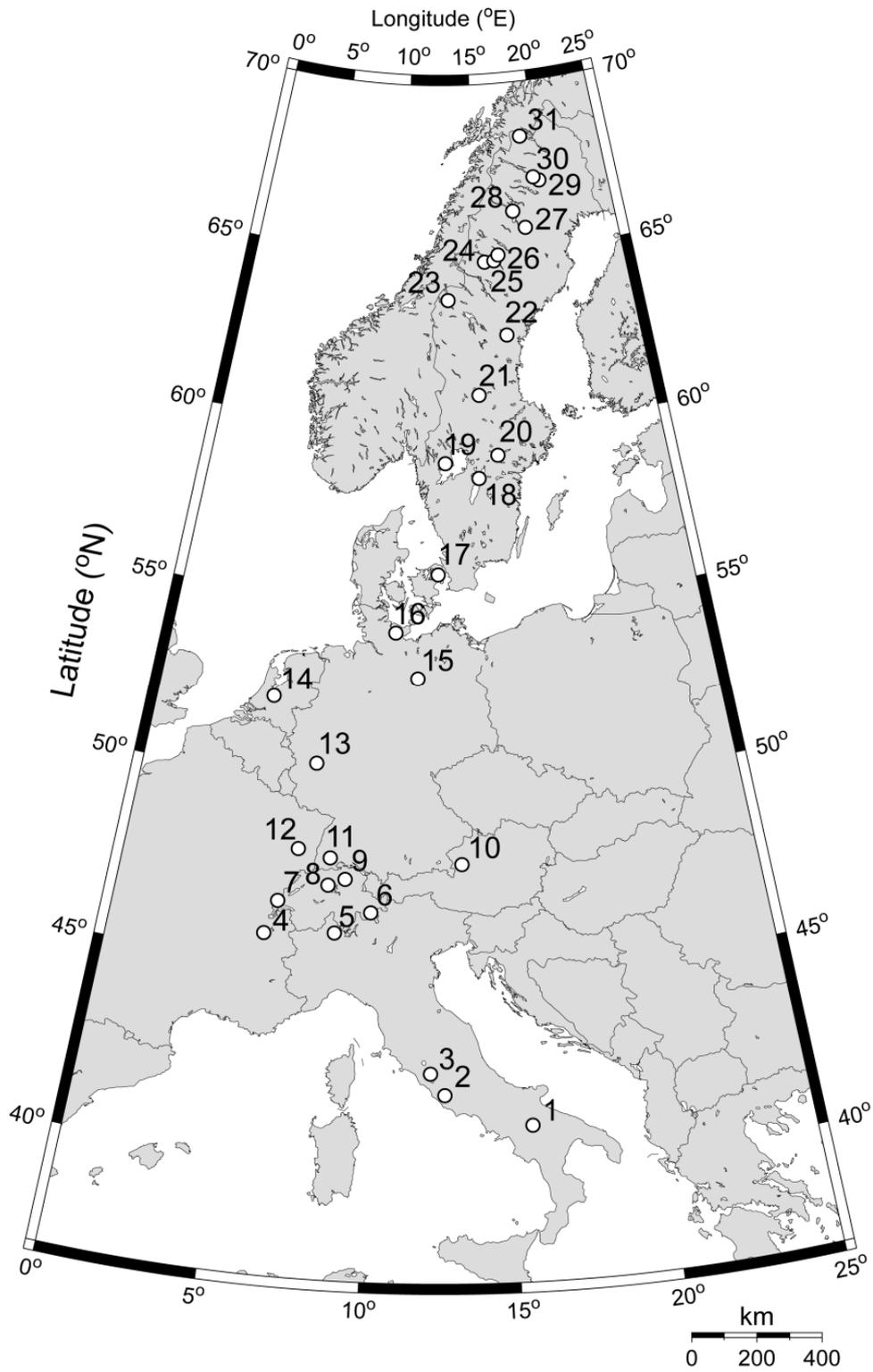
## Materials and methods

### Study area

Water samples as well as surface sediments were obtained from 31 lakes during two field campaigns in January and July 2006. The lakes are situated along an extensive north-south transect from 41° to 68°N latitude (Fig. 1 and Table 1). Most of the studied lakes are relatively large, deep, and have a stable stratification. All lakes south of 48°N, as well as Maarsseveense Plassen (the Netherlands) were sampled during the winter campaign, those north of 48°N during summer. The lakes are located in Italy (4 lakes), France (2), Switzerland (4), Austria (1), Germany (4), the Netherlands (1), Denmark (1), and Sweden (14).

### Material

Water samples were taken at a depth of 0.5 m using a 10 l water sampler (Uwitec, Austria) and stored in glass vials with no head space at 4°C until further analysis. Sediment cores were obtained using a gravity corer (Uwitec, Austria) in the profundal part of the lake basins



**Figure 1** Location of the 31 study lakes along a latitudinal transect through Europe. For lake names see Table 1

**Table 1** Characteristics of the 31 study lakes.  $\delta^{18}\text{O}$  in precipitation was calculated from [www.waterisotopes.org](http://www.waterisotopes.org). Littoral sediments were only used in lakes where littoral coring depth is indicated. MAAT: mean annual air temperature.

Lake number	Lake name	Latitude (N°)	Longitude (E°)	Elevation (m a.s.l.)	Open water area (km <sup>2</sup> )	Volume (km <sup>3</sup> )	Retention Time (years)	Maximum depth (m)	Profundal coring depth (m)	Littoral coring depth (m)	Sampling date	Sediment used (cm)	MAAT (°C)	$\delta^{18}\text{O}$ lake water (‰ V-SMOW)	$\delta^{18}\text{O}$ precipitation (‰ V-SMOW)
1	Lago Grande di Monticchio	40.93	15.61	656	0.4	0.004	7	36	33.3	N/A	22 Jan 2006	0-10	11.8	-2.6	-6.8
2	Lago di Albano	41.75	12.67	293	6	0.45	47	175	53	N/A	21 Jan 2006	0-5	13.9	N/A	-6.2
3	Lago di Vico	42.31	12.17	507	12.1	0.26	17	48.5	47.6	N/A	20 Jan 2006	0-4	13.2	-0.3	-6.8
4	Lac du Bourget	45.74	5.86	231	42	3.5	8	145	144	N/A	26 Jan 2006	0-10	10.3	-8.2	-7.1
5	Lago Maggiore	45.90	8.52	193	213	37.1	4	370	22	N/A	24 Jan 2006	0-3	12.1	-9.7	-7.1
6	Lej da San Murezzan	46.49	9.85	1768	0.78	0.02	0.11	44	44	N/A	2003	0-6	1.7	N/A	-10.5
7	Lac de Joux	46.63	6.26	1004	9	0.16	N/A	38	11.7	N/A	25 Jan 2006	0-1	6.6	-9	-9
8	Sempachersee	47.16	8.14	504	14.4	0.62	16.9	87	75.5	N/A	29 Jan 2006	0-5	9.2	-6.7	-8.4
9	Pfäffikersee	47.35	8.78	537	3.3	0.06	2.1	36	32	N/A	30 Jan 2006	0-5	8.4	-9	-8.5
10	Mondsee	47.82	13.38	481	14.2	0.51	1.7	68.3	47.6	N/A	16 Jan 2006	0-5	9.7	N/A	-8.4
11	Titisee	47.89	8.15	846	1.1	0.02	22.5	39.2	37.2	N/A	31 Jan 2006	0-5	5.6	N/A	-9.1
12	Lac de Gerardmer	48.07	6.85	660	1.2	0.02	2.1	38.4	32	N/A	13 May 2006	0-1	8.8	-8.5	-8.9
13	Laachersee	50.41	7.27	275	3.3	0.1	21	52	50.2	14	12 May 2006	0-3	8.9	-2.3	-8.5
14	Maarsseveense Plassen A	52.14	5.09	1	0.7	0.09	N/A	30.8	24	N/A	07 Nov 2005	0-5	9.8	-3.8	-8.2
15	Arendsee	52.89	11.49	23	5.2	0.15	114	48.7	43.3	N/A	04 Jul 2006	0-3	8.5	-2.9	-8.6
16	Grosser Plönersee	54.16	10.42	21	30	0.37	3	58	41	N/A	05 Jul 2006	0-3	8.4	-4.6	-9
17	Furesø	55.80	12.42	80	7.4	0.12	10.6	37.7	36.2	N/A	06 Jul 2006	0-3	8.0	-4.7	-9.6
18	Vättern	58.52	14.62	89	189	77.6	55.9	128	60.5	16.3	08 Jul 2006	0-2	6.5	-5.7	-10.6
19	Vänern	58.96	12.79	44	565	153	9	106	61.4	28.1	09 Jul 2006	0-5	6.4	-8	-10.4
20	Hjälmaren	59.15	15.71	22	483	3	3.5	22	10	N/A	10 Jul 2006	0-2	5.9	-6.9	-10.9
21	Siljan	60.89	14.80	161	292	8.09	N/A	134	82.6	10	11 Jul 2006	0-2	5.8	-11.5	-11.4
22	Navam	62.59	16.69	280	9.6	0.11	N/A	51.5	48.6	N/A	12 Jul 2006	0-2	2.7	-8.7	-12.4
23	Kallsjön	63.66	13.02	380	158	6.14	N/A	134	73.2	N/A	22 Jul 2006	0-5	2.5	-10.7	-12.7
24	Mellan Rissjön	64.75	15.52	443	1.6	0.02	N/A	29	6	N/A	21 Jul 2006	0-1	-0.1	-12.6	-13.2
25	Malgomaj	64.77	16.19	341	101	3.11	N/A	148	78	N/A	21 Jul 2006	0-5	0.4	-13	-13.1
26	Vojmsjön	64.94	16.49	420	70	3.11	N/A	145	71	N/A	20 Jul 2006	0-5	-0.1	-12.9	-13.2
27	Vuolgamjaure	65.67	18.54	437	2	0.01	N/A	15	15	N/A	19- Jul 2006	0-1	-0.8	-11.9	-13.5
28	Homavan	66.18	17.76	425	283	11.2	N/A	228	32	N/A	18 Jul 2006	0-1	-1.3	-13	-13.8
29	Hapsajaure	67.01	19.94	413	1.1	N/A	N/A	9.5	6	N/A	14- Jul 2006	0-1	-1.8	-12.1	-14.1
30	Stora Lulevatten	67.13	19.53	365	155	1.31	N/A	28.5	21.1	N/A	14- Jul 2006	0-2	-1.8	-12.8	-14
31	Tometräsk	68.36	18.85	341	332	17.1	N/A	168	29	8.2	15 Jul 2006	0-1	-0.5	-12.6	-14.2

and in some lakes also from a more littoral site. The top 10 cm of sediment was subsequently extruded and sliced into contiguous 1 cm increments and freeze-dried.

### Aquatic invertebrate remains

Prior to analysis the freeze-dried sediment samples were soaked in demineralized water for 30 minutes to facilitate sieving with a 200  $\mu\text{m}$  mesh-size sieve. Sieve residues were stored in vials with demineralized water. Chironomid head capsules and cladoceran ephippia were manually picked from the sieve residue under a stereo microscope (magnification 40x) using fine forceps. The top centimeter was initially used to pick aquatic invertebrate remains and if insufficient numbers of remains were found, the subsequent 1 cm slices were sorted (Table 1). In some cases, sediments from the deepest part of the lake basin did not yield enough remains and samples from the littoral were therefore also studied. A minimum of 40  $\mu\text{g}$  (dry weight) of each aquatic invertebrate type was picked directly into a pre-weighed silver cup, which was subsequently checked under a stereo microscope for the presence of contamination. Cups were allowed to dry for several days in a covered Petri dish. The silver cups were then again weighed and – if enough material was present – folded. Samples were subsequently measured for stable oxygen isotopes.

### Meteorological data

Climatic normals (1961-1990) were obtained for three sites close to each lake from national meteorological institutes. Mean annual air temperature (MAAT) data were averaged per lake and corrected for altitude ( $-0.6^\circ\text{C}/100\text{ m}$  elevation; Rolland, 2002).

### Stable oxygen isotopes

Stable oxygen isotopes in precipitation were calculated for our study sites using the Online Isotope in Precipitation Calculator (IOPC; [www.waterisotopes.org](http://www.waterisotopes.org); Bowen and Wilkinson, 2002; Bowen and Revenaugh, 2003; Bowen *et al.*, 2005), which was established based on a global GNIP data set (IAEA/WMO, 2006).

Stable oxygen isotopic ratios from the lake water samples were determined using a GasBench II system coupled to a Thermo Finnigan Delta<sup>plus</sup> XP isotope ratio mass spectrometer (IRMS). A vacutainer with 200 $\mu\text{l}$  of sample was flushed with 0.5%  $\text{CO}_2$  in helium. After 20 hours of equilibration the head space was sampled and measured. Measurements were calibrated against Vienna-Standard Mean Ocean Water (V-SMOW) and Standard Light Antarctic Precipitation (SLAP). Corrected delta values are expressed relative to the V-SMOW standard for  $\delta^{18}\text{O}$  (Coplen, 1996).

For aquatic invertebrate remains a high Temperature Conversion Elemental Analyzer (Thermo Finnigan) coupled to an Isotope Ratio Mass Spectrometer (TC-EA/IRMS; Thermo Finnigan Delta<sup>plus</sup>) was used to determine ratios of stable oxygen isotopes (50-100  $\mu\text{g}$  range;

Kornexl *et al.*, 1999). All samples were analyzed using 4- x 3.2-mm silver cups (Elemental Microanalysis Ltd, batch number 128843). Two blanks were measured at the start of every run. Standardization was achieved using a cellulose standard (IAEA-C3), a benzoic acid standard (HEKAtech, batch number 33822501), and two international potassium nitrate standards (IAEA-NO-3 en USGS-32).  $\delta^{18}\text{O}$  data are reported as per mille (‰) relative to the V-SMOW standard.

## Results

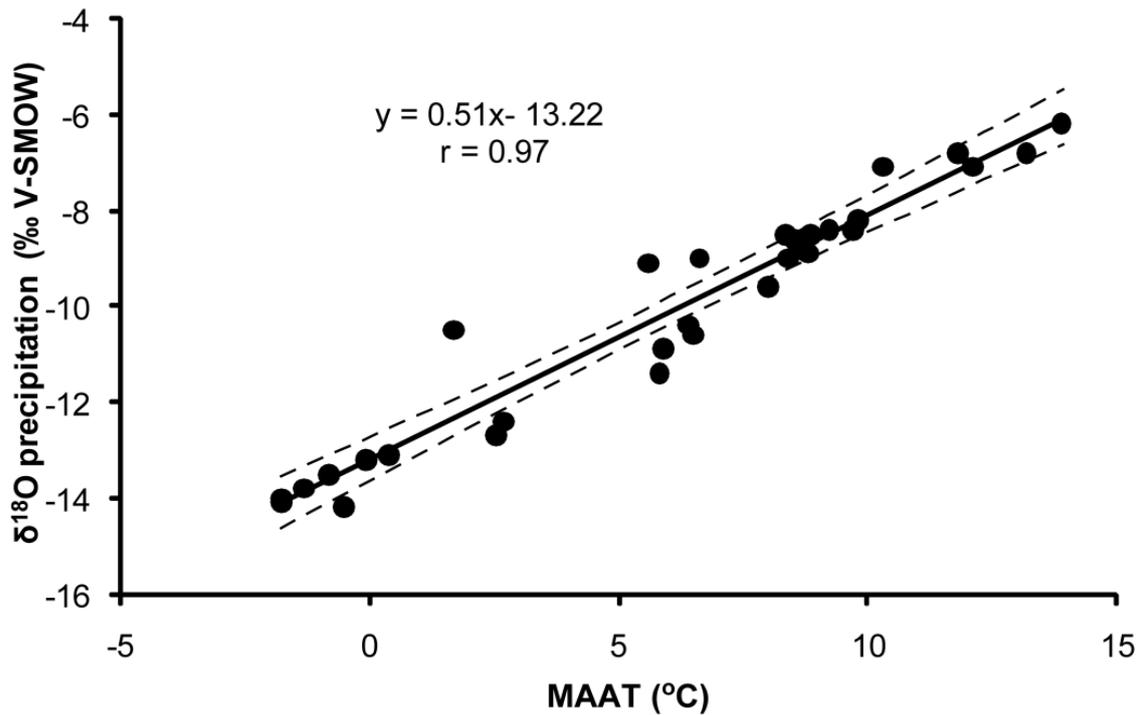
### Aquatic invertebrates

Surface sediments from 27 lakes contained sufficient invertebrate remains for  $\delta^{18}\text{O}$  analysis. Of these, 24 yielded sufficient chironomid head capsules and 20 yielded enough cladoceran ephippia. For the majority of the lakes the top three cm yielded enough remains, whereas for some the 3-4 or 4-5 cm fraction was also needed. For Lac du Bourget and Lago Grande di Monticchio the 5-10 cm sediment slice needed processing to get a sufficient sample size (Table 1). For Laachersee, Torneträsk, Siljan, Vänern, and Vättern littoral sediments were used in addition to the profundal sediments (Table 1).

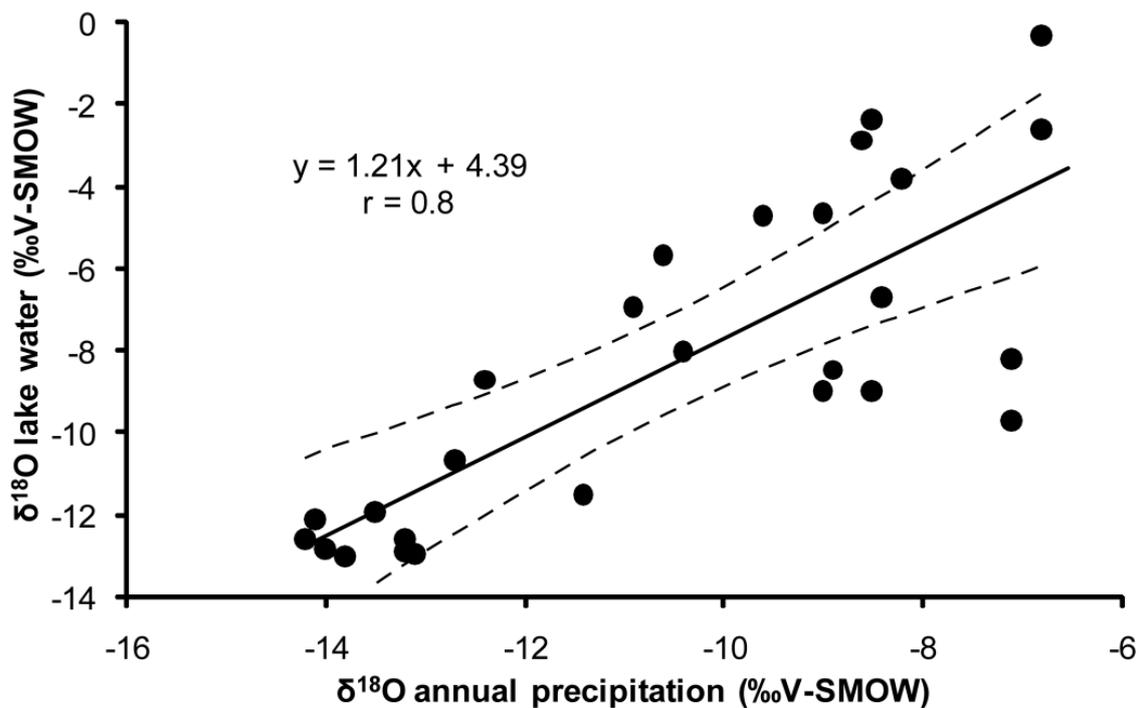
### Stable oxygen isotopes

Stable oxygen isotopes were successfully measured in lake water of 27 lakes, that is, in all lakes except Lago di Albano, Lej da San Murezzan, Mondsee and Titisee (Table 1). For Lej da San Murezzan no water sample was retrieved, whereas for the other lakes glass bottles to store lake water were damaged during transportation. Yearly averaged  $\delta^{18}\text{O}$  of precipitation varied between -6.2 and -14.2‰ along the transect and was positively correlated with MAAT ( $r=0.97$ ), which varied between -1.8 and 13.9°C (Fig. 2). Surface water  $\delta^{18}\text{O}$  from relatively large and deep lakes in Europe, in turn, was positively correlated with  $\delta^{18}\text{O}$  in precipitation (Fig. 3;  $r=0.8$ ). Generally, lake water  $\delta^{18}\text{O}$  was ~2‰ enriched relative to precipitation.

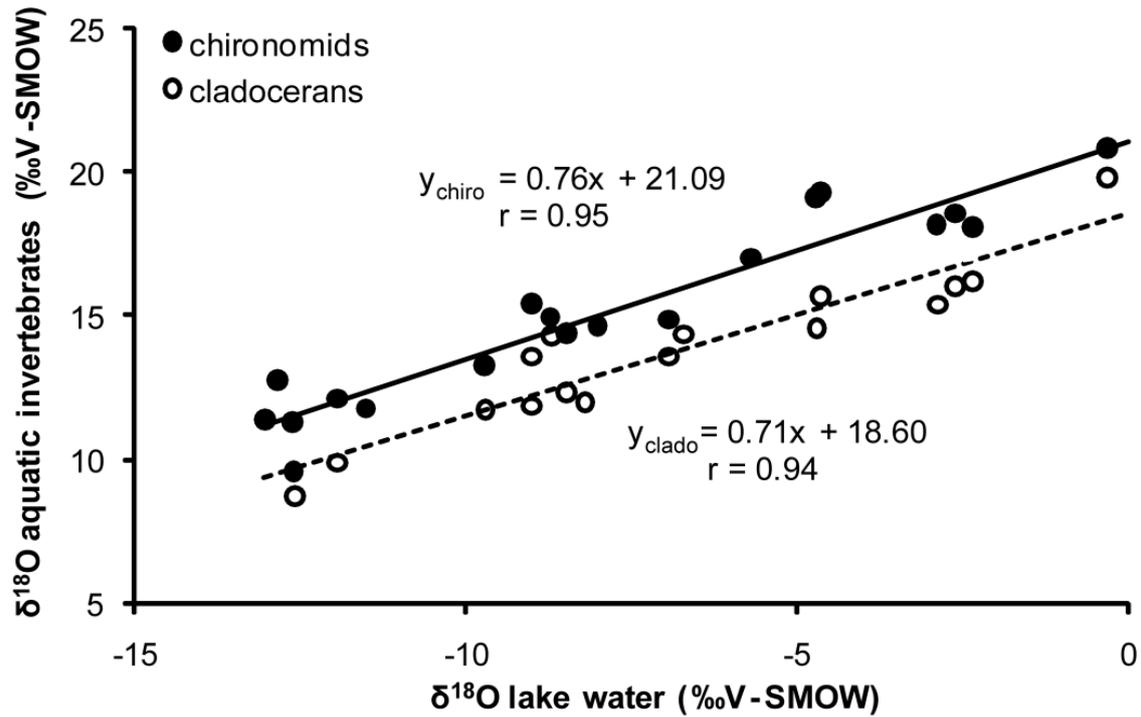
The stable oxygen isotopic composition of aquatic invertebrate remains was positively correlated with the  $\delta^{18}\text{O}$  of the lake water they lived in (Fig. 4). Chironomids showed the closest correlation ( $r=0.95$ ), whereas the relationship between cladoceran and lake water  $\delta^{18}\text{O}$  was slightly weaker ( $r=0.94$ ). Although the slope of the linear regression between lake water  $\delta^{18}\text{O}$  and the biota was similar for chironomids and cladocerans, an offset of approximately 2‰ can be seen between the  $\delta^{18}\text{O}$  values from the same lakes. Chironomids and cladocerans were ~23 and 21‰ enriched relative to lake water  $\delta^{18}\text{O}$ , respectively (Fig. 4). Chironomid and cladoceran  $\delta^{18}\text{O}$  showed a positive, linear, and statistically significant ( $P<0.01$ ) relationship with MAAT ( $r=0.81$  and 0.66, respectively; Fig. 5).



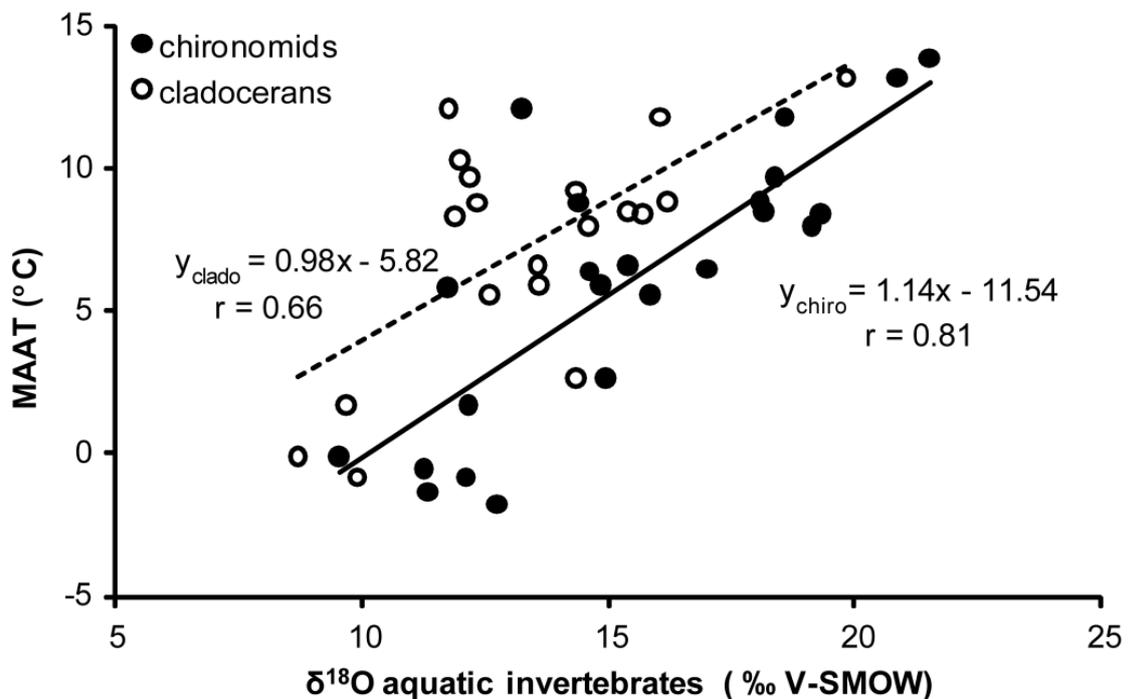
**Figure 2** Relationship between modeled stable oxygen isotopic composition of precipitation (calculated from [www.waterisotopes.org](http://www.waterisotopes.org)) and mean annual air temperature (MAAT). The trendline shows the best linear fit, with dotted lines representing the 95% confidence interval



**Figure 3** Relationship between stable oxygen isotopic compositions of precipitation (calculated from [www.waterisotopes.org](http://www.waterisotopes.org)) and lake water. The trendline shows the best linear fit, with dotted lines representing the 95% confidence interval



**Figure 4** Relationship between modeled stable oxygen isotopic composition of lake water, and chironomid head capsules (black circles), and ephippia of cladocerans (open circles). The black and dashed line show the linear regressions between lake water  $\delta^{18}\text{O}$  and the chironomid and cladoceran  $\delta^{18}\text{O}$ , respectively



**Figure 5** Relationship between modeled stable oxygen isotopic composition of chironomid head capsules (black circles), and ephippia of cladocerans (open circles), and mean annual air temperature (MAAT). The black and dashed line show the linear regressions between MAAT and  $\delta^{18}\text{O}$  of chironomid and cladocerans, respectively

## Discussion

Non-carbonate sediment components that have previously been used to develop records of lake water  $\delta^{18}\text{O}$  include aquatic cellulose (Wolfe *et al.*, 2001). However, in some cases the applicability of this proxy may be hampered by the presence of terrestrial cellulose (Sauer *et al.*, 2001), which is often enriched in  $^{18}\text{O}$  and more resistant to degradation.  $\delta^{18}\text{O}$  of diatom silica is another method often applied (e.g. Barker *et al.*, 2001). However, small amounts of contamination may influence diatom  $\delta^{18}\text{O}$  values (e.g. Leng and Barker, 2006) and therefore extensive sediment purification is required (e.g. Morley *et al.*, 2004). Remains of aquatic invertebrates are preserved in most lake sediments. Exoskeleton parts such as head capsules or mandibles of aquatic insects (e.g. chironomids), or resting eggs of cladocerans have the advantage that they originate from freshwater environments. Our results confirm the findings of Wooller *et al.* (2004) that there is a strong and systematic relationship between chironomid remains and lake water  $\delta^{18}\text{O}$ . Furthermore, a similar relationship exists between  $\delta^{18}\text{O}$  of cladoceran ephippia and lake water  $\delta^{18}\text{O}$  (Fig. 4). The larger number of sites examined in our survey allows a more detailed discussion of these relationships than has been possible in previous studies.

### Relationship between $\delta^{18}\text{O}$ in precipitation and lake water, and temperature

The spatial variation in  $\delta^{18}\text{O}$  in precipitation is strongly related to MAAT along the latitudinal transect, which is in agreement with previous studies (Dansgaard, 1964; Rozanski *et al.*, 1993; Fricke and O'Neil, 1999). Most moisture is taken up by air masses at low latitudes (Alley and Cuffey, 2001). As air masses move toward the poles, they are cooled and  $^{18}\text{O}$  enriched water is rained out preferentially. This results in higher  $\delta^{18}\text{O}$  values of precipitation at lower latitudes than at higher latitude regions. The high correlation between MAAT and  $\delta^{18}\text{O}$  in precipitation in our study ( $r=0.97$ ; Fig. 2) suggests that temperature is the dominant factor influencing  $\delta^{18}\text{O}$  in precipitation along the transect.

A weaker, but nevertheless statistically significant ( $P<0.01$ ), correlation was observed between precipitation and lake water  $\delta^{18}\text{O}$  ( $r=0.80$ ; Fig. 3). Some lakes, such as Lago Maggiore, Pfäffikersee, Lac de Joux, and Lac du Bourget were characterized by disproportionately low  $\delta^{18}\text{O}$  values. Lago Maggiore and Lac de Bourget are both lakes with large catchments that include high elevations, with large amounts of water originating from precipitation falling at high altitudes and from melt water in spring and early summer. Also, all four lakes were sampled during the winter campaign. Lakes that display relatively enriched lake water  $\delta^{18}\text{O}$  values include Lago di Vico, Laachersee, Arendsee, Vättern, Furesø, Hjälmaren, Navarn, and Grosser Plönersee. All lakes, except for Lago di Vico, were sampled during the summer campaign. Summer precipitation is isotopically enriched relative to winter precipitation (Siegenthaler and Oeschger, 1980; Rozanski *et al.*, 1993; Darling and Talbot, 2003). Seasonal changes can account for a difference of 1.5 to 5‰ in lake water  $\delta^{18}\text{O}$  (Rank *et al.*, 1984; Flora and Longinelli, 1989; von Grafenstein *et al.*, 1996, 2000). For Lago di Vico, local hydrological conditions, resulting in relatively high evaporation may be responsible for elevated lake water  $\delta^{18}\text{O}$  values. Lago di Vico has a relatively high water residence time

(17 years; Margaritora *et al.*, 2003), which enhances an evaporation effect on  $\delta^{18}\text{O}$  (Gibson and Edwards, 2002).

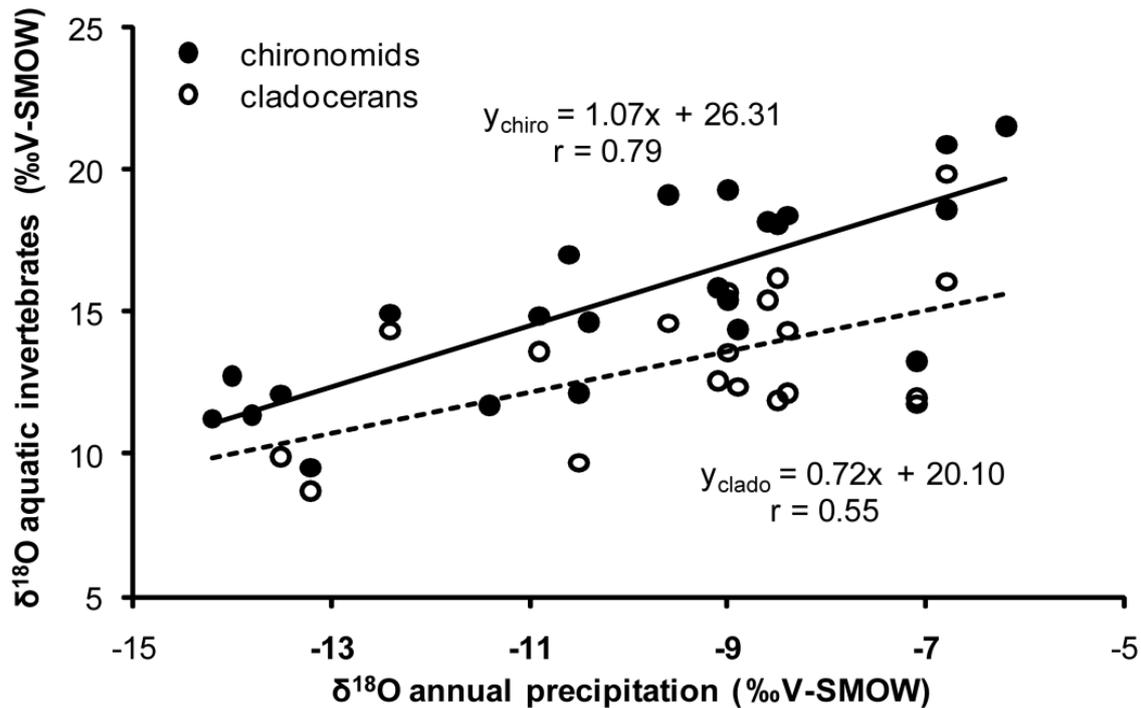
Overall, we observe a strong correlation between lake water  $\delta^{18}\text{O}$  and climate for deep lakes in Europe, which is not observed in more arid regions of the world, such as the western part of the United States of America (Henderson and Shuman, 2009). Our data suggest that lake water in large and deep European lakes mainly record the isotopic composition in precipitation. These large lakes are also less susceptible to seasonal lake water  $\delta^{18}\text{O}$  fluctuations due to their storage capacity (Darling, 2004).

### Relationship between $\delta^{18}\text{O}$ of lake water and aquatic invertebrates

A strong correlation is observed between lake water  $\delta^{18}\text{O}$  and the stable oxygen isotopic composition of subfossil invertebrates, which agrees with results of Wooller *et al.* (2004). For chironomid head capsules as well as for ephippia of cladocerans sufficient samples were obtained to determine a robust linear relationship between lake water and precipitation  $\delta^{18}\text{O}$  (Figs. 4 and 6, respectively). Interestingly, the relationship between  $\delta^{18}\text{O}$  in precipitation and the analyzed invertebrate remains is less strong, suggesting that the offset between our spot measurements of  $\delta^{18}\text{O}$  in lake water and estimated  $\delta^{18}\text{O}$  in precipitation may largely be a consequence of local hydrological conditions (e.g. evaporation rates, water residence times, inflow of water from higher altitudes) rather than the effect of seasonal variation in lake water  $\delta^{18}\text{O}$  between the two sampling campaigns. Schimmelmann and DeNiro (1986) indicated that stable isotopic compositions of chitinous remains from marine arthropods were not in equilibrium with their ambient water. Based on laboratory experiments, Wang *et al.* (2009) recently estimated that almost 70% of oxygen in chironomid head capsules was derived from ambient water. In our predominantly deep and stratified study lakes,  $\delta^{18}\text{O}$  of the food available for chironomid larvae has largely consisted of particles formed within the water column, such as remains of planktonic algae, bacteria, and zooplankton. Similarly, cladoceran ephippia, which in our samples predominantly originated from the planktonic genus *Daphnia*, were formed by individuals feeding on planktonic algae and bacteria. It can be expected that the  $\delta^{18}\text{O}$  of the diet of the studied invertebrate groups is thus also strongly related lake water  $\delta^{18}\text{O}$ . Based on our data it is, therefore, not possible to determine whether the strong relationship between chironomid and cladocerans, and lake water  $\delta^{18}\text{O}$  is mainly a consequence of the direct influence of lake water (Wang *et al.*, 2009) or whether it is also strongly influenced by the diet ingested by the animals. However, it is evident that in our dataset this relationship is strong, predictable, and represented in a range of lakes over a large climatic gradient.

### Offset between chironomids and cladocerans

For chironomid  $\delta^{18}\text{O}$  Wooller *et al.* (2004) reported an enrichment of 28.5‰ relative to regional precipitation. In our study we find an enrichment of 25‰ relative to precipitation (Fig. 6) and an enrichment of 22 to 24‰ relative to lake water  $\delta^{18}\text{O}$  (Fig. 4). Since Wooller



**Figure 6** Relationship between modeled stable oxygen isotopic composition of precipitation, and chironomid head capsules (black circles), and ephippia of cladocerans (open circles). The black and dashed line show the linear regressions between precipitation  $\delta^{18}\text{O}$  and the chironomid and cladoceran  $\delta^{18}\text{O}$ , respectively

*et al.* (2004) did not report lake water  $\delta^{18}\text{O}$ , we cannot compare it with our data. The relatively lower enrichment of chironomid  $\delta^{18}\text{O}$  relative to precipitation  $\delta^{18}\text{O}$  in our dataset than in measurements reported by Wooller *et al.* (2004) may be due to the difference in lake size between the two studies. Deep lakes as the ones studied here are expected to be less prone to evaporative fractionation (Darling, 2004), which may account for the lower enrichment between precipitation and chironomid  $\delta^{18}\text{O}$ . The slope of the regression between lake water and chironomid  $\delta^{18}\text{O}$  is very similar to the slope of the linear relationship between lake water and cladoceran  $\delta^{18}\text{O}$  (0.76 and 0.71, respectively; Fig. 4). The slopes are not statistically different from each other and both are significantly different from 1, suggesting that the relationship between  $\delta^{18}\text{O}$  of invertebrates and lake water in European lakes is different from the relationship suggested by Wooller *et al.* (2004) for eastern North American lakes. An offset of 2‰ is apparent between  $\delta^{18}\text{O}$  values of chironomids and cladoceran ephippia (Fig. 4). A number of reasons may potentially explain this difference. First of all, the season in which the remains of both aquatic biota are formed may differ. Cladocerans produce ephippia preferentially when conditions become unfavorable for survival (e.g. Tsugeki *et al.*, 2009), i.e. when lake water temperatures are low or food is scarce (e.g. Straile and Geller, 1998; Yoshida *et al.*, 2001). In many lakes ephippia are mostly produced in autumn and early winter (Alekseev and Lampert, 2001) and, therefore, may reflect an autumn/winter signal. In stratified lakes of temperate regions chironomid emergence mainly takes place in spring and autumn (e.g. Gerstmeier, 1989; Aagaard, 1978). Eggs are deposited by the females rapidly after emergence and the chironomid larvae will therefore start their early development either during the summer months or in late autumn or early winter. Summer precipitation is

generally isotopically heavier than winter precipitation (Siegenthaler and Oeschger, 1980; Rozanski *et al.*, 1993; Darling and Talbot, 2003). This leads to distinct seasonal differences in lake water  $\delta^{18}\text{O}$  (von Grafenstein *et al.*, 1996, 2000) which may explain part of the observed differences in  $\delta^{18}\text{O}$  between chironomids and cladocerans. Differences in diet and metabolism (vital effects), may have influenced  $\delta^{18}\text{O}$  in the two organisms as well. The habitats of cladocerans and chironomids also differ; planktonic cladocerans live in the pelagic part of the water column, whereas chironomid larvae are benthic organisms restricted to the lake bottom. However, Longinelli *et al.* (2008) found little oxygen isotopic difference in the water column of Lake Garda, a typical large, deep lake in Europe and concluded that no vertical isotopic stratification developed in this lake. Also, Mayr *et al.* (2007) studied vertical profiles of two deep lakes in Patagonia (Argentina) and found little (0.5‰) to no difference between bottom and surface water  $\delta^{18}\text{O}$ . In contrast, von Grafenstein *et al.* (1996) showed that  $\delta^{18}\text{O}$  in the upper water layers of Lake Ammersee (southern Germany) varied seasonally, whereas  $\delta^{18}\text{O}$  of the hypolimnion remained stable during the entire year. The temperature difference between deep, hypolimnetic and shallow epilimnetic water may be another reason for the observed difference in chironomid and cladoceran  $\delta^{18}\text{O}$ . A temperature-dependent fractionation between water and both groups of aquatic organisms may exist. Such a temperature-dependent fractionation is known to occur in carbonates (Friedman and O'Neil, 1977; Teranes *et al.*, 1999a, 1999b; von Grafenstein *et al.*, 2000). However, to the best of our knowledge this fractionation has not been studied in chironomids or cladocerans. Regardless of the exact cause, the offset between cladoceran and chironomid  $\delta^{18}\text{O}$  in our study lakes seems similar for most lakes, suggesting that it is related to fundamental differences in ecology, metabolism, or behavior between benthic chironomid larvae and planktonic cladocerans.

### Potential for down-core application

During past phases of climatic change atmospheric circulation patterns have shown distinct variations (e.g. Bond *et al.*, 1997; Renssen and Isarin, 2001). These changes were associated with variations in the spatial distribution of the stable oxygen isotopic composition of precipitation, and hence, of lake water  $\delta^{18}\text{O}$ . The relationship of  $\delta^{18}\text{O}$  of chitinous remains of cladocerans and chironomids with  $\delta^{18}\text{O}$  in lake water and precipitation can provide a basis for translating stable oxygen isotopic compositions of such fossils to estimates of past air temperatures. This would allow quantitative reconstruction of MAAT, similarly to the approaches used for fossil ostracod records from deep and stratified lakes (e.g. von Grafenstein *et al.*, 1999). Stable oxygen isotopic ratios can be measured reliably on fossil chironomid head capsules isolated from lacustrine sediments (Wooller *et al.*, 2004, 2008; Verbruggen *et al.*, in prep) provided they are not subjected to aggressive chemical pretreatment with acids or strong alkali (Verbruggen *et al.*, 2009). In our dataset, chironomid  $\delta^{18}\text{O}$  shows a stronger and more robust relationship to temperature than cladoceran  $\delta^{18}\text{O}$ . However, the relationship between cladoceran and lake water  $\delta^{18}\text{O}$  is also well expressed in the majority of our study lakes. Reconstruction methods based on chironomid or cladoceran  $\delta^{18}\text{O}$  therefore have the potential for complementing and expanding existing quantitative

chironomid-temperature inferences that are based on modern chironomid assemblage distributions (e.g. Heiri *et al.*, 2007; Ilyashuk *et al.*, 2009). Nevertheless, a number of open questions remain. First, it is unclear whether chironomid and cladoceran  $\delta^{18}\text{O}$  in deep and stratified lakes as the ones we examined, reflect  $\delta^{18}\text{O}$  during a particular part of the year or approximate an annual average. Second, vital effects and the effects of different environmental conditions in the habitats of chironomids and cladocerans on their  $\delta^{18}\text{O}$  values remain to be further studied. Finally, culturing experiments of these organisms under different temperatures are essential to determine whether  $\delta^{18}\text{O}$  in chironomids and cladocerans is affected by temperature-dependent fractionation, as observed in ostracods and gastropods (Xia *et al.*, 1997; von Grafenstein *et al.*, 1999; Wurster and Patterson, 2001). Once these effects have been unraveled, we will get a better understanding of the relevant processes that will allow a reliable interpretation of chironomid or cladoceran-based  $\delta^{18}\text{O}$  records as a palaeotemperature proxy.

## Acknowledgements

The help of Liesbeth Vissers and Koos Swart during fieldwork is gratefully acknowledged. Arnold van Dijk is cordially thanked for TC-EA/IRMS measurements and Maarten van Hardenbroek for fruitful discussions and helpful comments on this manuscript. This work was financially supported by the Darwin Center for Biogeosciences, Utrecht, the Netherlands and a Schure-Beijerink-Popping grant to AFL.

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# Chapter 4



# Which factors are driving late glacial and early Holocene chironomid community changes in the littoral of Rotsee (Switzerland)?

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*To be submitted to Journal of Quaternary Science*

## Abstract

Multi-proxy palaeoecological analyses have been carried out on late glacial and early Holocene sediments from the littoral zone of Rotsee (Switzerland). They included pollen, plant macrofossil, chironomid, loss-on-ignition, and stable oxygen isotope analyses to study leads and lags of biotic response to distinct changes in the late glacial climate, as well as to assess the importance of external and lake-internal forcing factors for changes in the littoral chironomid communities. Time-control of the record relies on a high-resolution chronology based on wiggle-match dating of 61 AMS radiocarbon dates on terrestrial plant remains. An open vegetation with many heliophilous herbs and dwarf shrubs characterized both regional and local vegetation before reforestation took place around 14,700 cal BP. Simultaneously, chironomid assemblages changed concurrent with a shift in stable oxygen isotopes, which is related to an increase in temperature. Cold-indicating chironomids were not abundant in the littoral zone of Rotsee during the Younger Dryas and early Holocene probably due to a lake level lowering, as suggested by the macrophyte communities. Independent zonation of biological proxies showed that many variations in terrestrial and aquatic communities occurred together with major shifts in stable oxygen isotopes. The effects of climate, catchment, and lake-internal processes on the composition of the littoral chironomid communities were assessed in a series of (partial) canonical correspondence analyses using one or several independent proxies as predictors. Unidirectional long-term processes explained a large part of the variance in the chironomid data from Rotsee. Climate forcing explained a significant amount of the variance but catchment processes and lake-internal dynamics explained more of the variation in the littoral chironomid record. This suggests that the effect of late-glacial climate change on chironomids from the littoral zone of Rotsee may have been overprinted by catchment and lake processes, especially during times of fluctuating lake levels.

# Introduction

The late Quaternary represents the most recent period in Earth's history that was characterized by dramatic natural changes in global climate. During these periods of climatic change the response of different compartments of system Earth, such as the atmosphere and hydrosphere, have led to a dynamic and substantial reorganization of both terrestrial and aquatic ecosystems on a global scale. On the landscape and ecosystem level, late Quaternary environmental change has influenced different organism groups in lakes, such as diatoms (e.g. Kirilova *et al.*, 2009), cladocerans (e.g. Hofmann, 2000), macrophytes (e.g. Birks, 2000), ostracods (e.g. Belis *et al.*, 2008), and chironomids (e.g. Heiri *et al.*, 2007; Ilyashuk *et al.*, 2009). Studies of the modern occurrence and distribution of different aquatic taxa in respect to climate can provide information that improves the understanding of lake ecosystems, and of the response of biota in respect to climatic variables (e.g. Lotter *et al.*, 1997; Brooks and Birks, 2001; Bigler and Hall, 2001; Bigler *et al.*, 2006). Alternatively, down-core records of remains of aquatic organisms can be studied to assess how they responded to past climatic changes in natural environments unaffected by human activities. Such studies can provide deeper insight into questions of vulnerability or resilience of ecosystems with regard to a changing environment. One of the major questions in respect to the effects of climate on lake ecosystems and lacustrine communities is whether biotic assemblages are affected by temperature directly, or whether other indirect changes associated with climatic change are responsible for the observed variations in assemblage composition and abundances. For terrestrial vegetation this has been debated for some time (e.g. Webb, 1986; Bradshaw, 1993) and for aquatic organisms, such as diatoms or chironomids a direct relationship with climate has been questioned as well (e.g. Anderson, 2000; Hann *et al.*, 1992). A sound understanding of the relationship between organisms and environmental factors affecting them is necessary to interpret biotic response to climate change in an adequate way or to reconstruct climate based on fossil records of aquatic organisms (e.g. Heiri and Lotter, 2005).

Climate directly influences lake systems by affecting lake water temperatures, as well as the length and extent of ice and snow cover, which in turn will change conditions within the lake, such as productivity, stratification, or oxygen availability. Moreover, climate is controlling catchment vegetation and soil processes, which have an influence on the input of minerogenic and organic matter and therefore also on the sediment as a substratum for aquatic organisms as well as on productivity via the nutrient content of the water (Birks *et al.*, 2000). Besides available autecological data, the distribution of subfossil remains of organisms in lake surface sediments can provide information on the relation of biota with environmental factors (e.g. Birks, 1998). These empirical relationships help to improve the understanding of relevant ecological forcing factors that govern the distribution and abundance of organisms. For aquatic organisms, such as chironomids (non-biting midges) lake water temperature, food, oxygen availability, substratum, and pH (Pinder, 1986) are such important ecological factors. Many chironomid taxa have well-defined optima and narrow tolerances in respect to these environmental parameters and the fossil remains of chironomid larvae are therefore often used to reconstruct past environmental conditions (Brodin and Gransberg, 1993; Brodersen and Lindegaard, 1999; Korhola *et al.*, 2000; Heiri

and Millet, 2005; Nyman *et al.*, 2005; Brodersen and Quinlan, 2006; Eggermont *et al.*, 2008; Luoto, 2009). The sensitivity of chironomids to climatic change has extensively been demonstrated for many regions of the world (e.g. Lotter *et al.*, 1999; Brooks, 2006; Walker and Cwynar, 2006; Eggermont *et al.*, 2009).

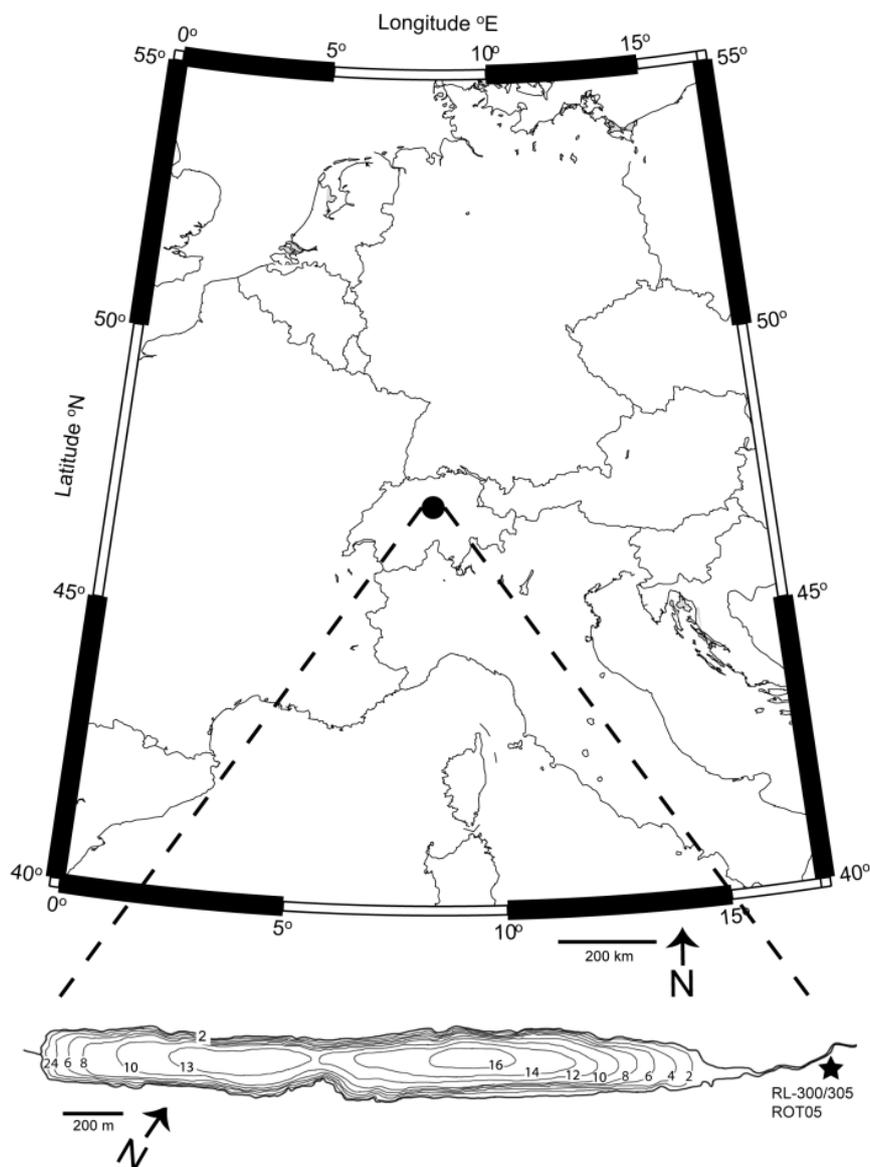
As in most palaeolimnological approaches using studies of lake surface sediments, the empirical relationships between chironomids and their environment are established for assemblages originating from the profundal zone of lakes. As a consequence most down-core chironomid records are from former deep-water environments of lakes. In contrast, studies focusing on past littoral chironomid communities are sparse (e.g. Brooks, 2000). The few late Quaternary chironomid records developed from littoral deposits have provided both records which provided clear evidence for changes in chironomid assemblages related to variations in temperature (e.g. Heiri and Millet, 2005) and records in which chironomid response to environmental change was more complex and probably a consequence of changing temperatures, water levels and habitat structure and availability (e.g. Brooks, 2000).

In this study we concentrate on the late glacial and early Holocene sediment record from the littoral zone of Rotsee (Switzerland) to study the influence of climate change on littoral chironomid assemblages. This period is characterized by distinct and well-documented climate shifts recorded in the stable oxygen isotope ( $\delta^{18}\text{O}$ ) records of carbonate-rich littoral sediments (e.g. Lotter *et al.*, 1992a; Finsinger *et al.*, 2008). The high-resolution radiocarbon chronology of the Rotsee sediments provides the opportunity to study rates of biotic change (e.g. Lotter *et al.*, 1992b) as well as leads and lags in biotic response to climate change (e.g. Ammann *et al.*, 2000). Next to fossil chironomid assemblages the Rotsee sediments have also been examined in respect to pollen, terrestrial plant macrofossils, aquatic macrophyte remains, organic matter and carbonate content, and bulk carbonate  $\delta^{18}\text{O}$ . The record therefore allows a comparison between the responses of different terrestrial and aquatic communities and allows applying a numerical hypothesis testing to examine how littoral chironomid assemblages reacted to changes in climate, the lake catchment, and the littoral habitat of the lake. Only a few studies have previously applied numerical approaches to test such palaeoecological hypotheses based on down-core lake sediment records (e.g. Lotter *et al.*, 1995; Birks *et al.*, 2000; Lotter and Birks, 2003; Bradshaw *et al.*, 2005). Specifically we examine whether terrestrial and aquatic biota in and around Rotsee reacted simultaneously to external climate forcing. Furthermore, we tested the importance of different external and internal forcing factors in explaining the variation in the chironomid record by using a data-splitting approach (e.g. Birks and Birks, 2006), i.e. by using one or several independent proxies as predictors to explain the response in the littoral chironomid communities.

# Material and methods

## Study area

Rotsee is situated on the Central Swiss plateau, in the vicinity of Lucerne (47°9'N; 8°20'E; Fig. 1) at an elevation of 419 m a.s.l. (Lotter and Zbinden, 1989). The lake is elongated and dates back to the end of the last glacial. At present, it reaches a maximum depth of 16 m (Lotter, 1990). Three cores (RL-300, RL-305, ROT05) were taken in close proximity to each other in the overgrown northeastern part of the lake basin using a modified Livingstone piston corer.



**Figure 1** Location of Rotsee in Switzerland (circle). Bathymetric map modified after Lotter (1990). All cores (RL-300, RL-305, and ROT05) were taken in the littoral zone of the northeastern part of the lake basin (star)

## Correlation of cores

Lithostratigraphies of cores RL-300 and RL-305 are similar to core ROT05 (Fig. 2). The three cores were correlated based on the major shifts in their bulk carbonate  $\delta^{18}\text{O}$  records, as well as conspicuous shifts in pollen and microfossil assemblages, the presence of the Laacher See Tephra, and lithological changes as documented in Lotter (1988). Eventually, the sample depths of cores RL-300 and RL-305 were converted into depths in ROT05 and used in this study.

## Sedimentological analyses

Loss-on-ignition (LOI) analysis was carried out on contiguous 1 cm intervals from core ROT05 according to Heiri *et al.* (2001). After leaving samples in ceramic crucibles at 105°C overnight, weight loss was recorded after combusting sediments for 4 h at 550°C (LOI550) and for 2 h at 950°C (LOI950) in a Barnstead Thermoline 48,000 furnace.

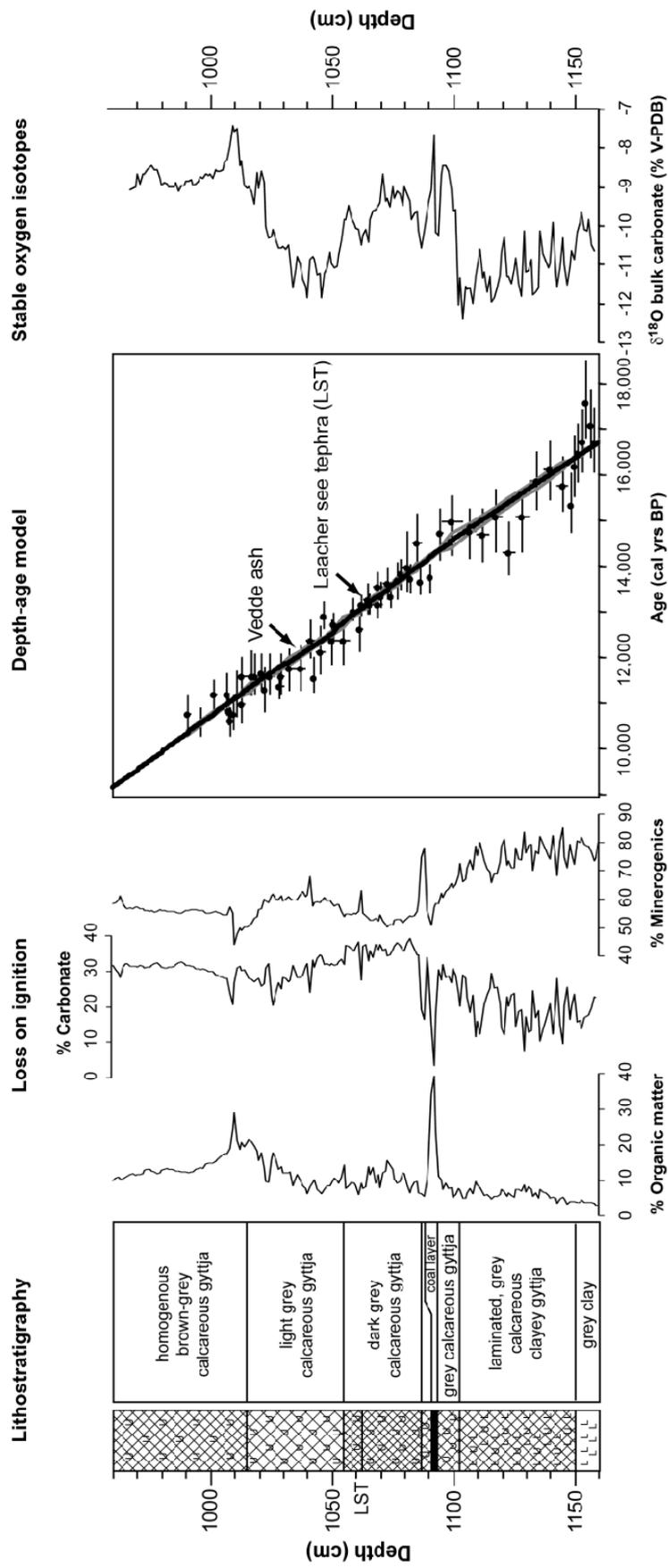
The stable oxygen isotopic composition ( $\delta^{18}\text{O}$ ) of bulk carbonates was measured on sediment samples taken every 5 to 10 cm in cores RL-300 and RL-305, whereas a continuous record was obtained from ROT05 sediments. Samples from RL-300 and RL-305 were analyzed according to Siegenthaler and Eicher (1986). Freeze-dried samples of ROT05 were grinded to a fine powder using an agate mortar and pestle. Next, stable oxygen isotopic ratios were measured on 500  $\mu\text{g}$  of sample using a VGI mass spectrometer equipped with an isocarb using a dual inlet system. Isotopic data are reported using the conventional  $\delta$  notation. Calibration against international standards Naxos and NBS-19 allowed  $\delta^{18}\text{O}$  data to be expressed as per mille (‰) relative to V-PDB (Vienna-Peedee Belemnite).

## Fossil biota

Sediment samples from all three cores (ROT05, RL-300, RL-305) were used for pollen analysis using a standard chemical treatment (HCl, KOH, HF, HCl, acetolysis, KOH; see Lotter, 1988) and were then embedded in glycerine. Pollen and spores were identified under a microscope (magnification 400x) with a counting sum of 500 arboreal pollen grains.

Plant macrofossil analysis was carried out on cores RL-300 and RL-305 that were cut into 5 and 4 cm segments, respectively. Samples were subsequently soaked in KOH for 24 h and in HCl until carbonates were dissolved. After sieving (0.5 and 0.315 mm mesh size), plant macroremains were sorted and identified under a stereo microscope (magnification 6-12x). The terrestrial plant macrofossils were used for accelerator mass spectrometry (AMS) radiocarbon dating (Zbinden *et al.*, 1989).

For chironomid analysis core ROT05 was sampled at 4 cm intervals. Samples (0.1-1.3 g of freeze dried sediment) were treated with 10% KOH for 2 h at room temperature to deflocculate organic material and subsequently sieved using a 100  $\mu\text{g}$  mesh sieve following Walker (2001) and Brooks *et al.* (2007; no acid treatment). Chironomids were sorted from this residue in a Bogorov tray under a stereo microscope (magnification 40x) and mounted



**Figure 2** Lithostratigraphy, loss-on-ignition, depth-age model, and stable oxygen isotope record of bulk carbonates in late glacial and early Holocene sediments of Rotsee

onto microscope slides with Euparal® as a mounting medium. A minimum of 40 head capsules was identified (Heiri and Lotter, 2001). Chironomids were identified under microscope (magnification 400x) following identification keys by Wiederholm (1983), Schmid (1993), Rieradevall and Brooks (2001) and Brooks *et al.* (2007).

## Zonation

The pollen record was subdivided into the regional pollen assemblages (PAZ) as described by Ammann *et al.* (1996) for the Central Swiss Plateau. The plant macrofossil and chironomid biostratigraphies were subdivided into assemblage zones using optimal sum-of-squares partitioning (Birks and Gordon, 1985) as implemented in the program ZONE (version 1.2, see Lotter and Juggins, 1991). Statistical significance of the number of zones was assessed using the broken stick model (Bennett, 1996).

## Multivariate statistics

### *Detrended correspondence analysis (DCA)*

Major trends of variation in the biotic proxies were summarized using DCAs (Hill and Gauch, 1980). Gradient lengths > 2.5 standard deviation units of all data sets justified the use of unimodal-based response models (ter Braak and Prentice, 1988). Percentage data (pollen and chironomids) and accumulation rate data (plant macrofossils) were square-root transformed, detrended by segments, and rare taxa were downweighted. Statistical significance of the number of DCA axes was assessed by comparison with the broken-stick model (Jolliffe, 1986; Legendre and Legendre, 1998)

### *Canonical correspondence analyses (CCA)*

A series of CCAs of the chironomid percentage data (square-root transformation, downweighting of rare taxa) with  $\delta^{18}\text{O}$ , LOI550, LOI950, and the significant DCA axes scores (1<sup>st</sup> and 2<sup>nd</sup> DCA axis) of aquatic and terrestrial plant macrofossils as explanatory variables (see Birks *et al.*, 2000; Lotter and Birks, 2003) were carried out to assess the amount of variance explained by these predictors. In a second run of CCAs with the same explanatory variables sample age was used as a co-variable to account for the effects of the strong temporal auto-correlation of stratigraphical data. The statistical significance of the CCAs was assessed by restricted Monte Carlo permutation tests (499 restricted permutations). All numerical analyses were carried out using CANOCO 4.51 (ter Braak and Šmilauer, 1998).

# Results and discussion

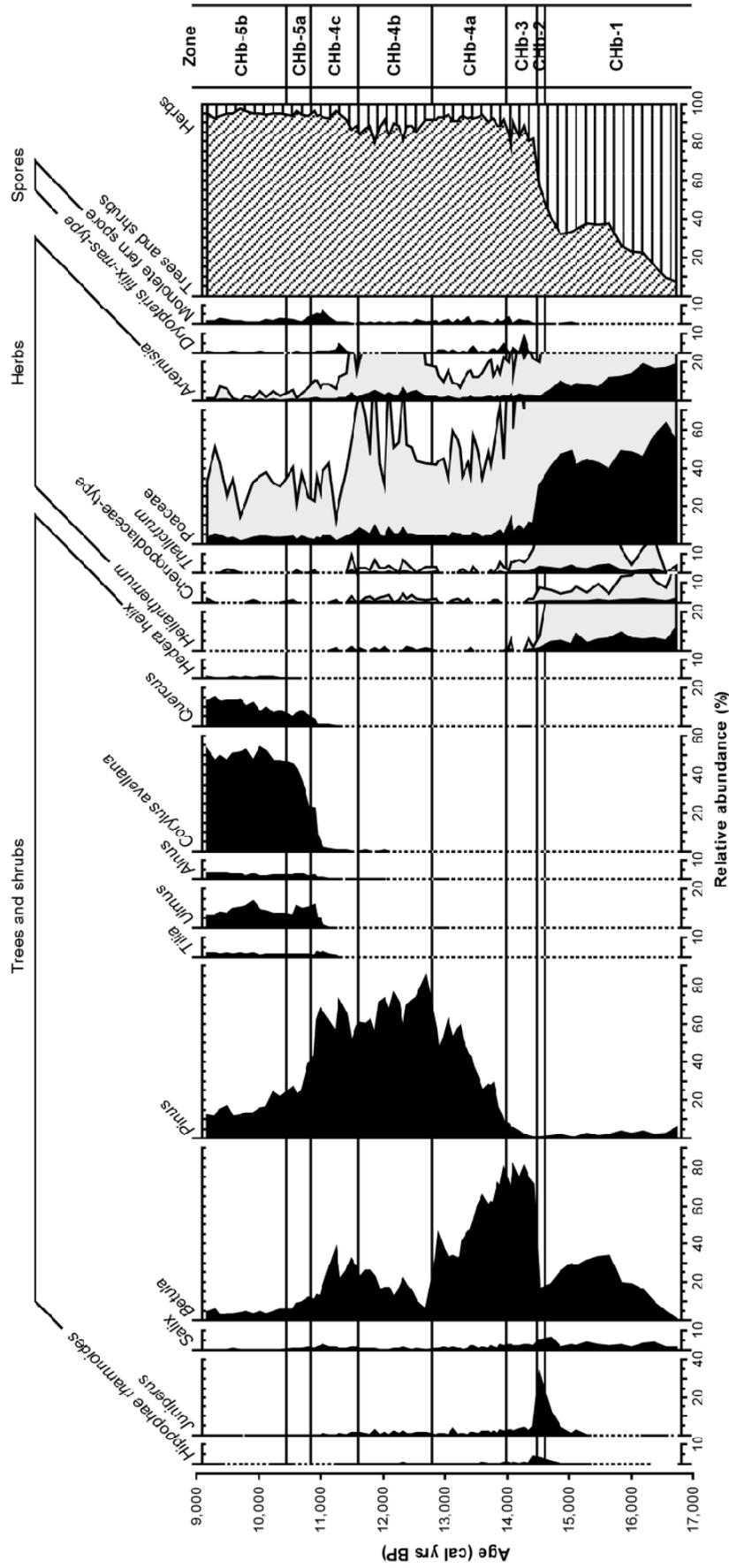
## Age model

The oxygen isotope record obtained from bulk carbonates from core ROT05 was visually matched to the NGRIP  $\delta^{18}\text{O}$  record from Greenland (Rasmussen *et al.*, 2006) using AnalySeries (Paillard *et al.*, 1996). Based on resulting differences in sedimentation rates, the core was subdivided into four different zones. Terrestrial plant remains, selected from cores RL-300 and RL-305 for Accelerator Mass Spectrometry (AMS) dating, have previously yielded 61 radiocarbon dates (Zbinden *et al.*, 1989). These  $^{14}\text{C}$  dates were wiggle-matched within each of the four zones to the IntCal04 calibration curve (Reimer *et al.*, 2004) following Blaauw *et al.* (2003). The resulting  $^{14}\text{C}$  ages for the Rotsee record were expressed as calibrated calendar years before present (cal yrs BP; Fig. 2). The presence of two tephra layers allowed an evaluation of our depth-age model. The Laacher See Tephra (LST) occurs at a depth of 1062 cm and is assigned an age of 13,057 cal yrs BP using our wiggle-match dated chronology. This is consistent with previous studies, which date the eruption of the Laachersee volcano in the Eifel (Germany) at 11,063  $\pm$ 12 conventional  $^{14}\text{C}$  yrs BP (i.e. 12,920-13,060 cal yrs BP; 2-sigma interval; Friedrich *et al.*, 1999). Furthermore, the Vedde ash has geochemically been verified in the interval between 1034 and 1036 cm (C. Lane and S. Blockley, pers. comm.). This interval corresponds to an age of 11,960-12,030 cal yrs BP using our age model, which agrees with the age estimated for the Vedde ash in other lake sediment records (11,832-12,225 cal yrs BP; Blockley *et al.*, 2007) and in the Greenland ice cores (12,121  $\pm$ 114 yrs BP; Mortensen *et al.*, 2005; Rasmussen *et al.*, 2006). To compare individual  $^{14}\text{C}$  ages with the age assigned to the record using wiggle-match dating, radiocarbon ages were separately calibrated using CALIB 5.0.2 (Stuiver and Reimer, 1986, 1993) (Fig. 2).

## Regional and local vegetation

The pollen record from Rotsee can be easily correlated with the regional PAZ defined for the Central Swiss Plateau (Ammann *et al.*, 1996) and therefore reflects the regional vegetation development of the lowland area between Lake Constance and Lake Geneva. The plant macrofossil data from the Rotsee sediments supplement the pollen information and allow characterizing the catchment vegetation. This is especially valuable for the late glacial period as it helps unequivocally determining the local immigration and establishment of tree populations that is otherwise often masked in the palynological data by long-distance pollen transport (Birks and Birks, 2000; Birks, 2001).

The record of both pollen and plant macrofossils at Rotsee goes back to the end of the last glaciation (i.e. >17,000 cal yrs BP; see also Lotter and Zbinden, 1989). The lowermost regional pollen assemblage zone (PAZ CHb-1, Fig. 3) represents a tree-less pioneer vegetation characterized by many heliophilous herbs with steppe and arctic/alpine affinities. Based on the low plant macrofossil accumulation rates in zone 1 (Fig. 4) this



**Figure 3** Relative abundances (%) of terrestrial pollen and spores in late glacial and early Holocene sediments of Rotsee. Abundances of herbaceous pollen are exaggerated by 10x (grey areas)

vegetation, also consisting of dwarf shrubs such as *Salix* and *Betula nana*, must have been rather open which is corroborated by the high amount of minerogenic input into the sediment (Fig. 2).

Between 14,700 and 14,600 cal yrs BP the regional vegetation rapidly changed into an open juniper scrub (PAZ CHb-2, Fig. 3). The sediments of both analyzed cores did not contain any *Juniperus* macrofossils as are sometimes found at other late glacial sites on the Swiss Plateau (e.g. Weber, 1980). However, dwarf birch and willow remains were locally still present (top of zone 1, Fig. 4) and the steadily declining minerogenic matter content of the sediment (Fig. 2) points to an increasingly denser vegetation cover. This zone is commonly considered as the onset of the Bølling biozone (see Ammann and Lotter, 1989) and coincides with the first major shift in  $\delta^{18}\text{O}$  towards less negative values (Lotter *et al.*, 1992a), which is interpreted as a climatic warming.

Around 14,470 cal yrs BP a fast increase in both *Betula* pollen (Fig. 3) and tree birch macro remains (Fig. 4) indicates the change to a birch woodland (PAZ CHb-3) that is initiating the reforestation on the Swiss Plateau. Minor fluctuations in the  $\delta^{18}\text{O}$  curve indicate the presence of an Aegelsee fluctuation. However, in contrast to sites at higher elevation on the Swiss Plateau and the Alps (e.g. Lotter *et al.*, 1992a; Lotter, 1999; CHb-3b, Ammann *et al.*, 1996) no conclusive evidence for such a climatic oscillation is visible in the Rotsee pollen record. From ca. 14,000 cal yrs BP onwards *Pinus sylvestris* populations established regionally (PAZ CHb-4, Fig. 3) as well as locally (Fig. 4) and formed a birch-pine woodland (PAZ CHb-4a, Fig. 3).

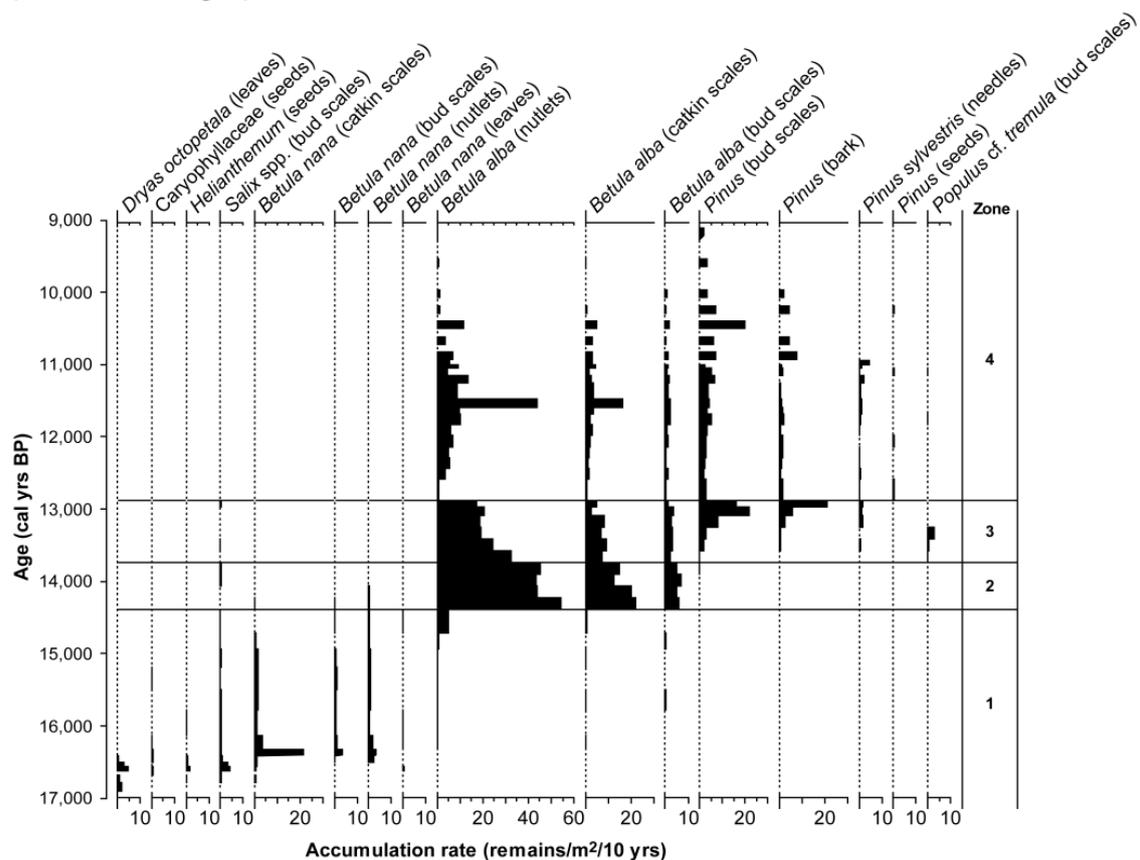


Figure 4 Accumulation rates of terrestrial plant macrofossils in Rotsee (as remains/m<sup>2</sup>/10 years)

Synchronous with the drop in  $\delta^{18}\text{O}$  values (Fig. 2), indicating the Younger Dryas cooling around 12,800 cal yrs BP both the *Betula* pollen and macrofossil abundances declined rapidly, whereas the abundance of pollen of grasses and of heliophilous herbs such as *Artemisia*, Chenopodiaceae, and *Thalictrum* increased (Fig. 3). Together with higher amounts of minerogenic matter in the sediment (Fig. 2), this points to an opening of the pine-birch woodland (PAZ CHb-4b) in the catchment of Rotsee.

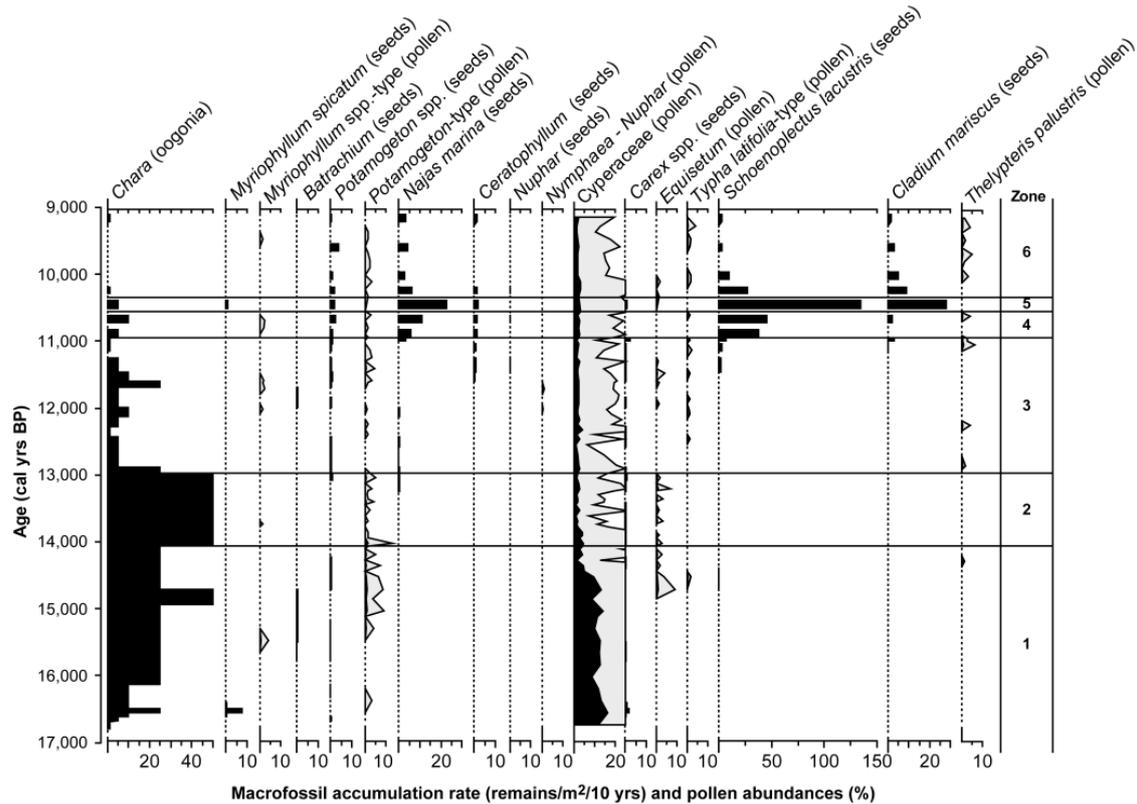
After the increase in  $\delta^{18}\text{O}$  values around 11,600 cal yrs BP both the relative amount of herb pollen (PAZ CHb-4c, Fig. 3) and the minerogenic matter content of the sediment decreased and the plant macrofossils point to a pine-birch forest in the catchment of Rotsee that is typical for the Preboreal biozone (see Ammann *et al.*, 1996) on the Swiss Plateau.

The early Holocene regional vegetation after about 10,900 cal yrs BP, as depicted from the palynological data, is characterized by the establishment and expansion of thermophilous deciduous trees and shrubs such as first *Corylus* (PAZ CHb-5a) and then *Tilia*, *Quercus*, *Ulmus*, and *Fraxinus* (PAZ CHb-5b). With the exception of sporadic occurrences of *Tilia platyphyllos* seeds (not shown, but see Lotter, 1988) the plant macrofossil record, however, suggest that the Rotsee catchment was covered by a pine-birch forest during the early Holocene.

### Aquatic vegetation

The plant macrofossil analyses also provide information on the late glacial and early Holocene aquatic vegetation and the zonation of macrophyte communities in Rotsee. Different life forms of macrophytes occur at different water depths and produce characteristic sediment types. Habitat (e.g. presence/absence, type of macrophytes), water depth, and substrate type are relevant environmental factors that can determine the composition of the chironomid fauna (e.g. Pinder, 1986; Langdon *et al.*, 2006). Fig. 5 shows the macrofossil influx together with the pollen percentages for different aquatic macrophyte taxa. After the lake was isolated from the direct influence of glacial melt water, evidenced by change from a pure clay to a calcareous clay gyttja (Fig. 2) about 16,900 cal yrs BP, stonewort algae (*Chara*) first colonize Rotsee together with other submerged macrophytes such as *Myriophyllum spicatum*, *Batrachium* spp., and *Potamogeton* spp. The presence of remains of plant communities of the *Charetea* indicate transparent water conditions (Ellenberg, 1982). Carbonate incrustations of the *Chara* algae were increasingly building up the sediment, especially between ca. 16,000 and 13,000 cal yrs BP (Fig. 5), and are responsible for its high carbonate content (Fig. 2). The Younger Dryas cooling that started around ca. 12,800 cal yrs BP strongly diminished the submerged Characeae communities, which did not reach comparable densities again during the Holocene. The occurrence of floating-leaved macrophytes such as *Potamogeton natans*, *Nuphar*, and *Nymphaea* indicate that Rotsee became shallower during the Younger Dryas. Given that floating-leaved plants of the *Nymphaeion* association typically occur at water depths of 3-4 m (e.g. Lachavanne and Klötzli, 1984) this is likely indicating the effect of a lake level lowering rather than the result of the natural infilling of the basin by several meters. Low lake levels are reported for many central European lakes during the Younger Dryas and the earliest part of the Holocene (e.g. Magny and Richoz, 2000; Magny, 2001; Magny *et al.*, 2006). Magny *et al.* (2007) indicate

that the Preboreal Oscillation (11,300-11,150 cal yrs BP) was characterized by a regional increase in lake levels. In the Rotsee record the enhanced occurrence of reed plants of the *Phragmites* association, such as *Schoenoplectus lacustris* and *Cladium mariscus* (Fig. 5) together with a lithological change and an increase in sedimentary organic matter (Fig. 2) points to a renewed drop in lake level that started around 11,000 and ended ca. 10,300 cal yrs BP. This is in agreement with findings by Magny (2004) and Magny *et al.* (2007), who found that lake levels are generally low in west-central Europe during this period.



**Figure 5** Accumulation rates of aquatic vegetation. Histograms show influx of aquatic plant macrofossils (as remains/m<sup>2</sup>/10 years) and line graphs show relative abundances of aquatic pollen (in %). Abundances of aquatic pollen are exaggerated 10x (grey areas). Zonation is based on macrofossils only

## Chironomid assemblages

A total of 40 chironomid taxa were identified in the studied sediment record. Given the high-resolution chronology available for Rotsee, the data were expressed as accumulation rates (Fig. 6). The chironomid biostratigraphy was divided into three statistically significant zones, two of which were further divided into subzones (Fig. 7).

Subzone 1a (>14,660 cal yrs BP) is characterized by a high influx of *Paratanytarsus austriacus*-type and *Psectrocladius sordidellus*-type. *Cricotopus intersectus*-type, *Corynoneura arctica*-type, *Einfeldia*, *Tanytarsus lugens*-type, *Chironomus anthracinus*-type, and *Ablabesmyia* are also abundantly present. Many of these taxa are associated with macrophytes (Cranston *et al.*, 1983; Brodin, 1986; Buskens, 1987; Moller Pillot and Buskens, 1990; Brodersen *et al.*, 2001). Especially *P. austriacus*-type, *C. arctica*-type, and

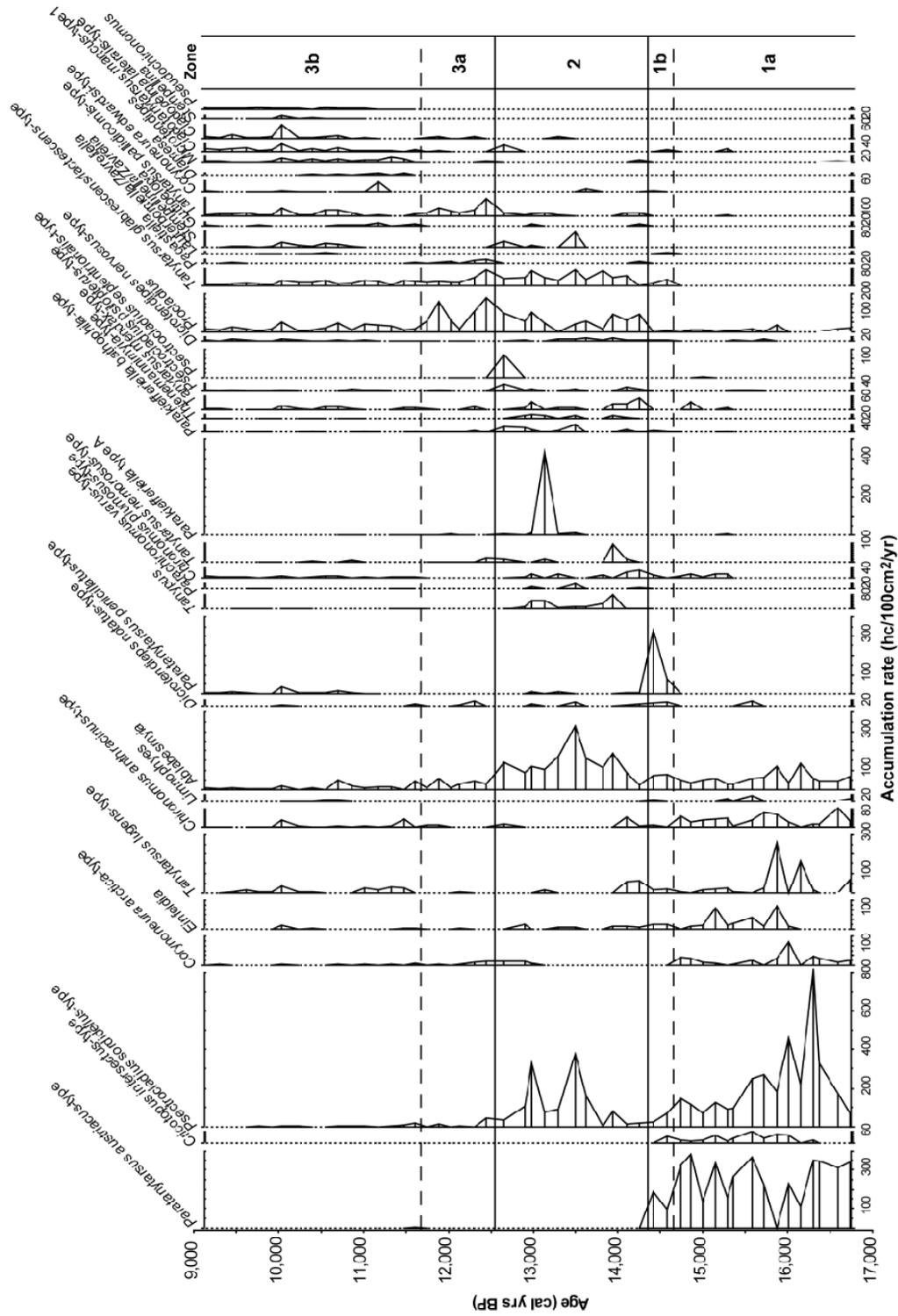


Figure 6 Accumulation rates of chironomid head capsules (as head capsules/100 cm²/yr)

*T. lugens*-type indicate that the climate was relatively cool (Brundin, 1956; Heiri and Lotter, 2003; Brooks *et al.*, 2007).

A peak in *Paratanytarsus penicillatus*-type is observed in subzone 1b (14,660-14,340 cal yrs BP). Furthermore, *P. austriacus*-type and *Ablabesmyia* are present. *Tanytarsus glabrescens/lactescens*-type had its first occurrence at the bottom of subzone 1b. *P. penicillatus*-type is described as being typical of warmer conditions (Brooks *et al.*, 2007). Also *Tanytarsus* is known to occur in the littoral zone of relatively warm lakes (Brooks *et al.*, 2007), and Heiri (2001) has shown that *T. lactescens* is abundant in temperate, carbonate-rich lakes. The increased abundance of taxa indicative of warmer conditions is in agreement with the warming inferred from the  $\delta^{18}\text{O}$  increase at the onset of the interstadial (Figs. 6 and 7).

Zone 2 (14,340-12,550 cal yrs BP) is characterized by an increased abundance of *Ablabesmyia* and *P. sordidellus*-type. A peak in *Parakiefferiella* type A is seen at 13,140 cal yrs BP. *Procladius*, *T. glabrescens/lactescens*-type, *Tanytus*, and *Parakiefferiella bathophila*-type were present. All these taxa are indicative of temperate or relatively warm temperatures (Brundin, 1949; Chernovskii, 1949; Saether, 1979; Hofmann, 1984; Kansanen, 1985; Brodin, 1986; Uutala, 1986; Bilyj and Davies, 1989; Brooks *et al.*, 2007). Most of this zone covers the interstadial, which is characterized by elevated temperatures as compared to the periods before and after it. The occurrence of chironomids that are indicative of temperate climates is in agreement with this.

Subzone 3a (12,550-11,680 cal yrs BP) is characterized by a relatively low influx of chironomid head capsules. *Procladius*, *T. glabrescens/lactescens*-type, and *T. pallidicornis*-type are present, as well as *Ablabesmyia*, the latter with a lower influx than in the previous zone. For the first time low accumulation rates of *P. sordidellus*-type are observed. Most of the dominant taxa are indicative of temperate to warm environments (Brodin, 1986; Uutala, 1986; Bilyj and Davies, 1989), although *Procladius* is found in lakes that cover a large range of trophic states and altitudes (Wiederholm, 1981, 1989; Lotter *et al.*, 1997, 1998). These results are rather unexpected as according to the pollen and oxygen isotope data (Fig. 3) this zone mostly corresponds to the Younger Dryas cold phase. Chironomid assemblages do change in the early part of the Younger Dryas. The absence of cold stenothermic chironomid taxa, however, suggests that either temperature changed, but was not low enough for these taxa to settle in the littoral zone of the lake during this interval, and/or that other factors than the direct effect of temperature influenced the chironomid assemblages in subzone 3a. Climate could have affected chironomids indirectly e.g. via changes in lake level, lake trophic state, substrate, as well as density and composition of macrophyte communities. The shift from submerged to floating-leaved macrophyte communities (Fig. 5) suggests a lake level lowering during the Younger Dryas, which is also observed in other lakes in central Europe (e.g. Magny *et al.*, 2001). A general increase in water temperature with decreasing water depth is often observed in lakes (e.g. Wetzel, 2001). Such a shallowing of Rotsee may have affected the mixing of the water column as well as the depth of the thermocline. This may have moderated the influence of cooling air temperatures and led to only a weak decrease of water temperature at the coring site. The lowered lake level of Rotsee may therefore explain the absence of cold-indicating chironomids in the littoral.

Subzone 3b (<11,680 cal yrs BP) is also characterized by a low head capsule influx, indicating that chironomid population density was relatively low during this period. The most

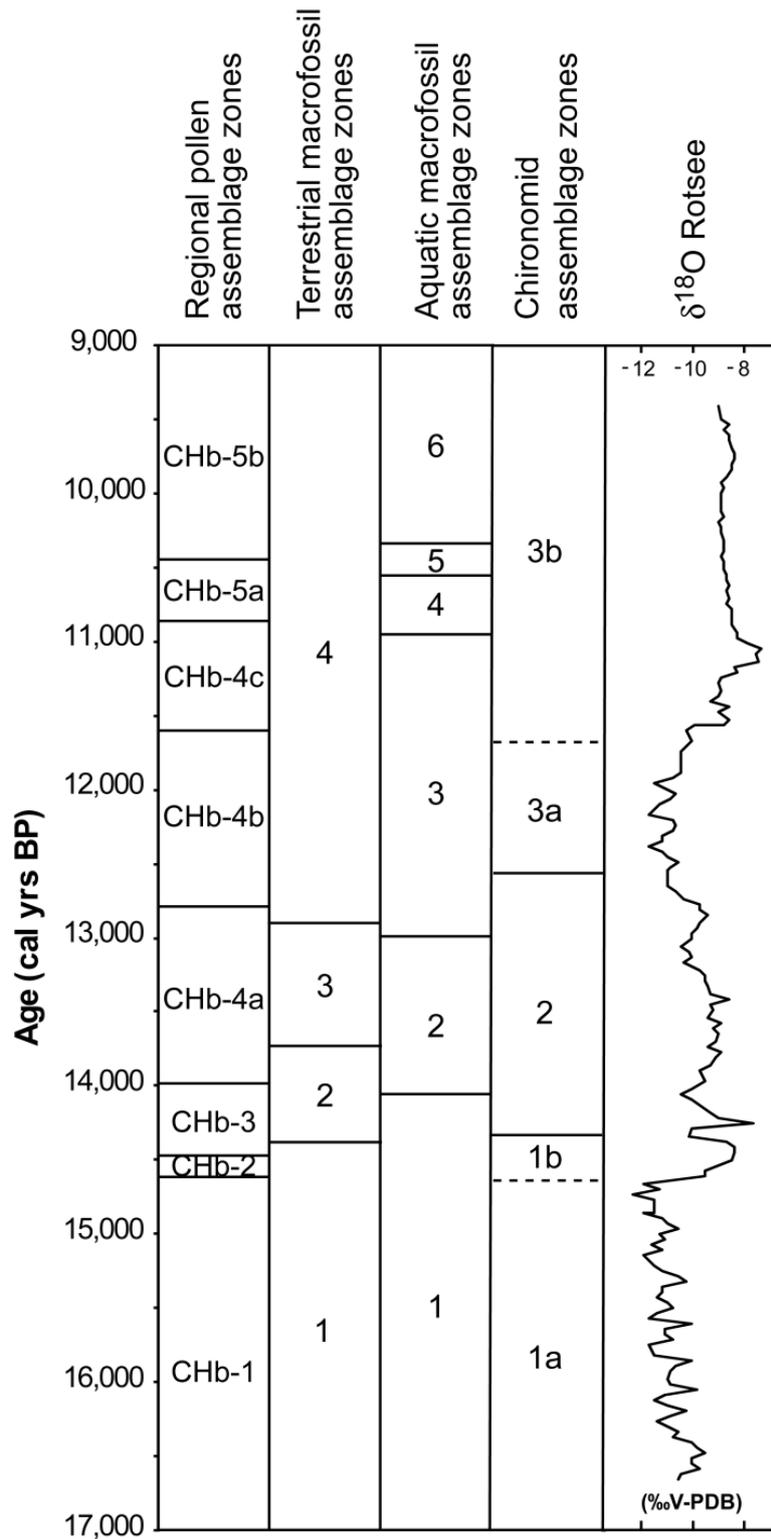
common taxa include *Procladius*, *T. glabrescens/lactescens*-type, *T. pallidicornis*-type, *Microtendipes*, *Cladotanytarsus mancus*-type 1, *Stempellinella/Zavreliella*, *Cladopelma lateralis*-type, *Stempellina*, and *Pseudochironomus*. The latter two morphotypes are restricted to this subzone. *T. lugens*-type became more abundant during this subzone. Most taxa are typically encountered in the littoral zone and are indicative of temperate to warm climates (Brundin, 1949; Saether, 1979; Brodin, 1986; Uutala, 1986; Bilyj and Davies, 1989; Walker *et al.*, 1991; Brooks and Birks, 2001).

## Leads and lags

Using a consistent zonation approach on the different proxies, the timing of changes in the biotic assemblages can be compared for the different studied organism groups. This allows the assessment of leads and lags in the response of different organisms to environmental change. If zone boundaries of various proxies coincide, it can be assumed that changes in a major environmental forcing led to taxonomic turnover (Lotter and Birks, 2003). In contrast, zone boundaries apparent in only one proxy record are likely to reflect environmental change on a lower level. However, different organism groups may need a different threshold to be exceeded before they will react to environmental forcing by changes in their community composition (e.g. Heegard *et al.*, 2006). Furthermore, chironomid, macrofossil, and pollen records were analyzed at different temporal resolutions and the response of the investigated biota can therefore at best be synchronous within one to two centuries.

Different intervals can be distinguished in the Rotsee profile that have coinciding zone boundaries for most fossil sequences (Fig. 7). The first one occurs around the onset of the interstadial as indicated by increasing  $\delta^{18}\text{O}$  values (ca. 14,650 cal yrs BP). Chironomids responded immediately to the temperature rise that is associated with this transition. The regional vegetation represented by the pollen record also responded at this transition (PAZ CHb-2/CHb-3), whereas the response in local terrestrial vegetation (terrestrial macrofossils) seems to have lagged the climatic change by approximately 250 years. This lag may be a consequence of the absence of juniper macrofossils in the Rotsee record. At the onset of the Younger Dryas (12,860 cal yrs BP) all vegetation proxies showed a change of assemblage composition within the uncertainty of the sampling resolution (Fig. 7). Chironomids, however, showed a lagged response by several centuries, which exceeds the delay that can be attributed to sampling resolution.

In contrast to these parallel changes in several organism groups most zonal boundaries detected in the proxy records are specific for one or two individual proxy indicators. For example, at the onset of the Holocene a distinct shift in regional terrestrial vegetation has been reported for the Swiss Plateau, which coincided with climatic changes (Lotter *et al.*, 1992a; Ammann *et al.*, 2000). However, neither records of local terrestrial or aquatic vegetation at Rotsee showed a response to this warming. Similarly, two zonal boundaries are detected in the record of aquatic vegetation between ca. 11,000 and 10,400 cal yrs BP. Regional vegetation seems to have reacted in this time interval as well, whereas both the terrestrial plant macrofossil and chironomid records are not providing evidence of major assemblage shifts during this period (Fig. 7).



**Figure 7** Summary of zonation of terrestrial pollen, terrestrial plant macrofossils, aquatic plant macrofossils, and chironomids, and the bulk carbonate stable oxygen isotope record of Rotsee. Regional pollen assemblage zones of terrestrial pollen following Ammann *et al.* (1996), the other fossil sequences are zones using optimal sum-of-squares partitioning. Dashed lines indicate important secondary zone boundaries

## Rates of compositional change

In an attempt to assess rates of change and more gradual variation patterns in the different biostratigraphies, that were not captured by numerical zonation, we performed a detrended correspondence analysis (DCA) on the fossil sequences. Sample scores of the most relevant DCA axes plotted against time can provide an approximation of the timing and magnitude of compositional change in biostratigraphical data (Birks *et al.*, 2000).

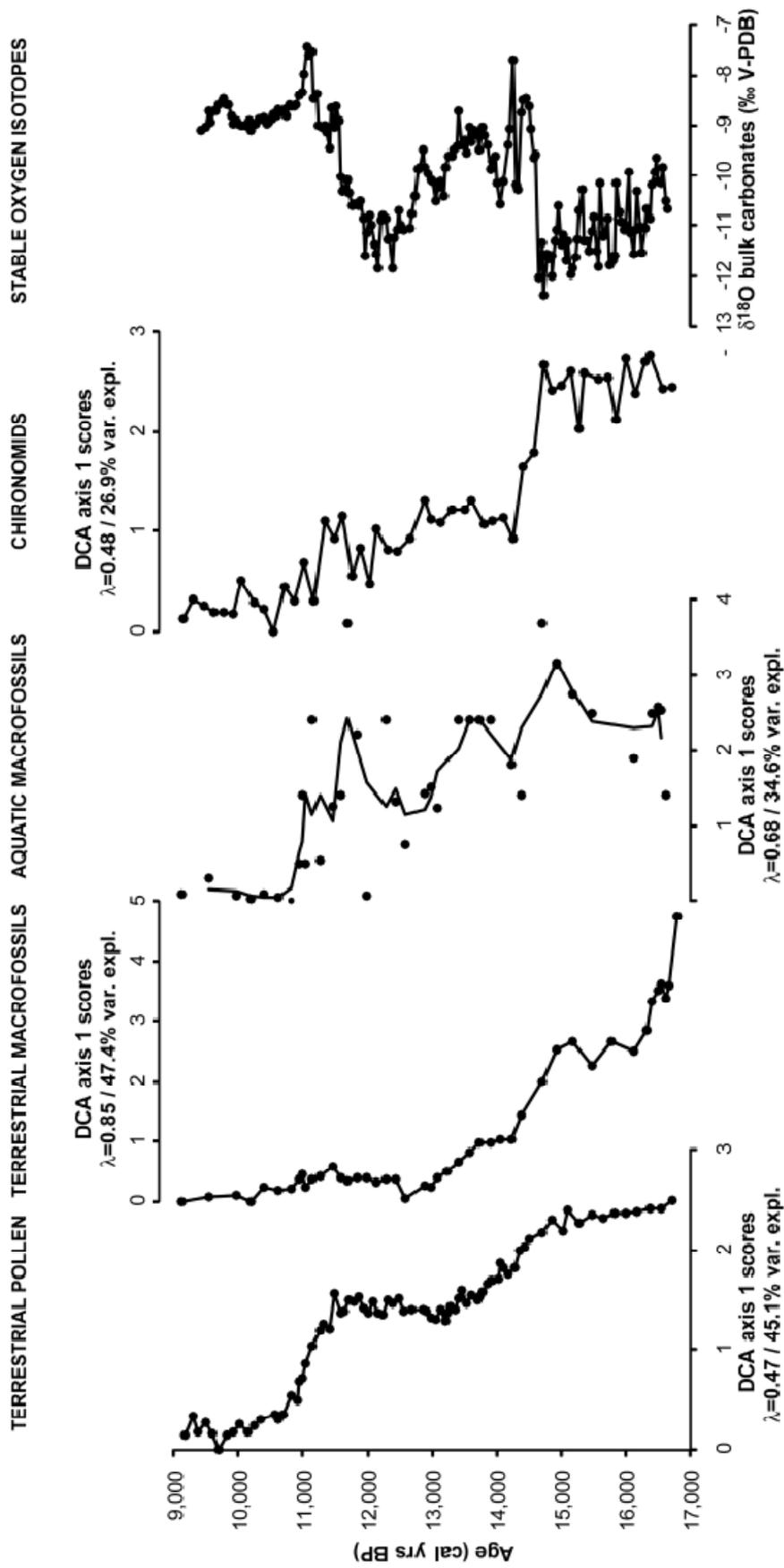
For all proxy records the first DCA axis showed a more or less unidirectional trend towards lower sample scores throughout the studied interval. For the pollen data, scores of DCA axis 1 generally declined with decreasing age. However, two periods can be discerned that are characterized by increased compositional change. The first period (14,520-13,200 cal yrs BP) showed a gradual shift in pollen assemblage composition and reflects the reforestation phase of the interstadial, when vegetation was consecutively dominated by juniper, birch, and pine. The second period (11,490-10,620 cal yrs BP) showed a more abrupt compositional change, which represents the change from a pine-birch woodland to a broadleaved forest during the early Holocene (Fig. 8).

Local catchment vegetation is characterized by abrupt compositional changes prior to 16,330 cal yrs BP (Fig. 8), which reflects rapid increases in *Betula nana*, *Dryas octopetala*, *Helianthemum*, and *Salix* (Fig. 4). More gradual changes in the local vegetation have taken place between 14,940 and 12,590 cal yrs BP. The onset of this decrease reflects decreases in taxa that characterize terrestrial macrofossil assemblage zone 1 (Fig. 4) and the subsequent rapid increase of *Betula alba* around 14,390 cal yrs BP. This gradual change continues as *Betula alba* remains decrease and *Pinus sylvestris* increases from 13,740 cal yrs BP onward. DCA axis 1 scores remain stable from 12,590 cal yrs BP onward, which coincides with terrestrial macrofossil assemblage zone 4. Relatively little compositional change is observed during this period, during which influx of *Betula alba* and *Pinus sylvestris* remained relatively stable.

DCA axis 1 scores for the aquatic macrofossil record generally displayed a high variability (Fig. 8). DCA axis scores generally decreased until approximately 13,000 cal yrs BP, with some positive excursions around 14,900 and 13,700 cal yrs BP. Between 11,000 and 10,830 cal yrs BP, another phase of rapid compositional change is distinguished, which is attributed to the increase of *Najas marina*, *Schoenoplectus lacustris* and *Cladium mariscus*. From 10,830 cal yrs BP onward, DCA sample scores remained stable indicating little compositional change.

The chironomid record showed relatively stable sample scores until 14,740 cal yrs BP (Fig. 8). A phase of rapidly decreasing DCA sample scores (14,740-14,260 cal yrs BP) coincided with the onset of the interstadial as evidenced by increased  $\delta^{18}\text{O}$  values. This decrease in scores continued into the early interstadial. DCA scores remained relatively stable until 12,900 cal yrs BP. From this time onward sample scores showed a decreasing trend.

Sample scores of the first DCA axis show that at the onset of the interstadial chironomid communities responded with the most pronounced and rapid compositional change, whereas proxies reflecting vegetation changed more gradually. Chironomids are known to react quickly to environmental change due to their relatively short generation time (e.g. Brooks and Birks, 2001, 2004). However, in contrast to other studies (e.g. Brooks and Birks, 2001; Heiri and Millet, 2005; Heiri *et al.*, 2007) no distinct and abrupt compositional



**Figure 8** Detrended correspondence analysis (DCA) axis 1 scores for terrestrial pollen, terrestrial plant macrofossils, aquatic plant macrofossils, and chironomids, compared to the stable oxygen isotope record of bulk carbonates. Aquatic plant macrofossil scores are smoothed with a 3-sample running average

change in chironomid assemblages is evident at the onset of the Younger Dryas cooling. This is attributed to the absence of cold-indicator taxa during the Younger Dryas, which we relate to environmental changes other than the direct effect of temperature. A lake level lowering as suggested by the macrophyte communities (Fig. 5) likely influenced the littoral chironomid communities. The similar trend in DCA axis 1 sample scores for both aquatic macrofossil and chironomids from 13,000 yrs BP onward suggests that the same within-lake changes were affecting macrophyte and chironomid communities.

### Climate - catchment - lake processes

Aquatic biota can be influenced by different environmental processes (e.g. Battarbee, 2000). Generally, three major forcing factors may influence aquatic organisms such as chironomid communities and can, therefore, affect their species composition:

1. Climate change as an external forcing may influence aquatic ecosystems in a direct or indirect way.
2. Catchment processes such as vegetation composition, type and cover, pedogenesis, or human impact that lead to the export of nutrients, ions, or erosion of organic and minerogenic matter.
3. Lake internal processes that can affect water chemistry (e.g. DOC, nutrients, pH, salinity), transparency, stratification, oxygen availability, type and cover of macrophyte vegetation, and sediment type.

However, only few palaeolimnological studies have tried to disentangle the influence of different drivers on aquatic ecosystems in the past (e.g. Lotter and Birks, 1997, 2003; Birks *et al.*, 2000). Here, we used a data splitting approach (see Birks and Birks, 2006), i.e. using one or several independent proxies as predictors or explanatory variables to assess their influence on a set of response variables. In our study we used the relative abundances of the chironomids as response variables in an attempt to test the null hypothesis that climate change, catchment, or lake internal processes had no significant influence on the chironomid communities during the late glacial and the early Holocene. In a series of CCAs we calculated the amount of variance in the chironomid data explained by these three processes and assessed their significance by restricted permutation tests. The significance level ( $P < 0.05$ ) is a measure of the likelihood of rejection of the null hypothesis.

We used the bulk carbonate  $\delta^{18}\text{O}$  values of Rotsee (Fig. 7) as a proxy for climate change, whereas catchment processes were modeled by the 1st and 2nd (significant) DCA axes of the terrestrial plant macrofossil data (Table 1). Lake processes were modeled by LOI950 (as a significant predictor in a CCA with forward selection) and the 1st and 2nd (significant) DCA axes of the aquatic plant macrofossil data. To assess lake internal processes that may have been relevant for the composition of chironomid assemblages more specifically, we also modeled the effects of substrate by using LOI950 (sedimentary carbonate content) and LOI550 (sedimentary organic matter content) and the effects of habitat type by using the 1st and 2nd (significant) DCA axes of the macrophyte remains as predictors (Table 1).

Lakes like other ecosystems are evolving through time and there are several long-term processes such as sediment infilling of the basin, succession of macrophyte communities, or changes in the chemical composition of the water column due to catchment processes. Such unidirectional processes explain 21.7% of the variance in the chironomid data if sample age is used as the sole explanatory variable (Table 1), showing that there is a significant and strong temporal trend in the data. We therefore also assessed the importance of the different external and lake-internal processes by allowing for such a long-term temporal trend and the auto-correlation between samples inherent to stratigraphical data by partialling out the effect of sample age in a series of partial CCAs (Table 1).

**Table 1** Partial CCA results showing the amount of variance explained in the relative abundances of chironomids by different predictor variables and by partialling out the effect of sample age as co-variable. All processes/forcings are statistically significant ( $P < 0.05$ ). LOI550, LOI950: Loss-on-ignition at 550° and 950°C, respectively.

Forcing	Predictor	Variance explained (%) (statistical relationship)			
				<i>age partialled out</i>	
Climate	$\delta^{18}\text{O}$	14.8	( $P=0.002$ )	6.1	( $P=0.008$ )
Catchment processes	Terrestrial macrofossils (DCA axes 1 and 2)	23.4	( $P=0.002$ )	13.9	( $P=0.002$ )
In-lake processes	LOI950, aquatic macrofossils (DCA axis 1 and 2)	16.4	( $P=0.002$ )	7.7	( $P=0.012$ )
Substrate	LOI950, LOI550	16.8	( $P=0.002$ )	7.2	( $P=0.018$ )
Habitat	Aquatic macrofossils (DCA axes 1 and 2)	15.2	( $P=0.002$ )	5.7	( $P=0.034$ )
Unidirectional processes	Sample age	21.7	( $P=0.002$ )		

The highest significant amount of variance in the Rotsee chironomid data is explained by catchment vegetation (23.4% or 13.9% if long-term trends are allowed for; Table 1), followed by lake internal processes (16.4% or 7.7% with age partialled out). Regarding the lake internal processes, the substratum as modelled by the amount of organic matter and carbonate content of the sediment explained a slightly higher amount of variance in the chironomid assemblages than the habitat summarized by the macrophytes (Table 1). Climate as approximated by the stable oxygen isotope record explains a significant but lower amount of variance in the chironomid data than the other processes. This is likely the effect of the location of the core in the former littoral zone of the lake, where, compared to the profundal zone, direct catchment processes and lake internal processes amplified by lake level fluctuations, are potentially masking the climate effects on the composition of chironomid communities.

## Conclusions

Shifts in the fossil records of various biotic groups reflect past environmental changes during the late glacial and early Holocene at Rotsee. Boundaries of zones of relative stability, as assessed by optimal sum-of-squares partitioning, coincide with shifts in the stable oxygen isotope record at the transitions to the interstadial and the Younger Dryas cold phase, suggesting that climate exerted a major impact on the composition of biotic assemblages. Partial CCA results show that, even when long-term trends of lake and catchment development are allowed for, climate plays a statistically significant role in explaining the variation in the Rotsee chironomid communities. However, because of the location of the coring site in the shallow, littoral zone of Rotsee and the influence of lake-level fluctuations as evidenced by the local macrophyte communities, lake-internal dynamics, and catchment-related processes seem to be important drivers that determine changes in the aquatic ecosystem. This may be the reason why catchment-related as well as lake-internal processes are explaining a larger amount of the total variance in the littoral chironomid communities than climate.

## Acknowledgements

Ton van Druuten and Friederike Wagner-Cremer are thanked for help during fieldwork. Jan van Tongeren is acknowledged for laboratory assistance and Anna Lyaruu for pollen analysis on core ROT05. Arnold van Dijk is acknowledged for stable oxygen isotope measurements. We thank the Darwin Center for Biogeosciences ([www.darwincenter.nl](http://www.darwincenter.nl)) for financial support.

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# Chapter 5



# Chironomid $\delta^{18}\text{O}$ as a proxy for past lake water $\delta^{18}\text{O}$ : a down-core record based on late glacial sediments from Rotsee (Switzerland)

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*To be submitted to Quaternary Science Reviews*

## Abstract

We explored whether the stable oxygen isotope composition ( $\delta^{18}\text{O}$ ) of fossil chironomid remains can successfully be used to reconstruct past lake water  $\delta^{18}\text{O}$  in late glacial and early Holocene sediments from Rotsee (Switzerland). A sediment core from the littoral zone of the former lake was examined since it contained both high concentrations of chironomid remains and abundant authigenic carbonates to allow a direct comparison of chironomid  $\delta^{18}\text{O}$  with values measured on bulk carbonates. Sediment carbonate particles adhering to chironomid remains can potentially affect measurements by contamination, we tested two methods to chemically remove residual carbonates in the chironomid samples. Treatment with hydrochloric acid promoted oxygen exchange between chironomid remains and the water used during pretreatment, whereas a 2M ammonium chloride ( $\text{NH}_4\text{Cl}$ ) solution did not seem to affect chironomid  $\delta^{18}\text{O}$  to a significant extent. Fossil chironomid  $\delta^{18}\text{O}$  was analyzed for the Rotsee record both using standard palaeoecological methods and pretreatment with  $\text{NH}_4\text{Cl}$ . Samples prepared using standard techniques showed a poor correlation with  $\delta^{18}\text{O}$  of bulk carbonate ( $r^2=0.14$ ) suggesting that carbonate contamination of the chironomid samples obscured the chironomid  $\delta^{18}\text{O}$  signature, whereas samples pretreated with  $\text{NH}_4\text{Cl}$  correlated well with bulk carbonate  $\delta^{18}\text{O}$  ( $r^2=0.67$ ) and successfully tracked the late glacial changes in sedimentary  $\delta^{18}\text{O}$  described for the Alpine region, such as depleted  $\delta^{18}\text{O}$  values during the Oldest Dryas period, the Aegelsee and Gerzensee Oscillations, and Younger Dryas period and the enriched  $\delta^{18}\text{O}$  values associated with sediments deposited during the late glacial interstadial and the earliest Holocene. Differences in the amplitude of variations in bulk sediment and chironomid  $\delta^{18}\text{O}$  are attributed to differential effects of temperature on oxygen isotope fractionation during the formation of bulk carbonates and chironomid head capsule

cuticles or seasonal changes of lake water  $\delta^{18}\text{O}$  within the former lake, potentially affecting  $\delta^{18}\text{O}$  of these two substances to a different extent. Our results indicate that chironomid  $\delta^{18}\text{O}$  can successfully reconstruct centennial to millennial-scale changes in lake water  $\delta^{18}\text{O}$  and that the method can be applied to lake marl records provided that care is taken to eliminate carbonate contamination from the samples.

## Introduction

Stable oxygen isotope ( $\delta^{18}\text{O}$ ) records can be obtained using sediments from hard water lakes by measuring  $\delta^{18}\text{O}$  on bulk carbonates (Eicher and Siegenthaler, 1976) or on carbonate remains originating from specific aquatic organism groups (e.g. ostracod valves, charophyte incrustations, mollusc shells, see e.g. Schwalb *et al.*, 1995; von Grafenstein *et al.*, 1999a; Anderson *et al.*, 2001; Coletta *et al.*, 2001; Schwalb, 2003). Such stable oxygen isotope records based on authigenic carbonates provide information on past  $\delta^{18}\text{O}$  of the lake water (Eicher and Siegenthaler, 1976), which in turn depends on the isotopic composition of precipitation (Gonfiantini, 1986). Precipitation  $\delta^{18}\text{O}$  is related to temperature at mid to high latitudes (Dansgaard, 1964; Rozanski *et al.*, 1993) due to the preferential rain-out of heavier isotopes during poleward air mass movement (e.g. Alley and Cuffey, 2001) and is also affected by the source area of the moisture (Alley and Cuffey, 2001). Moreover,  $\delta^{18}\text{O}$  of lake water can be influenced by changes in local hydrology, such as evaporation (e.g. Mayr *et al.*, 2007) and input of isotopically light ice and snow melt water (Bottomley *et al.*, 1986). However, the temperature dependence of the oxygen isotopic composition of precipitation implies that in many parts of the world reconstructions of past lake water  $\delta^{18}\text{O}$  can provide information about past climatic changes (Leng and Marshall, 2004). For example,  $\delta^{18}\text{O}$  measured on bulk carbonates or ostracod remains in lake sediments confirmed the presence of abrupt temperature changes in Europe during the late glacial period (~14,700-11,600 calibrated  $^{14}\text{C}$  years before present (cal yrs BP); e.g. Lotter *et al.*, 1992; von Grafenstein *et al.*, 1999a). These changes in lake water  $\delta^{18}\text{O}$  were shown to coincide with past variations in  $\delta^{18}\text{O}$  of precipitation over central Greenland (von Grafenstein *et al.*, 1999a). For the late glacial period,  $\delta^{18}\text{O}$  records from lake sediments also play an important role for correlating different stratigraphies within Europe (e.g. Lotter *et al.*, 1992; Finsinger *et al.*, 2008) and with the Greenland ice core records (e.g. Björck *et al.* 1998; Schwander *et al.*, 2000; Hoek and Bohncke, 2001).

A major limitation of  $\delta^{18}\text{O}$  records from bulk lake sediments is that carbonate-rich deposits are not always present. They are usually absent in lakes on silicate bedrocks and for these lakes  $\delta^{18}\text{O}$  needs to be measured on other sediment components. Diatom silica is an alternative to produce  $\delta^{18}\text{O}$  records (e.g. Barker *et al.*, 2001). This technique, however, requires extensive sediment purification (Morley *et al.*, 2004) since small amounts of contamination can have a large impact on the stable oxygen isotope measurements (Leng and Barker, 2006). In addition, dissolution may result in poor diatom preservation (e.g. Mackay, 2007) and diatom frustules can be difficult to separate from other silicate particles in the sediments (Rings *et al.* 2004; Morley *et al.*, 2005). Stable oxygen isotopes have also been measured on aquatic cellulose originating from freshwater algae (Wolfe *et al.*, 2007).

Terrestrial cellulose may be an important source of contamination in some sediments and may, therefore, in some instances hamper interpretation of aquatic cellulose records (Sauer *et al.*, 2001). Recently, fossil chironomids have been suggested as a source for obtaining  $\delta^{18}\text{O}$  records (Wooller *et al.* 2004).

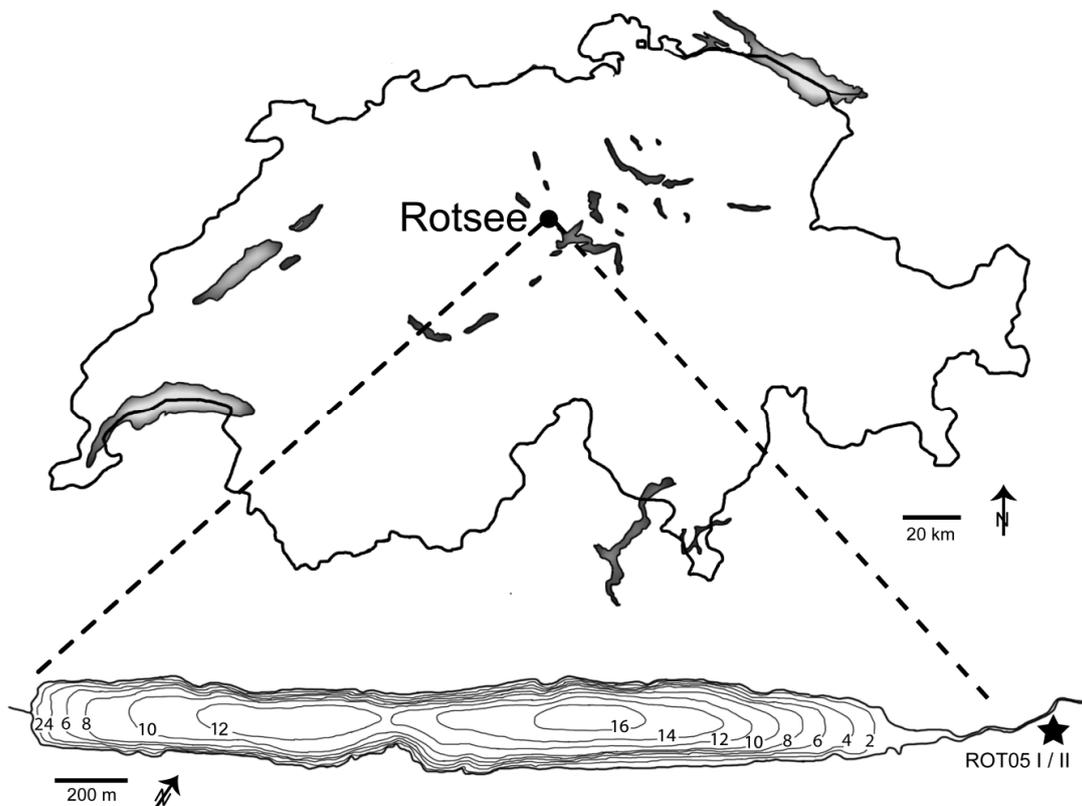
Remains of chironomid larvae and their strongly sclerotized head capsules in particular are well preserved in lake sediments (Brooks, 2003). Fossil chironomid assemblages have been used successfully for reconstructing temperatures based on the distribution and ecology of different chironomid morphotypes (e.g. Heiri *et al.*, 2007). Chironomid larvae live and grow in lakes and their  $\delta^{18}\text{O}$  values are therefore expected to reflect the isotopic signature of lake water. Furthermore, it can be expected that  $\delta^{18}\text{O}$  of aquatic organic matter is not strongly affected by temperature fractionation during its formation (e.g. DeNiro and Epstein, 1981; Wolfe *et al.*, 2001). Wooller *et al.* (2004) studied the relationship between  $\delta^{18}\text{O}$  in chironomids and precipitation in four lakes in the United States, Canada, and Greenland. They showed that chironomid  $\delta^{18}\text{O}$  is related to precipitation  $\delta^{18}\text{O}$ . More recently, Verbruggen *et al.* (in prep a) demonstrated a strong relationship between chironomid  $\delta^{18}\text{O}$  and the  $\delta^{18}\text{O}$  signature of lake water and precipitation in deep lakes in Europe ranging from southern Italy to northern Sweden. Effects of different analytical techniques and methods of sample preparation on chironomid  $\delta^{18}\text{O}$  measurements have been examined by Wang *et al.* (2008), who studied the effects of silver and tin cups used during analysis on chironomid  $\delta^{18}\text{O}$ , whereas Verbruggen *et al.* (2009) focused on the effects of different chemical pretreatments on  $\delta^{18}\text{O}$  values, chemical composition, and morphology of chironomid head capsules. Based on laboratory experiments with chironomids that were fed identical diets, but cultivated in water with different isotopic signatures, Wang *et al.* (2009) estimated that almost 70% of oxygen incorporated in head capsules is derived from the water the chironomid larvae lived in. The first down-core chironomid  $\delta^{18}\text{O}$  record was presented by Wooller *et al.* (2004), although it had a relatively low stratigraphic resolution. A second published chironomid  $\delta^{18}\text{O}$  record from Iceland showed distinct variations in chironomid  $\delta^{18}\text{O}$ , which the authors attributed to changes in seasonality of precipitation and the origin of air masses delivering precipitation to Iceland (Wooller *et al.*, 2008). Although it is clear that  $\delta^{18}\text{O}$  can be measured reliably on fossil chironomid assemblages and the first down-core records provided promising results (Wooller *et al.* 2004; 2008), the method remains to be tested against an independent, well-established  $\delta^{18}\text{O}$  method to demonstrate that fossil chironomid  $\delta^{18}\text{O}$  produces results comparable to  $\delta^{18}\text{O}$  records based on other sediment components.

Here, we present a high-resolution chironomid  $\delta^{18}\text{O}$  record from Rotsee (Switzerland). We analyzed bulk carbonate  $\delta^{18}\text{O}$  in the same sediment samples as chironomid  $\delta^{18}\text{O}$  to directly compare records developed from these two materials. The sediment sequence spans the late glacial and early Holocene, a period with distinct and rapid climatic shifts, which in this region are characterized by high-amplitude variations in  $\delta^{18}\text{O}$  (e.g. Lotter *et al.*, 1992; Schwander *et al.*, 2000). It therefore provides an opportunity to test whether chironomid  $\delta^{18}\text{O}$  tracks the well-documented oxygen isotope excursions in European lake sediment records that have been described for the Oldest Dryas cold period, late glacial interstadial, the Younger Dryas cold phase, and early Holocene warming.

# Materials and methods

## Study site

Rotsee (47°9'N; 8°20'E) is a lake situated near the city of Lucerne on the Swiss Plateau (Fig. 1) at an elevation of 419 m a.s.l. The lake was formed at the end of the last glaciation (Lotter and Zbinden, 1989). At present, it is 2.5 km long and 200 m wide (Lotter *et al.*, 1992). The lake has an average depth of 9 m, whereas its maximum depth is 16 m (Lotter, 1990). Having a surface area of 0.46 km<sup>2</sup>, its volume is 0.0039 km<sup>3</sup> with a catchment area of 4.6 km<sup>2</sup>. Climatic normals indicate that mean July and annual air temperatures are 15.8 and 9°C, respectively, for the period 1961-1990 (data from [www.MeteoSchweiz.ch](http://www.MeteoSchweiz.ch)). The lake sediments of Rotsee have previously been sampled and analyzed for fossil pollen, plant macrofossils, and diatom assemblages (Lotter, 1988, 1989, 1990; Lotter and Zbinden, 1989; Zbinden *et al.*, 1989).



**Figure 1** Location of Rotsee in Switzerland (top). Bathymetric map (bottom) modified after Lotter (1990). Asterisk indicates coring location

## Sediments

On the northeastern part of the lake two complementary cores consisting of littoral sediments (ROT05 I/II) were obtained in 2005 in one meter segments using a modified Livingston piston corer. Cores were taken in the littoral part of the former lake because of the high head capsule concentrations expected for these sediments and because littoral and sub-littoral lake marl deposits are well suited for bulk carbonate  $\delta^{18}\text{O}$  analysis. A slight vertical offset between the two cores ensured that the core segments overlapped partially, providing a continuous sediment sequence covering late glacial and Holocene deposits at Rotsee. Cores were visually correlated using distinct and well-identifiable layers, before being sliced into one-centimeter increments. Subsequently, sediments were freeze-dried. Analyses focused on sediments covering the late glacial from the Oldest Dryas (16,800 cal yrs BP) onwards to the early Holocene (9,400 cal yrs BP).

### Bulk carbonate $\delta^{18}\text{O}$ measurements

Stable oxygen isotopes were measured on bulk carbonates from each sample of the section to obtain a continuous record. Freeze-dried samples were ground to a fine powder using an agate mortar and pestle. Next, stable oxygen isotopic ratios were measured on 500  $\mu\text{g}$  of sample using a VGI mass spectrometer equipped with an isocarb using a dual inlet system. Isotopic data are expressed as per mille relative to V-PDB (Vienna Peedee Belemnite) and reported using the conventional  $\delta$  notation. Calibration was achieved against international standard NBS-19, and an in-house standard (Naxos) with a relative precision of 0.1‰.

### Chironomid $\delta^{18}\text{O}$ measurements

The lake marl deposits used in this study allowed us to obtain a high-resolution bulk carbonate record for the Rotsee sequence. The disadvantage of marls, however, is the high risk of contamination of chironomid head capsules with carbonate particles that may obscure the original chironomid  $\delta^{18}\text{O}$  signature. Initial screening of chironomids isolated from Rotsee sediments under a stereo microscope at 40x magnification indicated that it was difficult to entirely eliminate residual carbonate particles from samples by sieving and exposure in an ultrasonic bath. In addition to conventional methods for chironomid sample preparation we therefore also assessed whether chemical pretreatment to remove carbonates prior to  $\delta^{18}\text{O}$  analysis could improve the agreement between the chironomid and bulk carbonate  $\delta^{18}\text{O}$ . Two different methods to chemically remove carbonates, exposure to HCl and ammonium chloride ( $\text{NH}_4\text{Cl}$ ), were tested on head capsules from fresh larvae of *Chironomus riparius* (Discusfarm Marsilea, Lelystad, the Netherlands). Entire *Chironomus riparius* larvae were soaked in KOH 10% for 2h at 70°C to facilitate head capsule removal and eliminate soft tissue (Verbruggen *et al.*, 2009). The sclerotized head capsules were used in our tests. In a first experiment, two batches of head capsules of *Chironomus riparius* were exposed to demineralized water ( $\delta^{18}\text{O} = -7.6\text{‰}$ ) or water originating from a thawed section of a Greenland ice core ( $\delta^{18}\text{O} =$

-25.7‰). In both cases the water was acidified using hydrochloric acid (HCl) until a pH of 1 was reached. Triplicate samples were drawn from the acid solutions at 1, 2, 6, 25, 50, and 100 h, and subsequently rinsed ten times with MilliQ water prior to  $\delta^{18}\text{O}$  analysis. The aim of this test was to assess whether exposure to acid changed chironomid  $\delta^{18}\text{O}$  by facilitating oxygen exchange between water and chironomid cuticles.

In a second experiment, *Chironomus riparius* head capsules were exposed to a 2M  $\text{NH}_4\text{Cl}$  solution buffered with sodium hydroxide (NaOH) until a pH of 7.5 was reached. Triplicate samples were drawn from the  $\text{NH}_4\text{Cl}$  solution at 1, 2, 4, 8, 26, 50, and 100 h and were then carefully rinsed with MilliQ water before analysis of  $\delta^{18}\text{O}$ . The aim of this test was to assess whether exposure to  $\text{NH}_4\text{Cl}$  led to shifts in the  $\delta^{18}\text{O}$  of chironomid head capsules.

For down-core analysis chironomids were prepared using both conventional palaeoecological pretreatment of sediments for chironomid analysis (exposure to 10% KOH for 2 h) and pretreatment with  $\text{NH}_4\text{Cl}$  as described above. KOH is commonly used in chironomid analysis and does not seem to affect chironomid  $\delta^{18}\text{O}$  at low concentrations and temperatures (Verbruggen *et al.*, 2009). KOH treatment was applied to chironomid samples before sieving over a 200  $\mu\text{m}$  mesh-size sieve (van Hardenbroek *et al.*, 2009). During sieving, samples were treated in an ultrasonic bath with demineralized water for 10-20 seconds in order to eliminate as many fine particles as possible that may have been attached to the chironomid head capsules. Residues were rinsed extensively (10 times) and stored in demineralized water. Then, chironomid head capsules were isolated from the sieve residue under a stereo microscope (magnification 40x) using fine forceps. Head capsules were directly picked into pre-weighed 4- x 3.2-mm silver capsules (Elemental Microanalysis Ltd, batch number 128843) until a minimum weight of  $\sim 50 \mu\text{g}$  was reached (Verbruggen *et al.*, 2009). Fossil chironomids treated with  $\text{NH}_4\text{Cl}$  were soaked in demineralized water for 30 min, sieved, exposed to an ultrasonic bath, and rinsed in a similar way as described above. Chironomids were picked into Eppendorf vials, which were then exposed for exactly 2 h to a buffered 2M  $\text{NH}_4\text{Cl}$  solution (pH=7.5), while placed on a shaker apparatus. Next,  $\text{NH}_4\text{Cl}$ -pretreated head capsules were centrifuged (Thermo Heraeus Megafuge 1.0; 2500 rpm for 5 min) and the supernatant was discarded. After five rinses with MilliQ water the head capsules were picked into pre-weighed silver capsules. All samples were air-dried for several days prior to  $\delta^{18}\text{O}$  analysis.

Stable oxygen isotope ratios of chironomid head capsules were determined with a High Temperature Conversion Elemental Analyzer (Thermo Finnigan) coupled to an Isotope Ratio Mass Spectrometer (TC-EA/IRMS; Thermo Finnigan Delta<sup>plus</sup>), which was calibrated to specifically analyze samples of relatively small mass (50-100  $\mu\text{g}$  range; Kornexl *et al.*, 1999). Two blanks were measured at the start of every run. A cellulose standard (IAEA-C3), benzoic acid standard (HEKAtech, batch number 33822501), and two international potassium nitrate standards (IAEA-NO-3 en USGS-32) allowed  $\delta^{18}\text{O}$  data to be reported in per mille (‰) relative to the V-SMOW (Vienna-Standard Mean Ocean Water; Coplen, 1996) standard.

## Core description and age model

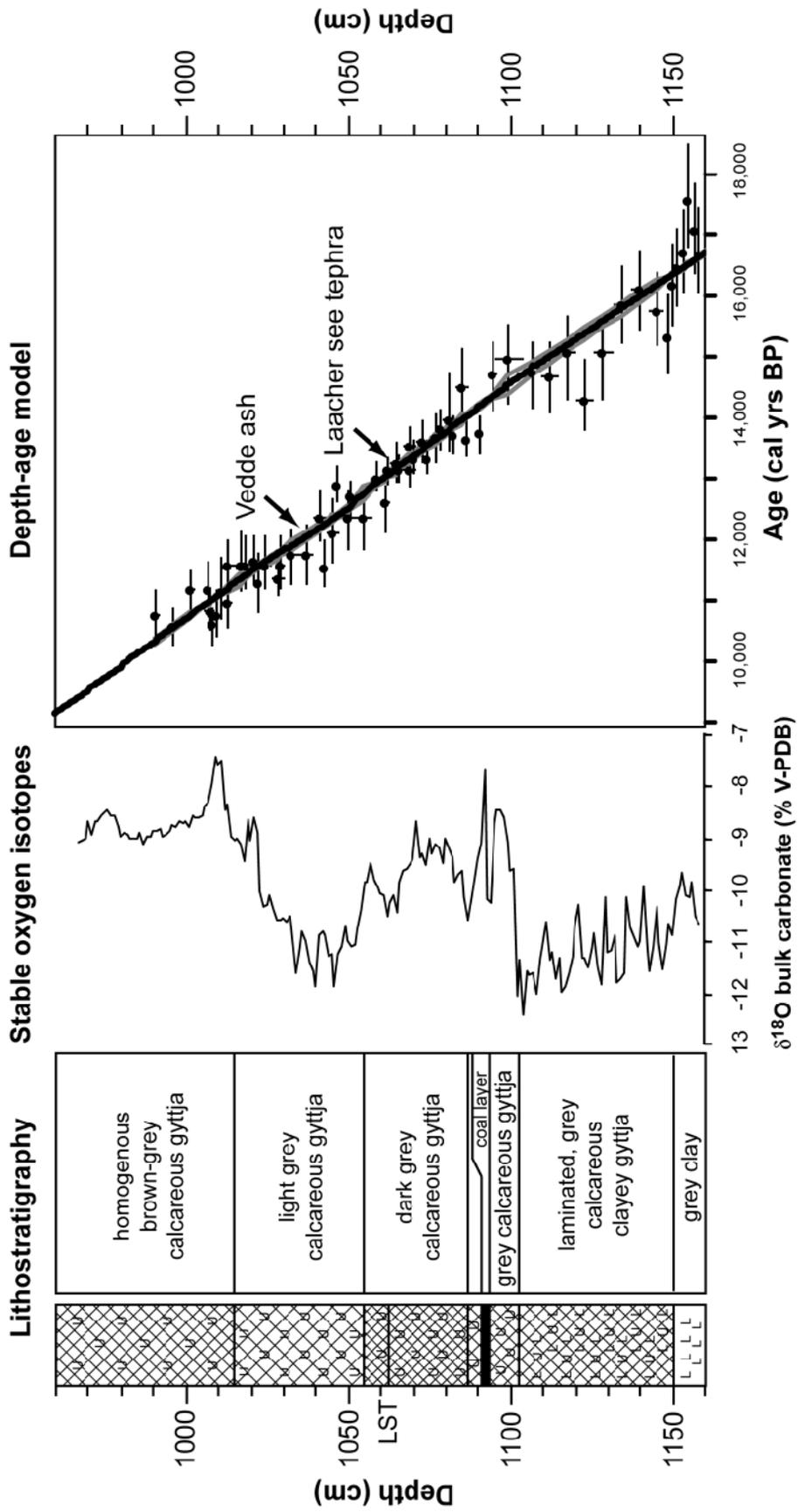
The lithostratigraphy of the Rotsee sediments allowed the ROT05 I/II sediment sequence to be subdivided into different units (Fig. 2). The stratigraphically lowest unit (depth: 1160-1150 cm) consisted of laminated clay. Between 1102 and 1087 cm, sediments consisted of a grey and laminated clayey calcareous gyttja interrupted by a coal-like layer between 1093 and 1091 cm. The next unit (1087-1014 cm) consisted of slightly darker calcareous gyttja, which became lighter at 1054 cm. From 1014 cm upward the sediments were no longer laminated, but changed into a more brownish and homogenous calcareous gyttja with few gradual color alternations.

A previous study of the Rotsee sediments yielded enough terrestrial plant macrofossils to obtain 61 AMS radiocarbon dates (Lotter and Zbinden, 1989; Zbinden *et al.*, 1989). This earlier record was correlated to ROT05 I/II based on bulk carbonate  $\delta^{18}\text{O}$ , as well as shifts in pollen assemblages, the presence of the Laacher See Tephra (LST), and lithological changes as documented in Lotter (1988). A depth-age model was constructed for ROT05 I/II by visually wiggle-matching these  $^{14}\text{C}$  dates to the IntCal04 calibration curve (Reimer *et al.*, 2004; Fig. 2) following Blaauw *et al.* (2003) and dates were expressed in calibrated calendar years before present (cal yrs BP). This depth-age model was further supported by the presence of the LST at a depth of 1062 cm as well as the Vedde ash deposited in the interval spanning 1034-1036 cm. The presence of the latter was verified to occur at the interval between 1034 and 1036 cm (C. Lane and S. Blockley, pers. comm.) using geochemical analyses as described in Blockley *et al.* (2007). The age model is described in detail in Verbruggen *et al.* (in prep b).

## Results and discussion

### Rotsee bulk carbonate $\delta^{18}\text{O}$ record

The stable oxygen isotopes measured on bulk carbonates from Rotsee showed distinct shifts congruent with the ones described from other lake marl records in the Alpine region (Eicher and Siegenthaler 1976; Lotter *et al.*, 1992; Eicher, 1994) and in the Greenland ice core  $\delta^{18}\text{O}$  records (e.g. Johnsen *et al.*, 2001; Rasmussen *et al.*, 2006; Lowe *et al.*, 2008). Stable oxygen isotope ratios in the Rotsee record (Fig. 2) were relatively depleted in sediments older than 14,650 cal yrs BP. During the late glacial interstadial (14,650-12,860 cal yrs BP) carbonate  $\delta^{18}\text{O}$  was enriched. These higher  $\delta^{18}\text{O}$  values were interrupted by negative oscillations during shorter-lived cold climatic oscillations, such as the Aegelsee (14,220-14,060 cal yrs BP) and Gerzensee oscillations (13,260-13,060 cal yrs BP). During the Younger Dryas (12,860-11,610 cal yrs BP) relatively depleted  $\delta^{18}\text{O}$  values were found in the carbonates, whereas they were progressively enriched from the onset of the Holocene onward (<11,610 cal yrs BP).

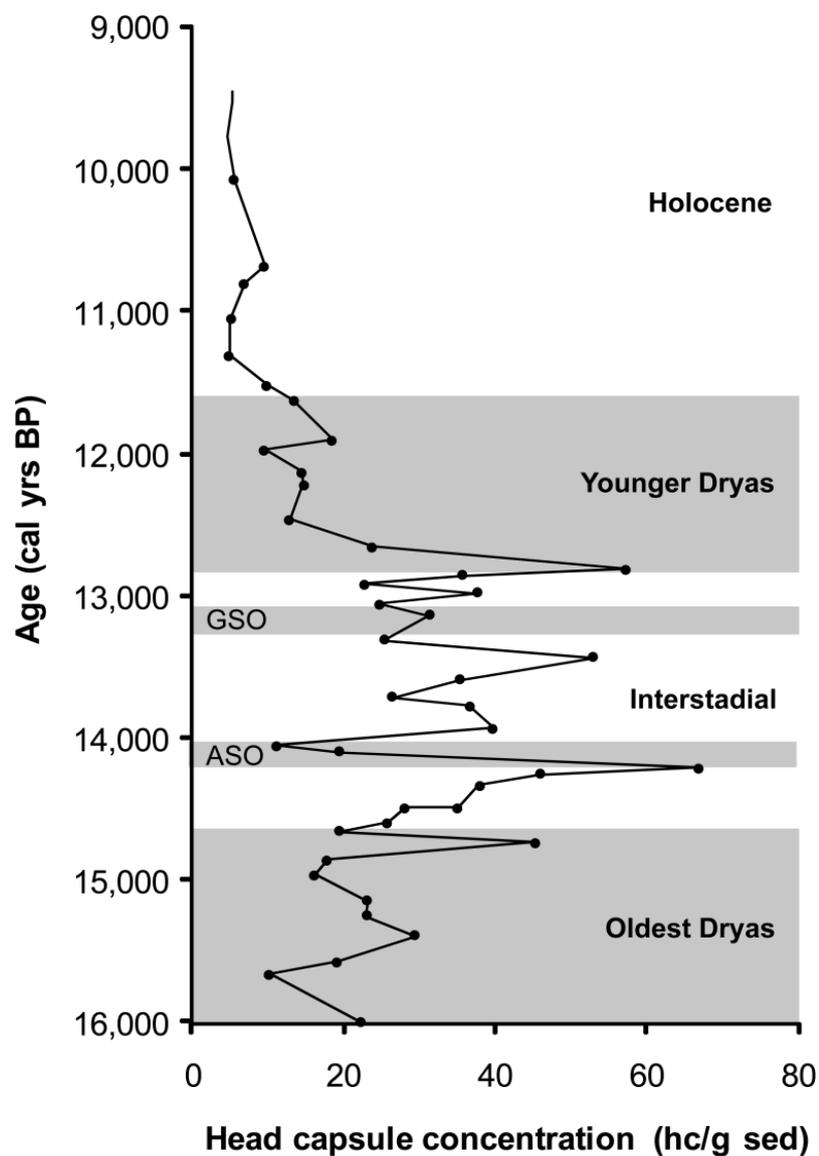


**Figure 2** Lithostratigraphic units of core ROT05 (left), stable oxygen isotope record measured on bulk carbonates (middle) and depth-age model (right) based on both AMS  $^{14}\text{C}$  dates that have been wiggle-matched onto the IntCal04 calibration curve and the presence of the Laacher See Tephra and Vedde ash (indicated by arrows) (for details see Verbruggen *et al.* in prep b)

## Chironomid $\delta^{18}\text{O}$ analysis

### *Chironomid concentrations*

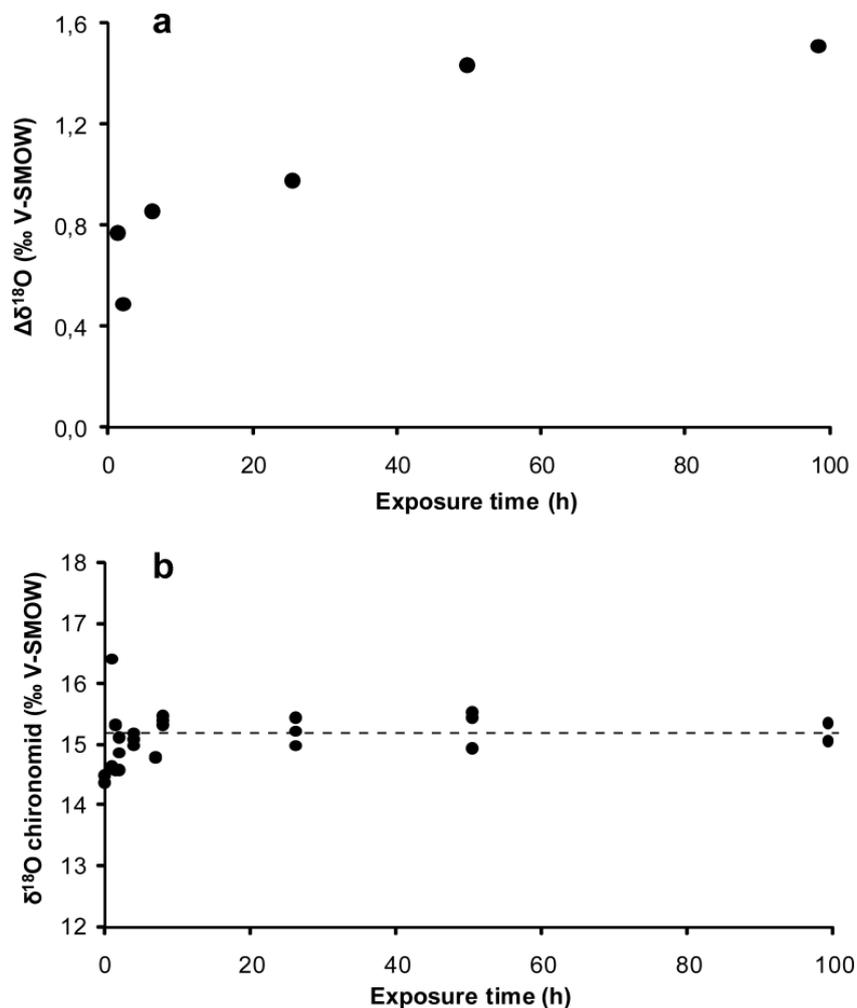
Concentrations of chironomid head capsules  $> 200 \mu\text{m}$  per gram sediment (dry weight) varied between 4 and 67 head capsules (hc)/g in the studied sediments (Fig. 3). In the interstadial sections head capsule concentrations were the highest with some decreases during the Aegelsee and Gerzensee oscillations. From the Younger Dryas onward, head capsule concentrations decreased distinctly and remained low for the rest of the analyzed sequence. For samples older than 12,600 cal yrs BP sediment intervals of 1 or 2 cm were needed to obtain sufficient head capsules for  $\delta^{18}\text{O}$  analysis. For younger periods head capsules were picked from 3 or, rarely, 4 cm intervals.



**Figure 3** Concentration of chironomid head capsules  $> 200 \mu\text{m}$  in Rotsee sediments. Grey areas indicate cold periods. GSO Gerzensee oscillation; ASO Aegelsee oscillation

*Effect HCl and  $\text{NH}_4\text{Cl}$  on chironomid  $\delta^{18}\text{O}$* 

Removing carbonates in palaeoecological studies primarily relies on the use of acids such as HCl (e.g. Walker, 2001; Brooks *et al.*, 2007). However, lowering of the sample pH promotes exchange of oxygen between organic matter and water (Roberts and Urey, 1939; Wedeking and Hayes, 1983). Additionally, acids may induce selective removal of chitin relative to proteins, which may also result in alterations of head capsule  $\delta^{18}\text{O}$  (Verbruggen *et al.*, 2009). In our experiment testing the effects of HCl on chironomid remains we found that the longer chironomid head capsules were exposed to acid solutions of differing  $\delta^{18}\text{O}$ , the more the samples differed in their  $\delta^{18}\text{O}$  values (Fig. 4a). Acid-mediated selective removal of chitin from the chironomid head capsules is expected to have affected  $\delta^{18}\text{O}$  in a similar way in the two batches, which were exposed to acid under identical pH and temperature conditions. The progressively larger offset in  $\delta^{18}\text{O}$  with time therefore indicates that the samples were affected by oxygen exchange with the acidified water used during the experiment.



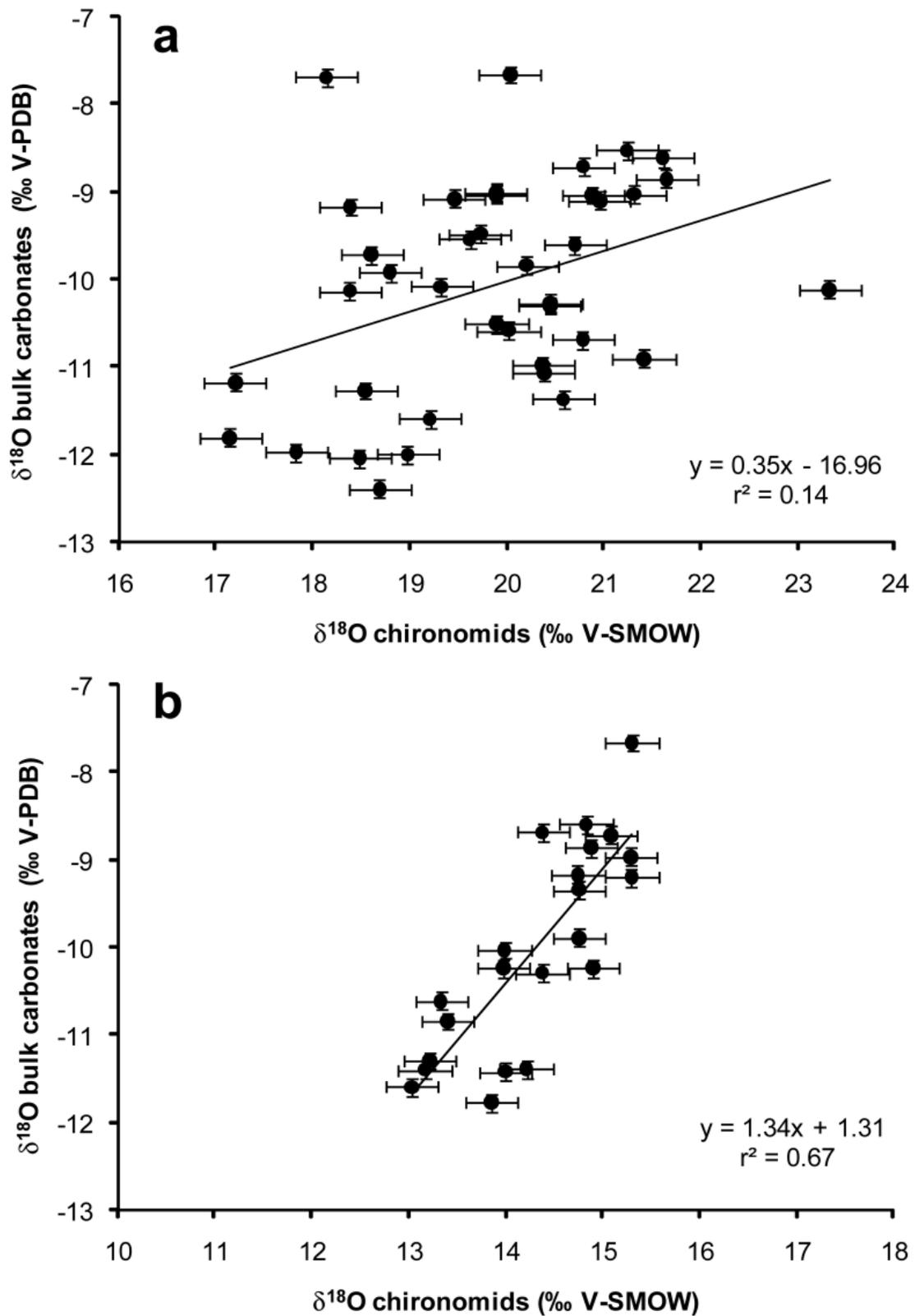
**Figure 4** a) Difference in chironomid  $\delta^{18}\text{O}$  pretreated with isotopically light acidified water ( $\delta^{18}\text{O}=-25.7\text{‰}$ ;  $\text{pH}=1$ ) and heavy acidified water ( $\delta^{18}\text{O}=-7.6\text{‰}$ ;  $\text{pH}=1$ ) plotted against exposure time. b) Effects of prolonged exposure to ammonium chloride ( $\text{NH}_4\text{Cl}$ ) on chironomid  $\delta^{18}\text{O}$ . Dotted line indicates average chironomid  $\delta^{18}\text{O}$  value of all samples

As an alternative to acid,  $\text{NH}_4\text{Cl}$  solutions can be used to eliminate carbonates (Hieltjes and Lijklema, 1980).  $\text{NH}_4\text{Cl}$  reacts with calcium carbonate to form calcium chloride and ammonium carbonate. Since this reaction does not involve acidification, this treatment can be implemented in a buffered environment at circum-neutral pH. The second experiment showed that  $\delta^{18}\text{O}$  measured on chironomid remains exposed to  $\text{NH}_4\text{Cl}$  for up to a few hours did not differ from the  $\delta^{18}\text{O}$  measured on chironomids exposed for several days (Fig. 4b). This suggests that the effect of  $\text{NH}_4\text{Cl}$  on chironomid  $\delta^{18}\text{O}$  is negligible as no long-term trend in chironomid  $\delta^{18}\text{O}$  was observed. Therefore, based on the two experiments,  $\text{NH}_4\text{Cl}$  was chosen to chemically eliminate carbonates from chironomid samples as this treatment seems not to have affected chironomid  $\delta^{18}\text{O}$ .

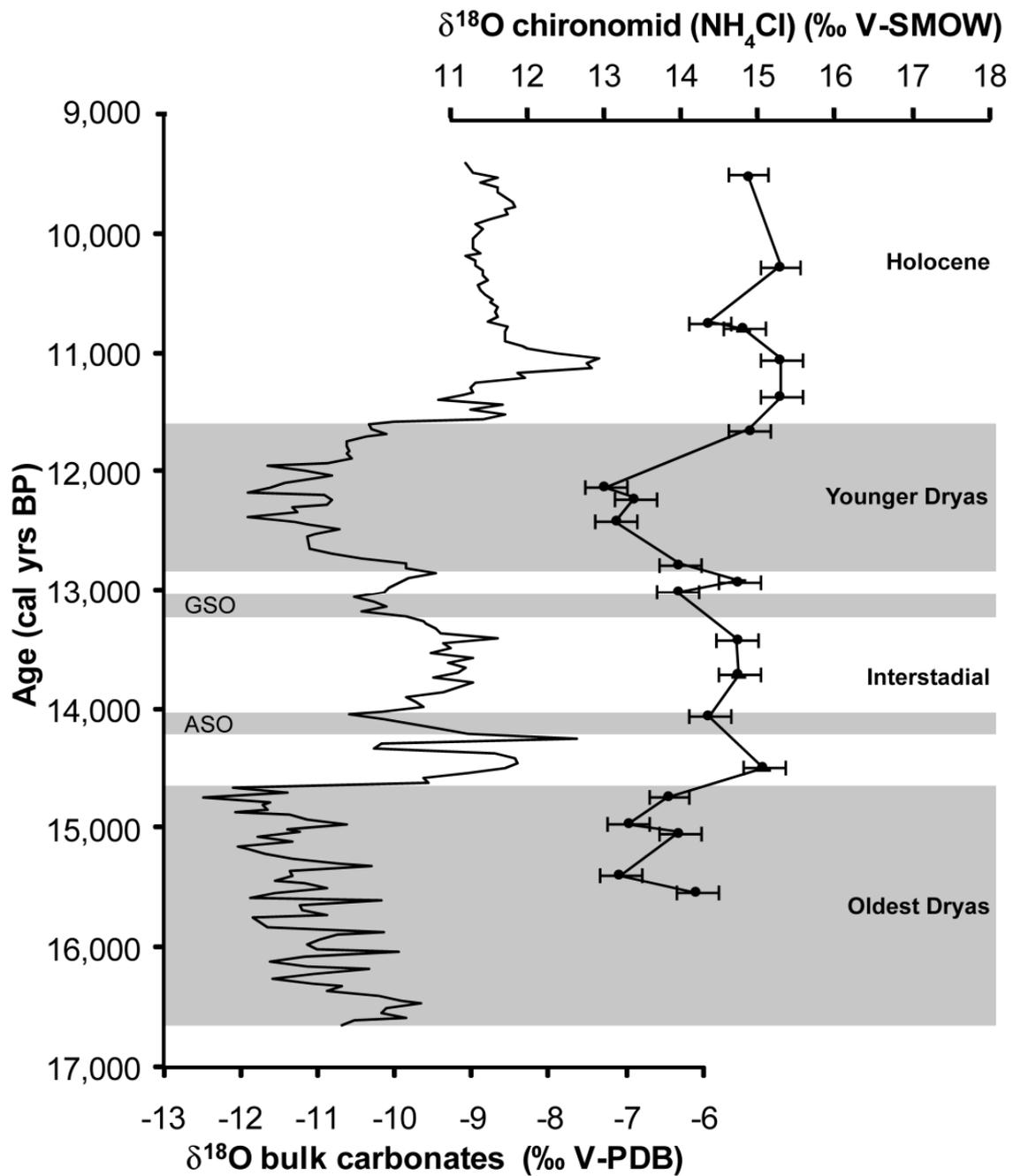
### *Down-core chironomid $\delta^{18}\text{O}$ record*

$\delta^{18}\text{O}$  values obtained from chironomids processed with standard palaeoecological methods correlated poorly with  $\delta^{18}\text{O}$  measured on bulk carbonates from the same sediment depths ( $r^2=0.14$ ;  $P=0.019$ ;  $n=39$ ; Fig. 5a).  $\delta^{18}\text{O}$  values of these chironomid samples were in a similar range as the ones measured on the bulk carbonates. However, large between-sample variability suggested that the  $\delta^{18}\text{O}$  signature of the chironomids was masked by contamination or uncontrolled fractionation during analysis of the samples. Carbonate removal using  $\text{NH}_4\text{Cl}$  resulted in an average weight loss of 28% of the chironomid samples from Rotsee. Furthermore, measured  $\delta^{18}\text{O}$  was on average  $\sim 6\text{‰}$  more negative in the down-core samples pretreated with  $\text{NH}_4\text{Cl}$  than in samples processed with standard palaeoecological methods (Fig. 5). The range of  $\delta^{18}\text{O}$  values in fossil chironomids treated with  $\text{NH}_4\text{Cl}$  (13-15.5‰) was closer to  $\delta^{18}\text{O}$  measured on modern chironomid head capsules used in our study (15.2‰; Fig. 4b) than in measurements without  $\text{NH}_4\text{Cl}$  treatment (17-23.5‰).  $\delta^{18}\text{O}$  obtained from  $\text{NH}_4\text{Cl}$ -pretreated chironomids is strongly correlated with bulk carbonate  $\delta^{18}\text{O}$  measured at the same sampling depths ( $r^2=0.67$ ;  $P<0.001$ ;  $n=22$ ; Fig. 5b). Down-core variations in chironomid  $\delta^{18}\text{O}$  pretreated with  $\text{NH}_4\text{Cl}$  closely followed the ones apparent in the bulk carbonate  $\delta^{18}\text{O}$  record (Fig. 6). During colder periods, such as the Oldest Dryas, the Aegelsee and Gerzensee oscillations, and Younger Dryas (grey areas in Fig. 6) chironomids display more negative  $\delta^{18}\text{O}$  values than in other sections of the record. The amplitude of oscillations in the chironomid and carbonate  $\delta^{18}\text{O}$  records is comparable (3.5 versus 2‰ in carbonates and chironomids, respectively, at the onset of the interstadial, and 2 and 1.5‰, respectively, at the Younger Dryas/Holocene transition), although variations are less pronounced in the chironomid record.

Our down-core chironomid  $\delta^{18}\text{O}$  results suggest that samples processed with standard palaeoecological methods were contaminated by residual carbonates, which affected the  $\delta^{18}\text{O}$  measurements and introduced a large amount of noise into the chironomid  $\delta^{18}\text{O}$  analyses. Samples in which carbonates had been eliminated by exposure to  $\text{NH}_4\text{Cl}$  solution provided a record with more realistic  $\delta^{18}\text{O}$  values, which followed the late glacial variations in  $\delta^{18}\text{O}$  recorded in bulk carbonates. These results indicate that chemical elimination of carbonates is an essential pretreatment step when analyzing  $\delta^{18}\text{O}$  in chironomid remains from lake marl records since carbonate particles can confound the  $\delta^{18}\text{O}$  signature recorded in these fossils.



**Figure 5** Correlation of  $\delta^{18}\text{O}$  values measured in bulk carbonates and chironomids pretreated with standard palaeoecological methods (a) and  $\text{NH}_4\text{Cl}$  (b) from late glacial and early Holocene sediments from Rotsee



**Figure 6** Stable oxygen isotope records of bulk carbonates (left curve) and chironomids pretreated with ammonium chloride (right curve). Grey areas indicate cold periods. GSO Gerzensee Oscillation, ASO Aegelsee Oscillation

## Differences in amplitudes between bulk carbonate and chironomid $\delta^{18}\text{O}$ records

Although the bulk carbonate and chironomid  $\delta^{18}\text{O}$  records are characterized by a similar pattern of variations, distinct differences are apparent in the amplitude of the  $\delta^{18}\text{O}$  changes. Carbonate formation is associated with a temperature-dependent fractionation of oxygen isotopes (e.g. Siegenthaler and Eicher, 1986; von Grafenstein *et al.*, 1999a; Leng and Marshall, 2004), leading to a reduced offset between  $\delta^{18}\text{O}$  in carbonate and lake water with increasing temperature. To date, no information is available about whether temperature affects oxygen isotope fractionation between chironomid head capsules and water. Studies on other aquatic organism groups such as aquatic mosses (DeNiro and Epstein, 1981) and algae (Wolfe *et al.*, 2001) indicate that  $\delta^{18}\text{O}$  of organic structures originating from freshwater organisms are not strongly affected by the temperature of the lake water. This suggests that the formation of the chironomid cuticle may also not be associated with a temperature-dependent fractionation of the stable oxygen isotopes. Lake water temperature has not been constant at our study site during the late glacial period, which was characterized by pronounced variations in summer and annual temperature (e.g. Lotter *et al.*, 1992, 2000; von Grafenstein *et al.*, 1999a; Heiri *et al.*, 2007). Decreasing water depth associated with sediment infilling and successional changes in littoral macrophytes may also have affected water temperatures at our relatively shallow coring site during the late glacial period. Hence, differential effects of temperature on  $\delta^{18}\text{O}$  during formation of chironomid exoskeletons and lake carbonates may explain the difference in the amplitudes of the chironomid and bulk carbonate  $\delta^{18}\text{O}$  records (Fig. 6). A second factor that can potentially explain the difference between the amplitude of variations in bulk carbonate and chironomid  $\delta^{18}\text{O}$  is the part of the seasonal cycle within which the chironomid exoskeletons and the bulk carbonates were formed. Carbonates in lakes are mainly formed during late spring and summer when lake water pH is elevated due to high photosynthetic activity (Wetzel, 2001). Several chironomid species can also complete their development during the spring and summer (e.g. Tokeshi, 1995). However, other littoral chironomid larvae thrive mainly during the period from September to December (Thorp and Bergey, 1981; Beattie, 1982; Drake and Arias, 1995). In lakes in the Alpine region  $\delta^{18}\text{O}$  of lake water may vary by as much as 2‰ over the seasonal cycle (von Grafenstein *et al.*, 2000). Therefore, chironomid and bulk carbonate  $\delta^{18}\text{O}$  may also differ since these sediment components may reflect lake water  $\delta^{18}\text{O}$  during different seasons of the year. A third factor that can explain differences between chironomid and bulk carbonate  $\delta^{18}\text{O}$  are vital effects during formation of the chironomid exoskeleton. Laboratory studies that examine the relationship between  $\delta^{18}\text{O}$  in chironomid head capsules and in water over a range of  $\delta^{18}\text{O}$  values are presently not available. Measurements of  $\delta^{18}\text{O}$  in subfossil chironomid assemblages in lake surface sediments indicate that chironomid  $\delta^{18}\text{O}$  is strongly related to lake water  $\delta^{18}\text{O}$  (Wooller *et al.*, 2004; Verbruggen *et al.*, in prep a). However, this relationship may be modulated to some extent by microhabitat effects and vital offsets that are, for instance, known to affect the  $\delta^{18}\text{O}$  in ostracod valves (von Grafenstein *et al.*, 1999b). Detailed laboratory studies assessing chironomid  $\delta^{18}\text{O}$  in water with different isotopic compositions and field studies of the relationship between chironomid and lake water  $\delta^{18}\text{O}$  during the seasonal cycle will be necessary to determine to which extent chironomid  $\delta^{18}\text{O}$  is affected by autecological and vital effects.

## Potential for reconstruction of past lake water temperature

Reconstructions of past lake water  $\delta^{18}\text{O}$  are now available based on bulk carbonates in lake sediments (e.g. Eicher and Siegenthaler, 1976; Lotter *et al.*, 1992), carbonate fossils of individual indicator groups such as ostracods or molluscs (Leng and Marshall 2004), and aquatic cellulose originating from algae (Wolfe *et al.* 2001) or aquatic mosses (Sauer *et al.* 2001). Together with previous studies (e.g. Wooller *et al.*, 2004, 2008; Verbruggen *et al.*, in prep a), our results indicate that chironomid  $\delta^{18}\text{O}$  can also be used to reliably reconstruct past changes in lake water  $\delta^{18}\text{O}$  provided that care is taken to eliminate carbonate contamination of chironomid samples and if treatments which mask the chironomid  $\delta^{18}\text{O}$  signature (e.g. acids, strong alkali treatments) are avoided. Inorganic materials which have been studied to reconstruct past variations in lake water  $\delta^{18}\text{O}$ , such as carbonates and diatom silica, are affected by a temperature-dependent fractionation of oxygen isotopes during formation of carbonate crystals or diatom valves (Brandriss *et al.*, 1998; Leng and Marshall, 2004). In contrast, fractionation during formation of organic materials produced within lakes is not expected to be affected by temperature. For example, DeNiro and Epstein (1981) studied the oxygen isotope fractionation between water and cellulose produced by aquatic vascular plants and determined that the effect of temperature on this process was small. Wolfe *et al.* (2001) reviewed the available literature describing the fractionation between water and cellulose produced by aquatic plants (macrophytes and algae) and similarly concluded that this process was not strongly affected by temperature. If oxygen isotope fractionation in chironomid chitin is also independent of temperature this would allow chironomid  $\delta^{18}\text{O}$  to be used in combination with  $\delta^{18}\text{O}$  analysis of inorganic autochthonous sediment components such as carbonate to reconstruct water temperature in lakes.  $\delta^{18}\text{O}$  of carbonate would then reflect the past  $\delta^{18}\text{O}$  of lake water modified by the effects of temperature on oxygen isotope fractionation, whereas chironomid  $\delta^{18}\text{O}$  would allow an estimate of past lake water  $\delta^{18}\text{O}$  unaffected by a temperature effect. Since the relationship between lake water  $\delta^{18}\text{O}$ , carbonate  $\delta^{18}\text{O}$ , and temperature is well studied and follows a straightforward relationship (Leng and Marshall, 2004) this would allow the water temperature during formation of lacustrine carbonate to be inferred. In contrast to other organic sediment components such as, e.g., algal cellulose, chironomid cuticles can be identified under a microscope and manually separated from the sediment and can therefore provide lake water  $\delta^{18}\text{O}$  records even in situations where the sediments are affected by significant input of terrestrial organic matter.

## Conclusions

We have shown that an excellent correlation exists between the stable oxygen isotope records measured on chironomid head capsules and on bulk carbonates in late glacial and early Holocene sediments from Rotsee. This indicates that chironomid  $\delta^{18}\text{O}$  provides an alternative to  $\delta^{18}\text{O}$  analyzed on sedimentary carbonates, diatoms, or algal cellulose when reconstructing past changes in lake water  $\delta^{18}\text{O}$  and when correlating late glacial lake sediment records from Europe amongst each other and with the  $\delta^{18}\text{O}$  record of the Greenland ice cores (e.g. Björck *et al.*, 1998; Hoek and Bohncke, 2001). Since chironomid remains are well-preserved in the sediments of lakes on carbonate-poor bedrock and with a high proportion of inorganic material, this approach, together with or as an alternative to  $\delta^{18}\text{O}$  analyzed on biogenic silica or aquatic cellulose, significantly expands the lake sediment records that can be studied for variations in past lake water  $\delta^{18}\text{O}$ . However, our results also indicate that care must be taken to clean chironomid samples from potential sources of contamination such as carbonate particles, especially when analyzing carbonate-rich deposits, such as lake marls. We demonstrated that acid treatment should be avoided since this leads to oxygen exchange between acid solutions and chironomid cuticles. However, buffered  $\text{NH}_4\text{Cl}$  solution performs well in eliminating carbonate contamination in chironomid samples from lake marl records and therefore provides an alternative to  $\text{HCl}$  for dissolving residual carbonates before analysis. The direct use of chironomid  $\delta^{18}\text{O}$  as a climate proxy would benefit from more information about factors influencing the stable isotope signature of lake-dwelling chironomid larvae. Besides establishing whether and to which extent chironomid  $\delta^{18}\text{O}$  is affected by temperature, an important next step will be to further expand the existing datasets documenting the relationship between lake water and chironomid  $\delta^{18}\text{O}$  (Wooller *et al.*, 2004; Verbruggen *et al.*, in prep a), and to study the variability in chironomid  $\delta^{18}\text{O}$  within lake basins, during the seasonal cycle, and between different species.

## Acknowledgements

We thank Ton van Druten and Friederike Wagner-Cremer for help during fieldwork. We acknowledge Arnold van Dijk for stable oxygen isotope measurements. The Institute for Marine and Atmospheric research Utrecht (IMAU) is cordially thanked for providing us with isotopically light water from ice cores for our experiments examining the effects of acid treatment on chironomid  $\delta^{18}\text{O}$ . Simon Blockley and Christine Lane are greatly acknowledged for geochemical analyses of the Vedde ash. This research was financially supported by the Darwin Center for Biogeosciences ([www.darwincenter.nl](http://www.darwincenter.nl)).

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# Algemene introductie, samenvatting en conclusies



# Algemene introductie

Het klimaat verandert snel. Het Intergovernmental Panel on Climate Change, het IPCC, heeft recentelijk een uitgebreid rapport uitgebracht, waarin staat dat de huidige opwarming van de Aarde onmiskenbaar plaatsvindt (IPCC, 2007). In dit rapport worden zowel mogelijke oorzaken, alsmede de grootte en gevolgen van deze klimaatsverandering beschreven. Veel ecosystemen zullen naar alle waarschijnlijkheid beïnvloed worden door veranderende milieuomstandigheden (bijv. Parmesan *et al.*, 1999; Walther *et al.*, 2002). Om een beter inzicht te krijgen in natuurlijke en door de mens veroorzaakte (anthropogene) klimaatvariabiliteit, evenals de mogelijke effecten van milieuverandering op ecosystemen in de toekomst, moeten we klimaatveranderingen en hun effect op de biosfeer in het verleden begrijpen. Om klimaatverandering in het verleden en in de toekomst beter te begrijpen moeten proxies ontwikkeld worden, die de mogelijkheid verschaffen tot klimaatreconstructies op langere tijdschalen. Instrumentale metingen van klimaatsvariabelen reiken namelijk vaak niet tot het begin van de anthropogene klimaatsveranderingen. Daarom moeten natuurlijke archieven bestudeerd worden om milieu's ouder dan ~100 jaar te kunnen reconstrueren. In dit proefschrift wordt de ontwikkeling van zo'n proxy besproken. Met behulp van deze proxy kan klimaatverandering in het verleden gereconstrueerd worden op basis van de stabiele zuurstofisotopensamenstelling van chironomiden, zoals in dit proefschrift gedaan is voor het laat glaciaal en vroeg Holoceen in Europa.

## Chironomiden en klimaat

Klimaatveranderingen worden nauwkeurig opgeslagen in meersedimenten. Het sedimentenarchief van Europese meren dateert vaak terug tot het einde van de laatste ijstijd, ongeveer 16,000 jaar geleden. De fossiele overblijfselen van verschillende aquatische organismen zijn de afgelopen decennia gebruikt om milieucondities in meren te reconstrueren (bijv. Smol, 2008). Chironomiden, of dansmuggen, zijn een voorbeeld van een dergelijke groep organismen die de laatste jaren veel aandacht heeft gekregen in de palaeolimnologie (Brooks en Birks, 2001; Brooks, 2006). Adulte dansmuggen leven in de lucht, alwaar ze zich voortplanten. De vrouwtjes leggen hun eitjes in water, waaruit larven komen. Dansmuglarven doorlopen vier larvenstadia voordat ze verpoppen en uitkomen als adulten. Chironomidenlarven komen op veel plekken op Aarde voor en zijn veelvoorkomend in zoetwatermeren. Hun kopkapsels blijven goed bewaard in sedimenten en zijn goed te identificeren. Veel chironomidentaxa tolereren slechts kleine wijzigingen in milieuvariabelen (stenotopisch) en reageren snel op milieuveranderingen doordat ze een snelle generatietijd en grote verspreidingsnelheid hebben (bijv. Brooks *et al.*, 2007).

Veel studies laten een empirisch verband zien tussen het voorkomen van bepaalde chironomiden en milieuomstandigheden. Zulke moderne 'training sets' zijn beschikbaar voor vele regio's, waaronder IJsland (Langdon *et al.*, 2008; Holmes *et al.*, 2009), Finland (Olander *et al.*, 1999; Luoto, 2009a), Zweden (Larocque *et al.*, 2001), het Verenigd Koninkrijk (Brooks *et al.*, 2001), Zwitserland (Lotter *et al.*, 1997, 1998; Heiri *et al.*, 2003; Bigler *et al.*, 2006),

Afrika (Eggermont *et al.* 2009), het Tibetaans Plateau (Zhang *et al.*, 2007), Nieuw Zeeland (Rees *et al.*, 2008), California (Porinchu *et al.*, 2002), Alaska (Kurek *et al.*, 2009), Beringia (Barley *et al.*, 2006) en Canada (Walker *et al.*, 1991; Quinlan *et al.*, 1998; Larocque *et al.*, 2006; Porinchu *et al.*, 2009). In elke regio wordt de distributie van chironomidentaxa beïnvloed door een reeks milieufactoren. Hoewel het effect van individuele variabelen kan verschillen per regio, wordt temperatuur over het algemeen beschouwd als de belangrijkste parameter die, zeker op grote geografische schalen, de chironomiden soortendistributie beïnvloedt (Brooks, 2006). Dit temperatuureffect kan direct zijn, door het veranderen van de watertemperatuur, of indirect zijn, via veranderingen in de zuurstof- en nutriëntenconcentratie in het hypolimnion. Hoeveelheden organisch materiaal in het sediment, opgelost organisch koolstof, pH en conductiviteit worden meestal gedreven door veranderingen in het stroomgebied van het meer en kunnen chironomidistributies op een lokaler niveau beïnvloeden (Pinder, 1995).

Zodra goed onderbouwde relaties tussen chironomiden en hun milieu zijn achterhaald en ge-evalueerd, kan deze informatie worden gebruikt om ecologisch relevante variabelen te reconstrueren met behulp van fossiele chironomidenassociaties, die bewaard zijn gebleven in meersedimenten. Dit wordt gedaan met zogenaamde 'transfer functions'. Inmiddels zijn er met behulp van fossiele chironomiden kwantitatieve reconstructies gemaakt van zomertemperatuur van meerwater en lucht (bijv. Heiri en Lotter, 2005; Heiri *et al.*, 2005; Heiri *et al.*, 2007), van trofische status en zuurstofniveau's (Brodersen en Quinlan, 2006), saliniteit (Eggermont *et al.*, 2009), zuurtegraad van het water (Brodin en Gransberg, 1993), chlorofyl  $\alpha$  concentraties (Brodersen en Lindegaard, 1999), hoeveelheid organisch materiaal in het sediment (Nyman *et al.*, 2005), en waterdiepte (Korhola *et al.*, 2000; Luoto, 2009b). Op chironomiden gebaseerde temperatuurreconstructies in het bijzonder werken goed in vele gebieden (Lotter *et al.*, 1999; Brooks, 2006; Walker en Cwynar, 2006).

## Stabiele zuurstofisotopen en klimaat

De temperatuur in Europa heeft sterk gefluctueerd sinds het Laatste Glaciale Maximum (~20,000 gecalibreerde jaren geleden). Vanaf deze periode hebben relatief koude perioden (stadialen) en relatief warme perioden (interstadialen) elkaar afgewisseld. Dit proefschrift focust op de periode van 17,000 tot 9,000 jaar geleden, die gekenmerkt wordt door twee stadialen en één interstadiaal. De laatste bevat op zijn beurt weer uit twee kleinschalige perioden van afkoeling. De opwarmingen en afkoelingen die deze periode kenmerken zijn afgeleid uit schommelingen in de zuurstofisotopencurve van de goed gedateerde ijskernen uit Groenland (Johnsen *et al.*, 2001). Dezelfde schommelingen zijn waargenomen in stabiele zuurstofisotopencurven gemeten aan meersedimenten uit het Europese continent (bijv. Eicher en Siegenthaler, 1976; Lotter *et al.*, 1992). Daardoor kunnen deze curven worden gebruikt om continentale archieven met elkaar te correleren.

De stabiele zuurstofisotopensamenstelling ( $\delta^{18}\text{O}$ ) van natuurlijke continentale archieven zoals ijskernen, speleothems en meersedimenten is veelal afhankelijk van de stabiele zuurstofisotopensamenstelling van neerslag, wat de belangrijkste bron van zuurstof is voor deze archieven. De ratio van zware ( $^{18}\text{O}$ ) en lichte ( $^{16}\text{O}$ ) zuurstofisotopen in neerslag wordt uitgedrukt in permil (‰) en is relatief ten opzichte van een standaard (bijv. Vienna-

Standard Mean Ocean Water, V-SMOW; Coplen, 1996). De  $^{18}\text{O}/^{16}\text{O}$  ratio wordt beïnvloed door verschillende factoren. Door neerslag regelmatig te onderzoeken op stabiele zuurstofisotopen, zoals gedaan wordt door de IAEA/WMO sinds de jaren '60 (IAEA/WMO, 2006) is aangetoond dat temperatuur de belangrijkste factor is die de  $\delta^{18}\text{O}$  van neerslag op middelhoge en hoge breedtegraden bepaalt (Dansgaard, 1964; Siegenthaler en Oeschger, 1980; Rozanski *et al.*, 1993). Omdat luchtmassa's afkoelen tijdens hun weg van lage breedtegraden naar de polen neemt de concentratie  $^{18}\text{O}$  sterk af doordat dit zware isotoop het eerst condenseert, waardoor er relatief meer van het lichte isotoop in de damp van de luchtmassa's achterblijft (Gat, 1996). Neerslag  $\delta^{18}\text{O}$  wordt ook beïnvloed door andere factoren, zoals het 'kwantiteitseffect' (plaatsen met meer neerslag zullen een lichter neerslag  $\delta^{18}\text{O}$  signaal hebben, zie Dansgaard, 1964), het 'hoogte-effect' (Poage en Chamberlain, 2001; Siegenthaler en Oeschger, 1980), en het 'continentale effect' (of afstand tot de kust, zie Alley en Cuffey, 2001). Hoewel het grootste gedeelte van het water in neerslag afkomstig is van oceaانverdamping op lage tot middelhoge breedtegraden (Rozanski *et al.*, 1993), kan ook de bron van water in deze luchtmassa's en de weg die deze luchtmassa's afleggen over de continenten de  $\delta^{18}\text{O}$  in neerslag beïnvloeden (Alley en Cuffey, 2001). Echter, in het algemeen zal neerslag  $\delta^{18}\text{O}$  afnemen naarmate gebieden kouder zijn, verder verwijderd zijn van de kust of op grotere hoogtes liggen (Fricke en O'Neil, 1999).

## Chironomiden en stabiele zuurstofisotopen

Neerslag is de belangrijkste bron van stabiele zuurstofisotopen in meerwater. Meerwater  $\delta^{18}\text{O}$  is daarom afhankelijk van neerslag  $\delta^{18}\text{O}$  (Gonfiantini, 1986), maar kan veranderen door veranderingen in lokale hydrologie. Zo zal verdamping bijvoorbeeld een verzwaring van meerwater  $\delta^{18}\text{O}$  teweegbrengen, omdat de lichte isotoop ( $^{16}\text{O}$ ) bij voorkeur verdampt (Gonfiantini, 1986) en zo zal de input van isotopisch licht ijs en sneeuwsmeltwater meerwater  $\delta^{18}\text{O}$  verlichten (Bottomley *et al.*, 1986).

Zuurstof wordt ingebouwd in de endo- en exoskeletten van aquatische organismen. De isotopensamenstelling van deze structuren is afhankelijk van de stabiele zuurstofisotopensamenstelling van het meerwater (Wang *et al.*, 2009). Delen van het exoskelet van enkele aquatische organismen blijven goed bewaard als fossielen in meersedimenten en kunnen derhalve gebruikt worden om meerwater  $\delta^{18}\text{O}$ , en daarmee klimaatverandering in het verleden te reconstrueren. Overblijfselen van verschillende groepen organismen, waaronder ostracoden (mosselkreeftjes), mollusken (weekdieren; von Grafenstein *et al.*, 1999; Schwalb, 2003) en diatomeeën (Barker *et al.*, 2001; Leng en Barker, 2006) zijn in het verleden gebruikt om  $\delta^{18}\text{O}$  curves te verkrijgen, naast de vaak toegepaste  $\delta^{18}\text{O}$  methode, namelijk aan carbonaten in meersedimenten. Echter, overblijfselen van ostracoden zijn niet altijd aanwezig in meersedimenten en een uitgebreide sedimentzuivering is onontbeerlijk om een op diatomeeën gebaseerde  $\delta^{18}\text{O}$  curve te verkrijgen, aangezien kleine hoeveelheden verontreiniging een groot effect kunnen hebben op de  $\delta^{18}\text{O}$  waarden (Leng en Barker, 2006). Naast bovengenoemde organismen kan ook aquatische cellulose gebruikt worden om meerwater  $\delta^{18}\text{O}$  te reconstrueren (Wolfe *et al.*, 2007). De interpretatie van sedimentaire cellulose wordt echter bemoeilijkt door de mogelijke aanwezigheid van terrestrische cellulose (Sauer *et al.*, 2001).

Chitine (Poly-*N*-acetyl-D-glucosamine) is qua chemische structuur vergelijkbaar met cellulose. Ook chitine blijft goed bewaard in sedimenten onder geschikte omstandigheden (Muzzarelli, 1977). Omdat chironomidenkopkapsels deels van chitine zijn gemaakt, hebben ze de potentie om gebruikt te worden om  $\delta^{18}\text{O}$  curves te genereren. Dit was voor het eerst bestudeerd door Wooller *et al.* (2004, 2008). Zij vonden een relatie tussen de  $\delta^{18}\text{O}$  van neerslag en de  $\delta^{18}\text{O}$  van overblijfselen van chironomiden. Wang *et al.* (2008) hebben een protocol opgesteld waarin de monsteropwerking, -opslag en  $\delta^{18}\text{O}$  meting van fossiele chironomiden beschreven staat. Bovendien lieten Wang *et al.* (2009) zien met een cultuurexperiment dat bijna 70% van de zuurstof in kopkapsels van chironomiden van het water waarin de muggenlarven leefden afkomstig was en dat de stabiele zuurstofisotopensamenstelling van chironomiden daardoor voor het grootste deel afhankelijk is van de  $\delta^{18}\text{O}$  van het water waarin ze leven. Echter, een methodologie wat betreft chironomiden  $\delta^{18}\text{O}$  analyse is nog steeds in ontwikkeling. Veel aspecten met betrekking tot het gebruik en de interpretatie van deze nieuwe klimaatsproxy moeten nog ontwikkeld en geëvalueerd worden.

## Onderzoeksdoel

Het belangrijkste doel van dit proefschrift was het verder ontwikkelen van het gebruik van stabiele zuurstofisotopen in chironomiden als een proxy voor meerwater  $\delta^{18}\text{O}$ , en daarmee, voor klimaatverandering in het verleden. Aangezien chironomiden eerder al zijn gebruikt om temperatuurverandering in het verleden te achterhalen met behulp van hedendaagse soort-milieu relaties, zou dit de mogelijkheid bieden om fossiele overblijfselen van één organisme te gebruiken voor het maken van twee, onafhankelijke klimaatreconstructies. De eerste reconstructie is gebaseerd op de identificatie van chironomidenfossielen in meersedimenten en de interpretatie van verschuivingen in de soortssamenstelling van chironomiden. De tweede is gebaseerd op het  $\delta^{18}\text{O}$  signaal van meerwater, wat opgeslagen is in fossiele overblijfselen van chironomiden. Als chironomiden met succes gebruikt kunnen worden om  $\delta^{18}\text{O}$  curves te genereren, dan zou het mogelijk worden om  $\delta^{18}\text{O}$  curves te verkrijgen van meren waar dat niet mogelijk is op basis van andere organismen, zoals in meren op een zure ondergrond, waar dikwijls kalkskeletjes van ostracoden of weekdieren ontbreken, of in meren waar een grote hoeveelheid terrestrische input het verkrijgen van betrouwbare diatomee- of cellulose  $\delta^{18}\text{O}$  curves onmogelijk maakt.

## Samenvatting

De eerste stap om dit doel te realiseren, is te achterhalen wat het effect is van verschillende chemicaliën, die vaak gebruikt worden in de palaeoecologie, op de  $\delta^{18}\text{O}$  waarde, de chemische samenstelling en morfologie van chironomidenkopkapsels. Ten tweede is een  $\delta^{18}\text{O}$  analyse gedaan aan subfossiele kopkapsels en ephippia van watervlooien (Cladocera)

die geïsoleerd zijn uit oppervlakesedimenten van dertig diepe en gestratificeerde meren in een transect dat zich uitstrekt van noord Zweden tot zuid Italië. De resultaten van deze analyse laten zien dat chironomiden  $\delta^{18}\text{O}$  sterk en positief gerelateerd is met  $\delta^{18}\text{O}$  in meerwater en neerslag. Als derde stap zijn stabiele zuurstofisotopen gemeten aan fossiele chironomiden, afkomstig uit laat glaciële en vroeg Holocene sedimenten van Rotsee in Zwitserland. De chironomiden  $\delta^{18}\text{O}$  curve is rechtstreeks vergeleken met de  $\delta^{18}\text{O}$  curve van bulk carbonaten gemeten aan dezelfde sedimenten om te zien of chironomiden de variatie in meerwater  $\delta^{18}\text{O}$  op eeuw- tot millenia-schaal konden volgen. Microscopisch kleine carbonaatdeeltjes bleven echter aan de kopkapsels hangen, waardoor het originele  $\delta^{18}\text{O}$  signaal in chironomiden gemaskeerd werd, wat op zijn beurt leidde tot een slechte correlatie tussen de  $\delta^{18}\text{O}$  curves gemeten aan chironomiden en aan carbonaten. Om die reden zijn de vervuilende carbonaten chemisch verwijderd in een pH neutraal milieu. Uiteindelijk is met behulp van chironomiden een betrouwbare laat glaciële en vroeg Holocene  $\delta^{18}\text{O}$  curve gegenereerd die in overeenstemming was met de curve gemeten aan de carbonaten. Hieruit is geconcludeerd dat chironomiden gebruikt konden worden om meerwater  $\delta^{18}\text{O}$  en dus klimaatvariabiliteit te reconstrueren. Daarnaast bood een chironomidenanalyse van oppervlakesedimenten de mogelijkheid om de relatie te bestuderen tussen het voorkomen van chironomidentaxa en hun omgeving op een continentale schaal, in dit geval in grote, diepe en gestratificeerde meren in Europa. Factoren die bestudeerd zijn, zijn onder andere temperatuur, nutriëntenconcentratie en zuurstofbeschikbaarheid. Tegelijkertijd bood het onderzochte sedimentenarchief van Rotsee de mogelijkheid tot het testen van verschillende hypothesen omtrent de drijfveren in en om Rotsee die het voorkomen van laat glaciële en vroeg Holocene chironomidentaxa beïnvloeden.

## Synopsis

Voorafgaand aan dit proefschrift was er slecht beperkte informatie beschikbaar over de effecten van chemische opwerkmethoden op de stabiele zuurstofisotopensamenstelling van kopkapsels van chironomiden. **Hoofdstuk 1** laat zien dat chemische behandeling met zuren, basen en natriumchloriet een effect heeft op de chemische samenstelling van exoskeletten van chironomiden, zoals bepaald door middel van Pyrolyse-gas chromatografie/massa spectrometrie (Py-GC/MS), op de stabiele zuurstofisotopensamenstelling van overblijfselen van chironomiden, zoals gemeten met High-Temperature Conversion-Elemental Analyzer/Isotopen Ratio Massa Spectrometrie (TC-EA/IRMS) en de morfologie van kopkapsels, zoals vastgelegd door middel van Scanning Electron Microscopie (SEM). In de gas chromatogrammen van pyrolyzaten is te zien dat hete basische oplossingen en natriumchloriet selectief eiwitten verwijderen uit kopkapsels, wat leidt tot relatief zwaardere  $\delta^{18}\text{O}$  waarden van chironomiden. De SEM foto's toonden aan dat lagen met chitinevezels uitstaken bij kopkapsels die behandeld waren met basische chemicaliën. Een chemische opwerking met zuren daarentegen, leidde tot relatief lichtere  $\delta^{18}\text{O}$  waarden bij chironomiden. Dit wordt gewijkt aan een uitwisseling van zuurstofisotopen tussen het water waarin het zuur is opgelost en de kopkapsels. Naast deze door zuren veroorzaakte uitwisseling, kan de-N-acetylatie van chitine tot chitosan, wat oplosbaar is in water, een rol hebben gespeeld bij de

verlaging van  $\delta^{18}\text{O}$  waarden van chironomiden. De selectieve verwijdering van chitine door zuren was ook evident in het bijbehorende gas chromatogram, waarin het grootste deel van de componenten van de kopkapsels bestond uit componenten die afkomstig waren van eiwitten. Een eiwitmatrix zonder uitstekende chitinevezels is dan ook te zien in de SEM foto van de kopkapsels opgewerkt met zuren. Gebaseerd op deze resultaten wordt het gebruik van sterke basen en zuren voor chironomiden  $\delta^{18}\text{O}$  analyse ten zeerste afgeraden.

Om empirische relaties tussen chironomidenassociaties en hun milieu te achterhalen worden voor verschillende gebieden in Europe zogenaamde moderne 'training sets' gebruikt. In veel van deze datasets wordt temperatuur gezien als belangrijkste variabele die het beste verschillen in chironomidistributies kan verklaren. In deze studies worden diepe en gestratificeerde meren echter vaak niet behandeld. Bovendien zijn de meeste calibratie sets gefocust op relatief kleine geografische gebieden en hebben ze geen continentale transecten omvat. In **Hoofdstuk 2** worden profundale chironomidenassociaties in oppervlakesedimenten van diepe meren in een transect van noord Zweden tot zuid Italië bestudeerd en gerelateerd met luchttemperatuur in Juli, zomertemperatuur van oppervlaktewater, totale fosforconcentratie, hypolimnetische zuurstofconcentratie, conductiviteit, pH, meeroppervlakte, maximale diepte, Secchi diepte, stroomgebiedoppervlakte, breedtegraad en hoeveelheid organisch materiaal in het sediment. Canonische correspondentieanalyses (CCA) tonen dat chironomidenassociaties significant gerelateerd zijn met breedtegraad, temperatuur, nutriënten- en zuurstofconcentraties in diepe meren. Deze variabelen covariëren met elkaar in deze dataset en zijn positief of negatief gecorreleerd met de eerste as van een detrended correspondentieanalyse (DCA) van de chironomidendata. Dit suggereert dat profundale chironomiden direct (via voedsel en zuurstof) en indirect (via temperatuur) beïnvloed worden door omgevingsfactoren. Ondanks de covariatie is er een significante relatie tussen temperatuur en het voorkomen van chironomidenassociaties in deze dataset, zelfs wanneer de effecten van nutriënten- en zuurstofbeschikbaarheid statistisch verwijderd zijn. Dit suggereert op zijn beurt dat indirecte effecten via trofische status niet de enige manier zijn waarop temperatuur chironomidenpopulaties beïnvloedt in diepe, gestratificeerde meren. Omdat trofische status een significant deel van de variantie in de chironomiden dataset verklaart, is een reconstructie van totale fosforconcentraties in het verleden gemaakt met behulp van de zogenaamde "Benthische Kwaliteits Index" (BQI). De BQI maakt gebruik van scores voor bepaalde gevoelige chironomidetaxa. BQI waarden berekend voor de bestudeerde meren zijn lineair gecorreleerd met log-getransformeerde totale fosforwaarden. Wanneer deze relatie op fossiele chironomidenassociaties van Päijänne, een diep meer in Finland, wordt toegepast, is een totale fosforreconstructie gegenereerd die goed overeenstemt met totale fosforwaarden die instrumentaal gemeten zijn in het meer voor de periode 1970-1990. Dit toont aan dat de BQI het potentieel heeft om kwantitatieve reconstructies te verschaffen die een inzicht geven in de trofische status van diepe, gestratificeerde meren.

Naast het bestuderen van relaties tussen subfossiele chironomiden uit diepe meren en hun milieu, zoals besproken in Hoofdstuk 2, is een studie gedaan naar de relatie tussen de stabiele zuurstofisotopensamenstelling van overblijfselen van aquatische ongewervelden en van het water waarin ze leefden. Het is noodzakelijk deze hedendaagse relaties tussen  $\delta^{18}\text{O}$

waarden van water en organismen te begrijpen, om klimaatreconstructies van het verleden op basis van fossiele organismen op de juiste manier te kunnen interpreteren. In **Hoofdstuk 3** wordt een verband gelegd tussen aan de ene kant neerslag en meerwater  $\delta^{18}\text{O}$  en aan de andere kant de  $\delta^{18}\text{O}$  van kopkapsels van chironomiden en ehippia van watervlooien in oppervlaktensedimenten van de bestudeerde diepe meren. Een duidelijke en statistisch significante relatie is te zien tussen neerslag  $\delta^{18}\text{O}$  en luchttemperatuur gemiddeld over het jaar. Neerslag  $\delta^{18}\text{O}$  is ook positief gecorreleerd met meerwater  $\delta^{18}\text{O}$ , hoewel deze relatie minder sterk is. De  $\delta^{18}\text{O}$  waarden van overblijfselen van chironomiden en watervlooien is positief en sterk gecorreleerd met meerwater  $\delta^{18}\text{O}$  en minder sterk met luchttemperatuur gemiddeld over het jaar. Hoewel de helling van de regressielijnen tussen  $\delta^{18}\text{O}$  van aquatische ongewervelden en meerwater vergelijkbaar waren voor zowel chironomiden als watervlooien, is er een systematische offset te zien tussen de  $\delta^{18}\text{O}$  waarden van deze twee organismen. Dit wordt gewijt aan verschillen in ecologie, stofwisseling en/of gedrag tussen chironomiden en watervlooien. Deze studie laat zien dat een betrouwbare  $\delta^{18}\text{O}$  analyse uitgevoerd kan worden aan zowel kopkapsels van chironomiden als ehippia van watervlooien en dat de  $\delta^{18}\text{O}$  waarden van deze organismen direct gerelateerd zijn met meerwater  $\delta^{18}\text{O}$ . Zuurstofisotopencurven die gemeten zijn aan chironomiden of watervlooien kunnen daarom gebruikt worden om meerwater  $\delta^{18}\text{O}$ , en daarmee temperatuur te reconstrueren in het verleden.

De volgende twee hoofdstukken spitsen zich toe op laat glaciale en vroeg Holocene meersedimenten (17,000-9,000 gecalibreerde jaren geleden). In **Hoofdstuk 4** zijn de resultaten te zien van een multi-proxy analyse van onder andere chironomiden, pollen en plant macrofossielen, aangevuld met analyses van stabiele zuurstofisotopen aan bulk carbonaten en van de hoeveelheid organisch materiaal en carbonaten in littorale sedimenten van Rotsee in Zwitserland. Het ouderdomsmodel is gebaseerd op 'wiggles-match daten' van koolstofdateringen op hoge resolutie en op tephrochronologie. Deze studie had als doel om 'leads en lags' (het relatief vroeg (lead), danwel laat (lag) voorkomen) te bestuderen wat betreft reacties van diverse biota op klimaatsveranderingen in het laat glaciaal. Tevens is het belang en gewicht van het effect van verschillende externe en interne processen in en om het meer achterhaald die mogelijk veranderingen in littorale chironomidenpopulaties kunnen verklaren. De timing en grootte van veranderingen in de soortensamenstelling van de verschillende organismen is bepaald met behulp van DCA's. Veranderingen in associaties van verschillende biota vonden gelijktijdig plaats met grote veranderingen in de  $\delta^{18}\text{O}$  curve tijdens de overgang naar het interstadiaal en naar de Jonge Dryas perioden. Dit suggereert dat klimaatverandering gemeenschappen van organismen heeft beïnvloed. Chironomiden die indicatief zijn voor koude periodes waren echter niet veelvoorkomend in het littoraal van Rotsee tijdens de Jonge Dryas en het vroeg Holoceen. Waarschijnlijk is dit het gevolg van een waterniveaudaling, zoals de macrofyten laten zien. Tenslotte zijn de effecten van klimaat, en stroomgebied- en meer-gerelateerde processen op de littorale chironomidenassociaties onderzocht met (partiële)CCA's waarin één of meerdere onafhankelijke proxies als weerspiegeling van deze processen gebruikt zijn. In volgorde van verklaringskracht zijn stroomgebied- en meerprocessen en klimaat statistisch significant in het verklaren van variatie in de chironomidendata, zelfs wanneer de effecten van langdurige trends statistisch verwijderd zijn. Door de boorlocatie in het ondiepe, littorale deel van Rotsee en de daarmee

samenhangende invloed van schommelingen in waterniveau lijken de interne dynamiek van het meer en stroomgebied-gerelateerde processen belangrijke rollen te spelen die veranderingen in het laat glaciaal aquatische ecosysteem van Rotsee teweegbrengen. Dit is waarschijnlijk de reden waarom zowel stroomgebied-gerelateerde als interne processen in het meer een groter deel van de variantie in de littorale chironomidenassociaties verklaren dan klimaat in deze periode.

Tenslotte is in **Hoofdstuk 5** de toepasbaarheid van  $\delta^{18}\text{O}$  analyse op fossiele chironomiden getest op sedimenten afgezet in Rotsee tijdens het laat glaciaal en vroeg Holoceen. Dit is een periode waarin duidelijke klimaatschommelingen goed gedocumenteerd zijn in de literatuur. Door hoge concentratie kopkapsels in deze littorale sedimenten is voldoende materiaal beschikbaar voor  $\delta^{18}\text{O}$  analyse. Het doel van dit onderzoek is een vergelijking te maken tussen  $\delta^{18}\text{O}$  waarden van carbonaten en van kopkapsels van chironomiden. Voorafgaand aan de  $\delta^{18}\text{O}$  analyse zijn de fossiele chironomiden blootgesteld aan een standaard palaeoecologische opwerkmethode (behandeling met een zwakke KOH oplossing, zeven, ultrasoon bad). De aanwezigheid van kalk in de sedimenten van Rotsee kan resulteren in verontreiniging van de kopkapsels en uiteindelijk leiden tot ongecontroleerde fractionatie tijdens chironomiden  $\delta^{18}\text{O}$  analyse. Om deze reden is een tweede chironomiden  $\delta^{18}\text{O}$  curve gegenereerd van kopkapsels die opgewerkt zijn met een methode om carbonaatdeeltjes te verwijderen. Een experiment liet zien dat het gebruik van zuren voorafgaand aan chironomiden  $\delta^{18}\text{O}$  analyse kan resulteren in zuurstofisotopenuitwisseling tussen overblijfselen van chironomiden en water, zoals beschreven in Hoofdstuk 1. Carbonaatdeeltjes zijn daarom verwijderd met behulp van een gebufferde ammoniumchloride ( $\text{NH}_4\text{Cl}$ ) oplossing in een pH neutrale omgeving. De  $\delta^{18}\text{O}$  curve gemeten aan kopkapsels die opwerkt zijn conform een standaard palaeoecologische methode komt niet overeen met de  $\delta^{18}\text{O}$  curve gemeten aan carbonaten. De  $\delta^{18}\text{O}$  curve verkregen door kopkapsels opgewerkt met  $\text{NH}_4\text{Cl}$  daarentegen laat een uitstekende correlatie zien met carbonaat  $\delta^{18}\text{O}$ , waarin koude en warme perioden gekenmerkt worden door relatief lichte, respectievelijk, zware  $\delta^{18}\text{O}$  waarden. Verschillen in de amplitude van schommelingen in de chironomiden en carbonaat  $\delta^{18}\text{O}$  curven worden toegeschreven aan verschillende effecten van een temperatuur-gerelateerde fractionatie van zuurstofisotopen tijdens de vorming van carbonaten en chironomidenkopkapsels. Ook kan meerwater  $\delta^{18}\text{O}$  per seizoen variëren en dit kan een verschillend effect hebben op carbonaten dan op chironomiden. De goede overeenstemming tussen de schommelingen in de  $\delta^{18}\text{O}$  curves gebaseerd op chironomiden en carbonaten suggereert dat deze methode uitermate zinvol kan zijn in verschillende settings. Zo kan chironomiden  $\delta^{18}\text{O}$  een goed alternatief zijn in meren waar carbonaten afwezig zijn of in meren met een hoge terrestrische input. Tenslotte kan chironomiden  $\delta^{18}\text{O}$  ook gebruikt worden in meren waar carbonaten wel aanwezig zijn, mits carbonaatdeeltjes adequaat verwijderd zijn met  $\text{NH}_4\text{Cl}$  voorafgaand aan  $\delta^{18}\text{O}$  analyse. Als een fractionatie in zuurstofisotopen bij chironomiden onafhankelijk is van temperatuur, dan zou chironomiden  $\delta^{18}\text{O}$  gebruikt kunnen worden in combinatie met een  $\delta^{18}\text{O}$  analyse aan carbonaat om watertemperatuur in meren te kunnen reconstrueren. Immers, carbonaat  $\delta^{18}\text{O}$  weerspiegelt meerwater  $\delta^{18}\text{O}$ , gemodificeerd door een temperatuur-afhankelijke fractionatie. Chironomiden  $\delta^{18}\text{O}$  daarentegen zou dan een weerspiegeling zijn van meerwater  $\delta^{18}\text{O}$ , die niet gewijzigd is door temperatuur. Aangezien de relatie tussen meerwater  $\delta^{18}\text{O}$ , carbonaat

$\delta^{18}\text{O}$  en watertemperatuur goed bestudeerd is en met een simpele relatie beschreven kan worden, zou op dergelijke wijze de watertemperatuur tijdens de vorming van carbonaten gereconstrueerd kunnen worden.

## Conclusies

Dit proefschrift laat zien dat betrouwbare metingen van stabiele zuurstofisotopen verkregen kunnen worden aan zowel verse, subfossiele en fossiele kopkapsels van chironomiden. De volgende conclusies kunnen getrokken worden wat betreft chironomiden  $\delta^{18}\text{O}$  analyse:

- 1) Chemische behandelingen worden afgeraden, omdat ze het  $\delta^{18}\text{O}$  signaal in chironomiden kunnen beïnvloeden. Echter, wanneer met carbonaat-rijke sedimenten gewerkt wordt, biedt een oplossing van ammoniumchloride een goede oplossing om carbonaatdeeltjes adequaat te verwijderen, zonder verstoring van het originele chironomiden  $\delta^{18}\text{O}$  signaal. Een opwerking met ammoniumchloride heeft geen substantieel effect op  $\delta^{18}\text{O}$  waarden van chironomiden.
- 2) In diepe Europese meren zijn meerwater en chironomiden  $\delta^{18}\text{O}$  sterk en positief gecorreleerd. Er is een duidelijke relatie tussen neerslag  $\delta^{18}\text{O}$ , meerwater  $\delta^{18}\text{O}$  en luchttemperatuur gemiddeld over het jaar. Dit suggereert dat chironomiden  $\delta^{18}\text{O}$  gebruikt kan worden om meerwater  $\delta^{18}\text{O}$  en dus klimaatverandering in het verleden te reconstrueren.
- 3) Een  $\delta^{18}\text{O}$  curve is met succes verkregen aan laat glaciële en vroeg Holocene chironomiden. Relatieve schommelingen in de chironomiden  $\delta^{18}\text{O}$  curve zijn in overeenstemming met die in de  $\delta^{18}\text{O}$  curve gemeten aan carbonaten. Dit toont aan dat klimaatsveranderingen de  $\delta^{18}\text{O}$  waarden van chironomiden hebben beïnvloed. Hieruit kan worden afgeleid dat overblijfselen van chironomiden gebruikt kunnen worden om  $\delta^{18}\text{O}$  curves te produceren, zelfs in meren waar geen carbonaat aanwezig is of waar sprake is van een hoge input van terrestrisch materiaal.
- 4) Er moet verder onderzoek gedaan worden naar deze nieuwe proxy voor meerwater  $\delta^{18}\text{O}$  en, derhalve, klimaat. Weinig tot geen informatie is momenteel beschikbaar over de mogelijke effecten van temperatuur op exoskeletten van chironomiden tijdens de vorming van kopkapsels, over de effecten van seizoensgebonden verschillen in meerwater  $\delta^{18}\text{O}$  op chironomiden  $\delta^{18}\text{O}$  en over de variabiliteit van chironomiden  $\delta^{18}\text{O}$  binnen een meer. Desalniettemin documenteert dit proefschrift het potentieel van deze nieuwe methode om reconstructies te genereren van temperatuur en hydrologie. Het levert het eerste bewijs dat chironomiden  $\delta^{18}\text{O}$  analyse de mogelijkheid biedt om reconstructies te maken van meerwater  $\delta^{18}\text{O}$ , die in overeenstemming zijn met reconstructies op basis van andere componenten in het sediment.



# Acknowledgements

As a 10-year old I had already made up my mind: I wanted to become an archeologist in the broadest sense of the word. Armed with a shovel I often dug holes to scavenge for old pipes, bones and tiles at the farm where I grew up. Somehow, almost 20 years later, I still manage to do this (though the backyard has expanded to many lakes around the world and fossil chironomids have temporarily replaced the bones) and even get paid to do so. Therefore, I must thank a lot of people.

First, but perhaps a bit unconventional, I need to thank my parents **Tony** and **Toos**. They kept on stimulating me to learn more, not only in a scientific sense, but also about the world, and about myself. Pap, mam: heel erg bedankt daarvoor! Sometimes it's good to remember that all you need to do, is to 'just keep breathing'.

The research I did over the past four years would not have been so succesfull if it hadn't been for my two supervisors, **Andy Lotter** and **Oliver Heiri**. Obwohl mein Schwyzerdütsch nach vier Jahren nicht weiter als 'Kuchikäschtli' reicht, habe ich ganz viel von Euch gelernt. Ich möchte Euch gerne für Eure Hilfe danken. Ihr habt mich immer ermutigt meinen Ideen zu folgen und zu verwirklichen, und dafür bin ich Euch sehr dankbar.

The advantage of science is that you cannot do it all by yourself (although a voice inside your head may tell you differently). In the past years I have had great cooperations, support, discussions and interest from several people that were involved in the 'chiro', 'oxygen', 'paleo' or 'chrono' business. For that, I thank **Gert-Jan Reichart**, **Jan de Leeuw**, **Klaas Nierop**, **Simon Blockley**, **Christine Lane**, **Jarmo Meriläinen**, **Maarten Blaauw**, **John Birks**, **Steve Brooks**, **Boris Il'yashuk**, the many contributors to the 'deep lakes dataset', and the people who made our two-month fieldwork frenzy a pleasant one by offering us a cup of tea or a place to sleep after a day's coring. It was greatly appreciated.

Whenever I was completely submerged in science or had to share whatever joy or frustration there were always these so-called 'lotgenoten' or 'socios' to count on, to lift me up or to share things with. I think it's amazing how close we are as a group of PhD students. **Emi** and **Peter S**, my first roomies, I would like to thank you for your neverending moral support and many laughs we shared (especially in that store in Minnesota, that 'soepjurk' looked great on you Peter). You're the most awesome, fan-tas-tic e-verrrrr. **Maarten**, Monsieur du Pantalon dur, Moartuh vèn Hèrdubroek, dank je wel voor je luisterend oor en schouder wanneer 't effies nodig was. Heel veel succes met jouw laatste loodjes, misschien een samenvatting in 't Utrechs? **Nina**, doctor(es?), het was mij een waar genoegen om met je te mogen werken, kletsen, drinken en dansen. Bedankt voor alle gezelligheid bij 'Paul en Paul'. **Judithchen**, lieverd, laat je nooit uit 't veld slaan; je bent geweldig! **Em(ster)**, ...da ge bedankt zet, da witte... Bedankt voor je lieve vriendschap! **Peter B**, niet alleen maar voor de Nature's gaan hè? Anders wordt je proefschrift zo dun. **Sander**, ook jij gaat 't nog ver schoppen. Sorry als ik je gepest heb met je rode haren. 't Is pure jaloezie. **Maud**, you rock! **Adriana**, gracias por ser más que una amiga, tu fuerza y amistad valen mucho para mi ¡te quiero mucho!

## Acknowledgements

**Cornelia, Liesbeth**, I have great memories of our trips. Thanks for the laughs and never forget 'Miss Milky', 'Probeer om te draaien', our personalized mugs, the attack of the swans in Monticchio, the scary hotel owners near Laachersee and the good times we had exploring some of the most breathtaking places in Europe. Good luck with your theses!

Many people helped me getting everyday things done in and around the lab. You are irreplaceable, **Marjolein** and **Leonard**. Many thanks also to **Arnold van Dijk**, **Jan van Tongeren**, **Natasja Welters**, **Gijs Nobbe**, **Jan Drenth**, **Ton van Druten**, **Koos Swart** and **Marjan Versluijs** for the assistance in the lab(s) and in the field.

The third floor of the Earth Sciences building is truly a great place to work. Thanks to people at LPP (ex-roomies **Waldemar Herngreen** and **Henk Visscher**, colleagues **Hanneke Bos**, **Henk Brinkhuis**, **Johan van der Burgh**, **Timme Donders**, **Walter Finsinger**, **Han van Konijnenburg-van Cittert**, **Wolfram Kürschner**, **Roel Janssen**, **Cindy Looy**, **Gianluca Marino**, **Francesca Sangiorgi**, **Appy Sluijs**, **Zwier Smeenk**, **Rike Wagner-Cremer**) and at Geosciences (**Lucy S** (for the virtual cookies!), **Elisabeth van B**, **Karoliina K**, **Tom J**, **Anja M**, **Shauna NF**, **Margreet B**, **Kees H**, **Aafke B**, **João TA**, **Hemmo A**, **Ivo D**, **Sander E**, **Tanja K**).

Many thanks to **Bert van der Zwaan** and **Arian Steenbruggen** for making the Darwin Center possible. To **Andy**, **Jaap Sinninghe Damsté** and **Riks Laanbroek** and everyone involved in this Darwin project for writing the proposal and for your guidance from the beginning to the end. Of course many thanks are due to the members of the reading committee of this thesis: **Prof. dr. Jan de Leeuw**, **Prof. dr. Melanie Leng**, **Prof. dr. Jack Middelburg** and **Prof. dr. Dirk Verschuren**.

When work is sometimes all I can think about, it's good to have a great back up team that noticeably starts yawning when I talk about chironomids. I thank **Wilma & Wilco**, **Antoine & Saskia L**, **Jorinde**, **Joyce**, **Jaap**, **Kim & Noud & Nena**, **Eef & Rogier** (en boon), **Agnes & Tycho**, **Saskia v H & Gieljan**, **Saskia K** (AKA 'mug'), **Jeroen**, the **Tilburg Sarto** clan, **Ariann**, **Marieke & Joost**, **Janneth**, **Patricia**, **Marieke**, **Guus**, **Ans**, and **Arda & Marcel & Ester** for being there in my life.

Even during the last day of my 'PhD business' I have my personal 'scientific and emotional' back-up, my paranymphs. Leeve **Irene**, ich dink dük mêt weemöod trök aan oos daag op de Warande, aan de Pleegirl sessie, Lotje en alle lol, die wae gehadj hōbben. Bedanktj des ze dit noe auch mêt mich deilf. Leeve **Micha**, van de afgeloupe veer jaor haj ich nieks wille misse. Doe waars der op de juuste momenten. Veur uch beide: Bedanktj veur dees vrundjsjap!

**Robin**, van jou heb ik veel geveergd de afgelopen tijd. Dank voor je geduld en begrip in tijden dat ik dat niet had. Dank voor de liefde die je me al die jaren gegeven hebt. Ik heb er geen woorden voor.

Dixit.



## Acknowledgements

# Curriculum Vitae

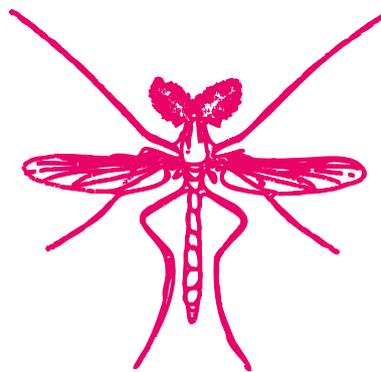
Frederike Verbruggen was born on July 29, 1980 in Eindhoven and grew up in Beek en Donk, the Netherlands. After graduating at the 'gymnasium' level at Lorentz Lyceum, Eindhoven in 1998 she went to Tuxtla Gutiérrez, Chiapas, Mexico for one year to learn Spanish. She also gave English classes at a private school. In 1999 she enrolled in biology at Utrecht University (UU), where she specialized in biogeology. She conducted three internships and one literature thesis during her Masters. She reconstructed the late glacial and Holocene vegetation history using fossil pollen at Lac de Blanchemer, France (Palaeoecology, UU), middle Miocene bottom water conditions in the Mediterranean using fossil foraminifera (Stratigraphy and Paleontology, UU) and made a review of the use of ancient DNA in paleoecology (Palaeoecology, UU). Her final internship she conducted in the United States of America, where she used fossil pollen to make a biostratigraphy for the lower Paleocene of the Denver Basin (Denver Museum of Nature & Science, US Geological Survey, University of Colorado at Boulder). This resulted in a *cum laude* Master graduation in December 2004. In 2005 she had the honor to accept the Unilever Research Prize. After her graduation she worked for six months at the Stratigraphy and Paleontology group at Utrecht University coordinating various courses at Bachelor and Master level. In October 2005 she started as a PhD student in the Palaeoecology group at UU within a multi-disciplinary project funded by the Darwin Center for Biogeosciences where she explored the potential of using stable oxygen isotopes in chironomids as a new proxy for past temperatures. The results of those four years lie in front of you. At present, she works at ADC ArcheoProjecten, Amersfoort, as a palynologist and archeobotanist.











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