Stable carbon isotopes of invertebrate remains:
do they reveal past methane release from lakes?

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LPP Foundation 2010

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**Stable carbon isotopes of invertebrate remains:
do they reveal past methane release from lakes?**

**Stabiele koolstofisotopen van invertebratenresten:
onthullen ze vroegere methaanuitstoot uit meren?**

(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 maandag 10 januari 2011 des middags te 4.15 uur

door

Maarten Reinier Baron van Hardenbroek van Ammerstol

geboren op 2 mei 1982 te Utrecht

Promotor: Prof.dr. A.F. Lotter

Co-promotor: Dr. O.M. Heiri

This thesis is dedicated to

Pim Tiggelman

4 juni 1961 – 12 augustus 2009

Samoerai en Vriend,
Kunstenaar, Romanticus,
Geliefde blijft hij.

IF

If you can keep your head when all about you
Are losing theirs and blaming it on you,
If you can trust yourself when all men doubt you,
But make allowance for their doubting too;
If you can wait and not be tired by waiting,
Or being lied about, don't deal in lies,
Or being hated, don't give way to hating,
And yet don't look too good, nor talk too wise:

If you can dream - and not make dreams your master;
If you can think - and not make thoughts your aim;
If you can meet with Triumph and Disaster
And treat those two impostors just the same;
If you can bear to hear the truth you've spoken
Twisted by knaves to make a trap for fools,
Or watch the things you gave your life to, broken,
And stoop and build 'em up with worn-out tools:

If you can make one heap of all your winnings
And risk it on one turn of pitch-and-toss,
And lose, and start again at your beginnings
And never breathe a word about your loss;
If you can force your heart and nerve and sinew
To serve your turn long after they are gone,
And so hold on when there is nothing in you
Except the Will which says to them: 'Hold on!'

If you can talk with crowds and keep your virtue,
' Or walk with Kings - nor lose the common touch,
if neither foes nor loving friends can hurt you,
If all men count with you, but none too much;
If you can fill the unforgiving minute
With sixty seconds' worth of distance run,
Yours is the Earth and everything that's in it,
And - which is more - you'll be a Man, my son!

Rudyard Kipling

Contents

General introduction and synopsis

Methane (CH_4) is an important greenhouse gas in the atmosphere, accounting for approximately 20% of the greenhouse effect (IPCC 2007; Wuebbles and Hayhoe 2002). Regional and global estimates indicate that lakes can be important sources of methane (Huber et al. 2006; IPCC 2007; Zimov et al. 1997) and that lakes can contribute up to 16% of natural methane emissions (Bastviken et al. 2004), yet they are often ignored in studies that model greenhouse gas emissions. In order to understand increasing methane emissions in the present, it is important to study their variations during past periods of climate change. However, records of methane release from lakes over time scales longer than a few years are extremely rare. In this thesis a method is explored to reconstruct past methane availability in lakes based on the stable carbon isotope composition ($\delta^{13}C$) of aquatic invertebrate remains. The relationship between methane fluxes and $\delta^{13}C$ of invertebrate remains was studied in laboratory experiments and field surveys in Sweden and Siberia. Finally, this approach was applied to sediment records to provide information about methane release from lakes in relation to climate change in the past.

Methane from lakes

Atmospheric methane concentrations have been ranging between 350 and 800 parts per billion (ppb) during the past 800,000 years (Loulergue et al. 2008), but increased rapidly to 1750 ppb in the past two centuries. Understanding release and uptake of methane from different compartments of the global carbon cycle is important to predict the effect of changing environmental conditions on global methane dynamics. Wetlands and lakes are identified as one of the most important sources for methane to the atmosphere (IPCC 2007) that may have been underestimated in terms of surface area and methane production per unit of surface area (Downing et al. 2006; Kankaala et al. 2007b). Methane emissions from wetlands and lakes can have an important positive feedback to climate change, especially in sensitive regions such as the arctic (Walter et al. 2006; Wuebbles and Hayhoe 2002). However, very few estimates exist of past methane output from lakes (Walter et al. 2007) and the response of methane emissions from individual lakes to changing environments is still poorly studied. At present it is uncertain how methane output per unit area of lake surface will vary with changing climate and in different climate zones. Therefore, additional methods to investigate past methane output from lakes are urgently needed.

Methane as carbon source for aquatic invertebrates

In lakes, methane is produced by methanogenic bacteria during the degradation of organic matter in the deep, anoxic waters or in anoxic lake sediment (Bartlett and Harriss 1993; Rudd and Taylor 1980). This methane is characterized by distinctly depleted $\delta^{13}C$ values of -75 to -50‰ VPDB (Hornibrook et al. 2000; Whiticar 1999). Methane can be transported from the sediment into the lake water and further into the atmosphere by a range of processes such as diffusion, ebullition (percolating of bubbles) through the sediment and water column, or transport via vascular plants (Bastviken et al. 2008; Kankaala et al. 2007b; Walter et al. 2007). During transport from the sediment considerable amounts of this methane may be oxidized by chemical processes or methane oxidizing bacteria (Bastviken 2009; Frenzel et al. 1990; Kankaala et al. 2007b). Methane oxidizing bacteria (MOB) utilize methane in aerobic sediments and the water column and produce biomass 12-30‰ lower in $\delta^{13}C$ values than their methane source (Jahnke et al. 1999), resulting in $\delta^{13}C$ of MOB biomass as low as -100 to -65‰ VPDB (Fig. 1). Several invertebrates can incorporate methanogenic carbon originating from MOB or protozoans feeding on them, leading to very depleted $\delta^{13}C$ values in invertebrate tissues of some groups of cladocerans (water fleas) and chironomids (non-biting midges) (Bastviken et al. 2003; Deines et al. 2007; Jones et al. 2008; Kankaala et al. 2007a). Even animals on higher trophic levels such as fish that feed on invertebrates can incorporate significant amounts of methane-derived carbon (Harrod and Grey 2006; Ravinet et al. 2010). Analyses of the $\delta^{13}C$ values of animals can, therefore, provide insights on the relative importance of MOB in their diet, and indirectly on methane availability in lake food webs.
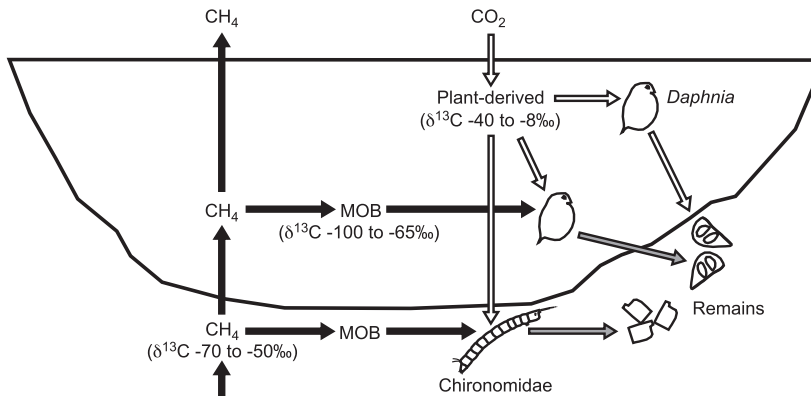
Fig. 1 Schematic overview of carbon sources for aquatic invertebrates in a simplified lake ecosystem with chironomids, Daphnia, and their chitinous remains. Methane-derived carbon is indicated by black arrows ($\delta^{13}C$ values are based on Jahnke et al. 1999; Whiticar 1999; Hornibrook et al. 2000). Plant-derived carbon is indicated by white arrows ($\delta^{13}C$ values based on Yoshioka et al. 1994; Grey and Jones 1999; Jones et al. 1999; Bade et al. 2006; Vuorio et al. 2006; Kankaala et al. 2010). $\delta^{13}C$ of chironomids and Daphnia can be expected to vary depending on the availability of methane- and plant-derived organic matter in different compartments of the lake, as indicated by grey arrows. All $\delta^{13}C$ values are expressed relative to VPDB. Modified version of an unpublished figure by O. Heiri.
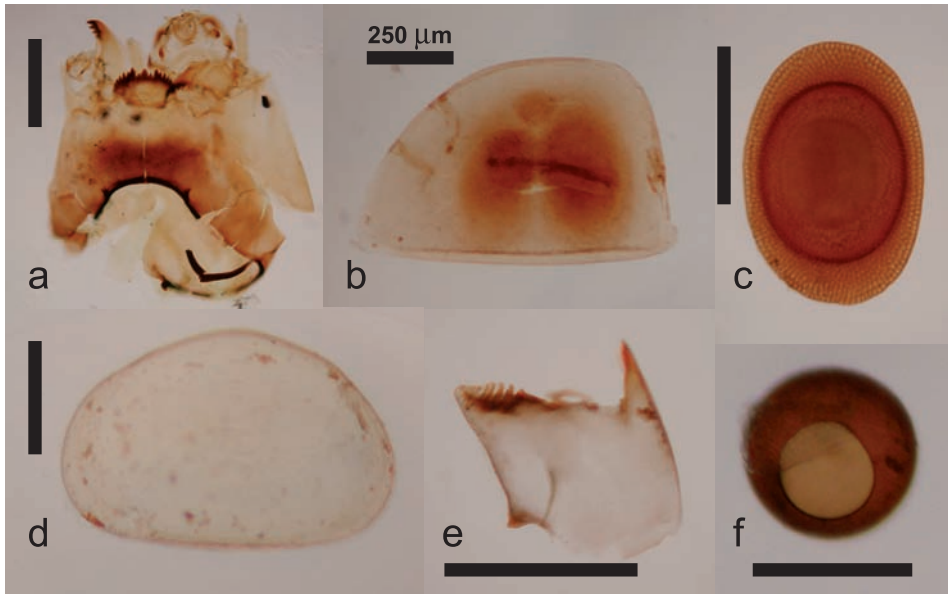
Fig. 2 Examples of chitinous invertebrate remains analyzed in this thesis including (a) head capsule of a chironomid larva (Chironomidae; non-biting midges), (b) resting stage (ephippium) of *Daphnia* (Cladocera; water fleas), (c) resting stage (statoblast) of *Plumatella* (Bryozoa; moss animals), (d) chitinous remain of ostracod carapace (Ostracoda: seed shrimps), (e) mandible of ephemeropteran (Ephemeroptera; mayflies), and (f) cocoon of Rhabdocoela (Turbellaria; flatworms). Scale bars represents 250 μm.

$\delta^{13}C$ analysis of invertebrate remains

A number of invertebrate taxa produce robust chitinous structures that preserve well in lake sediments (Fig. 2). Remains of several invertebrate groups have been used in palaeoecological studies (Smol et al. 2001) using relationships between environmental variables and modern species distributions to reconstruct environmental conditions based on past changes in the taxonomic composition of invertebrate assemblages. Hardly any studies, however, have used stable carbon isotope analysis of chitinous invertebrate remains in lake sediments to reconstruct carbon cycling and food web functioning in the past, compared with the large number of stable isotope studies available that examine modern lake ecosystems. One of the first studies analyzing $\delta^{13}C$ of chitinous remains from freshwater invertebrates is a recent study by Wooller et al. (2008) that compared $\delta^{13}C$ in chironomid remains and bulk sediment organic matter from Stora Vidarvatn (Iceland). Perga (2010) demonstrated strong correlations between $\delta^{13}C$ values of living water fleas of the genera *Bosmina* and *Daphnia* and stable isotope values of their carapaces. In her study, Perga also measured $\delta^{13}C$ on *Bosmina* carapaces of a sediment record from Lake Annecy. However, only very few studies have investigated how well carbon sources of freshwater invertebrates can be traced by $\delta^{13}C$ of their remains and how well methane-derived carbon is recorded in them.

11

Carbon sources available for lacustrine invertebrates have specific ranges of $\delta^{13}C$ values (Fig. 1). Methane-derived carbon can have $\delta^{13}C$ values as low as -100 to -65‰, but $\delta^{13}C$ of plant-derived carbon is much higher. Terrestrial plants and animals transported into the lake, and organic matter produced by aquatic macrophytes and algae in the littoral typically have $\delta^{13}C$ values between -33 and -8‰ (Meyers and Teranes 2001). $\delta^{13}C$ of organic matter produced by phytoplankton is usually between -35 and -25‰ (Bade et al. 2006; Grey and Jones 1999; Vuorio et al. 2006; Yoshioka et al. 1994), but in oligotrophic lakes phytoplankton can reach -40‰ (Jones et al. 1999; Kankaala et al. 2010). Several lacustrine invertebrate groups are likely to assimilate carbon from different sources including both plant- and methane-derived carbon (Fig. 1), resulting in lower $\delta^{13}C$ values if their diet contains more methane-derived carbon. However, it is unknown if differences in $\delta^{13}C$ values between invertebrate species that are observed in modern ecosystem studies can also be found in invertebrate remains and whether these remains faithfully record past changes in $\delta^{13}C$ of invertebrate biomass.

Aim and outline of the thesis

The main aim of this thesis was to examine whether $\delta^{13}C$ values of invertebrate remains can be used to indicate the past abundance of methane-derived carbon in lake food webs. Major questions were:
- Is it possible to trace methane-derived carbon in the fossilizing structures of invertebrates using stable carbon isotope analysis?
- How are invertebrate remains affected by *post-mortem* processes (taphonomy, diagenesis, and sample processing) and how reproducible are stable carbon isotope measurements on invertebrate remains?
- Does a relationship exist between methane fluxes from lakes and $\delta^{13}C$ values of invertebrate remains obtained from surface sediments of these lakes?
- Can $\delta^{13}C$ analysis of invertebrate remains from sediment records be used for reconstructing past changes in the contribution of methane-derived carbon to lake food webs?
- Is it possible to infer changes in methane availability in lakes, and indirectly methane release, over decadal to centennial time scales?

The first chapters of this thesis address methodological aspects regarding stable carbon isotope analysis of invertebrate remains and examine how well assemblages of fossil invertebrate remains represent the living invertebrate community. In **Chapter 1** it is demonstrated that methane-derived carbon is incorporated into chironomid head capsules, using culturing experiments with MOB grown on ^{13}C-labelled methane. Also, it was shown that chemical pre-treatments that are commonly used for sediment processing, including 2h 10% KOH, 2h 10% HCl, and 18h 40% HF, do not have a noticeable effect on $\delta^{13}C$ values of chironomid remains. Only a combination of boiling, accelerated solvent extraction, and heavy chemical oxidation had a clear but relatively minor effect on $\delta^{13}C$ values.

Furthermore, it was determined that a minimum of 20 µg chitinous material is required for reproducible $\delta^{13}C$ measurements on the equipment used, based on a mean 40% carbon content of chironomid remains. The presented results indicated that taxon-specific $\delta^{13}C$ values of invertebrate remains from fossil sediment samples give reproducible results. $\delta^{13}C$ of head capsules generally ranged from -28 to -25.8‰ in sediment samples from three examined lakes, although $\delta^{13}C$ values as low as -36.9‰ were observed, suggesting that methane-derived carbon may have been incorporated into some of the chironomid remains.

Processing of sediment sample for stable isotope analysis of chitinous remains can be time consuming, since invertebrate remains need to be separated manually from other fragments contained in sieved sediment fractions. Therefore, the effect of sieving with different mesh sizes on the efficiency of processing fossil chironomids for isotope analyses was investigated in **Chapter 2**. Large remains are less common in sediment samples, but contribute more to the minimum mass required for isotope analysis compared with small remains. The results produced in a series of experiments indicate that the time required to separate a given mass of chironomid head capsules from other sieve residue is disproportionately long for sieves with mesh sizes <200 µm. Processing time could be decreased by 30-58% for the studied sediment samples by employing sieves with a 200 µm mesh rather than the 100 µm mesh commonly used for standard palaeoecological analyses and this will increase the number of samples that can be analyzed if time is limited.

In **Chapter 3** the distribution of life assemblages of benthic invertebrates in a lake basin (Lake De Waay, the Netherlands) is described and compared to the distribution of their subfossil remains. The focus of the chapter is on Chironomidae, but other benthic invertebrate groups that produce chitinous remains were also studied. These include Bryozoa, Ceratopogonidae, *Chaoborus*, Coleoptera, *Daphnia*, Ephemeroptera, Heteroptera, Limoniidae, Malacostraca, Odonata, Oribatida, and Trichoptera. Most living benthic invertebrates collected in the lake in different seasons were constrained to the littoral zone (e.g. chironomids of the tribe Orthocladiinae, Odonata, and Trichoptera), with the exception of a few taxa (*Chaoborus flavicans*, *Chironomus*) that are adapted to low oxygen conditions occurring in the seasonally anoxic profundal zone of Lake De Waay. In contrast, subfossil assemblages in lake surface sediments were similar in the entire lake basin, suggesting that considerable numbers of invertebrate remains were transported and redeposited off-shore in De Waay. These processes were probably facilitated by the lake's steep bathymetry.

Good agreement was observed between the living and subfossil assemblages in the lake, except for some groups that lack identification keys for subfossil remains. Of the total 44 chironomid taxa, 30 taxa occurred both as living and subfossil specimens and, on average, these 30 taxa represent 94% of the specimens encountered in a sediment sample. Five rare chironomid taxa were only found in the life assemblages, whereas eight rare and four common taxa were only found as subfossil remains. These results indicate that the subfossil assemblages in

surface sediments provide a spatially integrated and representative sample of the life assemblage in Lake De Waay. Also, it is suggested that a combination of these approaches can give a more complete understanding of the benthic invertebrate community.

The second part of this thesis focuses on the relationship between methane fluxes and invertebrate $\delta^{13}C$ and attempts to use $\delta^{13}C$ measured on invertebrate remains in sediment records to infer past changes in methane availability in lakes. In **Chapter 4** the results from seven Swedish lakes are presented in which methane fluxes were measured and compared with $\delta^{13}C$ of the chitinous remains of several chironomid taxa (Chironomini, Orthocladiinae, Tanypodinae, and Tanytarsini), bryozoans (*Cristatella mucedo* and *Plumatella*), the cladoceran genus *Daphnia*, Ephemeroptera, and ostracods (Fig. 2). Relative differences in $\delta^{13}C$ values between taxa were similar in samples from different lakes and different water depths, which can be explained by different dietary preferences and habitats of the examined invertebrate groups. For example, $\delta^{13}C$ values of Orthocladiinae and Ephemeroptera were always between -31.3 and -27.0‰, likely reflecting plant-derived carbon, whereas ostracods and *C. mucedo* had $\delta^{13}C$ values as low as -39 and -38.1‰, respectively, likely reflecting a methane-derived carbon source.

Negative correlations were observed between diffusive methane fluxes in the seven lakes and $\delta^{13}C$ values in remains of several invertebrate taxa (Chironomini, *Daphnia*, Tanypodinae, and Tanytarsini). This correlation was distinct for *Daphnia* and statistically significant (r = -0.90, p = 0.0062) for the Chironomini. The relationship between methane release and $\delta^{13}C$ of invertebrate remains was studied in more detail in two of the studied lakes in which several sediment cores were taken along transects from the littoral to the deeper part of the lake. $\delta^{13}C$ of Chironomini, *Daphnia*, and *C. mucedo* from these sediments were negatively correlated to methane release from the sediment cores, and for Chironomini this correlation was also statistically significant (r = -0.67, p = 0.025). Therefore, it is suggested that incorporation of methane-derived carbon by Chironomini, Tanytarsini, Tanypodinae, *Daphnia,* and possibly by *C. mucedo* can explain the pattern in $\delta^{13}C$ of the taxa for the studied lakes. These results indicate that $\delta^{13}C$ analysis of invertebrate remains reveals taxon-specific information about $\delta^{13}C$ of the diet of these organism groups that can be applied when interpreting palaeoecological records. $\delta^{13}C$ of Chironomini and *Daphnia* remains appear to be particularly useful to trace past changes in methane availability in lakes.

Chapter 5 focuses on a ca. 140-year sediment record from Strandsjön, one of the lakes studied in Chapter 4. Analyses of bulk sediment geochemistry ($\delta^{13}C$, $\delta^{15}N$, C:N ratio, relative abundance of organic carbon) are compared with $\delta^{13}C$ values of invertebrate remains in the record. The invertebrate remains included head capsules of four chironomid groups (Chironomini, *Chironomus*, Tanytarsini, and Tanypodinae), resting stages of bryozoans (*Plumatella* and *C. mucedo*) and cladocerans (*Daphnia*), and cocoons of Turbellaria. $\delta^{13}C$ values of the four chironomid groups showed a strong co-variation, and revealed several periods (dated ca. AD 1890, 1920, 1950, and 1990) with 2-3‰ lower $\delta^{13}C$ values

of chironomid remains. In contrast, δ^{13}C values in remains of filter-feeding non-chironomid invertebrates (*Daphnia*, *C. mucedo*, and *Plumatella*) were constant in the sediment record, with a clear decrease in the youngest sediments (since ca. AD 1960). δ^{13}C of Turbellaria cocoons had constant values of ca. 29‰ throughout the sediment record. The deviation between δ^{13}C of remains of chironomids and non-chironomid filter-feeding invertebrates suggests that δ^{13}C of food resources in Strandsjön varied differently in benthic habitats and the open water column during the past 140 years.

A distinct 10‰ increase in bulk sediment δ^{13}C values at the end of the nineteenth century may have coincided with an artificial 0.5-1 m lowering of the water level in Strandsjön. Possibly, this measure caused increased water column mixing and nutrient availability in the photic zone and the production of ^{13}C-enriched algae. However, a similar shift in δ^{13}C values was not observed in remains of any invertebrate group, suggesting that δ^{13}C of organic matter in the water column was not affected. Alternatively, bulk sediment δ^{13}C may have been affected by post-depositional processes in the sediment (e.g. methanogenesis, methanotrophy, or other chemoautotrophic processes) that cause low bulk sediment δ^{13}C values. In lakes where post-depositional processes affect δ^{13}C values of bulk sediment, δ^{13}C of chitinous invertebrate remains may be more suitable for reconstructing the past carbon isotopic composition of organic matter within the lake ecosystem than bulk organic matter analyses. Furthermore, the presented results indicate that taxon-specific δ^{13}C analysis of invertebrate remains can provide more detailed information about δ^{13}C of organic matter in benthic and open water compartments than bulk sediment δ^{13}C.

Ten lakes in arctic Siberia were studied in **Chapter 6** to explore the relationship between diffusive methane fluxes and δ^{13}C of chitinous invertebrate remains in lake surface sediments. This information was then used to interpret a downcore record of taxon-specific δ^{13}C of invertebrate remains from a Siberian thermokarst lake with the aim to infer past changes in methane availability within the lake ecosystem. The Siberian Arctic is a region sensitive to climate change where increasing temperature is expected to have a strong positive effect on methane emissions from wetlands and lakes. Relatively high δ^{13}C values between -30.8 and -27.1‰ were measured in Orthocladiinae, a group of chironomids that typically feeds on macrophytes and algae. These high δ^{13}C values suggest that this taxon incorporates more ^{13}C-enriched plant-derived carbon than other taxa in this study. In contrast, chitinous remains of *Chironomus* and ostracods had the lowest δ^{13}C values, ranging from -35.9 to -29.6 and from -36.9 to -30.9‰, respectively, indicating a more ^{13}C-depleted carbon source. Negative correlations were observed between diffusive methane fluxes measured in the examined lakes and δ^{13}C of *Chironomus,* Chironomini, Tanytarsini, Tanypodinae, *Daphnia*, and ostracods. These correlations were significant for Chironomini (r = -0.65, p = 0.040) and *Daphnia* (r = -0.66, p = 0.039). Also, correlations between δ^{13}C values of invertebrate remains and other physical or chemical parameters of the lakes (e.g. dissolved organic carbon and δ^{13}C of bulk sediment) are less strong than the correlations with diffusive methane fluxes. This suggests that methane availability

affects δ¹³C values of these organisms more strongly than other parameters.

Investigation of the ca. 1400-year sediment record obtained from one of the thermokarst lakes indicated that δ¹³C values of chitinous remains of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* are lowest in sediment sections deposited between ca. AD 850 and 1150 and since ca. AD 1970, which coincided with higher temperatures as inferred in proxy-records from this and other high-latitude regions in the northern hemisphere. Orthocladiinae, typical inhabitants of the littoral in the examined thermokarst lakes, have δ¹³C values that are more similar to the bulk sediment δ¹³C record and probably more indicative of δ¹³C values of plant-derived material. The combined surface sediment and downcore data suggest that *Chironomus*, Chironomini, Tanytarsini and *Daphnia* incorporated more methane-derived carbon during warmer periods in the lake selected for the down-core sediment study, and that methane may have been more abundant during these periods than during colder periods.

Synopsis

Only very few reconstructions exist that infer past changes in methane emissions from lakes, although it is estimated that they are responsible for a significant part of natural methane emissions to the atmosphere. In this thesis, the relationship between methane fluxes from lakes and δ¹³C of chitinous invertebrate remains was studied, to explore the potential for reconstructing past methane fluxes based on these remains in lake sediment records. Culturing experiments demonstrated that methane-derived carbon is incorporated into chironomid head capsules. Also, δ¹³C values of chironomid head capsules are not noticeably affected by mild chemical pre-treatment methods that are commonly used to process sediment samples for palaeolimnological studies, and δ¹³C values of invertebrate remains could be measured sufficiently precise to differentiate between methane- and plant-derived carbon sources.

A number of invertebrate taxa had systematic differences in δ¹³C values of their remains in all surface sediment samples (Fig. 3). Ephemeroptera and Orthocladiinae always had relatively high δ¹³C values (mean values of -29.2 and -29.3‰, respectively), suggesting that they feed primarily on plant-derived carbon sources. This corresponds with observations that the majority of species contained in these groups feed on living and dead algae, diatoms, and macrophytes in the littoral zone of lakes. Therefore, it is suggested that remains of Ephemeroptera and Orthocladiinae could be used as indicator of the δ¹³C values of past phosynthetically produced organic matter, and may be more reliable for this purpose than bulk sediment δ¹³C that provides an integrated δ¹³C signal of allochtonous and autochtonous material. Chitinous remains of *Cristatella mucedo* and ostracods always had relatively low δ¹³C values compared to other invertebrate taxa (mean values of -34.4 and -35.1‰, respectively), indicating that these taxa assimilated a more ¹³C-depleted carbon source than other invertebrates. Likely, ostracods and *C. mucedo* incorporated methane-derived carbon. Benthic ostracods can bury into the sediment, where they may access MOB, and filter-
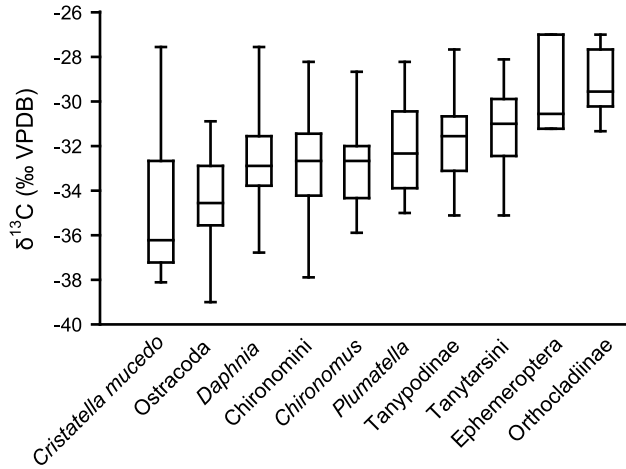
Fig. 3 δ¹³C values of invertebrate remains from 28 surface sediment samples from 17 lakes in Sweden and Siberia. Boxplots indicate median, quartiles, minimum, and maximum values.

feeding *C. mucedo* selectively ingests small particles and can feed on bacteria, including MOB. $\delta^{13}C$ of remains of other invertebrates, including *Chironomus*, Chironomini, Tanytarsini, Tanypodinae, *Daphnia*, and *Plumatella* was more variable in surface sediments of different lakes (Fig. 3), which indicate that these taxa can access a wider variety of carbon sources, of which some are more ^{13}C-depleted than others.

Taxon-specific $\delta^{13}C$ values of invertebrate remains from sediment records can provide information about changing $\delta^{13}C$ values of carbon sources in different compartments of lakes. For example, in the sediment record from Strandsjön $\delta^{13}C$ of sediment-dwelling chironomids co-varied and showed a number of negative shifts indicating variations in $\delta^{13}C$ of their diet in the sediment. In contrast, $\delta^{13}C$ of remains of filter-feeding *Daphnia*, *Plumatella*, and *C. mucedo* showed a very different pattern in the 140-year record, suggesting that filter-feeders incorporated different organic matter that may largely reflect $\delta^{13}C$ of phytoplankton in the water column of Strandsjön. Information about $\delta^{13}C$ values in different compartments of lakes cannot be derived from bulk sediment $\delta^{13}C$ records and can be used to construct a more detailed overview of past changes in lake food webs. Furthermore, bulk sediment $\delta^{13}C$ can be affected by post-depositional processes. In such situations, e.g. in the sediment record from Strandsjön, $\delta^{13}C$ of chitinous invertebrate remains may be the more suitable approach for reconstructing past $\delta^{13}C$ of carbon sources in lake ecosystems than bulk organic matter analyses.

Methane-derived ^{13}C-depleted carbon can be an alternative carbon source to plant-derived material for invertebrates in the sediment and water column of lakes (Fig. 1). In the study lakes from Sweden and Siberia, $\delta^{13}C$ values of chitinous remains of several invertebrate taxa were negatively correlated with diffusive methane fluxes in surface sediments, suggesting the incorporation of ^{13}C-depleted methane-derived carbon. These taxa included Chironomini, Tanytarsini, Tanypodinae, and *Daphnia* in seven Swedish lakes and *Chironomus*,
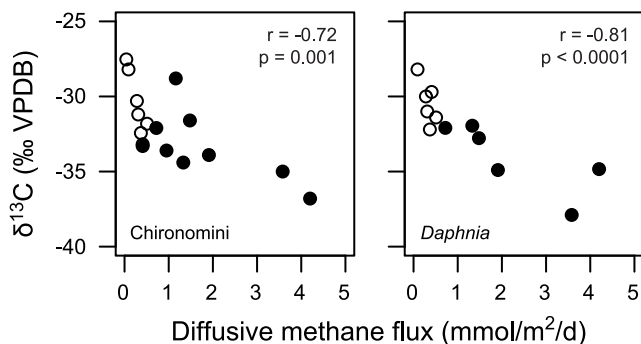
Fig. 4 Relationship between diffusive methane fluxes measured at the lake surface and $\delta^{13}C$ in remains of Chironomini and *Daphnia* from surface sediments. Correlations are based on combined data from surface sediments from seven lakes in Sweden (white points) and ten lakes in Siberia (black points).

Chironomini, Tanytarsini, Tanypodinae, *Daphnia*, and ostracods in ten Siberian lakes. If the data sets from both regions are combined, strong and highly significant correlations are observed between diffusive methane fluxes and $\delta^{13}C$ of Chironomini and *Daphnia* remains (r = 0.72, p = 0.001 and r = 0.81, p < 0.0001, respectively, Fig. 4). A similar pattern was also observed within two Swedish lakes, in which surface sediment cores were obtained along depth transects. $\delta^{13}C$ values of Chironomini, *Daphnia,* and *C. mucedo* were lower in sections of the lake basin in which the sediments had higher methane release rates.

The above suggests that $\delta^{13}C$ in remains of several invertebrates is affected by methane-derived carbon. $\delta^{13}C$ of Chironomini and *Daphnia* remains is sensitive to availability of methane-derived carbon. The habitat and feeding mode of Chironomini and *Daphnia* provide a likely explanation for this sensitivity. Several Chironomini larvae build tubes in the sediment that can be an ideal microhabitat for MOB, providing the larvae with a ^{13}C-depleted carbon source if MOB are abundant. Filter-feeding *Daphnia* are selective for small particles and can feed on bacteria, including MOB when these are abundant in the water column.

In the sediment record from a Siberian thermokarst lake $\delta^{13}C$ values of the remains of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* generally co-varied. All five taxa showed relatively large negative $\delta^{13}C$ shifts in warm periods compared with $\delta^{13}C$ values of Orthocladiinae remains and bulk sediment. It is suggested that the difference between taxa is caused by the incorporation of methane-derived carbon by *Chironomus*, Chironomini, Tanytarsini, and *Daphnia*, whereas $\delta^{13}C$ of Orthocladiinae and bulk sediment mainly reflect the carbon isotopic value of plant-derived carbon. Co-variation of $\delta^{13}C$ values of the chironomid taxa *Chironomus*, Chironomini, Tanytarsini, and Tanypodinae was also observed in the sediment record from Strandsjön. However, in this record it is not clear if $\delta^{13}C$ changes in the chironomid taxa or in other invertebrates like *Daphnia* are reflecting the availability of methane-derived carbon.

This thesis demonstrates the potential to use taxon-specific stable carbon isotopes analysis of invertebrate remains for qualitative reconstructions of

methane availability in lakes, based on the observed correlations between diffusive methane fluxes and $\delta^{13}C$ in remains of *Chironomus*, Chironomini, Tanytarsini, Tanypodinae, ostracods, *Daphnia*, and *C. mucedo* in the study lakes. These correlations are particularly strong and statistically significant for Chironomini and *Daphnia*. Qualitative reconstructions of methane availability using $\delta^{13}C$ of invertebrate remains in sediment records can provide information about past methane dynamics in lakes under changing environmental conditions. The results in the record from a Siberian thermokarst lake suggest that methane becomes more available in the lake during warmer periods. This is in agreement with models that suggested higher methane release from tropical and high-latitude wetlands during warmer and wetter periods in the past (Brook et al. 2000; Huber et al. 2006).

Future directions

Further study is required to assess how widespread the relationship between methane fluxes and $\delta^{13}C$ of invertebrate remains is and whether it can be found in other regions and lake types than the ones studied here, such as lakes in tropical, warm temperate, or alpine zones. Another important aspect that needs to be addressed in more detail is to what extent (local) environmental conditions affect the relationship between methane fluxes and $\delta^{13}C$ of remains. Also, a larger set of lakes needs to be analyzed to quantify the relationship between $\delta^{13}C$ values in invertebrate remains and methane fluxes and rigorously test this relationship for statistical significance. The $\delta^{13}C$ values measured in this study are not as low as reported for some living *Chironomus*, Chironomini, and *Daphnia* specimens (Jones et al. 2008; Kankaala et al. 2010), which hinders unravelling the relative importance of methane- and plant-derived carbon for invertebrates based on $\delta^{13}C$ values of their remains. One explanation for this could be that in the examined lakes $\delta^{13}C$ of the living invertebrates were also not as low as reported in some previous studies. Another explanation could be that lake sediments, since they are integrated over time, lead to relatively high time-averaged $\delta^{13}C$ values in samples of invertebrate remains and can not reach the extremely low values recorded in some individual live specimens. Therefore, future studies should include lakes with living invertebrate communities that are known to have markedly ^{13}C-depleted values (below -45‰). Such lakes will give the opportunity to observe how well methane-derived carbon is reflected by time-integrated samples of invertebrate remains. Culturing experiments are another approach to further investigate and constrain the relationship between $\delta^{13}C$ values of diet, living specimens, and fossilizing remains – especially for invertebrate groups that, based on the presently available results, seem to be able to incorporate methane-derived carbon, e.g. Chironomini, *Daphnia*, bryozoans, and ostracods. Ultimately, this would lead to a more complete understanding of the extent to which methane-derived carbon fuels lake food webs and would contribute to the development of a tool for more quantitative reconstructions of methane fluxes from lakes.

Chapter 1

Fossil chironomid δ¹³C as a proxy for past methanogenic contribution to benthic food webs in lakes?

Abstract

We examined in a series of experiments whether stable carbon isotope analysis of modern and fossil larval head capsules of chironomids allowed the tracing of their dietary carbon source. Our main focus was to assess whether carbon from naturally ^{13}C-depleted methane oxidizing bacteria (MOB) can be traced in chironomid cuticles using stable carbon isotope analysis. Firstly, we showed that a minimum sample weight of ~20 µg was required for our equipment to determine head capsule δ^{13}C with a standard deviation of 0.5‰. Such a small minimum sample weight allows taxon-specific δ^{13}C analyses at a precision sufficient to differentiate whether head capsules consist mainly of carbon derived from MOB or from other food sources commonly encountered in lake ecosystems. Secondly, we tested the effect of different chemical pre-treatments that are commonly used for sediment processing on δ^{13}C measurements on head capsule. Processing with 10% KOH (2 h), 10% HCl (2 h), or 40% HF (18 h) showed no detectable effect on δ^{13}C, whereas a combination of boiling, accelerated solvent extraction, and heavy chemical oxidation result in a small (0.2‰), but statistically significant decrease in δ^{13}C values. Thirdly, using culturing experiments with MOB grown on ^{13}C-labelled methane, we demonstrated that methanogenic carbon is transferred not only into the larval tissue, but also into chironomid head capsules. Fourthly, we analyzed taxon-specific δ^{13}C of fossil chironomid head capsules from different lake sediments. δ^{13}C of head capsules generally ranged from –28 to –25.8‰, but in some instances we observed δ^{13}C values as low as –36.9 to –31.5‰, suggesting that carbon from MOB is traceable in fossil and subfossil chironomid remains. We demonstrate that stable carbon isotope analyses of fossil chironomid head capsules can give insights into dietary links and carbon cycling in benthic food webs in the past and that the method has the potential to reconstruct the importance of MOB in the palaeo-diet of chironomid larvae and, indirectly, to infer past changes in methane flux at the sediment water interface in lakes.

Introduction

Non-biting midges (Insecta: Diptera: Chironomidae) are sensitive indicators for a variety of environmental parameters. The chitinous remains of chironomid larvae preserve well and remain identifiable in lake sediments. Consequently, fossil chironomid assemblages can be used to reconstruct the past chironomid fauna of lakes and infer past changes in physical and chemical variables from lake sediments. Examples of such chironomid-based environmental inferences include the reconstruction of air or water temperature (Brooks 2006; Walker and Cwynar 2006; Heiri et al. 2007), total phosphorus (Brooks et al. 2001; Langdon et al. 2006), chlorophyll a (Brodersen and Lindegaard 1999), oxygen availability (Quinlan et al. 1998), or lake depth (Korhola et al. 2000). Recently, the potential of fossil chironomids for isotope studies has been demonstrated with regard to ^{14}C dating (Jones et al. 1993; Fallu et al. 2004) and δ^{18}O as a palaeotemperature proxy (Wooller et al. 2004, 2008). In contrast, the composition of stable carbon isotopes in fossil chironomids has received less attention for reconstructing past environmental change. In a recent palaeolimnological study, Wooller et al. (2008) measured the carbon isotope ratio (δ^{13}C) as well as δ^{15}N and δ^{18}O in chironomid fossils but for the most part they used chironomid δ^{13}C to constrain the interpretation of other stable isotopes measured on the same material, rather than as an independent geochemical proxy.

Chironomid larvae are benthic animals and the habitat of many species includes the interface between sediments and the water column, where the larvae either live on the sediment surface or in the uppermost few cm of the sediments. Therefore, they have access to freshly deposited algal material sinking down from the water column as well as to organic matter and associated microorganisms in and on the sediments. The carbon isotopic signature of insects is determined by their diet (DeNiro and Epstein 1978) and it can therefore be expected that δ^{13}C of chironomid tissue reflects the isotopic values of the food ingested by the larvae and will give insight in the carbon cycling within lake food webs. Algal biomass δ^{13}C commonly ranges between –30 and –25‰ (Boutton 1991; Meyers and Lallier-Vergès 1999). A stable isotope study of lake ecosystems worldwide (Jones et al. 2008) revealed that many chironomid larvae dwelling in or on sediments have carbon isotope values that are substantially ^{13}C-depleted (δ^{13}C as low as –64‰) relative to the algae or bulk sediment on which they feed. Such extremely low δ^{13}C values are commonly found in biogenic methane as produced in lake sediments by methanogenic archaea (Whiticar 1999) as well as in methane oxidizing bacteria (MOB) that utilize methane as their carbon source (Templeton et al. 2006). Molecular studies of two contrasting German lakes (Eller et al. 2005) and laboratory experiments with ^{13}C-labelled methane (Deines et al. 2007b) have recently provided more direct evidence that such low δ^{13}C values of chironomid larvae are derived from MOB.

The relation between chironomid δ^{13}C, MOB, and methane production has only recently been discovered and bears great potential for applications in palaeoenvironmental studies. Since the carbon isotopic signature of MOB is distinct and is incorporated into chironomid biomass, it can be expected that fossil

chironomid cuticles also preserve this signal. As a consequence, $\delta^{13}C$ in subfossil and fossil chironomid remains may provide a proxy for tracing the importance of MOB in the past diet of chironomid larvae. The abundance of MOB themselves is dependent on methane originating from the sediment (Templeton et al. 2006; Deines et al. 2007b). Therefore, $\delta^{13}C$ in fossil chironomids could potentially provide information on past changes in methane production from lake sediments without the need for long term monitoring.

However, a number of uncertainties and challenges remain before $\delta^{13}C$ in fossil chironomids can be used to reconstruct the carbon isotopic signature of their ingested food, and the importance of MOB in their diet. First, it is unclear how much material is necessary to measure fossil chironomid $\delta^{13}C$ using the available analytical methods. Ideally the method should be applicable to small sample weights so that taxon specific measurements are possible. This would allow $\delta^{13}C$ of deposit feeders to be compared with values measured on chironomids with a different feeding behaviour (e.g. grazers or predators). Second, it has not yet been explored whether the chemical pre-treatment and processing methods commonly used for preparing sediment for fossil chironomid analysis affect $\delta^{13}C$. Third, the extent to which the carbon isotopic signature of MOB is expressed in the larval exoskeletons and consequently in the fossil record is still unclear. Insect cuticle formation is complex with the new cuticle formed both from material re-digested from the old exoskeleton and from material assimilated by the larvae prior to moulting. Although it has been shown that soft tissue in chironomid larvae can reach $\delta^{13}C$ values close to those of MOB (Deines and Grey 2006) it remains to be examined whether the chironomid exoskeleton and fossil chironomid remains can attain similar values.

Here, we assess the minimum sample weight required for $\delta^{13}C$ analysis of chironomid head capsules using standard-sized combustion columns and test if different sediment processing and pre-treatment steps affect $\delta^{13}C$ values of head capsules. Furthermore, we use culturing experiments with living chironomids to demonstrate that the carbon isotopic signature of methane can be traced into chironomid exoskeletons. Finally, we report stable carbon isotope values from subfossil and fossil invertebrate remains confirming that chironomid head capsules may contain methane-derived carbon and indicate next steps necessary for further developing fossil chironomid $\delta^{13}C$ as a proxy for reconstructing the past importance of methanogenic carbon in the diet of chironomid larvae.

Methods

Sample size and chemical sample pre-treatment

In order to develop the methodology for measuring $\delta^{13}C$ on head capsules of larval chironomids, several analytical tests were carried out on cuticles originating from modern chironomid larvae. For this purpose we obtained cultured larvae of *Chironomus riparius* from a local aquarium food supplier (Marsilea Lelystad,

the Netherlands). Except where indicated otherwise the larvae were chemically fossilized before further chemical treatment by removing soft tissue by heating in 10% KOH for 1 h at 70°C. In all of these tests the head capsules were manually separated from the cuticle of the larval body after rinsing twice in demineralized water and isolated for further treatment.

First, we determined the minimum weight required for $\delta^{13}C$ analyses. For this purpose samples ranging from 7 to 100 μg were carefully weighed into tin capsules and analysed. Based on past experience with the Elemental Analyser – Isotope Ratio Mass Spectrometer (EA-IRMS) equipment we used, a minimum current of 1.0 V is required for reproducible $\delta^{13}C$ results. One of the aims of our chironomid $\delta^{13}C$ analyses was therefore to assess how much mass and how many individuals of chironomid head capsules were necessary to produce this voltage during measurement. In a second step, we tested whether different methods that are commonly used for chemical pre-treatment of sediment samples in palaeolimnological or organic geochemical studies affect $\delta^{13}C$ measurements of the head capsules. These pre-treatments included exposure at room temperature (1) for 2 h in 10% KOH, (2) for 2 h in 10% HCl and (3) for 18 h in 40% HF solution. For comparison, we also assessed the $\delta^{13}C$ of head capsules of larvae allowed to decay in demineralized water for three months without any additional treatment or chemical fossilization. Finally, a combination of techniques was used to degrade chemically fossilized head capsules: First, head capsules were boiled in demineralized water for 2 h. Second, we used accelerated solvent extraction (ASE) to imitate the effect of elevated temperature and pressure with liquid solvents (Richter et al. 1996). Third, we used a solution of sodium chlorite and glacial acetic acid as described for the processing of cellulose by Leavitt and Danzer (1993) to assess the effect of heavy oxidation on head capsules. Head capsules were wrapped in Whatman GF/C filters for this combined treatment.

Samples were analyzed for $\delta^{13}C$ on a Fisons NA 1500 NCS Elemental Analyser coupled to a Thermo Electron Delta plus isotope ratio mass spectrometer. All resulting $\delta^{13}C$ values are expressed relative to Vienna Pee Dee Belemnite (VPDB) in units of per mille (‰). The reference material used was a secondary standard of known relation to the international standard of Vienna Pee Dee belemnite. Replicate sample measurements (n=20) on the internal standard gave an analytical error of ±0.26‰ (2 SD). We used an unpaired t-test (95% confidence interval) in Prism 5 for Windows to test for significant differences in $\delta^{13}C$ values between the standard 10% KOH treatment and the other chemical treatments.

Culturing experiment

In addition to using *Chironomus riparius* larvae obtained from commercial sources for our experiments we also cultured *Chironomus riparius* larvae from egg masses in the laboratory. Egg masses were obtained from a local ecotoxicology laboratory (Grontmij | Aquasense, Amsterdam, the Netherlands). Larvae were grown from eggs in tap water at 27°C on a 16:8 h light:dark rhythm and fed on fish food (Tetramin, manufactured by Tetra GmbH, Melle, Germany) with an isotopic

composition of –22.3 ± 0.2‰ (VPDB). One sub-sample was grown until the larvae reached the final larval stage. Subsequently, the larvae were sieved from the sediments and placed into filtered tap water for 24 h to allow gut clearance (Feuchtmayr and Grey 2003). Excess faecal material was removed periodically during this step to prevent coprophagy.

A second sub-sample of larvae was transferred to a series of experimental glass tubes once they reached the second larval stage to determine whether the carbon isotopic signature of methane was taken up into soft larval tissue and cuticles. Each glass tube was prepared following Deines et al. (2007b): Sediment was collected from Ranworth Broad, UK (52°28′N, 1°42′E), and was sieved with a 0.5 mm mesh to remove macroinvertebrates and debris. Five 1 L screw cap glass bottles each were filled with 0.6 L of the pre-sieved sediment and closed with butyl rubber stoppers. From the headspace, 180 ml air was removed and replaced by 200 ml of a mixture of $70\%_{vol}$ $^{12}CH_4$ and $30\%_{vol}$ $^{13}CH_4$ (99% pure $^{13}CH_4$; Isotec, Miamisburg, Ohio, supplied by Sigma-Aldridge, Zwijndrecht, the Netherlands). This mixing ratio was used to prevent an inhibition of methane oxidation by too high concentrations of $^{13}CH_4$. The resulting $\delta^{13}C$ value of the CH_4 was 12,200‰ (VPDB). Pre-incubation of the sediment was carried out at 20°C on a rotary shaker. After 5 days, considered to be sufficient for a MOB-enriched micro-flora to develop, the sediment from all five bottles was combined, mixed and used to fill three experimental tubes (diameter: 4.5 cm, height: 38 cm; Ochs, Bovenden, Germany) to half of their total volume. Tubes were filled up with 100 μm filtered rain water and 20 ml of the $^{12/13}CH_4$-mixture was injected in reservoirs under the tubes. This methane was allowed to diffuse through a glass-sintered separation into the tubes to provide MOB with a ^{13}C labelled CH_4 source during the experiment (Deines et al. 2007b). The sediment was allowed to settle for 24 h before the experiment was started by placing 40 2nd instar chironomid larvae into each tube. Oxygen supply to the water column and the sediments was ensured by aerating the water using an aquarium air stone placed 5 cm above the sediment surface. Larvae were allowed to feed on and grow in these sediments from second to final (4th) larval stage for a period of 22 days at 20°C after which the 4th instar larvae were sieved from the sediments, allowed to clear their guts and freeze-dried. The larval heads were separated from the rest of the body with a surgical lancet, a sample of muscle tissue was obtained from each larva, and head capsules were cleaned in 10% KOH for 2 h to remove the remaining soft tissue. Muscle tissue, head capsules, and sediment samples were analysed separately for stable carbon isotopes on a Euro Vector Elemental Analyser coupled to a Thermo Electron Delta V advantage IRMS. The reference material used was a secondary standard of known relation to the international standard of Vienna Pee Dee belemnite. Replicate sample measurements (n=8) on the internal standard gave an analytical error of ±0.15‰ (2 SD).

δ¹³C of fossil head capsules

Head capsules of chironomid larvae from three different sediments were analysed to obtain a first estimate of the range of $\delta^{13}C$ in fossil material. Sediments with high head capsule concentrations and similar taxa were selected to compare interspecific and intraspecific variability. Surface sediment was collected from a tundra pond in arctic Siberia, located near the River Elon, 25 km from the town of Chokurdakh, Yakutia. Sediment was also analysed from subalpine Hinterburgsee, Switzerland (Heiri et al. 2003), from a depth of 5-7 cm below the sediment water interface, representing an age of ca. 30 years. The oldest sediment originated from the Slotseng lake basin at an archaeological site in Denmark and has an age in the range of 14,800-12,800 calibrated ^{14}C yrs BP (Mortensen 2008). Details for the different sediments can be found in Table 1.

The sediments were deflocculated in 10% KOH for 2 h at room temperature, sieved with 200 μm and 100 μm sieves, and rinsed with demineralized water. Head capsules were picked with a forceps and identified to the highest taxonomic level possible under a dissecting microscope at 40-100× magnification following Brooks et al. (2007). Samples were placed into Eppendorf tubes, treated with ultra clean 1% HCl for 2 h at room temperature to remove all carbonate particles and rinsed twice with ultrapure water (milliQ) using a centrifuge (5 min at 2300 rpm) to concentrate the material. The head capsules were then transferred into pre-weighed ultraclean tin cups and dried on a hotplate at 50°C for 6 h after which the tin cups were re-weighed and crimped for stable isotope analysis. Samples were analyzed on a Fisons NA 1500 NCS Elemental Analyser coupled to a Thermo Electron Delta plus IRMS. The reference material used was a secondary standard of known relation to the international standard of Vienna Pee Dee belemnite. Replicate sample measurements (n=10) on this internal standard gave an analytical error of ±0.06‰ (2 SD).

Table 1 Main characteristics of the lake sediments analyzed for fossil chironomids in this study.

Location	Tundra pond Russia	Hinterburgsee Switzerland	Slotseng Denmark
Coordinates	70°49′46″ N 147°29′13″ E	46°43′5″ N 8°4′2″ E	55°19′48″ N 9°16′17″ E
Approximate age	Modern	20th century	14.8-12.8 cal. ^{14}C ky BP
Altitude (m a.s.l.)	50	1515	40
Dry weight of analyzed sample (g)	0.60	1.37	1.98
Number of head capsules per g dry weight	476	64	979

Results and discussion

Sample size and chemical sample pre-treatment

According to our measurements a minimum sample size of ~20 µg of larval head capsules is needed to generate a signal of at least 1.0 V, the minimum voltage considered to produce reliable $\delta^{13}C$ analyses with the EA-IRMS equipment used for our experiment (Fig. 1). 20 µg corresponds to a minimum of approximately 10 large head capsules such as the 4th instar head capsules of *Chironomus riparius*. However, since our measurements relating the weight of chironomid head capsules with the measured current show a considerable scatter (Fig. 1) we recommend using a larger minimum sample size of at least 30 µg if enough material is available. Wang et al. (2008) observed a systematic error in $\delta^{18}O$ measurements for samples of fossil chironomids of low weight. We could not find evidence for a similar bias in $\delta^{13}C$ measured on chironomid samples with a low weight (Fig. 2), although our samples produced a higher voltage than the threshold of 0.5 V below which Wang et al. (2008) observed their bias.

The percentage of carbon (%C) in the samples is 46.0 ±2.1 %C (n=5) for head capsules that had decayed naturally, 36.5 ±11.3 %C (n=27) for the KOH treatment, 52.5 ±11.3 %C (n=3) for the HCl treatment, 43.3 ±4.0 %C (n=3) for the HF treatment, and 28.9 ±3.7 %C (n=8) for the combined boiling, ASE, and oxidation treatment. $\delta^{13}C$ measurements of head capsules produce a standard deviation of 0.23‰ (n=5) for head capsules that had decayed naturally, 0.26‰ (n=27) for the KOH treatment, 0.15‰ (n=3) for the HCl treatment, 0.31‰ (n=3) for the HF treatment, and 0.09‰ (n=8) for the combined boiling, ASE, and oxidation treatment. All measurements regardless of chemical treatment fall within a range
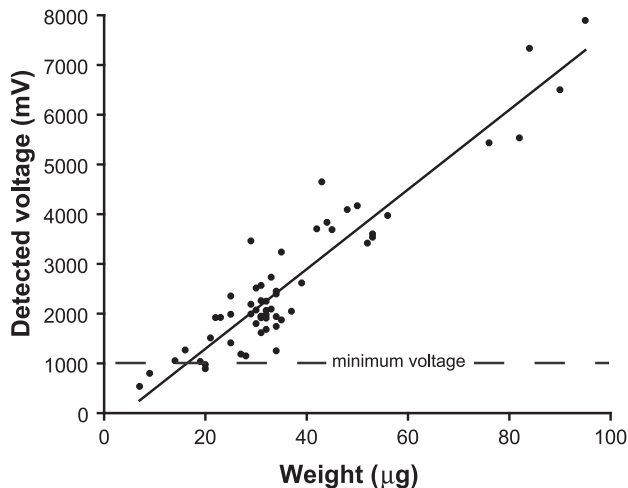

Fig. 1 Detected voltage measured during EA-IRMS for chironomid samples of different weight. The minimum voltage of 1.0 V is based on previous measurements with the analytical equipment used.
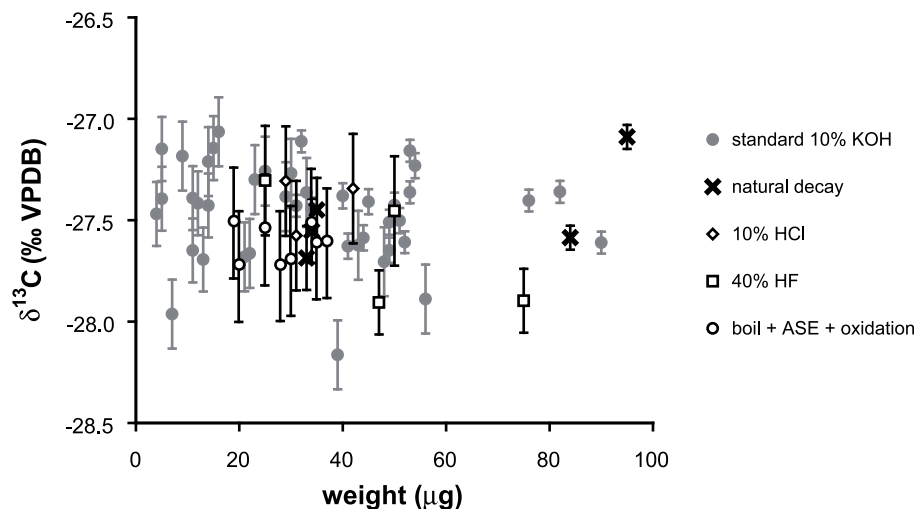
Fig. 2 $\delta^{13}C$ in head capsules of chironomid larvae from the same commercial source processed with different chemical treatments: standard 10% KOH (grey circles); natural decay (black crosses); 10% HCl (open diamonds); 40% HF (open squares); combination of boiling, accelerated solvent extraction (ASE) and oxidation (open circles). Error bars indicate reproducibility of laboratory standards measured for the different EA-IRMS runs.

of ±0.5‰ from the average (Fig. 2) indicating that $\delta^{13}C$ in head capsules can be measured with high precision relative to the values of -70 to -20‰ we expect to encounter in natural chironomid populations (Jones et al. 2008). To some extent the observed variability in $\delta^{13}C$ may be a consequence of the natural variability between chironomid specimens used for our experiments. Although we have no information about the diet of the chironomids supplied from an external source, all chironomid larvae originate from the same batch. We therefore consider it unlikely that the variability of these supplied chironomids exceeds variability of larvae feeding on a similar diet in natural systems. Goedkoop et al. (2006) found a similar ±0.53‰ variation in $\delta^{13}C$ of *C. riparius* larvae that were grown on artificial sediment consisting of ground peat, sand, kaolin clay, and $CaCO_3$. Of the chemical treatments tested in our experiments only the combination of boiling, ASE, and oxidation showed a small, but significant decrease in $\delta^{13}C$ of 0.2‰ (t-test, p = 0.02 at 95% confidence) relative to the standard method consisting of exposure of the head capsules to 10% KOH for 2 h. However, the effect on chironomid $\delta^{13}C$ values was small since head capsules treated in this manner had only on average 0.14‰ lower $\delta^{13}C$ than the mean $\delta^{13}C$ value of all the measurements (Fig. 2).

Schimmelmann and DeNiro (1986a) treated chitin with solutions of 2N HCl at room temperature for 14 h and 1N NaOH at 100°C for 0.5 h and found a change of less than 0.2 ±0.1‰ in $\delta^{13}C$ values. However, long term (up to 600 h) deproteination/deacetilation in a 1N NaOH solution at 25°C caused a 1.5‰ increase in $\delta^{13}C$, although the authors highlight that these treatments were tested on powdered crab chitin that is not as sclerotized and robust as chitin in insect cuticles. (Schimmelmann and DeNiro 1986a). Furthermore, Schimmelmann et

al. (1986) tested the effects of biodegradation on chitin in crustacean carapaces allowed to decay for 10 weeks in moist soil and marine sediment. They found a maximum increase in $\delta^{13}C$ values of 0.4 ±0.2‰ in the decayed carapaces relative to untreated exoskeletons. However, this difference in $\delta^{13}C$ is smaller than the 0.5‰ standard deviation in $\delta^{13}C$ values that we found in analyses of chironomid head capsules.

Based on the close agreement between $\delta^{13}C$ of chironomid head capsules processed with different chemical treatments we conclude that standard palaeoecological pre-treatment methods, such as exposure to 10% KOH for 2 h at room temperature to deflocculate sediments, or elimination of carbonate particles with 1% HCl for 2 h at room temperature, were not influencing $\delta^{13}C$ of head capsules to a significant extent. Our results and those of others described in the literature (e.g. Schimmelmann and DeNiro 1986a) suggest that more intensive chemical pretreatment such as strong oxidation or extended deproteinization/deacetilization would be necessary to produce a noticeable bias in chironomid $\delta^{13}C$.

Culturing experiment

In order to assess the influence of a MOB-enriched diet on $\delta^{13}C$ of chironomid cuticles we cultured chironomid larvae on sediments enriched with MOB grown on ^{13}C-labelled methane. The switch to a MOB-enriched diet had a strong effect on $\delta^{13}C$ of larvae and exoskeletons. On average, $\delta^{13}C$ values of tissue and head capsules of 4th instar larvae cultured on labelled MOB from the 2nd larval instar
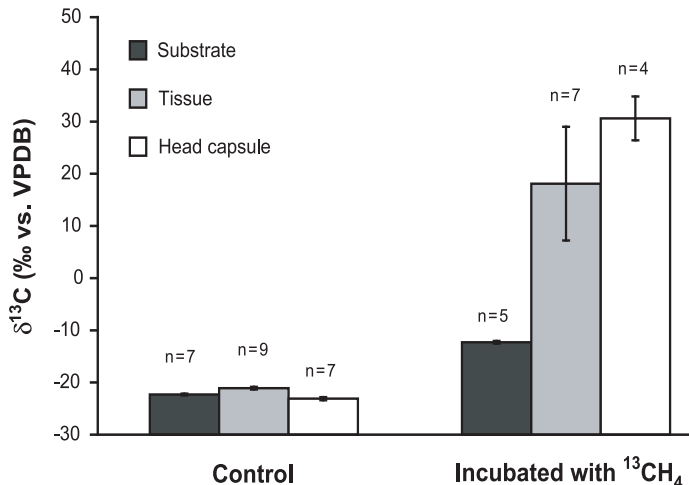
Fig. 3 Effect of labelled, methanogenic carbon in the diet of chironomid larvae on $\delta^{13}C$ of larval tissue and head capsules. The left bar graph shows replicate samples from a control group of fourth instar larvae that were cultured on a diet of commercially available fish food ($\delta^{13}C$ = –22.3 ± 0.2‰). The bar graph on the right indicates replicates from a group of fourth instar larvae that was first raised on a diet of fish food before being cultured from the second larval stage onwards in sediments incubated with ^{13}C-labelled methane ($\delta^{13}C$ of labelled CH_4 = 12,200‰).

onwards became 39 ±10.9‰ and 53 ±4.2‰ enriched, respectively, relative to δ¹³C of larvae fed with the same standard diet during their entire development (Fig. 3). In an almost identical experimental setup, Deines et al. (2007b) found a somewhat larger (52 ±20‰) shift in δ¹³C in larval tissue. However, there is a clear overlap of the standard deviations of the different measurements and this apparent difference is therefore not considered significant.

Previous studies have indicated that bulk tissues of lacustrine chironomid larvae often can be found with markedly depleted δ¹³C values typical of methanogenic carbon (Deines and Grey 2006; Jones et al. 2008). In this study we have demonstrated experimentally that a shift in δ¹³C in the diet of chironomids is transferred not only into the larval tissue but also into the sklerotized head capsules of the larvae. This implies that unless taphonomic processes affect the chemistry of chironomid cuticles, the δ¹³C signal of formerly ingested food will be preserved in the head capsules when they fossilize in lake sediments. It has been shown that chitinous insect cuticles are very resistant to degradation and can be recovered from sediments up to 25 million years old (Stankiewicz et al. 1997). Remains of chironomid larvae have been reported from sediments as old as early Oxygen Isotope Stage-3 (Helmens et al. 2007) or the Eemian Interglacial (Brodersen and Bennike 2003; Ilyashuk et al. 2006) and are common in many younger lake sediment records (e.g. Walker and Cwynar 2006; Brooks 2006). Therefore, δ¹³C of chironomid exoskeletons may provide insights into the past contribution of methanotrophic carbon to benthic food webs of lakes over a range of timescales. In our culturing experiment, neither larval tissue nor cuticles approached isotopic equilibrium with the methane on which we grew the MOB. Partially, this may be due to our experimental design in which larvae were first grown on a diet of fish food before being transferred to the MOB-enriched sediments. However, it is also likely that the *Chironomus riparius* larvae ingested other food items available in our experimental containers (e.g. other organic sediment components and microorganisms growing on them). Furthermore, it is conceivable that fractionation processes disproportionately affect chironomid δ¹³C if the larvae feed on substrates with unnaturally high δ¹³C such as the 12,200‰ of the methane used to culture MOB in our experiments. An important next step would therefore be to explore the relationship between δ¹³C of MOB and chironomids feeding on them if these micro-organisms form the diet of the larvae throughout their entire development.

δ¹³C of fossil head capsules

We measured δ¹³C values of (sub)fossil chironomid head capsules and other chitinous remains from three different lake sediments selected based on their high chironomid content (Table 1). Since our initial test indicated that δ¹³C can be measured at weights as low as 20-30 µg we prepared samples consisting of head capsules from a single species or morphotype where possible and samples consisting of individuals from the same tribe or subfamily from sediments in which fossil head capsules were sparser (Table 2). A sample of *Chironomus anthracinus*-

Table 2 δ¹³C values and carbon content of chironomid head capsules and Cladocera ephippia from different lake sediments. Each row represents one sample.

Material	Location	Age	Number of head capsules in sample	Weight (µg)	δ¹³C (‰ PDB)	%C
Chironomus anthracinus-type	Tundra pond	Modern	21	30	–36.9	46.7
Tanypodinae	Hinterburgsee	20th century	44	30	–31.5	38.1
Tanytarsini	Hinterburgsee	20th century	18	18	–30.4	22.3
Chironomus anthracinus-type	Slotseng	Late-Glacial (14.8-12.8 cal ¹⁴C kyr BP)	25	32	–26.9	39.8
Tanypodinae	Slotseng	Late-Glacial	24	31	–25.8	41.8
Corynocera ambigua	Slotseng	Late-Glacial	26	32	–27.0	35.5
Corynocera ambigua	Slotseng	Late-Glacial	22	26	–27.1	43.2
Corynocera ambigua	Slotseng	Late-Glacial	20	30	–27.2	38.3
Corynocera ambigua	Slotseng	Late-Glacial	21	28	–27.1	37.5
Corynocera ambigua	Slotseng	Late-Glacial	22	28	–27.2	42.0
Corynocera ambigua	Slotseng	Late-Glacial	20	26	–27.1	45.2
Corynocera ambigua	Slotseng	Late-Glacial	20	30	–27.0	32.4
Daphnia ephippia	Slotseng	Late-Glacial	11	32	–28.0	43.1
Daphnia ephippia	Slotseng	Late-Glacial	18	52	–26.5	38.1

type head capsules with a δ¹³C value of –36.9‰ was found in sediments from a Siberian tundra pond. This is comparable to δ¹³C values reported in living *Chironomus* larvae from Arctic lakes in Alaska, Sweden and Finland (Hershey et al 2006; Jones et al 2008). *Chironomus* species are the taxa most commonly reported to incorporate methane-derived carbon by feeding on MOB (Grey et al 2004; Jones et al. 2008). The δ¹³C value of –36.9‰ measured on *C. anthracinus*-type subfossils from Siberia, distinctly lower than the –30 to –18‰ typical of algal biomass reported in the literature (Boutton 1991; Meyers and Lallier-Vergès 1999), suggests a contribution from methane-derived carbon to this taxon's diet in the studied lake ecosystem.

In contrast, the –26.9‰ measured for a sample of head capsules of *C. anthracinus*-type from the Slotseng site does not indicate depleted values. The δ¹³C value at this site is within the range typical for lacustrine algae, suggesting a food source without or with a very low proportion of methanogenic carbon. Tanypodinae and Tanytarsini in Hinterburgsee also show lighter δ¹³C values (–31.5 and –30.4‰, respectively) than comparable taxa in Slotseng (-25.8 and -27.1‰ respectively; value for Tanytarsini calculated as the average of δ¹³C of *Corynocera ambigua*). The former values are low enough to suggest a contribution from a methane-derived carbon source in the diet of chironomid larvae. Tanypodinae are mostly predators and it is not likely that they assimilate MOB directly. However, they may feed on smaller chironomid larvae or oligochaete worms which inhabit the hypoxic sediments and may ingest MOB. Jones et al. (2008) also report values as low as –36.1‰ for the tanypodine genus *Procladius* in Blelham Tarn. At this site *Chironomus* is even further depleted in ¹³C. Possibly

methane-derived carbon is passed on to higher trophic levels in Blelham Tarn by predation on *Chironomus* larvae (Jones et al. 2008). Tanytarsini are generally considered to be collectors-gatherers (Ferrington et al. 2008) and are not known to prey on other invertebrates. The low $\delta^{13}C$ values of Tanytarsini head capsules isolated from the Hinterburgsee sediments suggest that methanogenic carbon may also be incorporated by these chironomids to some extent.

For the Slotseng sediments it was possible to measure replicate samples of head capsules of *C. ambigua*, the dominant chironomid taxon. $\delta^{13}C$ values were very similar for the different replicates, with a mean value of -27.1 ±0.08‰ (n=7). 20-26 head capsules were amalgamated per replicate sample (Table 2). This certainly evens out any marked variability between individuals (see, for example, Grey et al. 2004). Nonetheless, these replicate measurements demonstrate that $\delta^{13}C$ can be analysed on fossil chironomid head capsules from an individual species with a high degree of reproducibility.

Potentially, other chitinous invertebrate remains in lake sediments can be analysed in a similar fashion as chironomid head capsules to study trophic links and carbon cycling within a lake ecosystem in the past. Amongst such remains are cladoceran ephippia, head capsules of non-chironomid midge larvae such as Ceratopogonidae, Simuliidae, or Thaumaleidae, and mites. Indeed, methane-derived carbon has been traced in *Daphnia* biomass in modern lake ecosystems with $\delta^{13}C$ values reported as low as –50.3‰ (Jones et al. 1999; Kankaala et al. 2006, Taipale et al. 2007). Fossil ephippia of the cladoceran *Daphnia* from the Slotseng sediments show $\delta^{13}C$ values of between –28.0 and –26.5‰ (Table 2). This range of 1.5‰ is larger than the difference between Tanypodinae and *C. ambigua* in the same sediments. Such a relatively large variability may be explained by the lack of taxonomic control in our analyses, since the ephippia may originate from different species of *Daphnia*. Alternatively, these cladocerans may feed on algae in different compartments of the water column or the range of ephippial $\delta^{13}C$ may represent seasonal changes in the carbon isotopic signature of particulate organic matter in the water column (Bernasconi et al. 1997). Irrespective of the cause for the larger spread in *Daphnia* $\delta^{13}C$, the carbon isotopic composition of these cladoceran remains indicate that benthic and planktonic organisms in Slotseng fed on food items with a similar carbon isotopic composition, with no evidence that methanogenic carbon played an important role for macroinvertebrates in this lake ecosystem.

Conclusions

We showed that $\delta^{13}C$ is measurable on chironomid head capsules with a high precision (± 0.5‰) relative to the 20–40‰ difference between methanogenic carbon and other carbon sources in lacustrine ecosystems. $\delta^{13}C$ analyses on chironomid head capsules are possible on samples with a comparatively low weight (20-30 μg), allowing taxon specific measurements of chironomid remains belonging to the same species, morphotype, subfamily or tribe. Our culturing experiments with labelled methane clearly show that $\delta^{13}C$ values of food are

not only transferred to the larval tissue but also recorded in the chironomid cuticles. Moreover, $\delta^{13}C$ analyses on fossil chironomid head capsules showed depleted $\delta^{13}C$ values as low as –36.9‰ in one of our sediments, which confirms that methanogenic carbon is incorporated in chironomid exoskeletons. This demonstrates the potential to reconstruct the importance of MOB in the palaeo-diet of chironomid larvae and, indirectly, to provide information on past changes in methane flux from lake sediments.

Although the relation between chironomid larvae and their ingested food should be explored by more extensive rearing experiments, our results show that stable carbon isotopes in fossil chironomid head capsules can give insight in dietary links and carbon cycling in benthic food webs in lakes in the past. An important question that remains is whether it will be possible to quantify the relation between $\delta^{13}C$ in head capsules and the relative amount of MOB ingested by the larvae. Assuming at least a semi-quantitative relationship between the abundance of MOB and the methane production from sediments, this would ultimately allow an estimation of methane flux at the sediment-water interface based on $\delta^{13}C$ of fossil chironomids. To further develop chironomid $\delta^{13}C$ as a potential proxy for past benthic food web changes additional experiments and analyses are required to test the effect of preferential feeding, temperature dependent fractionation, and species-specific fractionation on $\delta^{13}C$ values of head capsules in lakes with different environmental conditions.

Acknowledgements

Chapter 2

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

Abstract

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

Introduction

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

The potential of fossil chironomids for isotope studies has first been shown for ^{14}C dating (Jones et al. 1993; Fallu et al. 2004). High-latitude or high-altitude sites are often devoid of terrestrial plant remains and bulk ^{14}C dates from lake sediments are often too old due to either contamination by allochtonous material or hard-water effects (Olsson 1991; Abbott and Stafford 1996). In such circumstances chironomids can be one of the few reliable sources of carbon available for dating. Recently, chironomid fossils have also been used in stable isotope studies, such as δ^{18}O-based temperature reconstruction (Wooller et al. 2004, 2008) or the reconstruction of lake productivity using stable carbon and nitrogen isotopes (Wooller et al. 2008). One of the major difficulties in all attempts to measure isotopes in chironomid fossils is to attain the required minimum sample mass for isotope analyses. The amount of chironomid material necessary for Accelerator Mass Spectrometry (AMS) ^{14}C dates was reported by Jones et al. (1993) as being 250-400 µg carbon (~800 head capsules), and Fallu et al. (2004) used between 180 and 370 µg chironomids (in their case equivalent to ~1300-2500 head capsules from unsieved sediment). The amount of larval chironomid head capsules necessary for an oxygen isotope measurement is approximately 100 µg (300-700 head capsules; Wooller et al. 2004) with a minimum of 50 µg (approximately 120 head capsules) reported by Wang et al. (2008).

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

Missing certain small taxa due to sieving with a too wide a mesh and excluding them from numerical analyses may not only have considerable effects on the palaeoecological interpretation of the assemblage but can also significantly bias quantitative chironomid-based environmental reconstructions (Heiri and Lotter 2001; Quinlan and Smol 2001a). For isotope analyses, however, mass is often more relevant than the number of individuals, at least when the isotopic composition of chironomids is expected to be similar within a lake basin and

between species of different size. It may thus be beneficial to select a processing method that optimises the preparatory process, i.e. that yields the greatest mass of chironomid fossils in the shortest processing time.

A method to concentrate chitinous fossils from lake and stream deposits is the floatation of insect remains in a denser, apolar organic liquid. Using kerosene, a grade mineral oil, insects can be concentrated from any sediment material (Coope 1986). However, Rolland and Larocque (2007) recently demonstrated that this method yields reduced amounts of large head capsule types such as the 4th instars of *Chironomus* because these are often filled with sediment and are therefore heavier. This is unfortunate because large, heavily sclerotized head capsules provide a disproportionately large share of the chironomid sample mass available for isotope analyses. An additional drawback for carbon and hydrogen isotope analysis is the introduction of carbon and hydrogen from kerosene. Although it may be possible to remove the kerosene by chemically cleaning the samples, the additional time needed for such a cleaning step and the introduction of potential contaminants makes this approach unattractive for isotope studies.

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

Methods

Three types of lake sediments were selected that differ in age, water content, chironomid fossil concentration, and geographic setting (Table 1). Sediment A comes from an unnamed tundra pond in arctic Siberia, collected near the River Elon and the town of Chokurdakh, Yakutia (van Huissteden et al. 2005). Sediment B was collected in subalpine Hinterburgsee, Switzerland (Heiri et al. 2003). Sediment C was collected from the former Slotseng lake basin, an archeological site in Denmark (Mortensen 2008). Further details on the different sediments are given in Table 1.

A known weight of freeze-dried sediment was rehydrated with 10% KOH for 2 hours at room temperature and subsequently sieved with tap water

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

Sediment	A	B	C
Site	Unnamed tundra pond Russia	Hinterburgsee Switzerland	Slotseng Denmark
Latitude	70°49′46″ N	46°43′5″ N	55°19′48″ N
Longitude	147°29′13″ E	8°4′2″ E	9°16′17″ E
Sediment age	Modern	20th century	12.8-14.8 cal.[14]C kyr BP
Altitude (m a.s.l.)	50	1515	40
Water content (%)	98	69	50
Total number of head capsules>100 μm in sample	287	176	2258[*]
Dry weight of analyzed sample (g)	0.60	1.37	1.98
Number of head capsules per g dry weight	476	64	979
Total weight of head capsules >100 μm in sample (μg)	794	118	3550[*]

* based on analysis of half of the 100 and 150 μm fraction. Fractions were subsampled following Heiri et al (2003)

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

Results and discussion

The number and mass of chironomid head capsules in each size fraction vary between the three analyzed sediment types. The total mass of the head capsules per gram dry weight of sediment is very similar in sediments A and C (1317

Table 2 Number of larval head capsules (HC), HC mass, and sorting time for 1.0 g dry weight of sediment samples A, B, and C. Values are calculated for the individual size fractions as well as cumulative for all fractions larger than a given mesh size. The three final columns show the efficiency of sorting fossil chironomids for the different fractions expressed as mass and number of HC per time, and the average mass per HC.

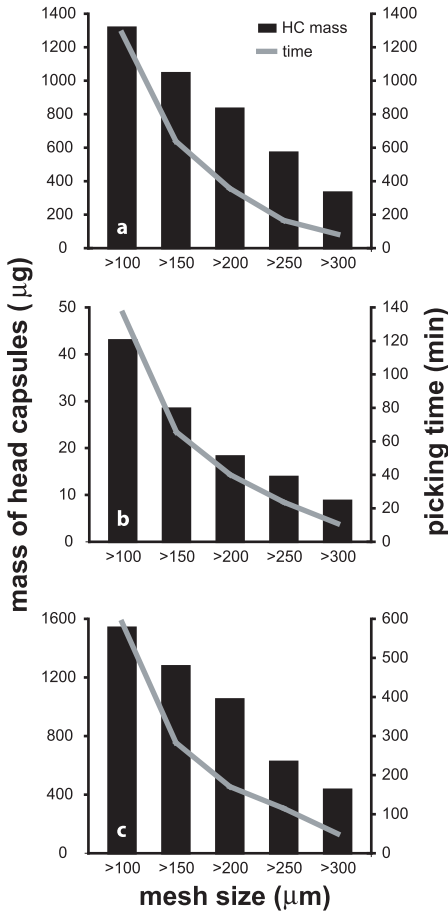|	Mesh size (µg)	# of HC	Cumulative # of HC	Weight (µg)	Cumulative weight (µg)	Sorting time (min)	Cumulative sorting time (min)	Efficiency of sorting (µg/min)	Efficiency of sorting (HC/min)	HC weight (µg/HC)
Sediment A	300	40	40	332	332	83	83	4.00	0.48	8.33
	250	38	78	239	570	83	166	2.88	0.46	6.26
	200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

Fig. 1 Mass of chironomid head capsules (HC) isolated from 1.0 g of dry sediment sieved with a 100, 150, 200, 250, and 300 μm mesh size sieve (black bars) and the associated sorting time (grey line). Values are calculated based on cumulative data from Table 2; a-c refer to the sediment types A-C.
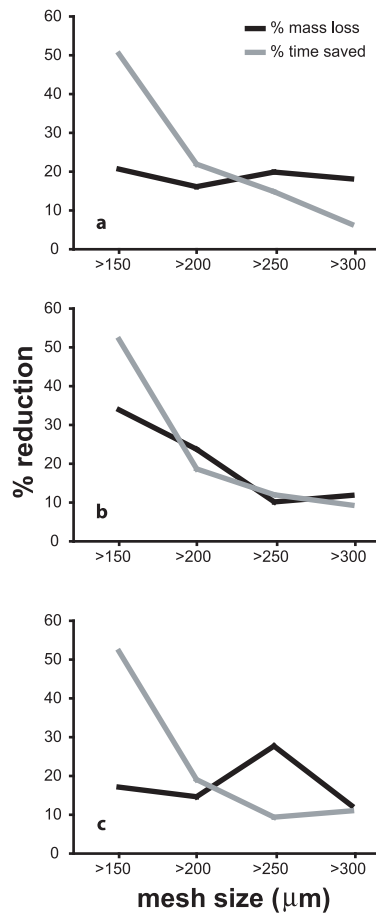
Fig. 2 Relative amount of time saved (grey) and head capsule mass lost (black) when using a mesh size of 150, 200, 250, and 300 μm for sample preparation relative to the sorting time and the head capsule mass retained when using a mesh size that is 50 μm smaller. Values are calculated based on cumulative weight and cumulative sorting time from Table 2; a-c refer to the sediment types A-C.

and 1539 μg, respectively), but only 43 μg in sediment B (Table 2). The highest concentration of head capsules was found in sediment C, which contains 980 head capsules per gram dry weight. This is twice as much as in sediment A and more than 15 times the concentration found in sediment B (Table 2). Furthermore, the average weight of a head capsule is higher in sediment A (4.1 μg) compared with sediments B and C (0.9 and 1.6 μg, respectively), indicating that the average mass of individual head capsules is site-specific.

Processing time for the cumulative sieve fractions decreased exponentially

with increasing mesh size (Fig. 1). The 100 and 150 µm sieve fractions uniformly require 50-52% and 20-22% of overall picking time, respectively, in all three sediments (Table 2). This is disproportional to the mass these fractions yield (Fig. 1). Quantitatively, smaller head capsules dominate in sediments A-C, but their weight contribution to the combined weight of all size fractions varies between sediments (Table 2): in sediment A each size fraction contains a similar mass of head capsules, in sediment B the small size fractions contain a larger mass than the large size fractions, and in sediment C the small size fractions contain a smaller mass than the large size fractions. Table 2 also indicates that the sorting efficiency (fossil mass isolated per unit of time) generally increases with mesh size for each sediment type analyzed in this study. The only clear exception is the >250 µm fraction of sediment C, for which the sorting efficiency is lower than for the >200 and the >300 µm fractions. A possible explanation of this pattern is the relatively large number of light, weakly sclerotized Tanypodinae remains found in the 250 µm size fraction of sediment C. Our results indicate that the time necessary for sorting all chironomids in a sample of sieve residue is reduced by 50-52%, if a 150 µm instead of a 100 µm mesh is used (Table 2). This reduction is very similar to the 50% reduction reported by Verschuren and Eggermont (2007) for African lake sediments.

Smaller mesh size sieves retain more debris particles that can obscure chironomid head capsules. Furthermore, the smaller head capsules are harder to see and handle than the large head capsules retained in large mesh sieves. This explains the exponential increase in picking time if smaller mesh size sieves are used. Larger mesh sizes have the advantage of saving time, but also the disadvantage of losing material that could be used for isotopic analyses. Therefore, a balance must be sought between the reduction of time and the mass loss associated with choosing coarser sieves for sample preparation. In order to find the optimal mesh size, we examined the relative decrease in sorting time and retained mass with increasing mesh sizes. The percentage of time and mass that is reduced by a given mesh size compared with the mesh that is 50 µm smaller is plotted in Fig. 2 for the tested mesh sizes of 150, 200, 250, and 300 µm. As long as the proportion of processing time saved by selecting a larger mesh size is larger than the relative amount of mass that is lost, it seems favorable to use the coarser mesh size. For sediments A and C a mesh size of 200 µm seems optimal, since the proportion of material lost is less than the proportion of sorting time gained by choosing this coarser mesh size. The somewhat different pattern for sediment B suggests that the optimal mesh-size for isolating chironomid remains from sediments depends on the size distribution and morphology (e.g. sclerotization) of head capsules in a given sediment type. However, overall choosing a 200-µm sieve increased the sorting efficiency for all three sediments we examined, and decreased picking time of the head capsules retained in a sample by 71-72% compared to sorting through all material retained in a 100-µm sieve.

Our results have major implications for the potential of fossil chironomids in stable isotope and radiocarbon studies. Fallu et al. (2004) reported that 1300-2400 chironomid head capsules were necessary to obtain 180-370 µg fossils for radiocarbon dating using unsieved lake sediments. Based on the cumulative

data provided in Table 2, the sorting of 180-370 µg of chironomid head capsules processed with a 100 µm sieve would have required 2.9-6.0, 9.5-19.6, and 1.2-2.4 hours of sorting time for sediments A, B, and C, respectively, if head capsules of all sizes would have been picked (Table 3). With the use of a 200 µm sieve the sorting time for the same mass of chironomid head capsules could be reduced to 1.3-2.6, 6.7-13.7, and 0.5-1.0 h, respectively, which is equivalent to a reduction of the sorting time by 56, 30, and 58% (Table 3). This shorter processing time would make it feasible to use chironomid head capsules for ^{14}C analysis at relatively high temporal resolution. Similarly, the isolation of 100 µg of chironomid remains recommended for δ^{18}O analysis by Wooller et al. (2004) would have taken us 1.6, 5.3, and 0.6 h for sediments A, B and C, respectively, if they were sieved with a 100 µm sieve, whereas processing time could have been reduced to 0.7, 3.7, and 0.27 h, respectively, to retrieve the same sample mass after sieving with a 200 µm sieve (Table 3).

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

Our results indicate that sieving of chironomid samples with mesh sizes in the range of 150-200 µm can significantly reduce processing time compared

Table 3 Sorting time needed for isolating the minimum weight of 100 µg of fossil chironomids recommended for δ^{18}O analysis (Wooller et al. 2004) and 180-370 µg recommended for ^{14}C dating (Fallu et al. 2004) if samples are sieved with 100 or 200 µm mesh sieves. Values are calculated using the cumulative data from Table 2.

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

with samples sieved with the standard mesh size of 90-115 μm. Selection of coarser sieve for chironomid sample preparation will therefore enhance the temporal resolution that can be achieved in studies of the isotopic composition of fossil chironomid assemblages. An important caveat, however, is that mesh-size will potentially affect results if different chironomid size classes or taxa are characterized by different isotopic values. For example, the concentration of the stable isotope ^{13}C can be very variable in different chironomid taxa within a lake basin (Grey et al. 2004; Chapter 1), with strongly depleted values reported for some chironomids. A selective enrichment or elimination of head capsules of ^{13}C-depleted chironomids associated with choosing a certain mesh-size would therefore lead to biased isotopic measurements on fossil chironomid samples. In contrast, Fallu et al. (2004) assumed that for ^{14}C analyses the isotopic composition of chironomid fossils indiscriminately reflects isotope concentrations in the lake water. Similarly, Wooller et al. (2004) demonstrated that chironomid δ^{18}O is equilibrated with the δ^{18}O of lake waters in which larvae live, if lakes with short residence times are examined. In these situations it can be expected that mesh-size will have a minor effect on isotopic measurements of fossil chironomid assemblages, although it remains to be demonstrated whether chironomid δ^{18}O is unaffected by the vital effects (e.g. habitat, instar effects, temperature fractionation) which have been described for inorganic remains of lacustrine invertebrates (e.g. Ito 2001).

Acknowledgements

Chapter 3

How representative are subfossil remains of Chironomidae and common benthic invertebrates for the living fauna of Lake De Waay, the Netherlands?

Abstract

The distribution of benthic invertebrates and their subfossil remains was examined within the basin of De Waay, a dimictic, eutrophic lake in the Netherlands. We focused on Chironomidae, but also report the abundances of 11 invertebrate groups that potentially produce chitinous remains that are preserved in the fossil record, although their remains could only be identified at a coarser taxonomic resolution. Most living invertebrates sampled in different seasons were constrained to the littoral zone, with the exception of a few taxa (Ceratopogonidae, *Chaoborus flavicans*, and *Chironomus*) that are adapted to low oxygen conditions in the seasonally anoxic profundal zone. In contrast, assemblages of invertebrate remains in lake surface sediments were similar in the entire lake basin, suggesting that considerable numbers of invertebrate remains are transported and redeposited off-shore in Lake De Waay, due to its steep bathymetry. These results indicate that a single sediment sample obtained from the centre of this lake contains subfossil invertebrate remains originating from the entire lake basin. In Lake De Waay, the majority of taxa found in the living assemblages were identified as remains in lake surface sediments, at least for the Chironomidae that could be identified at a similar taxonomic level in living and subfossil assemblages. Of the total 44 chironomid taxa found in Lake De Waay, 35 taxa occurred in the living assemblages and 34 taxa occurred in the subfossil assemblages. Thirty chironomid taxa occurred both as living and subfossil specimens, and on average these 30 taxa represent 94% of the specimens encountered in a sediment sample. Five rare chironomid taxa present as living larvae were not detected in the subfossil assemblages. Conversely, eight rare and four common chironomid taxa were found in subfossil remains, but not in living assemblages. Our results indicate that subfossil assemblages in surface sediment samples provide spatially integrated and representative samples of the living assemblage. However, a combined approach examining both the living benthic invertebrate fauna and invertebrate remains in lake surface sediments will potentially give a more complete and detailed overview of benthic invertebrates in a lake ecosystem than an approach based exclusively on one of these groups.

Introduction

Invertebrates are ubiquitous in lakes and their remains are preserved in lake sediments. Several groups of aquatic invertebrates have been shown to be sensitive to changes in their environment (Frey 1964; Smol et al. 2001) and are, therefore, often used as indicators in biomonitoring studies (Rosenberg and Resh 1993; Free et al. 2009). Not only is the living community indicative of the present state of lakes, but fossil invertebrate assemblages can also provide information about past changes in lake ecosystems (Frey 1964; Rumes et al. 2005). In addition, the analysis of subfossil invertebrate assemblages in lake surface sediments has potential to be used in biomonitoring, either as a quick screening tool to identify lakes with unusual invertebrate assemblages or as a supplementary method to be used together with sampling of living invertebrates. Lake surface sediments provide spatially and temporally integrated samples (Frey 1988) and, depending on the sedimentation rate, the top cm of sediment may represent several seasons to years of deposited material. Since material is usually transported from shallower sections of lakes towards the deepest parts, sediments obtained from the lake centre will typically include invertebrate remains originating from the entire lake basin, at least in small lakes (Smol 2008).

Several aquatic invertebrate groups have been used in palaeoenvironmental studies (Smol et al. 2001). Of these groups, the chironomids are one of the most abundant benthic macroinvertebrate groups in aquatic ecosystems (Pinder 1995) and have increasingly attracted attention in palaeolimnology over the past two decades. Their larval head capsules often occur in high abundances in sediments and have been used to reconstruct a range of environmental variables such as temperature (Heiri et al. 2007; Ilyashuk et al. 2009), lake trophic status (Lotter et al. 1998; Langdon et al. 2006), water depth (Korhola et al. 2000; Kurek and Cwynar 2009a), and oxygen availability (Quinlan and Smol 2001a; Heiri and Lotter 2003; Verbruggen et al. 2010a).

In many palaeolimnological studies, one sediment core is used to reconstruct past environmental changes for the entire lake system. This approach involves a number of assumptions about how well the subfossil assemblage represents the living community. Good agreement between living and subfossil assemblages have been observed for a number of palaeoecological indicator groups, including, e.g., *Chaoborus* (Quinlan and Smol 2010), Chironomidae (Iovino 1975), and Cladocera (Kattel et al. 2007). Several biotic and abiotic factors can influence the within-lake distribution of subfossil assemblages. Temporal and spatial variation in the living assemblages may result from specific habitat preferences (Frey 1988; Hofmann 1988; Moog 2002). Later, diagenetic processes and transport may affect certain taxa more strongly than others (Walker et al. 1984; Frey 1988; Brodersen and Lindegaard 1999; Eggermont et al. 2007). For chironomids, it has been shown that different subfossil assemblages can be found in different locations within a lake basin (Schmäh 1993; Heiri 2004; Eggermont et al. 2007; Kurek and Cwynar 2009a,b).

In this study, we examined to what extent subfossil remains of common invertebrate groups in a small, stratified Dutch lake accurately represent living

communities. We focused on aquatic invertebrates with subfossil chitinous remains that were retained in a 100 µm-mesh sieve. These included several groups of Diptera (especially chironomids), Bryozoa, Coleoptera, and Oribatida. Subfossil assemblages in surface sediments were compared with living specimens that were collected in the same lake. Information on how well subfossil assemblages represent the living community is vital to accurately interpret downcore palaeolimnological records and to compare assessments based on lake surface sediments with other biomonitoring methods (Sayer et al. 2010).

Methods

Surface sediments and samples of living invertebrates were obtained from Lake De Waay, a hypertrophic hardwater lake in the Netherlands with a maximum water depth of 15 m and a surface area of 1.3 ha (see Table 1 and Fig. 1 for location and general characteristics). The lake is a scour hole that was formed

Table 1. Physical characteristics and water chemistry of Lake De Waay. The range of measured values (n=3) is indicated and average values are given in parentheses.

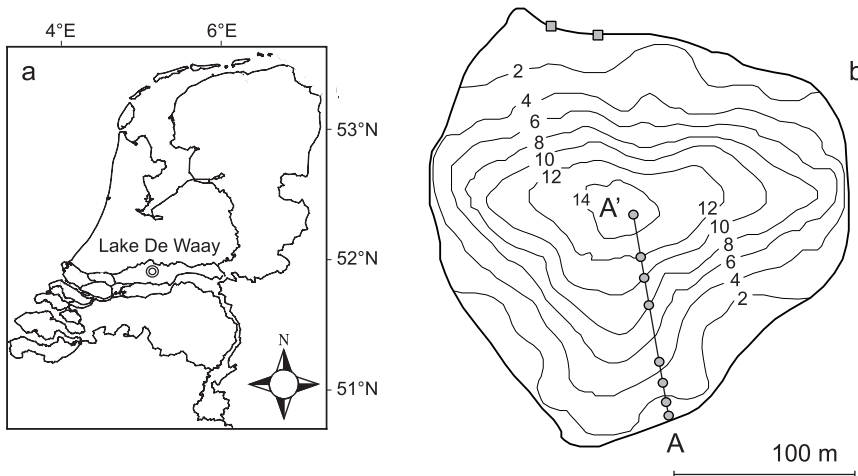| Location	51°55'55" N /5° 8'59" E
Area (ha)	1.3
Maximum depth (m)	15
Secchi Depth (m)	1.2 0 - 2.15 (1.43)
pH	7.5 - 8.6 (7.9)
Conductivity (μS cm^{-1} at 25 °C)	441 - 544 (474)
Total P (μg L^{-1})	105 - 119 (112)
Total N (mg L^{-1})	1.17 - 2.08 (1.82)
Dissolved organic carbon (μmol L^{-1})	468 - 553 (493)

Fig. 1. (a) Location of Lake De Waay in the Netherlands, and (b) Bathymetry of the basin and location of the sediment samples along a transect (A-A') from the littoral to the profundal. Grey circles indicate sampling stations along the transect and grey squares indicate locations of additional kick net samples.

by a dike breach in AD 1496 (Kirilova et al. 2010) and is partly surrounded by dykes at present. Agriculture, recreational activities, overwintering waterfowl, and local water management in the catchment heavily influence the lake, and sedimentation rates in the central part of the lake basin are high, approximately 2.3 cm per year (Kirilova et al. 2010). De Waay is a dimictic lake with a seasonally anoxic hypolimnion. The lake is usually stratified from April to October with a thermocline between 3 and 6 m water depth (Fig. 2; Leentvaar 1958). It could, therefore, be expected that distinct changes in the invertebrate community occur with water depth.

Samples of living invertebrates were collected in 2007-2009 once at the end of summer (13 September 2007), once at the beginning of spring (22 April 2008), and once in winter (17 February 2009). These samples were taken with a 15 x 15 x 15 cm Ekman grab at eight stations along a depth transect and immediately sieved

Fig. 2 Oxygen and temperature profiles of Lake De Waay after stratification took place (April 22, 2008).

with a 0.5 mm mesh-size net. A single grab of 3.5 L of sediment per station was taken from the littoral and sublittoral zones (<4 m depth) and double grabs from the profundal (>4 m depth) part of the basin. Additional kick net samples were taken from the shoreline between emerging macrophyte vegetation with a 0.5 mm net. All invertebrate samples were sorted the day after fieldwork in the laboratory, identified following Grontmij (2010) and then preserved in alcohol. Samples taken at different seasons were pooled at each station along the transect to allow for numerical analysis and plotting, and to include similar time intervals for living assemblages (representing 3 seasons in 1.5 years) and subfossil assemblages (representing ca. 1-2 years in the top 2 cm of sediment; Kirilova et al. 2010).

Surface sediments for obtaining subfossil invertebrate remains were sampled at the same eight stations along the depth transect with a HTH sediment corer (Renberg and Hansen 2008). Sediment cores were subsampled in the field into 2 cm slices and only the top 2 cm slice was used, except at 15 m water depth where the top 4 cm was used because of low concentrations of subfossils. Sediments were deflocculated for 2 h in 10% KOH at room temperature and sieved over a 100 μm sieve. Subfossil remains were picked out individually using a stereo microscope (25-40x magnification) with fine forceps. Between 36 and 72 chironomid head capsules for each sample were identified using a light microscope at 60-600x magnification. Remains of chironomids were identified following Wiederholm (1983), Rieradevall and Brooks (2001) and Brooks et al. (2007). Other invertebrate remains were identified at a coarser taxonomic resolution following Frey (1964), Reusch and Oosterbroek (1997), Vandekerkhove et al. (2004), Wood and Okamura (2005), and Brooks et al. (2007).
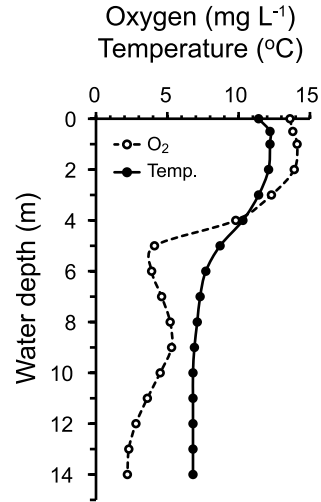
To make the data of living and subfossil assemblages comparable, modern and subfossil taxonomy were harmonized by pooling equivalent taxa in living and subfossil assemblages following Tables 2 and 3. This resulted in a harmonized taxonomy of 47 invertebrate taxa that could be applied to both living and subfossil assemblages. Furthermore, we transformed the data into percentages (Clarke and Warwick 2001). Detrended correspondence analysis (DCA) was performed using the program CANOCO version 4.52 (ter Braak and Šmilauer 2003) to explore patterns in the distribution of invertebrate taxa. DCA was run on the harmonized taxonomy that included 47 invertebrate taxa, with detrending by segments, square-root-transformation of species abundances and down-weighting of rare taxa.

Results

Occurrence of taxa in Lake De Waay

A total of 101 invertebrate taxa were identified in Lake De Waay, 84 taxa in the living assemblages and 45 taxa in the subfossil assemblages (Tables 2 and 3). A number of invertebrate taxa was exclusively encountered in the living assemblages (24 taxa) or in the subfossil assemblages (18 taxa). The number of chironomid taxa found in the living assemblages (35 taxa) and in the subfossil assemblages (34 taxa) was similar. In Table 2, we compare corresponding chironomid taxa in living and subfossil assemblages. Of the total 44 chironomid taxa found in Lake De Waay, 30 taxa occurred both as living and subfossil specimens. On average, these 30 taxa represented 94% (SD = 7.1%) of the specimens encountered in a sediment sample.

Five rare chironomid taxa, occurring once in the living assemblages, were not found as subfossils (*Apsectrotanypus trifascipennis, Stictochironomus, Tanypus kraatzi, Tanypus vilipennis,* and *Tanytarsus eminulus* gr.*)*. Eight rare chironomid taxa, occurring once or twice in the subfossil assemblages, were not found as living organisms (*Cladopelma laterialis*-type, *Dicrotendipes notatus*-type, *Kiefferulus, Metriocnemus eurynotus*-type, *Microtendipes rydalensis*-type, *Paramerina, Psectrocladius sordidellus*-type, *Tanytarsus pallidicornis*-type, and *Xenochironomus xenolabis*). Also, four common chironomid taxa in subfossil assemblages (*Corynoneura edwardsi*-type, *Endochironomus tendens*-type, *Limnophyes,* and *Tanytarsus pallidicornis*-type) were not found as living organisms. Living invertebrates were only sampled in the sediment and no epiphytic or planktonic samples were taken. Therefore, we did not include any Daphniidae or Bryozoa in the live assemblages, although their resting stages were encountered in subfossil assemblages. Taxonomic resolution of non-chironomid invertebrate remains was much coarser than that of chironomid head capsules and several invertebrate taxa found in living assemblages (Coleoptera, Heteroptera, Malacostraca, and Odonata) were not recognized in subfossil assemblages.

Table 2 Comparison of chironomid taxa identified in living and subfossil assemblages in Lake De Waay, with references for identification of subfossil remains. Taxa found in the living assemblages are aligned with their equivalent subfossil taxa. Taxa that only occur in either living or subfossil assemblages are printed in bold.

Living assemblages	Subfossil assemblages	Reference subfossil ID
Ablabesmyia longistyla	*Ablabesmyia*	Rieradevall and Brooks (2001)
Apsectrotanypus trifascipennis		
Chironomus annularius agg *Chironomus commutatus*	*Chironomus anthracinus*-type	Brooks et al. (2007)
Chironomus muratensis *Chironomus plumosus agg.*	*Chironomus plumosus*-type	Brooks et al. (2007)
	Cladopelma lateralis-type	Brooks et al. (2007)
Cladotanytarsus	*Cladotanytarsus mancus*-type1	Brooks et al. (2007)
	Corynoneura edwardsi-type	Brooks et al. (2007)
Cricotopus intersectus *Cricotopus intersectus agg.*	*Cricotopus intersectus*-type *Cricotopus laricomalis*-type	Brooks et al. (2007) Brooks et al. (2007)
Cricotopus sylvestris gr.	*Cricotopus sylvestris*-type	Brooks et al. (2007)
Cryptochironomus	*Cryptochironomus*	Brooks et al. (2007)
Dicrotendipes nervosus	*Dicrotendipes nervosus*-type	Brooks et al. (2007)
	Dicrotendipes notatus-type	Brooks et al. (2007)
Endochironomus albipennis	*Endochironomus albipennis*-type	Brooks et al. (2007)
	Endochironomus tendens-type	Brooks et al. (2007)
Glyptotendipes paripes	*Glyptotendipes barbipes*-type	Brooks et al. (2007)
Glyptotendipes pallens agg.	*Glyptotendipes pallens*-type	Brooks et al. (2007)
	Kiefferulus	Wiederholm (1983)
	Limnophyes	Brooks et al. (2007)
	Metriocnemus eurynotus-type	Brooks et al. (2007)
Microtendipes chloris *Microtendipes chloris gr.*	*Microtendipes pedellus*-type	Brooks et al. (2007)
	Microtendipes rydalensis-type	Brooks et al. (2007)
Nanocladius bicolor agg.	*Nanocladius rectinervis*	Brooks et al. (2007)
Parachironomus arcuatus *Parachironomus arcuatus gr.*	*Parachironomus varus*	Brooks et al. (2007)
	Paramerina	Rieradevall and Brooks (2001)
Phaenopsectra	*Phaenopsectra* type A *Phaenopsectra flavipes*-type	Brooks et al. (2007) Brooks et al. (2007)
Polypedilum nubeculosum	*Polypedilum nubeculosum*-type	Brooks et al. (2007)
Polypedilum sordens	*Polypedilum sordens*-type	Brooks et al. (2007)
Procladius (Holotanypus)	*Procladius*	Rieradevall and Brooks (2001)
	Psectrocladius sordidellus-type	Brooks et al. (2007)
Stictochironomus		
Tanypus kraatzi **Tanypus vilipennis**		
Tanytarsus eminulus gr.		
Tanytarsus excavatus *Tanytarsus excavatus gr.* *Tanytarsus lestagei agg.* *Tanytarsus mendax* *Tanytarsus mendax gr.* *Tanytarsus mendax/occultus*	*Tanytarsus mendax*-type	Brooks et al. (2007)
	Tanytarsus pallidicornis-type	Brooks et al. (2007)
Tribelos intextum	*Tribelos*	Brooks et al. (2007)
	Xenochironomus xenolabis	Brooks et al. (2007)

Table 3 Non-chironomid taxa found in the living and subfossil assemblages of Lake De Waay, with references for identification of subfossil remains. Taxa found in the living assemblages are aligned with their equivalent fossil taxon. Taxa that only occur in either living or subfossil assemblages are printed in bold. Taxon names for groups not found in subfossil assemblages are given in parentheses.

| Living assemblages	Subfossil assemblages	Reference subfossil ID
Arrenurus crassicaudatus *Arrenus latus* *Hydrodoma despiciens sensu lato* *Hygrobates lonipalpis* *Limnesia maculata sensu stricto* *Limnochares aquatica* *Neumania vernalis* *Piona imminuta* *Pionidae* *Unionicola*	Oribatida	Frey (1964)
Haliplus **Hyphydrus ovatus** **Noterus clavicornis** **Stenopelmus rufinasus**	(Coleoptera)	
Gammarus pulex **Gammarus tigrinus** **Asellus aquaticus**	(Malacostraca)	
Ceratopogonidae	Ceratopogonidae	Brooks et al. (2007)
Chaoborus flavicans	*Chaoborus spp.*	Brooks et al. (2007)
(Bryozoa)	**Cristatella mucedo** **Plumatella spp.** **Lophopus crystallinus**	Wood and Okamura (2005)
(Daphniidae)	**Ceriodaphnia** **Daphnia**	Vandekerkhove et al. (2004)
(Limoniidae)	**Limoniidae**	Reusch and Oosterbroek (1997)
Caenis horaria *Caenis luctuosa* *Caenis robusta* *Cloeon dipterum* *Cloeon simile*	Ephemeroptera	Brooks et al. (2007)
Callicorixa praeusta **Hesperocorixa linnaei** **Huydrometra stagnorum** **Micronecta** **Notonecta glauca** **Sigara falleni** **Sigara lateralis** **Sigara striata**	(Heteroptera)	
Aeshna mixta **Coenagrionidae** **Ishmura elegans** **Zygoptera**	(Odonata)	
Anabolia nervosa *Cyrnus flavidus* *Cyrnus trimaculatus* *Ecnomus tenellus* *Limnephilidae* *Lype paeopa* *Molanna angustata* *Mystacides nigra* *Oecetis furva* *Orhotrichia costalis* *Polycentropodidae* *Tinodes waeneri* *Triaenodes bicolor*	Trichoptera	Frey (1964)
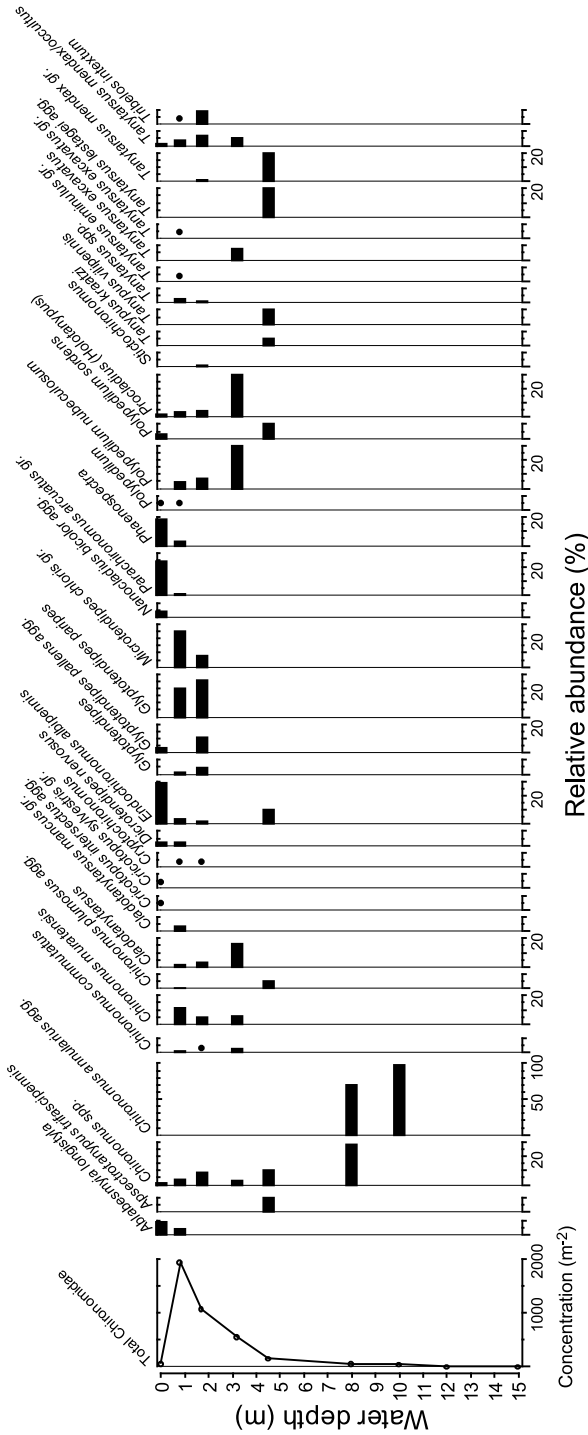
Fig. 3 Relative abundances of living chironomid larvae in Lake De Waay at different water depths. All taxa are plotted, and low abundances (<1%) are indicated by black dots. Maximum values of the x-axes are 10% unless labeled otherwise. The values are averages of three sampling campaigns (13 September 2007, 22 April 2008, and 17 February 2009).

Fig. 4 Total number of chironomid head capsules per gram sediment and relative abundance of all identified subfossil remains in the sediments of Lake De Waay at different water depths. Maximum values of the x-axes are 10% unless labeled otherwise.

Distribution of taxa along a depth transect and DCA

The highest concentrations of living invertebrates (2963 individuals m^{-2}) were found in the sample at 0.8 m water depth, and abundances decreased with depth (Figs. 3 and 5). The only taxa found in the deepest part were larvae of *Chironomus annularius* at 8 and 10 m water depth, Ceratopogonidae at 10 and 12 m water depth, and *Chaoborus flavicans* larvae that were present at high absolute abundances (ca. 350-1600 larvae m^{-2}) in samples taken at 8 m water depth and below. Subfossil assemblages were similar in samples from different water depth (Figs. 4 and 5) and the highest concentrations of subfossil invertebrate remains were found at 4.5 m water depth (95 remains g^{-1} dry sediment).

These patterns are apparent in the DCA, which was run using the harmonized invertebrate taxonomy (Fig. 6). The first and second DCA axes accounted for 30.6% and 9.5% of the variance within the faunal data, respectively. The samples of living assemblages showed a range of 4.3 and 1.4 standard deviation units along Axis 1 and 2, respectively, and the samples of subfossil assemblages showed a range of 0.4 and 0.3, respectively. In general, the nine samples of living assemblages were arranged along axis 1 according to water depth, the deepest sample at 15 m being most different from the shoreline sample at 0 m. The eight samples with subfossil assemblages on the other hand were remarkably similar in the ordination plot and no clear depth-related pattern was observed in the invertebrate assemblages of these samples. The DCA sample scores indicated that the difference between living and subfossil assemblages became larger with increasing water depth as the subfossil assemblages were most similar to the living assemblages collected in the littoral zone between 0.8-3.2 m water depth.

Chaoborus flavicans was characterized by the lowest DCA axis 1 scores followed by Ceratopogonidae, *Chironomus anthracinus*-type, and *Chironomus* spp. (Fig. 6) and these taxa plotted relatively close to samples taken at 8 m water depth and below. Most taxa of Chironomidae, Coleoptera, Oribatida, Ephemeroptera, and the remains of Daphniidae and *Plumatella* were characterized by higher DCA axis 1 scores and plotted close to the sample of the living assemblage at 0-3.2 m water depth in the DCA plot. Other invertebrate groups, including Heteroptera, Limoniidae, Malacostraca, Odonata, Trichoptera, and the remains of *Cristatella mucedo* and *Lophopus crystallinus* plotted close to samples of living assemblages from the shallowest samples (0-1.7 m) in the DCA plot.

Discussion

Occurrence of taxa in Lake De Waay

Overall, the taxonomic composition of the living and subfossil invertebrate assemblages in Lake De Waay was similar, at least for chironomids that could be identified at a comparable taxonomic level in living and subfossil assemblages.

However, a number differences existed between living and subfossil assemblages because some taxa were exclusively encountered as living animals or as subfossil remains. There are a number of potential reasons for these differences that we will discuss here.

Remains that are thin and easily damaged or broken may cause an underrepresentation compared with living invertebrates. For example, Walker et al. (1984) suggested that *Procladius* may be poorly preserved in some lake sediments. However, in our study, remains of *Procladius* are common in both living and subfossil assemblages (Fig. 7) and other tanypodine chironomid taxa that produce thin remains are either present in both living and subfossil assemblages (*Ablabesmyia*), or have only a single occurrence in the living assemblages (*Apsectrotanypus*).

Taxa occurring in low numbers in Lake de Waay, such as Coleoptera, and the chironomid tribes Orthocladiinae and Tanypodinae, may have been missed due to limited sampling. For example, of the six taxa of Tanypodinae found as living larvae only three (*Ablabesmyia, Paramerina, and Procladius*) were represented as subfossils. Conversely, of the eight taxa of Orthocladiinae found as subfossils, only *Nanocladius* and three taxa in the genus *Cricotopus* were found as living larvae (Table 2). However, the number of living invertebrates found at each sampling station was high (49-1173, with the exception of 23 specimens at 4.5 m water depth), and the number of identified chironomid head capsules (36-72) from subfossil samples should provide representative counts and include the most dominant chironomid taxa (Heiri and Lotter 2001; Quinlan and Smol 2001b).

Several genera that were encountered only in subfossil assemblages such as *Corynoneura*, *Dicrotendipes*, *Endochironomus*, and *Metriocnemus* are often associated with macrophytes (Pinder and Reiss 1983; Brodin 1986; Brodersen et al. 2001; Merritt et al. 2008; Moller Pillot 2009). Other groups, such as Bryozoa, are associated with woody substrates or aquatic macrophytes (Wood and Okamura 2005) and *Xenochironomus xenolabis* is known to be a parasite on Spongillidae (Pinder and Reiss 1983; Moog 2002). More extensive sampling for living invertebrates on specific substrates could have added some of these taxa to the living assemblages. Similarly, certain taxa may not have been sampled because sampling of living invertebrates took place on only three dates. The timing of these sampling days allowed us to collect specimens at different moments in the seasonal cycle, but ideally, the living assemblages should have been sampled more often to obtain a more complete overview.

A reason for the absence of some invertebrate groups (Coleoptera, Heteroptera, Malacostraca, Odonata) in the subfossil assemblages might be the lack of keys for the identification of characteristic remains. This is also the reason for the loss of taxonomic detail for Ephemeroptera, Oribatida, and Trichoptera. However, detailed keys are available for chironomid remains and in this group similar taxa are encountered in living and subfossil assemblages, although the taxonomic detail was greater in living assemblages (Table 2). The majority of chironomid taxa occurred in both living and subfossil assemblages. In living assemblages, only five rare taxa were found of which no remains were
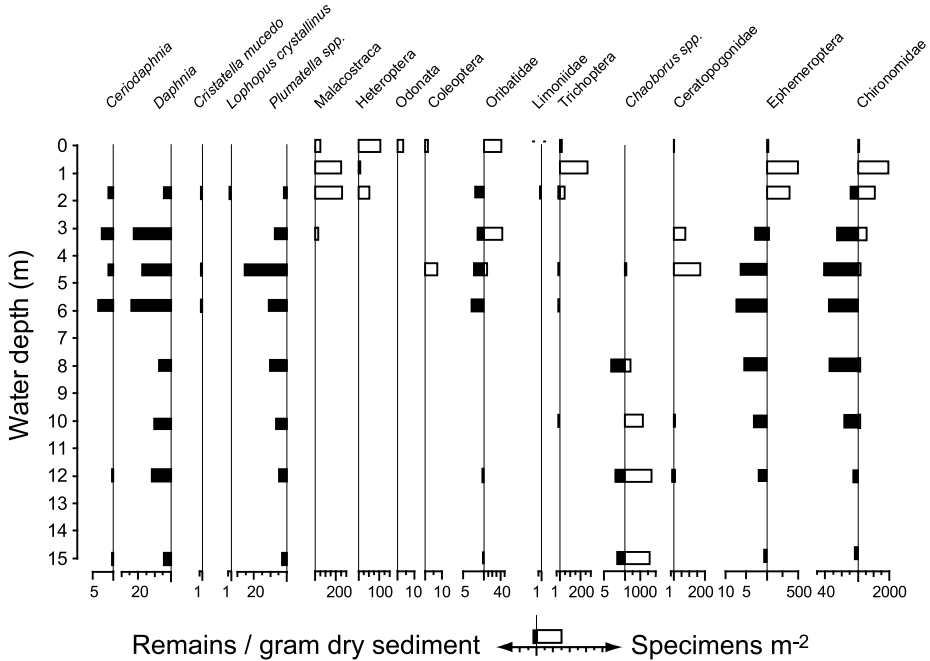
Fig. 5 Comparison between concentrations of all living invertebrates and their remains identified in Lake De Waay at different water depths. The values for living larvae are averages of three sampling campaigns on 13 September 2007, 22 April 2008, 17 February 2009.

encountered. However, eight rare and four common chironomid taxa in the subfossil assemblage were not found in the living assemblage. This suggests that in Lake De Waay the palaeolimnological approach provided a more complete overview of chironomid taxa than the sampling of living larvae during three seasons.

Distribution of taxa along depth transect

The only taxa found in living assemblages in the deepest part of the lake were larvae of Ceratopogonidae, *Chironomus annularius*, and *Chaoborus flavicans* (Figs. 3 and 5). The latter two of these taxa are exceptionally well adapted for surviving at low oxygen levels in the hypolimnion (Hofmann 1986; Jager and Walz 2002; Luoto and Nevalainen 2009). Oxygen availability is one of the key factors for the distribution of invertebrates in stratified lakes (Pinder 1995; Jager and Walz 2002). In Lake De Waay, oxygen levels decrease with depth (Fig. 2) and during summer stratification the profundal is anoxic. Therefore, changes in the invertebrate community with water depth, indicated by the position of samples along DCA axis 1 (Fig. 6), are likely to be related to oxygen availability. The living assemblages in shallow water samples (≤3.2 m water depth) are distinctly different from the living assemblages in deep water samples (≥8 m water depth),

and it is interesting to note the sample at 4.5 m water depth, located approximately at the thermocline, fell in between. These findings are in line with several studies that have indicated the potential to use subfossil chironomid assemblages to infer hypolimnetic oxygen levels (Quinlan and Smol 2001a; Brodersen and Quinlan 2006).

Water temperature may also have affected the in-lake distribution of invertebrates. In dimictic lakes, such as Lake De Waay, water temperatures show large annual variations in the littoral and sublittoral of the lake basin, whereas the deeper section of the basin will remain cool during the summer months (in De Waay typically not exceeding 8°C). A number of invertebrate taxa are known to be restricted to low temperatures during their larval development and their larvae are limited to the cool hypolimnion during the summer months, whereas other taxa are dependent on warmer temperature to complete their development (Brinkhurst 1974; Pinder 1995; de Mendoza and Catalan 2010).

Substrate composition also has a strong influence on chironomid distribution (Pinder 1986; de Mendoza and Catalan 2010) and changed within Lake De Waay from the shore towards the lake centre. In the deepest section of the lake, the only substrate available for benthic invertebrates consists of soft organic mud. In shallower sections of the lake, particle size of the sediments becomes coarser and habitats are supplemented by macrophytes, leaf litter, and dead wood. Several authors observed that locations with macrophytes generally have a greater complexity of habitats available for aquatic invertebrates as well as more diverse subfossil assemblages than macrophyte-free areas (Pinder 1995; Eggermont et al. 2007, 2008; de Mendoza and Catalan 2010). Macrophytes are an
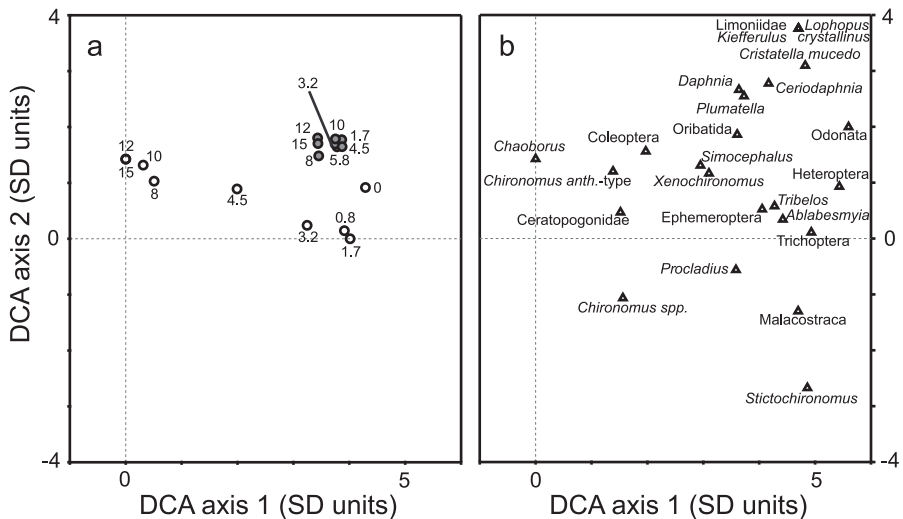

Fig. 6 Detrended correspondence analysis (DCA) plot based on living and subfossil assemblages from different water depths in Lake De Waay. (a) Samples of living assemblages (open circles) and subfossil assemblages (grey circles) with their respective depth indicated. (b) Invertebrate taxa occurring in both living and subfossil assemblages.

important food source and provide habitats for many taxa, and may also provide shelter or escape from predation (Merritt et al. 2008). Eggermont et al. (2008) investigated a depth transect in Lake Tanganyika, East Africa, and observed the highest densities of living chironomid larvae at 3-5 m depth at locations with macrophytes. Rumes (2010) collected both living invertebrates and their remains in 61 African crater lakes and observed that a significant part of the variation in living and subfossil assemblages was explained by the diversity of macrophytes. Several groups of aquatic insects and *Plumatella* were found clinging to or living between submerged or emerging macrophytes (Rumes 2010). Although we did not extensively sample macrophytes for living invertebrates, we did observe high invertebrate diversity and abundances in the sediment samples from the macrophyte zone (0-1.7 m) in Lake De Waay.

Taphonomy of invertebrate remains

The distribution of subfossil assemblages along the sampling transect in Lake De Waay was distinctly different from the distribution of the living invertebrates from which the remains originate. For the majority of taxa, the subfossil remains had a remarkably homogeneous distribution compared to living assemblages. This is demonstrated in the DCA plot, in which samples of living assemblages are distributed according to water depth along axis 1 (Fig. 6), whereas samples of subfossil assemblages plot together. The subfossil assemblages in Lake De Waay are most similar to the living invertebrate community in the littoral zone, between 0.8-3.2 m water depth. Similar results were found by Brodersen and Lindegaard (1999) who observed that the subfossil assemblages sampled in the lake centre reflected the chironomid communities in the littoral at a depth of 2-7 m in four Danish lakes.

The discrepancy between living and subfossil assemblages (Figs. 5 and 7) could be partly explained by the limited temporal and spatial sampling of living invertebrates as indicated above. However, this cannot explain the high number of subfossil remains of littoral taxa in the profundal of Lake De Waay, which can be explained (at least in part) by transportation of subfossils (Frey 1988). Physical lake characteristics such as lake surface area, volume, morphometry, and sediment type determine the influence of in-lake currents and, hence, can have a large influence on the amount of transport of chitinous remains that occurs (Hilton 1985; Frey 1988). Redistribution of littoral remains off-shore depends strongly on basin morphometry (Frey 1988; Eggermont et al. 2007). In our dataset, we have only found evidence for off-shore displacement of remains from the location at which the corresponding living organisms were found, and never in a shoreward direction. For example, living larvae of *Dicrotendipes nervosus-type, Endochironomus albipennis*-type, Ephemeroptera, and Oribatida were found only in samples from ≤4.5 m water depth. However, their remains were abundant in profundal sediments (Figs. 5 and 7). The steep basin morphology of Lake De Waay may be an important factor in explaining the transportation of invertebrate remains as sediment focusing is more pronounced in basins with steep slopes
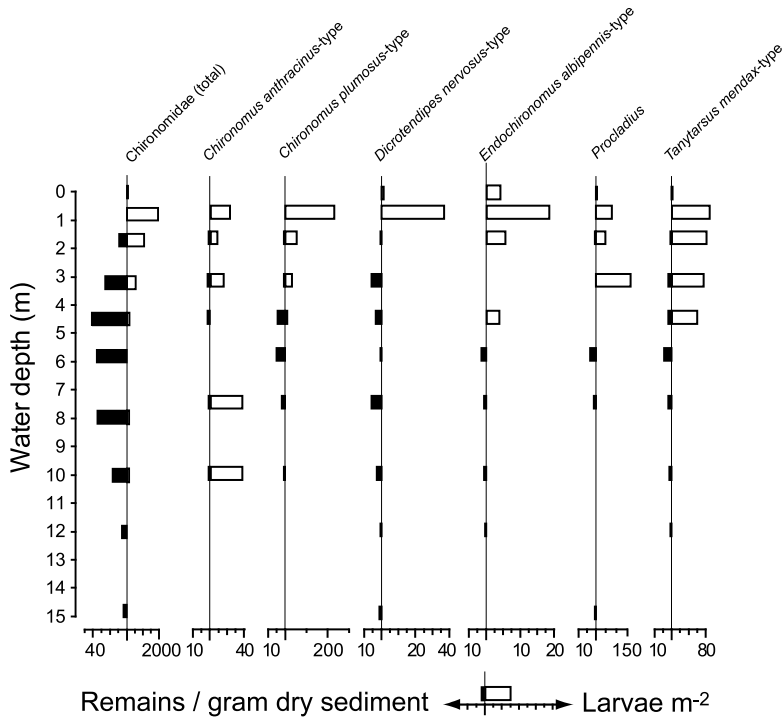
Fig. 7. Comparison between concentrations of six chironomid taxa and their remains in Lake De Waay at different water depths. The values for living larvae are averages of three sampling campaigns on 13 September 2007, 22 April 2008,and 17 February 2009.

(Kansanen 1986).

Overall, this is in agreement with the results of previous studies that found evidence for off-shore transport of invertebrate remains. Evidence for transport of chironomid remains to deeper sections of lakes was observed in shallow and continually mixing lakes (Iovino 1975; Hofmann 1986; Eggermont et al. 2007; Holmes et al. 2009) as well as in deeper, stratifying lakes (Wiederholm 1979; Schmäh 1993; Brodersen and Lindegaard 1999; Heiri 2004). In a survey of African crater lakes, more invertebrate taxa were recovered as subfossil remains from surface sediments than by collecting living invertebrates (Rumes et al. 2005; Rumes 2010), which may also be the result of the limited spatial and temporal sampling of living invertebrates. It is unlikely, however, that transport of remains can cause a complete integration of littoral and profundal communities (Frey 1988; Heiri 2004). Moreover, transport of remains does not play an important role in all lakes (Iovino 1975; Walker et al. 1984; Kurek & Cwynar 2009b; Luoto 2010). The most detailed study previously available that compared living and subfossil assemblages of chironomids by Iovino (1975) demonstrated that communities and their remains were qualitatively and quantitatively similar in a number of lakes. In the lakes studied in this survey, the ratio between the mean number of larvae per m² and the number of remains per 100 ml surficial sediment was

approximately 0.1, although higher ratios were also observed. However, in lakes that are prone to wind-driven currents, Iovino (1975) observed transportation of subfossil remains from typical littoral taxa to the sublittoral and from sublittoral taxa to the profundal. The importance of wind for transport of remains is also recognized by others (Schmäh 1993; Eggermont et al. 2007; Holmes et al. 2009). Similarly, currents by inflowing streams can transport lotic and littoral remains into deeper parts of the basin (Heiri 2004; Bigler et al. 2006; Heiri and Lotter 2007; Luoto 2010).

　　　　The highest concentrations of living invertebrates in Lake De Waay were observed at 0-3.2 m water depth, whereas the highest concentrations of subfossil remains were observed at 4.5 m water depth in the sublittoral. This pattern may be explained by different sedimentation rates within the basin and, possibly, by the location of the thermocline around 5 m water depth in Lake De Waay. Iovino (1975) observed a similar pattern that was most clear in transects influenced by wind-generated currents. Wind and wave action will be weaker below the thermocline and a relatively large number of subfossil remains could be expected to be redeposited at the thermocline. Schmäh (1993) also observed a peak in concentrations of chironomid subfossils in the sublittoral zone of Lake Constance. This peak occurred at a depth of approximately 4-11 m. It is unclear, however, whether this coincided with the depth of the thermocline during stratification or with the depth at which the slope of the lake bottom became steeper. There was no indication of a thermocline effect on the concentrations of chironomid remains studied by Heiri (2004). Highest subfossil concentrations were found in the profundal of four of the five relatively shallow Norwegian lakes that he studied. Only one dystrophic lake with low oxygen conditions in the hypolimnion had highest concentrations at the thermocline depth in the sublittoral, but this was likely due to limited production of chironomid remains in the profundal. Kurek and Cwynar (2009b) sampled chironomid remains at different water depths in three lakes in western Alaska that have a comparable bathymetry to Lake De Waay. Only in one of these three lakes did they observe a significant change in the composition of chironomid assemblages at the depth of the thermocline.

Conclusions

The overall taxonomic composition of living and subfossil invertebrate assemblages in Lake De Waay was similar, at least for chironomids that could be identified at a comparable taxonomic level in living and subfossil assemblages. A total of 44 chironomid taxa were found in Lake De Waay, of which 30 taxa occurred in both living and subfossil assemblages. These 30 taxa included, on average, 94% of the specimens in the studied samples. This demonstrates that biomonitoring approaches based on benthic samples containing living chironomids and palaeolimnological approaches examining subfossil chironomid remains in lake surface sediments will give comparable assemblage compositions. In contrast to samples of living assemblages, surface sediments analyzed for

invertebrate remains also represent the invertebrate fauna occurring in the lake over a period of seasons to years and in a range of (micro)habitats. However, systematic identification keys for subfossil remains of several non-chironomid groups are still urgently required to allow identification of subfossil assemblages at a comparable taxonomic level for all invertebrate groups.

We observed a distinct difference in distribution between living invertebrate larvae and subfossil remains within the basin of Lake De Waay. Living invertebrates were mostly constrained to the littoral and sublittoral zone with the exception of a few taxa (*Chaoborus flavicans* and *Chironomus*) that are adapted to low oxygen conditions in the profundal of this eutrophic lake. Our results suggest that remains are transported and redeposited off-shore in Lake De Waay due to its steep bathymetry. A single sediment sample obtained from the centre of this lake contained subfossil invertebrate remains originating from the entire lake basin. In contrast to Luoto (2010), our results support previous observations indicating that, in palaeolimnological studies, one profundal core can provide an integrated assessment of benthic invertebrate remains originating from the entire lake basin, especially in relatively small lakes with a steep bathymetry. Examining both living and subfossil assemblages will provide more complete and detailed information about the invertebrate community in a lake ecosystem than examination of only one of these assemblages.

Acknowledgements

Chapter 4

Relationship between δ¹³C in invertebrate remains and methane flux in Swedish lakes

Abstract

Methane-derived carbon can be an important carbon source for aquatic food webs via methane-oxidizing bacteria, leading to ^{13}C-depleted stable carbon isotopic signatures in invertebrates feeding on these microorganisms. We measured methane fluxes from seven lakes in Sweden and along transects from the littoral to the profundal in two of these lakes. Methane fluxes were compared with the stable isotopic composition (δ^{13}C) of chitinous remains of ten invertebrate taxa obtained from surface sediment samples. These included several groups of chironomids (*Chironomus*, Chironomini, Orthocladiinae, Tanypodinae, and Tanytarsini), bryozoans (*Plumatella* and *Cristatella mucedo*), the cladoceran genus *Daphnia*, Ephemeroptera, and ostracods. The taxon-specific δ^{13}C measurements indicated that relative differences in δ^{13}C values between taxa are similar in samples from different lakes and different water depths along transects. Remains of Orthocladiinae and Ephemeroptera had relatively high δ^{13}C values between -31.3 and -27.0‰, reflecting plant-derived carbon. In contrast, chitinous remains of ostracods and *Cristatella mucedo* had δ^{13}C values as low as -39 and -38.1‰, respectively, suggesting these taxa incorporated methane-derived carbon. For several invertebrate groups, including Chironomini, Tanytarsini, Tanypodinae, and *Daphnia* a negative correlation was observed between δ^{13}C values of their remains and diffusive methane fluxes from the seven lakes. This correlation was distinct but not statistically significant for *Daphnia* (r = -0.57, p = 0.18) and strong and significant for Chironomini (r = -0.90, p = 0.0062). Similarly, within-lake variability of methane release from surface sediment cores taken along transects in two lakes was negatively correlated with δ^{13}C of Chironomini, *Daphnia*, and *Cristatella mucedo* remains. This correlation was statistically significant for Chironomini (r = -0.67, p = 0.025). We suggest that incorporation of methane-derived carbon in these invertebrate taxa can explain these correlations. Our results indicate that δ^{13}C analysis of invertebrate remains can reveal taxon-specific information about the carbon sources of the organisms involved. Furthermore, if our results are corroborated in more extensive studies of the relationship between methane flux and δ^{13}C of invertebrates obtained from lake surface sediments, δ^{13}C of Chironomini, *Daphnia*, and other sensitive invertebrate groups may potentially be used to reconstruct past changes in methane availability in lakes.

Introduction

Methane-derived carbon has recently been identified as an alternative, non-photosynthetic carbon source for lacustrine food webs (Bastviken et al. 2003; Jones et al. 2008; Taipale et al. 2007). Methane oxidizing bacteria (MOB) utilize methane, usually in aerobic sediment layers or in the oxic parts of the water column, and incorporate methanogenic carbon into their biomass. MOB can provide an important source of food for some animal groups living in lakes such as the larvae of non-biting midges (Chironomidae) or planktonic water fleas (Cladocera) of the genus *Daphnia* (Kankaala et al. 2007a; Jones et al. 2008). The distinct ^{13}C-depleted carbon isotopic composition of methane is further depleted by MOB and passed on to animals feeding on them. Recent studies have revealed exceptionally low δ^{13}C in a number of lacustrine invertebrate taxa with values lower than can be expected for organic matter produced exclusively by photosynthesis. For example, larvae of the chironomid genera *Chironomus* and *Stictochironomus* from a number of lakes in the UK, Finland, Germany, Japan, and Sweden have been reported with values as low as -72 and -64‰, respectively (Kiyashko et al. 2001; Jones and Grey 2004; Jones et al. 2008). Similarly, planktonic cladocerans of the genus *Daphnia* have been reported with δ^{13}C as low as -47.3 ±0.6‰ (Kankaala et al. 2010). These studies suggest that methanogenic carbon can enter lake food webs in a range of lake ecosystems and under different environmental circumstances.

A number of invertebrate groups (e.g. aquatic insects, planktonic crustaceans, moss animals), produce robust chitinous structures such as exoskeleton fragments and resting stages that preserve well in lake sediments. The carbon isotopic signature of invertebrate tissues is strongly related to the δ^{13}C of their food sources (DeNiro and Epstein 1978). It can therefore be expected that, if methanogenic carbon is incorporated by the studied invertebrate groups, the δ^{13}C of their fossilizing structures will be more ^{13}C-depleted in lakes with a high methane production than in those producing less methane. In a labelling experiment it was recently demonstrated that if larvae of the chironomid *Chironomus riparius* feed on MOB this affects the carbon isotopic signature of their exoskeleton (Chapter 1). It seems therefore likely that similar processes allow methanogenic carbon to be incorporated into exoskeletons and resting stages of other chironomids and non-chironomid invertebrate taxa as well. This raises the possibility of analysing δ^{13}C of chitinous invertebrate remains to trace past changes in methane production and oxidation in lakes. δ^{13}C of invertebrates in lake surface sediments sampled from lakes with different levels of methane production can be assessed to determine which invertebrate taxa are exceptionally prone to incorporate carbon originating from methane and MOB.

We present a study of δ^{13}C of invertebrate remains in the surface sediments of seven lakes from southern central Sweden (Table 1) and compare the carbon isotopic measurements with estimates of the methane output of the lakes. The top 2 cm of sediments in lakes in the area typically incorporate particles deposited during the past 2-5 years (Gaillard et al. 1991; Routh et al. 2007; Chapter 5) providing temporally integrated samples of the fossilizing invertebrates of

the studied lake ecosystem. Next to examining the between-lake variability of invertebrate $\delta^{13}C$ we also present more detailed studies comparing the pattern of invertebrate $\delta^{13}C$ and methane flux within two of the studied lake basins.

Materials and methods

Study sites

Surface sediment samples were obtained from the seven study lakes in Central Sweden with a gravity corer (Table 1). Cores for surface sediment samples were taken from the deepest part of each lake basin in June 2008. In addition, three replicate sediment cores were taken in Strandsjön and Långsjön at five and six locations, respectively, along a transect from the littoral to the deepest zone (Table 2). These cores were taken in April 2009 when both lakes were completely mixed with an oxic water column (dissolved oxygen at maximum water depth >9.5 mg L^{-1}).

Sediment characteristics

The top 0-2 cm of sediment from all gravity cores were sampled and an aliquot was taken to determine the concentration of sedimentary organic matter using loss-on-ignition (LOI) at 550 °C following Heiri et al. (2001). Sub-samples for stable isotope analysis were exposed to 2.5% HCl for 15 minutes to remove carbonates, rinsed three times with demineralized water, centrifuged 4 min at 2000 rpm to remove excess water, and freeze-dried. C:N ratios and $\delta^{13}C$ of bulk sediment organic matter were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS. Two secondary standards of known relation to international standards for VPDB were used as references. Replicate sample measurements on standards gave analytical errors (2 SD) of ±0.05‰ (n = 43).

$\delta^{13}C$ of invertebrate remains

The surface sediment samples used for the analysis of invertebrate remains were deflocculated in 10% KOH for 2 h at room temperature and sieved with 200- and 100-µm sieves (Chapter 2). Sieve residues were exposed to 2.5% HCl for 15 minutes, rinsed three times and stored in demineralized water in the dark. Remains were identified under a dissecting microscope at 40–100x magnification following Wood and Okamura (2005) for Bryozoa, Vanderkerkhove et al. (2004) for the resting stages of *Daphnia,* and Brooks et al. (2007) for remains of dipteran larvae. Furthermore, the chitinous remains of ostracod carapaces were collected. Remains were sorted with forceps according to their taxonomic group and transferred directly into pre-weighed ultraclean tin cups. These were dried on a hotplate at 50°C for 24 h after which the tin cups were re-weighed and crimped

Table 1. Physical characteristics (Surface area, maximum depth), water chemistry (Diffusive methane flux (Diffusive methane flux, Total methane flux*, pH, DOC, TP, TN), and bulk sediment characteristics (Loss-on-ignition at 550°C, $\delta^{13}C$, and C:N ratio) of study lakes. Methane fluxes are average values of replicate measurements in the measuring periods in June-July 2008.

Lake	Gäddtjärn	Långsjön	Lötsjön	Lilla Sången	Skottjärn	Strandsjön	Svarttjärn
Coordinates	59°51'32" N 15°10'58" E	59°53'12" N 17°57'16" E	59°52'1" N 17°56'51" E	59°54'10" N 15°23'37" E	59°56'30" N 15°23'49" E	59°52'28" N 17°10'5" E	59°53'25" N 15°15'27" E
Surface area (ha)	7	83	63	23.8	2.8	130	0.7
Max depth (m)	10	5	11.2	20	5	4	7
Diffusive flux (mmol m^{-2} d^{-1})	0.28 ±0.08 (n=35)	0.36 ±0.13 (n=42)	0.51 ±0.23 (n=28)	0.04 ±0.01 (n=50)	0.08 ±0.02 (n=44)	0.41 ±0.18 (n=16)	0.31 ±0.12 (n=31)
Total flux* (mmol m^{-2} d^{-1})	0.67 ±1.72 (n=52)	0.90 ±1.39 (n=63)	1.33 ±1.38 (n=61)	0.04 ±0.01 (n=52)	0.17 ±0.23 (n=56)	1.27 ±1.97 (n=51)	0.40 ±0.31 (n=37)
pH	4.5	7.5	7.6	6.3	4.6	-	4.8
DOC (mg L^{-1})	14.9	15.0	12.1	6.5	20.5	20.8	28.0
TP (µg L^{-1})	9.0	37.0	28.1	11.4	15.0	41.3	15.1
TN (µg L^{-1})	392	1321	905	275	632	-	502
LOI$_{550}$ (%)	47.4	8.2	21.2	45.1	76.6	29.5	68.6
$\delta^{13}C$ bulk sediment (‰ VPDB)	-29.0	-31.1	-30.9	-27.5	-28.1	-31.8	-27.8
C:N bulk sediment	15.5	8.3	10.1	13.8	20.0	7.9	18.4

*Total methane flux = Diffusive methane flux + ebullition

Table 2. Water depth, methane flux under aerobic conditions, and bulk sediment characteristics of cores from transects in Lakes Långsjön and Strandsjön (carbon content, $\delta^{13}C$, and C:N ratio). Average methane release from replicate cores (n=3) at each depth are reported ±standard error.

	Långsjön						Strandsjön				
Water depth (m)	1.0	1.5	2.0	2.5	3.0	3.5	1.3	1.5	1.9	2.2	2.4
Methane release (mmol m^{-2} d^{-1})	1.65 ±0.29	2.87 ±1.98	1.03	0.94 ±0.05	0.99 ±0.12	1.31 ±0.27	6.15 ±5.06	1.32 ±0.38	0.93 ±0.02	1.88 ±1.65	0.88 ±0.06
Carbon content bulk sediment (%)	17.7	3.2	2.6	7.8	9.8	9.7	18.5	15.8	14.0	13.2	12.6
$\delta^{13}C$ bulk sediment (‰ VPDB)	-33.7	-31.2	-28.5	-31.9	-32.8	-33.1	-32.5	-31.5	-32.5	-32.5	-32.8
C:N ratio	11.5	10.2	8.7	9.3	9.2	9.1	11.0	11.0	9.6	9.3	9.1

for stable isotope analysis. Control samples of water from sieve residues were evaporated in tin cups and no carbon contamination was detected.

Invertebrate samples were analyzed on a Fisons NA 1500 NCS Elemental Analyzer interfaced to a Thermo Electron Delta plus IRMS. The reference material used was a secondary standard of known relation to international standards for VPDB. Replicate sample measurements (n = 82) on this internal standard gave an analytical error of ±0.09‰ (2 SD). In sediment samples with enough material, replicates of individual taxa were analyzed and weight-corrected average δ¹³C values for these samples are reported here. Taxon specific analyses were made for the chironomid groups *Chironomus* spp., Chironomini (excluding *Chironomus*), Tanytarsini, Orthocladiinae, and Tanypodinae. Furthermore, taxon-specific analyses were made for Ephemeroptera, the cladoceran genus *Daphnia*, the chitinous remains of Ostracoda, and the Bryozoa taxa *Plumatella* and *Cristatella mucedo*. As 10-100 individual remains (20 µg) are required for one stable carbon isotope analysis not enough material could be collected for all taxa in each sediment sample. For example, remains of *Chironomus plumosus*-type were encountered in sediments, but only at low abundances, therefore only few samples could be analyzed for δ¹³C and *Chironomus* is not included in Figures 1-4 or analyzed statistically. Statistical analyses were performed using PAST v2.00 (Hammer et al. 2001).

Methane fluxes and water chemistry

Methane emissions from water surface to atmosphere for Strandsjön, Lötsjön, and Långsjön were measured on 10-18 June and 8-16 July 2008 and for Svarttjärn, Lilla Sången, Gäddtjärn, and Skottjärn on 23 June-2 July 2008. Measurements of methane fluxes at each site were performed using static floating chambers as described by Bastviken et al. (2010), which allowed diffusive flux and total flux (also including ebullition) to be separated. In total 14 chambers were used with between three and five chambers deployed in each of the following depth zones: 0-1 m, 1-2 m, 2-4 m, and >4 m. Gas from the chambers was sampled after 24 h and CH_4 concentrations were measured in the laboratory by gas chromatography using a flame ionization detector (Shimadzu 8A) with a Poropack Q column. Depth profiles of oxygen concentrations and temperature in the water column were measured in Strandsjön, Lötsjön, and Långsjön on 16 June and 16 July 2008 and in Svarttjärn, Lilla Sången, Gäddtjärn, and Skottjärn on 23 June 2008 using a Hach Lange HQD 40D oxygen-temperature meter with an optical Intellical DO sensor. In addition, pH, concentrations of dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) were measured in surface water samples of all lakes. The pH was measured using a Hach-Lange HQD 40D pH meter with an Intellical gel-filled pH electrode. Concentrations of DOC and TP were analyzed following Pace and Cole (2002).

Relationships between the δ¹³C values of the invertebrate remains and methane fluxes were also examined in more detail in Långsjön and Strandsjön. The variability of methane release and δ¹³C of invertebrate remains within

these lakes were analyzed by collecting sediment cores from transects from the shore to the centre of the lakes and measuring $\delta^{13}C$ of invertebrate remains and methane release from these cores. Triplicate cores were retrieved at 5-6 locations on transects of increasing water depth, transported to the laboratory, and kept near *in situ* temperatures in dark climate rooms. Water was removed until 2-3 mm water remained above the sediment to avoid drying out of the surface sediments. Methane release from the sediments was measured after 12 h in the dark according to Conrad and Rothfuss (1991) using a carbon dioxide and methane analyzer (Los Gatos Research Inc.) instead of a gas chromatograph. For measurement the cores were capped with rubber stoppers that were pierced by two needles and connected by PVC tubing to the in- and outlet of the gas analyzer to create a closed circuit. Methane concentrations in the core headspace were monitored for 3-15 minutes in each core. Methane fluxes were calculated from the linear increase in methane concentrations over the measured time in three replicate cores taken at the same water depth and average values are reported ± standard errors.

Results

Average values of diffusive methane fluxes measured at the lake surface ranged from 0.08 ±0.02 to 1.33 ±1.38 mmol m^{-2} d^{-1} in the seven studied lakes (Table 1). Diffusive methane fluxes were closely and significantly correlated with average values of total methane fluxes (diffusive fluxes + ebullition) measured at the lake surface (r = 0.94, p = 0.0018). Within Strandsjön and Långsjön, the two lakes studied in detail, highest methane release was measured in littoral sediments (Fig. 2a and 3a). High methane release was also measured in cores taken from the deepest section of Långsjön (Fig. 2a), whereas methane release was relatively low in cores from intermediate water depth.

$\delta^{13}C$ of a number of invertebrate remains were negatively correlated with methane fluxes observed for the different study lakes (Table 3a). For example, $\delta^{13}C$ of Chironomini, *Daphnia*, Tanypodinae, and Tanytarsini were all negatively correlated with diffusive methane flux measured at the lake surface. Of these $\delta^{13}C$ of Chironomini was significantly correlated with diffusive methane fluxes from the lake (r = -0.90, p = 0.0062) and *Daphnia* was nearly significantly correlated (r = -0.57, p = 0.18). $\delta^{13}C$ of other invertebrate remains (Orthocladiinae, Ostracoda, *C. mucedo*, *Plumatella*, and Ephemeroptera) were not or only weakly correlated (-0.25 < r < 0.25) with diffusive methane flux.

In Strandsjön and Långsjön $\delta^{13}C$ of a number of different invertebrate taxa showed clear variations with water depth. For example, in Långsjön $\delta^{13}C$ of Tanytarsini was lower in shallower parts of the lake than in deeper sections, whereas the opposite was true for $\delta^{13}C$ of Tanypodinae and Ostracoda (Fig. 2). Most other invertebrate remains showed more moderate variations in $\delta^{13}C$ with water depth. In Strandsjön $\delta^{13}C$ of Tanypodinae, *Daphnia*, *Plumatella*, and *C. mucedo* had lowest values in the shallowest part of the transect, whereas the relationship between $\delta^{13}C$ and water depth is less clear for Chironomini,

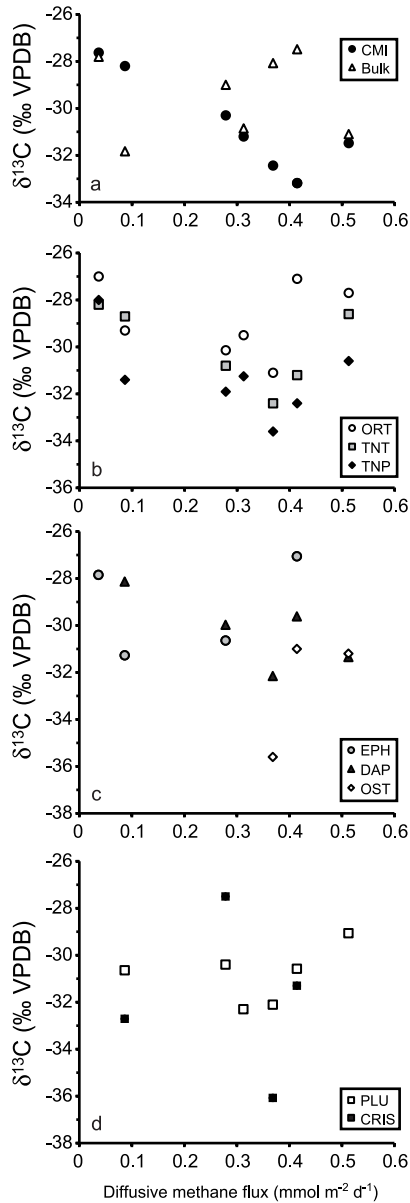**Fig. 1** $\delta^{13}C$ values of bulk sediment and invertebrate remains from surface sediments in the deepest part of the seven studied lakes plotted against mean diffusive methane fluxes. (a) Bulk sediment (white triangles) and Chironomini (black circles), (b) Orthocladiinae (white circles), Tanytarsini (grey squares), and Tanypodinae (black diamonds), (c) *Daphnia* (black triangles), Ostracoda (white diamonds), and Ephemeroptera (grey circles), and (d) *Plumatella* (white squares), and *Cristatella mucedo* (black squares).
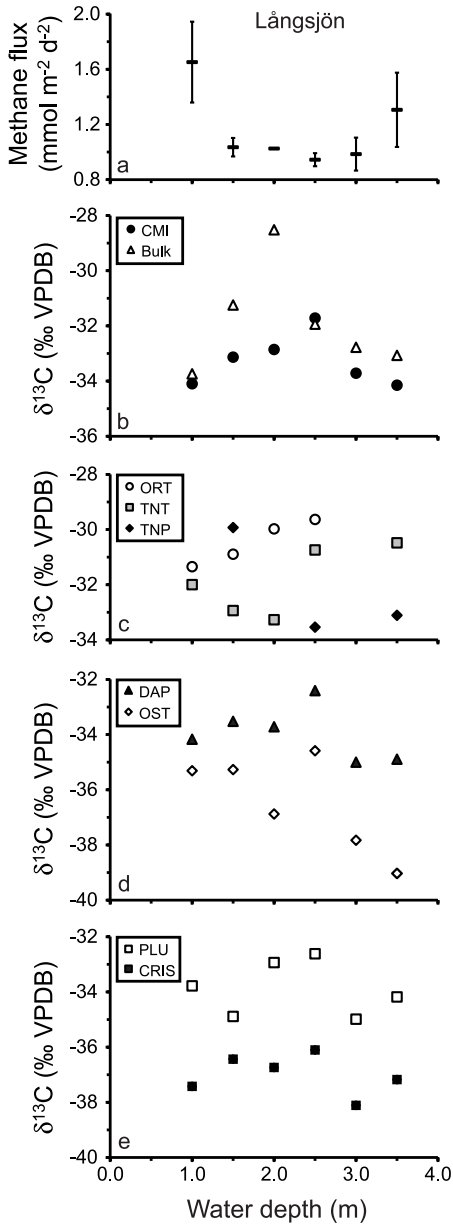
Fig. 2 Relationship between methane flux and δ13C of invertebrate remains in lake surface sediments within Långsjön. (a) Methane release from sediments (mean ± SD in three replicate cores) indicated by horizonal bars (scale is provided by left y-axis). (b-e) Mean δ13C values of bulk sediment and invertebrate remains (scale is provided on right y-axes). Legend identical to Fig. 1
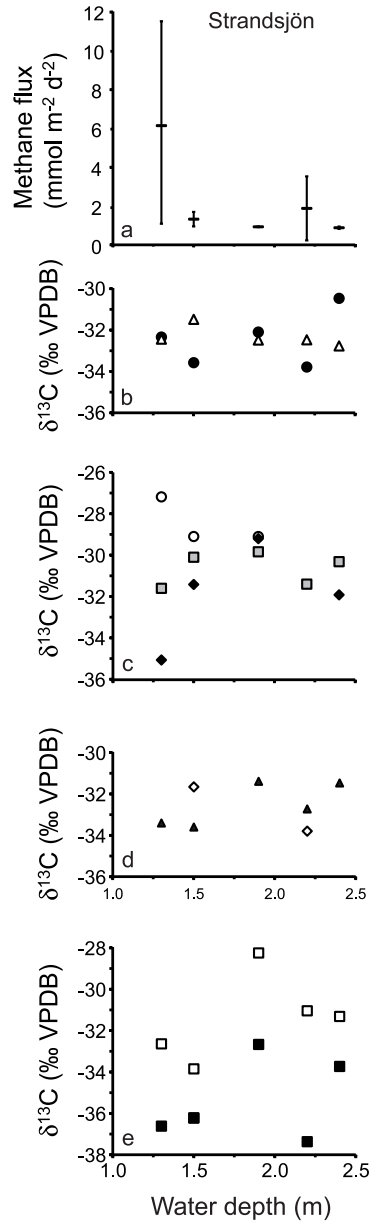
Fig. 3 Relationship between methane flux and δ13C of invertebrate remains in lake surface sediments within Strandsjön. (a) Methane release from sediments (mean ± SD in three replicate cores) indicated by horizonal bars (scale is provided by left y-axis). (b-e) Mean δ13C values of bulk sediment and invertebrate remains (scale is provided on right y-axes). Legend identical to Fig. 1
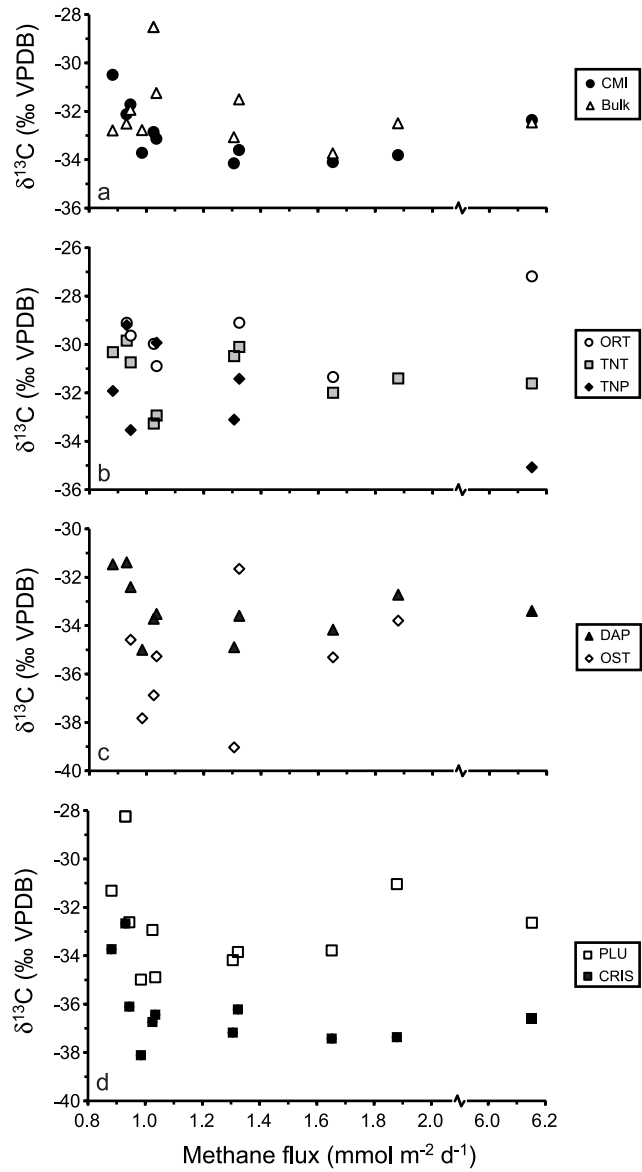
Fig. 4 δ¹³C values of bulk sediment and invertebrate remains from surface sediment transects in Långsjön and Strandsjön plotted against mean methane release from sediments. Note discontinuous x-axis. Legend identical to Fig. 1

Orthocladiinae, Tanytarsini, and Ostracoda (Fig. 3).

Of the taxa present in at least five samples from the transect in Långsjön $\delta^{13}C$ of Chironomini showed the strongest negative correlation with methane release measured from the sediments (r = -0.69, p = 0.13), followed by *Daphnia* (r = -0.36, p = 0.48), and *C. mucedo* (r = -0.34, p = 0.51, see also Table 3b). Of the taxa present in all five samples from the transect in Strandsjön, $\delta^{13}C$ values of *C. mucedo* showed the strongest correlation with methane release from the sediments (r = -0.87, p =0.057), followed by Chironomini (r = -0.83, p = 0.084), Tanytarsini (r = -0.64, p = 0.24), and *Daphnia* (r = -0.57, p = 0.31). The small number of data points and the small amplitude of changes in $\delta^{13}C$ makes the detection of significant relationships between methane fluxes and the carbon isotopic composition difficult in the two studied transects. However, if the data from the two transects are combined more robust relationships become apparent (Fig. 4, Table 3c). The strongest (and significant) negative correlation is apparent between methane release from the sediment cores and $\delta^{13}C$ in Chironomini (r = -0.67, p = 0.025). Non-significant negative correlations were found for *C. mucedo* (r = -0.51, p = 0.11) and *Daphnia* (r = -0.27, p = 0.42).

Interestingly, relative differences in $\delta^{13}C$ values between taxa are broadly comparable in all study lakes and showed a similar pattern as $\delta^{13}C$ of these taxa in all the examined samples (Fig. 5). The highest $\delta^{13}C$ values were always observed in Orthocladiinae (-31.2 to -27.0‰) and Ephemeroptera (-31.3 to -27.0‰), whereas lowest values were observed in *C. mucedo* (-38.1 to -27.5‰) and Ostracoda (-39.0 to -31.6‰).

Discussion

δ¹³C of invertebrate taxa

The carbon isotopic signature of aquatic invertebrates and their chitinous remains mainly reflect the $\delta^{13}C$ values of their diet (DeNiro and Epstein 1978). Food sources available for lacustrine invertebrates include terrestrial plants and animals transported into the lake, organic matter produced by aquatic macrophytes and algae in the littoral, and organic matter produced by phytoplankton. $\delta^{13}C$ of the first two of these food sources typically range between -33 and -8‰ (Meyers and Teranes 2001) and $\delta^{13}C$ of phytoplankton is mostly between -35 and -25‰ (Bade et al. 2006; Grey and Jones 1999; Vuorio et al. 2006; Yoshioka et al. 1994), only occasionally reaching -40‰ in oligotrophic lakes (Jones et al. 1999; Kankaala et al. 2010). In addition, MOB can also contribute considerably to the diet of some invertebrate taxa (Jones et al. 2008; Taipale et al. 2007). $\delta^{13}C$ of MOB values are 12-30‰ lower than their methane source (Jahnke et al. 1999), resulting in $\delta^{13}C$ of MOB biomass as low as -100 to -65‰. Since many lacustrine invertebrates ingest carbon from different sources it can be expected that their $\delta^{13}C$ is characterized by more moderate values than these extremes.

$\delta^{13}C$ of invertebrate remains analyzed in the seven lakes in this study vary substantially between taxa (Fig. 5 and Heiri et al. (2009)). Within the Chironomidae,

lowest values were reported for Chironomini (excluding *Chironomus*) (-33.2 to -27.6‰) and Tanypodinae (-33.6 to -28.0‰). Chironomini larvae are largely mud-dwellers, making tubes on the lake bottom and feeding on organic matter sinking from the water column either as filterers or deposit feeders (Moller Pillot 2009). Several species have high tolerances to low oxygen conditions (Quinlan and Smol 2001b; Saether 1979). The tubes of some Chironomini can be an ideal microhabitat for MOB (Deines et al. 2007a). Experiments have shown that *Chironomus* larvae incorporate methane-derived carbon (Deines et al. 2007b; Chapter 1). *C. plumosus* larvae in some lakes have been reported with δ¹³C values as low as -72‰ (Jones et al. 2008). In eight French lakes Borderelle et al. (2008) observed lower δ¹³C values in profundal Chironomini larvae (including *Chironomus*) compared to littoral specimens. This difference became larger with increasing hypolimnetic anoxia, possibly as a result of methane-derived carbon incorporation. Other Chironomini (e.g. *Stictochironomus*) have also been reported with δ¹³C values as low as -64‰ (Kiyashko et al. 2001), but a number of Chironomini also feed as grazers and miners (Mihuc and Toetz 1994; Moller Pillot 2009; Moog 2002) that incorporate mostly photosynthically-derived carbon.

Tanypodinae larvae are mostly predators on protozoans and small larvae of crustaceans and dipterans (Merritt et al. 2008; Vallenduuk and Moller Pillot 2007). Jones et al. (2008) observed 0-10‰ lower δ¹³C in *Procladius* larvae than δ¹³C of bulk sediment, and Bunn and Boon (1993) and Borderelle et al. (2008) also reported δ¹³C-depleted Tanypodinae larvae in lakes. The low δ¹³C values in Tanypodinae head capsules collected in our study lakes suggests that they can feed on δ¹³C-depleted prey items.

Tanytarsini are general collector-gatherers that feed mainly on detritus (Moog 2002), and a number of taxa are known to be tube building filter feeders (Merritt et al. 2008). In our study lakes δ¹³C values of Tanytarsini are on average 1.6‰ lower than Orthocladiinae and within the range of plant-derived carbon sources. However, the negative correlation between δ¹³C of their head capsules and diffusive methane fluxes, may suggest that they can incorporate methane-derived carbon.

Orthocladiinae are mostly taxa living in the littoral, feeding on living and dead algae, diatoms, and macrophytes (Merritt et al. 2008). Some taxa live in deep-water environments but are usually considered to be less well adapted to burrowing into the sediments than other chironomid taxa. In our sediment samples remains of Orthocladiinae are characterized by relatively high δ¹³C values (Fig. 5) suggesting that their diet mainly consists of organic matter produced by algae and aquatic macrophytes, which was shown for *Orthocladius* by Mihuc and Toetz (1994). This suggests that it may be possible to use fossil remains of Orthocladiinae as an indicator of average δ¹³C values of past primary production in lakes.

Of the non-chironomid taxa in this study, remains of Ephemeroptera are characterized by the highest δ¹³C values (Fig. 1 and 5a). Like Orthocladiinae, Ephemeroptera larvae are mainly littoral dwellers and feed on available plant detritus and algae (Vander Zanden and Rasmussen 1999). Reports of the stable isotopic composition of the chitinous remains of ostracod carapaces are not
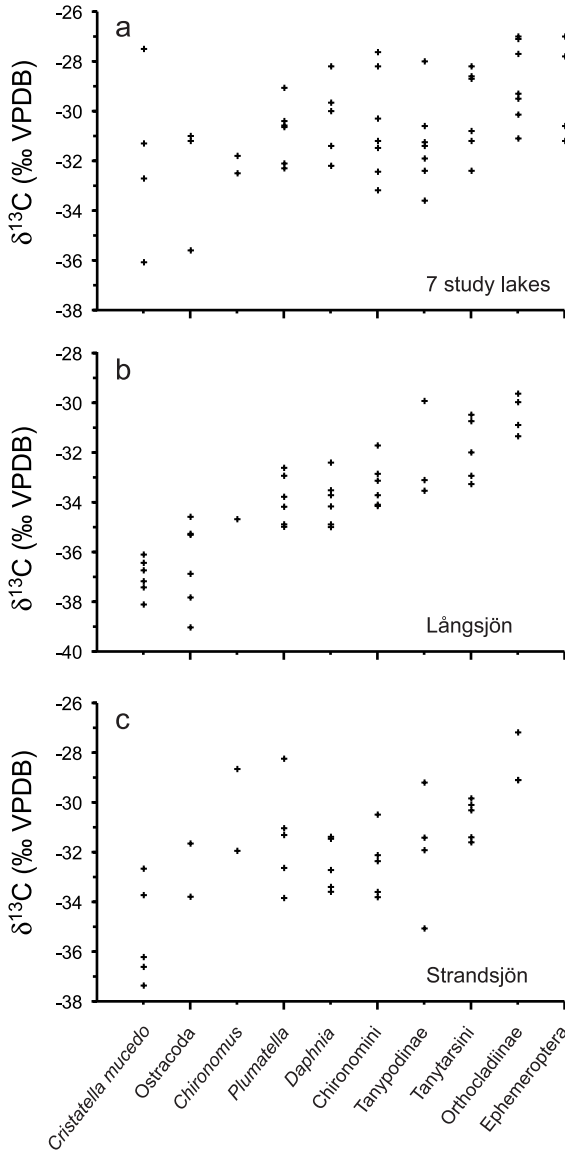
Fig. 5 δ¹³C values of invertebrate remains with taxa for (a) surface sediments in the deepest part of the seven study lakes, (b) surface sediment transect in Långsjön, and (c) surface sediment transect in Strandsjön.

available since the chitinous elements are usually removed before stable isotope measurements on the calcareous valves (Keatings et al. 2006). Chitinous remains of ostracod carapaces analyzed in our study were characterized by clearly lower δ¹³C values than most other taxa (Fig. 1 and 5). The low δ¹³C found, with the minimum value of -39‰, is below the values of other invertebrate remains in this study (Fig. 5) and also below the usual range expected for littoral and planktonic

algae. This strongly suggests that ostracods can incorporate methanogenic carbon originating from MOB or methanogenic bacteria in the sediments or invertebrates such as protozoans feeding on MOB. Such a carbon source can be available when ostracods burrow into the sediments (Griffiths and Martin 1993). Evidence for methane-derived and -affected carbon has also been suggested by studies of δ^{13}C of benthic ostracod calcite (Curry et al. 1997; Schwalb 2003).

Bryozoa live predominantly as colonies attached to hard substrates feeding on suspended nannoplanktonic algae (Kaminski 1984; Okamura and Hatton-Ellis 1995). This feeding mode is reflected by the relatively high δ^{13}C values in *Plumatella* statoblasts that correspond to values reported for algae. In contrast, statoblasts of *C. mucedo* have on average 2.8‰ lower δ^{13}C values than *Plumatella* with values reaching as low as -38.1‰ (Fig. 5), which suggests that *C. mucedo* has a different carbon source. Kaminski (1984) demonstrated that *C. mucedo* selects small seston (<7 µm in diameter) which can include bacteria, whereas *Plumatella repens* prefers slightly larger particles (ranging from 5 to 17 µm in diameter). The observation in this study that *C. mucedo* has distinctly lower δ^{13}C values than remains of other planktivorous taxa (e.g. *Daphnia*) and taxa predominantly feeding on littoral vegetation (e.g. Orthocladiinae and Ephemeroptera) support this interpretation.

Several studies have reported low δ^{13}C values in *Daphnia*, caused by the incorporation of ¹³C-depleted MOB (Bastviken et al. 2003; Taipale et al. 2007) or previously respired and ¹³C-depleted CO_2 (Rau 1978; Lennon et al. 2006). We found a negative correlation between diffusive methane fluxes and δ^{13}C of *Daphnia* ephippia, but the δ^{13}C values of their remains are not exceptionally low and within the range expected for phytoplankton.

Relations with methane fluxes

δ^{13}C values of most invertebrate remains studied in our lakes are negatively correlated with diffusive methane fluxes (Table 3a). If the δ^{13}C values of remains obtained from the surface sediments of the seven study lakes are examined, Chironomini show the strongest negative correlation with diffusive methane flux followed by *Daphnia*, Tanypodinae, and Tanytarsini. This suggests that δ^{13}C-depleted methane-derived carbon is incorporated in their tissues, which has been observed in living Chironomini larvae (Kiyashko et al. 2001; Jones et al. 2008) and *Daphnia* (Taipale et al. 2007; Kankaala et al. 2010). It is of interest that remains that are characterized by exceptionally low δ^{13}C values, such as *C. mucedo* statoblasts or chitinous ostracod remains do not show the strongest or most consistent relationship with our methane flux data. This suggests that these organisms may be actively harvesting methanogenic microorganisms, MOB or other organisms feeding on these, leading to relatively ¹³C-depleted carbon in a range of environments. It seems that δ^{13}C values of the tribe Chironomini, which includes many species with deposit-feeding and filter-feeding larvae, and possibly also filter-feeding *Daphnia*, are exceptionally sensitive to the presence of methane and microorganisms feeding on methanotrophic carbon.

Table 3a Correlations between $\delta^{13}C$ values of invertebrate remains and diffusive methane fluxes as well as total methane flux (diffusive flux and ebullition) in the seven study lakes.

	Diffusive methane flux			Total methane flux		
	r	p	n	r	p	n
Chironomini	-0.90	0.0062	7	-0.86	0.014	7
Tanytarsini	-0.46	0.30	6	-0.44	0.32	6
Orthocladiinae	-0.05	0.92	7	0.11	0.82	7
Tanypodinae	-0.54	0.21	7	-0.52	0.23	7
Daphnia	-0.57	0.18	5	-0.47	0.29	5
Ostracoda	0.18	0.70	3	0.26	0.57	3
Plumatella	0.22	0.63	6	0.40	0.37	6
Cristatella mucedo	-0.05	0.91	4	-0.03	0.95	4
Ephemeroptera	0.24	0.61	4	0.33	0.47	4

Table 3b Correlations between $\delta^{13}C$ values of invertebrate remains and methane release from sediment cores taken along transects in Långsjön and Strandsjön.

	Methane release Långsjön			Methane release Strandsjön		
	r	p	n	r	p	n
Chironomini	-0.69	0.13	6	-0.83	0.084	5
Tanytarsini	0.14	0.79	5	-0.64	0.24	5
Orthocladiinae	-0.74	0.091	4	0.13	0.84	3
Tanypodinae	-0.07	0.89	3	-0.25	0.69	4
Daphnia	-0.36	0.48	6	-0.57	0.31	5
Ostracoda	-0.03	0.96	6			2
Plumatella	-0.06	0.92	6	-0.30	0.63	5
Cristatella mucedo	-0.34	0.51	6	-0.87	0.057	5

Table 3c Correlations between $\delta^{13}C$ values of invertebrate remains and methane release from sediment cores from transects in Långsjön and Strandsjön combined.

	Methane release transects		
	r	p	n
Chironomini	-0.67	0.025	11
Tanytarsini	-0.11	0.75	10
Orthocladiinae	-0.17	0.61	7
Tanypodinae	-0.15	0.65	7
Daphnia	-0.27	0.42	11
Ostracoda	0.29	0.38	8
Plumatella	-0.07	0.84	11
Cristatella mucedo	-0.51	0.11	11

Within-lake variations

In Långsjön and Strandsjön methane release from sediment cores was exceptionally high in the littoral zone. This could be explained by the large amount of decomposing plant debris deposited in littoral environments, resulting in the highest amount of organic matter and higher C:N ratios in the littoral cores (Table 2) that reflect input from catchment vegetation (Meyers and Teranes 2001). Furthermore, solar radiation can reach the sediments in the littoral

Chapter 5

Past changes in dietary δ¹³C of lacustrine invertebrates indicated by taxon-specific δ¹³C analysis of their remains in a sediment record from Strandsjön, Sweden

Abstract

Taxon-specific stable carbon isotope (δ¹³C) analysis of chitinous remains of invertebrates can provide valuable information about their diets, derived from specific elements in lake ecosystems. This is complementary to bulk sediment δ¹³C, which provides an integrated signal of a lake system and its catchment. Here we present bulk sediment geochemistry (δ¹³C, δ¹⁵N, C:N, organic matter content) and δ¹³C values of invertebrate remains in a sediment record from Strandsjön, Sweden. Evidence for periodic incorporation of more δ¹³C-depleted carbon by benthic chironomids (Chironomini, *Chironomus*, Tanytarsini, and Tanypodinae) was indicated by their δ¹³C values that fluctuated simultaneously between -34.7 and -30.5‰ (VPDB), with minima observed ca. AD 1890, 1920, 1950, and 1990. This pattern was not observed in remains of non-chironomid filter-feeders (*Daphnia*, *Plumatella,* and *Cristatella mucedo*) whose δ¹³C values showed few high frequency variations and only a clear 2-3‰ decrease since ca. AD 1960. Rhabdocoela cocoons had relatively high δ¹³C values (-30.4 to -28.2‰) that were stable throughout the core. This may indicate that δ¹³C of remains of benthic chironomids, non-chironomid filter-feeders, and Rhabdocoela each provide information about past δ¹³C values of organic matter in different compartments of the lake. Bulk sediment δ¹³C values show a marked 10‰ increase at 27 cm depth, dated to ca. AD 1895, which is accompanied by a moderate increase in bulk sediment C:N ratio. This shift may be explained by a 0.5-1 m decrease in water level that took place in the second half of the nineteenth century. Possibly, this resulted in increased water column mixing and nutrient availability in the photic zone and the production of ¹³C-enriched algae. However, δ¹³C of invertebrate remains in this section of the sediment record do not shift like bulk sediment δ¹³C, suggesting that δ¹³C of organic matter in the water column was not affected. An alternative hypothesis for the large shift in bulk sediment δ¹³C is that post-depositional processes in the sediment have affected bulk sediment δ¹³C. ¹³C-depleted microbial biomass may have influenced δ¹³C of bulk sediment below 27 cm depth. This study indicates the potential of taxon-specific δ¹³C analysis of invertebrate remains to distinguish former dietary carbon sources available in deepwater benthic habitats (using δ¹³C of sediment-dwelling chironomids) from carbon sources in the open water column (using δ¹³C of non-chironomid filter-feeders), that cannot be separated

by analyzing $\delta^{13}C$ of bulk sediment. In Strandsjön, where bulk sediment $\delta^{13}C$ may be affected by post-depositional processes, $\delta^{13}C$ of invertebrate remains may provide the more suitable approach to reconstruct past variations in the carbon isotopic composition of organic production within the lake ecosystem than analysis on bulk organic matter.

Introduction

The stable isotopic composition of organic matter in lake sediments can provide information on past changes in climate, productivity, origin of organic matter, pollution and in-lake carbon cycling (Meyers and Lallier-Vergès 1999; Leng et al. 2005; Herzschuh et al. 2010; Verbruggen et al. 2010b). The stable carbon isotopes composition ($\delta^{13}C$) of bulk organic matter can be interpreted as an integrated estimate of the $\delta^{13}C$ value of different organic matter sources in a lake and its catchment (Meyers and Teranes 2001). The stable carbon isotopic composition of organic remains and substances formed within lakes can be affected by a number of processes such as preferential ^{12}C-uptake by planktonic algae during photosynthesis (Fogel and Cifuentes 1993), respiration by algae, zooplankton and bacterioplankton in the water column (France et al. 1997; Lennon et al. 2006) and methanogenesis and methanotrophy by microorganisms in the water column and the sediments (Eller et al. 2005; Taipale et al. 2007). The latter two processes and differential preservation of organic matter (Meyers 1997) imply that $\delta^{13}C$ of bulk organic matter can still change after deposition. These problems can be partly circumvented by analysing the chitinous remains of selected invertebrate taxa, such as exoskeleton fragments of aquatic insects, resting eggs of planktonic Crustacea or resting stages of moss animals (bryozoans) (Wooller et al. 2008; Chapter 1 and 4). These remains largely consist of a composite of proteins and chitin and are chemically robust and resistant to microbial degradation, especially if buried in anoxic lake sediments (Verbruggen et al. 2010c). They can by identified under the microscope and associated with a particular group of organisms. Therefore, it can be ensured that the analyzed organic remains originate from the lake itself. Since different invertebrate groups are characterized by different feeding modes (e.g. filter-feeders, deposit-feeders, algivores) and habitat preferences (planktonic or benthic, shallow water or deep water), taxon-specific analyses of invertebrate $\delta^{13}C$ can potentially provide insights on processes affecting the lacustrine carbon cycle that are active in different compartments of lakes.

Chitinous invertebrate remains in lake sediments have received surprisingly little attention for stable isotope analysis until very recently. Wooller et al. (2004) showed the potential to measure stable oxygen isotopes ($\delta^{18}O$) of chironomid head capsules from sediment records. The methodology for $\delta^{18}O$ analysis of chironomid head capsules was improved and applied on a Lateglacial record that showed very similar patterns in $\delta^{18}O$ of chironomid head capsules and authigenic carbonates (Verbruggen et al. 2010b, c). Furthermore, Wooller et al. (2008) analyzed $\delta^{13}C$ and $\delta^{15}N$ of chironomid head capsules in a lake sediment sequence from Iceland and compared values with $\delta^{13}C$ and $\delta^{15}N$

of bulk organic matter in the sediments. Griffiths et al. (2010) used $\delta^{15}N$ of bulk sediments, chironomid head capsules, and *Daphnia* ephippia to demonstrate the presence of nutrient input by bird colonies in two arctic ponds. Perga (2010) revealed that strong correlations exist between $\delta^{13}C$ and $\delta^{15}N$ values of tissue of living cladocerans (*Bosmina* and *Daphnia*) and their isolated chitinous carapaces. Furthermore, she analyzed $\delta^{13}C$ and $\delta^{15}N$ of *Bosmina* carapaces in sediment samples from Lake Annecy. In Chapter 1 we showed that $\delta^{13}C$ of the head capsules of chironomid larvae is influenced by their diet and that methanogenic carbon can influence the carbon isotopic signature of chironomid exoskeletons. However, down-core studies examining $\delta^{13}C$ in the remains of multiple planktonic and benthic invertebrate groups simultaneously are not yet available. This approach would allow the taxon-specific records to be compared in order to detect variations in $\delta^{13}C$ common to several indicator group. Hence, past changes in the carbon cycle of lakes tracked by invertebrates originating from individual compartments of lakes could be separated from background variations in $\delta^{13}C$ common to all indicator groups and from high-frequency noise in individual records.

Here we present a palaeolimnological study that reports $\delta^{13}C$ values in the chitinous remains of four chironomid subfamilies, cladocerans of the genus *Daphnia*, two Bryozoa genera, and Rhabdocoela in a dated ca. 140 year-record from Strandsjön in southern central Sweden. We compare taxon-specific invertebrate $\delta^{13}C$ and bulk sediment geochemistry and discuss the results in the context of past changes in $\delta^{13}C$ of organic matter in different compartments of the lake.

Methods

Site and sediment characteristics

Strandsjön is a shallow lake located in South-central Sweden (59°52′28″ N, 17°10′5″ E, 51 m a.s.l., Fig. 1) with a surface area of 130 ha. The water level was artificially decreased by 0.5-1 m at the end of the nineteenth century to its present maximum depth of 4 m (Brunberg and Blomqvist 1998). The catchment contains pine forest and pastures, some with dairy farming since the beginning of the twentieth century. Nutrient input from farming decreased after introduction of waste water treatment in the 1970s (Brunberg and Blomqvist 1998). In summer 2008 dissolved organic carbon (DOC) concentrations were 20.8 mg L^{-1} and total phosphorous concentrations are 41.3 µg L^{-1} (Chapter 4). The coring location was chosen at 2.4 m water depth in open water. This location represents the deepest part of a transect of surface sediment samples obtained from the lake in which $\delta^{13}C$ of invertebrate remains were analyzed previously (Chapter 4). A 36 cm long sediment core was obtained and sub-sampled in the field at 1 cm resolution. The samples were stored in plastic bags and kept cool and dark until freeze-drying upon arrival in the laboratory. Freeze-dried samples were used for dating by
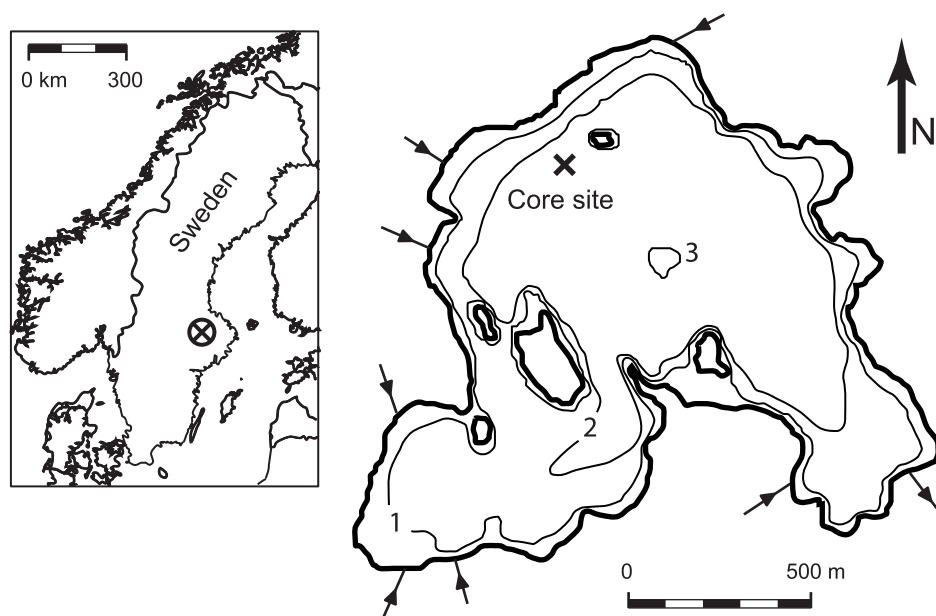
Fig. 1 Site location and bathymetric map of Strandsjön (redrawn after Brunberg and Blomqvist 1998). Coring site is indicated by X.

gamma spectrometry using a Canberra low-background Ge-well detector at the University of Copenhagen's Gamma Dating Center. ^{210}Pb was measured via its gamma-peak at 46,5 keV, ^{226}Ra via the granddaughter ^{214}Pb (peaks at 295 and 352 keV), and ^{137}Cs via its peak at 661 keV.

Samples for stable isotope analysis of bulk organic matter were exposed to 2.5% HCl for 15 minutes to remove carbonates, rinsed three times with demineralized water, centrifuged 4 min at 2000 rpm to remove excess water, and freeze-dried. C:N ratios, as well as stable carbon and nitrogen isotopes of bulk sediment organic matter were analyzed on a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 IRMS. Two secondary standards of known relation to international standards for VPDB (δ^{13}C) and AIR (δ^{15}N) were used as references. Replicate sample measurements on standards (n = 43) gave analytical errors (2 SD) of ±0.05‰ for δ^{13}C and ±0.05‰ for δ^{15}N.

δ^{13}C of invertebrate remains

Samples for stable carbon isotope analysis of chitinous invertebrate remains were deflocculated in 10% KOH for 2 h at room temperature and sieved with 200- and 100-µm sieves (Chapter 2). Sieve residues were soaked in 2.5% HCl for 15 minutes, rinsed three times and stored in demineralized water in the dark. Remains were identified under a dissecting microscope at 40–100x magnification following Wood and Okamura (2005) for Bryozoa, Vanderkerkhove et al. (2004)

for the resting stages of *Daphnia,* and Brooks et al. (2007) for chironomid head capsules. Remains were separated into the chironomid groups *Chironomus* spp., Chironomini (excluding *Chironomus*), Tanytarsini, Orthocladiinae, and Tanypodinae, the cladoceran genus *Daphnia*, and the Bryozoa taxa *Plumatella* and *Cristatella mucedo*. Furthermore, the chitinous cocoons of free-living flatworms (Turbellaria: Rhabdocoela) were collected (Frey 1964).

    After identification, remains were transferred with forceps directly into pre-weighed ultraclean tin cups. Tin cups were dried on a hotplate at 50°C for 24 h after which they were re-weighed and crimped for stable isotope analysis. Control samples of water from sieve residues were evaporated in tin cups and no carbon contamination was detected. Samples of invertebrate remains were analyzed on a Fisons NA 1500 NCS Elemental Analyzer coupled to a Thermo Electron Delta plus IRMS. A secondary standard of known relation to international standards for VPDB was used as reference. Replicate sample measurements of the secondary standard (n = 30) gave an analytical error (2 SD) of ±0.05‰.

Results

Age-depth model
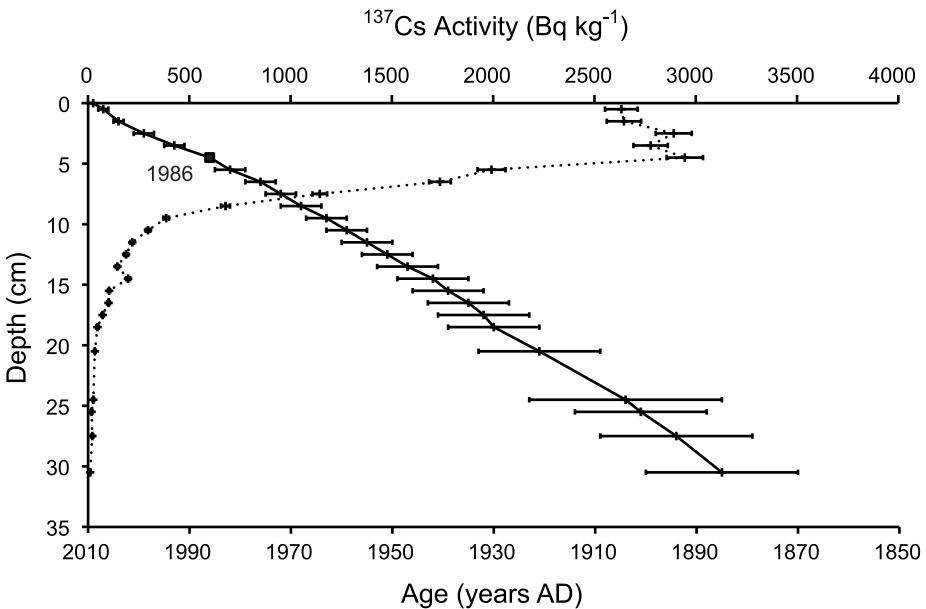
Fig. 2 Age model for the sediment record from Strandsjön based on a constant rate of supply model for ²¹⁰Pb with the 4.5 cm peak in ¹³⁷Cs activity as reference point for the 1986 Chernobyl accident.

Highest ^{137}Cs-activity was measured at 4.5 cm sediment depth in the Strandsjön sediments. ^{137}Cs-profiles measured in European lake sediments typically feature two maxima of ^{137}Cs-activity at AD 1986 and 1963, coinciding with the Chernobyl reactor accident and the 1963 peak in above ground nuclear bomb testing (Appleby 2001). In lakes with a high degree of sediment mixing these peaks can be smoothed out, sometimes leading to a single peak of activity centred on AD 1986 (Bigler and Hall 2003). This suggests that 4.5 cm sediment depth in the Strandsjön sediments is equivalent to AD 1986. Age-depth modelling using ^{210}Pb was based on a modified constant rate of supply (CRS) model (Appleby and Oldfield 1978) with 4.5 cm fixed at 1986 as a reference point (Fig. 2). This model was extrapolated for samples below 31 cm, resulting in an estimated bottom age of AD 1870 ±15 years for this core.

Bulk sediment geochemistry

δ^{13}C values at the base of the analysed sediment core fluctuate around -41‰. A major shift in δ^{13}C values of bulk sediment is observed at 27 cm depth (ca. AD 1895) leading to values of ca. -31‰ (Fig. 3). Simultaneously the C:N ratio of organic matter shifts from 8.1 to 10.5. δ^{13}C and C:N remain relatively constant in the younger part of the profile although C:N gradually decreases again towards 9.1 at the top of the record. We did not observe major shifts in the percentage of sedimentary organic carbon or bulk sediment δ^{15}N that coincide with the 10‰ shift in bulk sediment δ^{13}C. Carbon content of bulk sediment is 15-17% in the lower part of the core until 15cm depth, then decrease to 10.5% at 10 cm depth, and increase again to 12.6% at the top of the record.

δ^{13}C of invertebrate remains

δ^{13}C of larval remains of chironomids range between -34.6 and -30.3‰ with lowest values recorded by *Chironomus* (-34.6 to -30.8‰), followed by Tanytarsini (-33.9 to 30.3‰), Tanypodinae (-33.8 to -31.0‰) and Chironomini (-33.5 to -30.5‰). Relative shifts in δ^{13}C values between all four chironomid groups are very similar at different depths and have relative minima in δ^{13}C values at 2.5-4.5, 12.5, 20.5, and 27.5 cm sediment depth (Fig. 3). In contrast, δ^{13}C values of non-chironomid invertebrates do not show this high-frequency variability. Of non-chironomid remains, statoblasts of *Cristatella mucedo* have lowest δ^{13}C values (-35.1 to -31.2‰), followed by *Daphnia* ephippia (-32.8 to -30.5‰). Remains of Rhabdocoela (-30.2 to -28.4‰) and *Plumatella* (-33.1 to -28.0‰) have δ^{13}C values around -29‰ throughout most of the core. A general decrease of 2-3‰ in the top 10 cm of the core are observed in δ^{13}C of bulk sediment, *Daphnia*, *C. mucedo* and *Plumatella*, but not in Rhabdocoela or any of the chironomid taxa.
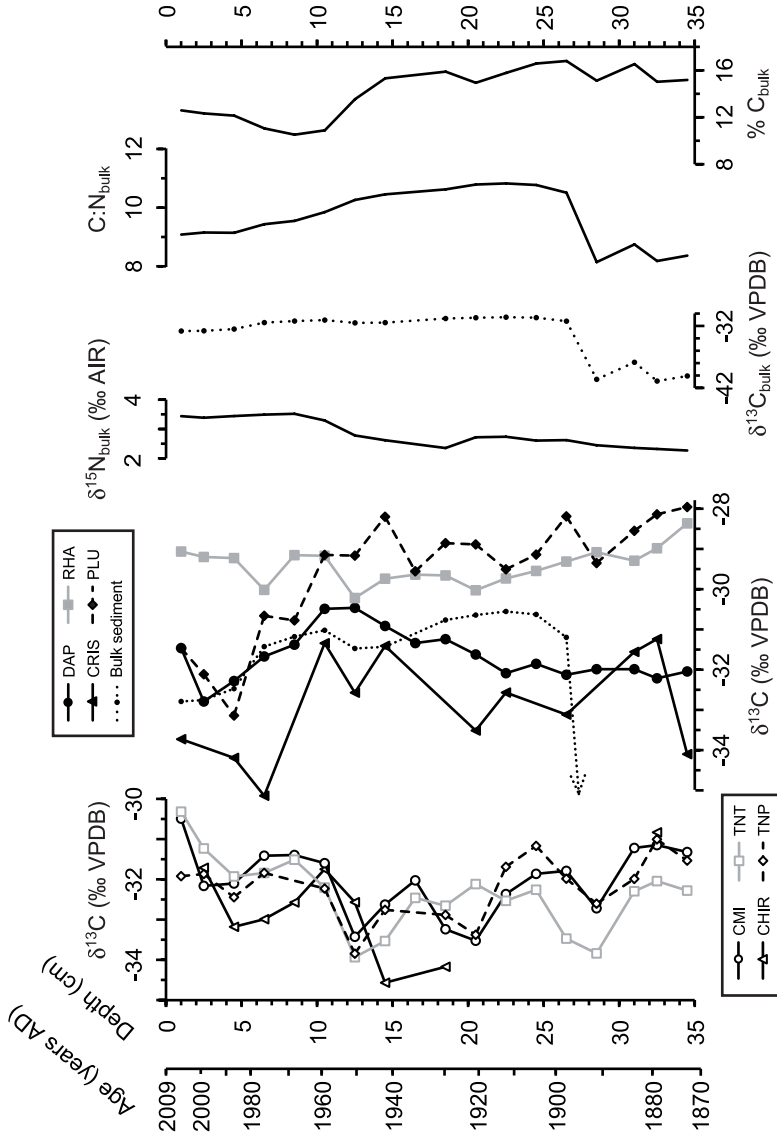
Fig. 3 δ¹³C values of invertebrate remains and bulk sediment geochemistry (δ¹³C, δ¹⁵N, C:N ratios, and organic carbon content) in the sediment record from Strandsjön. Invertebrate taxa are indicated by open triangles (CHIR: *Chironomus*), open circles (CMI: *Chironomini*), open grey squares (TNT: Tanytarsini), open diamonds (TNP: Tanypodinae), solid triangles (CRIS: *Cristatella mucedo*), solid circles (DAP: *Daphnia*), solid grey squares (RHA: Rhabdocoela), and solid diamonds (PLU: *Plumatella*).

Discussion

Bulk sediment δ¹³C

The δ¹³C of bulk organic matter shows a major shift at 27-28 cm depth, which is dated to ca. AD 1895. Possibly, this coincided with a lowering of the water level in Strandsjön by 0.5-1 m, which has been reported at the end of the nineteenth century (Brunberg and Blomqvist 1998). The lower water level may have resulted in increased water column mixing and higher availability of nutrients in the photic zone. Also, the lower water level may have caused increased influx of allochtonous organic material. It is likely that both processes would have had a profound effect on the organic matter in the lake ecosystem. Prior to the lake level lowering Strandsjön was a clear water lake (Brunberg and Blomqvist 1998). The dramatic lowering of the water table may have been the beginning of increased mixing of the water column and increased photosynthetic primary production in the lake. The sedimentation of ¹³C-enriched biomass from algae in the epilimnion may have resulted in higher δ¹³C values of sedimentary organic carbon above 27.5 cm depth (dated ca. AD 1895). A similar process leading to a 4‰ rise in bulk sediment δ¹³C was described for Lake Greifen by Hollander and Smith (2001). However, the shift towards higher C:N ratios at this point does not support the idea of increased production of aquatic algae, since lacustrine algae have C:N ratios <10 (Meyers and Teranes 2001). Furthermore, we find no evidence of this event in δ¹³C values of any of the invertebrate remains or the organic matter content in the sediments (Fig. 3). This indicates that if water table lowering has affected the composition of organic matter in the lake it did not have a major effect on δ¹³C of food sources available for the invertebrate groups assessed in this study.

An alternative mechanism for the low δ¹³C values of bulk sediment below 27.5 cm depth could be the *in situ* production of ¹³C-depleted organic matter by microorganisms in the sediments. This process would only affect δ¹³C of bulk sediment and not δ¹³C of chitinous invertebrate remains. Biomass of methanogenic and methane-oxidizing bacteria is characterized by δ¹³C-depleted carbon. For example, bacterial lipids with δ¹³C values between -40 and -90‰ have been described in the sediments of eutrophic lakes (Hollander and Smith 2001) and laboratory cultures (Jahnke et al. 1999; House et al. 2003).

δ¹³C of invertebrate diet

Few studies are available that describe δ¹³C of invertebrate remains in lake sediments, although it is generally assumed that δ¹³C of invertebrates and their chitinous fossils resembles the carbon isotopic composition of the food the animals ingested (DeNiro and Epstein 1978; Schimmelmann and DeNiro 1986b). Hence, variations in fossil invertebrate δ¹³C can provide information about changing δ¹³C of their diet in the past. δ¹³C of the remains of benthic chironomid larvae shows a very similar pattern for the different chironomid groups with several minima in

$\delta^{13}C$ during the past 140 years. In contrast, $\delta^{13}C$ of filter-feeding, non-chironomid invertebrates (*Daphnia*, *Cristatella mucedo*, and *Plumatella*) was very constant during much of the record, with a clear decrease in the youngest sediments. This deviation between $\delta^{13}C$ of chironomid and filter-feeding, non-chironomid taxa suggests that $\delta^{13}C$ of food resources in benthic habitats and the open water column of Strandsjön has varied in different ways during the past 140 years.

Wooller et al. (2008) found similar trends in $\delta^{13}C$ and $\delta^{15}N$ of chironomid remains as in $\delta^{13}C$ and $\delta^{15}N$ of bulk sediment and in Chapter 6 we observed similar trends in $\delta^{13}C$ values of chitinous remains of chironomids, *Daphnia*, and ostracods that were similar to trends in bulk sediment $\delta^{13}C$. However, in this sediment record chironomid taxa reveal higher frequency variations in $\delta^{13}C$ values than bulk sediment and other invertebrates. Griffiths et al. (2010) observed that chironomid-based $\delta^{15}N$ profiles displayed higher variability than a *Daphnia*-based $\delta^{15}N$ profile and suggested that inter-specific differences or temporal fluctuations in the availability of light isotopic sources, such as bacteria, may explain some of the observed variability. However, in our study all analyzed chironomid groups featured the same type of high frequency signal. It seems very unlikely that this $\delta^{13}C$ signal is related to changes in taxonomic composition within all four of the analyzed chironomid groups.

Incorporation of ¹³C-depleted carbon originating from methanogenic and methane-oxidizing bacteria has been observed for several chironomid species (Kiyashko et al. 2001; Borderelle et al. 2008; Jones et al. 2008). Many chironomids build tubes in soft sediments that can be an ideal microhabitat for methanotrophic bacteria (Deines et al. 2007a). In Chapter 4 and 6 correlations were observed between methane availability in lakes and $\delta^{13}C$ of Chironomini, *Chironomus*, and Tanytarsini. Based on this relationship it seems likely that increased methane production and oxidation in Strandsjön could have caused the lower $\delta^{13}C$ values of chironomid remains at 2.5-4.5, 12.5, 20.5, and 27.5 cm in the sediment record. Temperature may affect the availability of methane-derived carbon as warmer temperatures will stimulate bacterial methane production (Bastviken 2009). Furthermore, stronger stratification of the water column during warm summers and longer periods of ice cover during cold winters can lead to reduced oxygen availability. This can be expected to increase methane production, and will relocate methane-oxidizing bacteria to the oxycline in the water column. However, we did not observe any clear relationships between $\delta^{13}C$ values of chironomid or non-chironomid remains and mean temperatures in winter (December, January, and February), or summer (June, July, and August) recorded in Uppsala (Bergström and Moberg 2002), 25 km to the east (Fig. 4).

An alternative source of ¹³C-depleted carbon for invertebrates is the incorporation algae that utilized heterotrophically respired carbon. Recycling of carbon originating from heterotrophic organisms can lead to lower $\delta^{13}C$ values in dissolved inorganic carbon and lower $\delta^{13}C$ values in photosynthetic primary production (Rau 1978) and *Daphnia* feeding on ¹³C-depleted phytoplankton, which is strongest in lakes with higher DOC concentrations (Lennon et al. 2006). Lower $\delta^{13}C$ values caused by increased carbon recycling in periods with higher DOC concentrations could be expected to affect $\delta^{13}C$ of bulk sediment

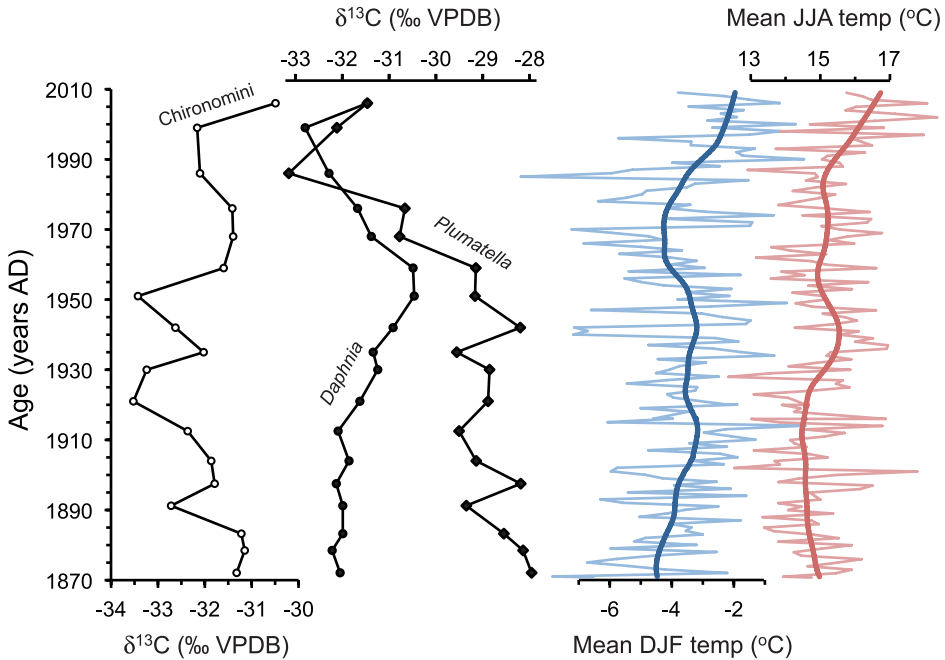**Fig. 4** δ¹³C values of selected invertebrate remains, mean temperature of December, January, and February (DJF), and mean temperature of June, July, and August (JJA). Chironomini are indicated by open circles, *Daphnia* by solid circles, and *Plumatella* by solid diamonds. Mean temperatures are derived from the Uppsala temperature record (Bergström and Moberg 2002) and plotted with locally weighed regression smoothing (LOESS, span 0.2).

organic matter and most invertebrates in a similar way. This would be especially likely for taxa that feed on seston such as Bryozoa and *Daphnia*. However, this is clearly not the case in our record: statoblasts of *Plumatella* have stable δ¹³C values throughout most of the analyzed sediment sections and their relatively high δ¹³C values reflect carbon from algae that is not ¹³C-depleted. Furthermore, variations in δ¹³C values of *Daphnia* ephippia and bryozoan statoblasts do not match δ¹³C of chironomid remains. Only in the top 10 cm of the record do we observe a synchronous decrease in δ¹³C values of bulk sediment, and remains of filter-feeding *Daphnia*, *Plumatella,* and *C. mucedo* that could possibly be ascribed to increased carbon recycling.

A final explanation for the variations in δ¹³C values of chironomid remains would be changes in δ¹³C associated with changes in in the rate of primary production by lacustrine algae. δ¹³C of algae in productive meso- to eutrophic lakes is often higher than δ¹³C of algae growing in oligotrophic conditions (Hollander et al. 1993; Brenner et al. 1999). Detailed reconstructions of the nutrient level and trophic state of Strandsjön are not available to examine this potential explanation in more detail. Possibly the lower δ¹³C observed in filter-feeding indicator groups and chironomids at 2-5 cm depth could be explained by changes in the trophic conditions of Strandsjön. However, during the remaining

sections of the record $\delta^{13}C$ of chironomids and non-chironomid filter-feeders do not coincide, as one would expect if changes in algal primary productivity would be driving the variations in within-lake $\delta^{13}C$.

In contrast to the remains of other invertebrate taxa, $\delta^{13}C$ values of Rhabdocoela cocoons have stable $\delta^{13}C$ values throughout the sediment record. Rhabdocoela are active predators feeding on small invertebrates and large ciliate protozoans (Jennings 1957). In contrast to benthic chironomids and non-chironomid filter-feeders they are not expected to be directly affected by changes in $\delta^{13}C$ values of phytoplankton or bulk sediments, which may explain the relatively stable $\delta^{13}C$ values of Rhabdocoela cocoons compared with $\delta^{13}C$ of other invertebrate groups in this study.

Conclusions

The $\delta^{13}C$ analyses from Strandsjön discussed here present one of the first taxon-specific stable isotope records available for chitinous aquatic invertebrate remains in lake sediments. Our results indicate that invertebrate remains can track $\delta^{13}C$ changes that do not co-vary with shifts in $\delta^{13}C$ of bulk organic matter. In lakes where bulk organic matter $\delta^{13}C$ is modified by post-depositional processes in the sediments (e.g. methanogenesis, methanotrophy, or other chemoautotrophic processes), $\delta^{13}C$ of chitinous invertebrate remains may be the more suitable parameter for reconstructing past variations in the carbon isotopic composition of organic matter within lake ecosystems than bulk organic matter analyses. Furthermore, our results indicate that remains of benthic chironomids and non-chironomid filter-feeding invertebrate groups show different $\delta^{13}C$ variations within the examined lake ecosystem. This suggests that organic matter in benthic environments (providing food for chironomid larvae), and suspended material (providing food for filter-feeding invertebrates) were affected by different processes during the past 140 years. Considering that methanogenic carbon has been observed to influence the carbon isotopic composition of chironomid larvae in a number of previous studies, and that invertebrates feeding on benthic food sources clearly record a different pattern of $\delta^{13}C$ changes than planktivorous filter-feeding taxa, it seems that changes in the abundance of methane and methane-oxidizing bacteria in the sediments and the sediment-water interface of the lake are the most likely explanation for the observed changes in fossil invertebrate $\delta^{13}C$. However, more information on the relationship between limnological conditions (e.g., trophic state, water depth, habitat structure, methane availability) and $\delta^{13}C$ of the different invertebrate groups explored within Strandsjön are necessary to corroborate this interpretation.

Acknowledgements

Chapter 6

Past variations in methane availability in a Siberian thermokarst lake implied by changes of δ^{13}C in chitinous invertebrate remains

Abstract

Increasing temperature is expected to have a strong positive effect on methane emissions from Arctic wetlands and lakes. Understanding methane dynamics in such systems in the past is crucial for estimating future methane release, but methane fluxes from lakes have received relatively little attention in the past and only few reconstructions of methane output are available for lakes. In this study, we propose a new method to assess changing methane availability, and indirectly methane production, of lakes in the past. We measured diffusive methane fluxes from the surface of ten lakes in Arctic Siberia and compared them to taxon-specific δ^{13}C values of chitinous invertebrate remains from lake sediments to investigate whether these invertebrates assimilated strongly ^{13}C-depleted carbon, typical for methane. Variations in δ^{13}C values between remains of invertebrate taxa could be explained by different habitats and feeding preferences. Remains of littoral invertebrates such as the chironomid tribe Orthocladiinae that assimilate plant-derived carbon generally had higher δ^{13}C values than remains of other invertebrate groups. δ^{13}C of chitinous remains of several chironomids groups (*Chironomus*, Chironomini, Tanytarsini, and Tanypodinae), cladocerans (*Daphnia*), and ostracods were lower in lakes with higher diffusive methane fluxes. δ^{13}C of remains of Chironomini and *Daphnia* correlated significantly with diffusive methane fluxes (r = -0.65, p = 0.040 and r = -0.66, p = 0.039, respectively). No significant correlations were observed between these two taxa and other physical or chemical parameters of the lakes (e.g. dissolved organic carbon, bulk sediment δ^{13}C, and nutrient concentrations), suggesting that δ^{13}C values in these invertebrates are more strongly affected by methane availability than other parameters. In a sediment record obtained from one of the studied lakes that covers the past ca. 1400 years, δ^{13}C values of remains of several invertebrate taxa were measured. Remains of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* have lowest δ^{13}C values in sediments deposited between AD 850 and 1150 and after AD 1970. In these periods higher temperatures are reconstructed in proxy-based temperature records in the study region and other Arctic locations. Our results suggest higher methane availability and methane output of our study lake during warmer periods than during cooler periods and suggest that thermokarst lakes can respond dynamically in their methane output to changing environmental conditions.

Introduction

Methane is an important greenhouse gas in the atmosphere, its radiative effect accounting for approximately 20% of the greenhouse effect (Wuebbles and Hayhoe 2002; IPCC 2007). Predictions of future climate change are highly dependent on accurate predictions of the release and uptake of methane within different compartments of the global carbon cycle under changing environmental conditions. Wetlands, including lakes, are amongst the most important sources of methane to the atmosphere (IPCC 2007) and regional and global estimates indicate that lakes play an important role in natural methane emissions (Zimov et al. 1997; Huttunen et al. 2003; Bastviken et al. 2004; Huber et al. 2006). It has been estimated that lakes can contribute up to 16% to the global methane burden (Bastviken et al. 2004).

Atmospheric methane has been fluctuating dynamically, with concentrations ranging between 350 and 750 ppbv over the past glacial-interglacial cycles (Brook et al. 1996; Loulergue et al. 2008). Although it is expected that wetlands and lakes can exert an important positive feedback to climate change, especially in sensitive regions such as the Arctic (Wuebbles and Hayhoe 2002; Walter et al. 2006) very few estimates of past methane emissions from lakes are available (Walter et al. 2007a). Most available reconstructions of methane release from lake ecosystems are based on estimates of past changes in the overall surface area comprised of lakes and assume a similar methane release per unit area in the past as measured at present. However, the individual response of lakes in their methane output to changing environments is still poorly studied and at present it is uncertain how methane output per unit area of lake surface will vary with changing climate.

In lakes, methane is produced by methanogenic bacteria during the degradation of organic matter in anoxic waters or in anoxic sediment (Rudd and Taylor 1980; Bartlett and Harriss 1993). This methane is characterized by distinctly depleted stable carbon isotope values relative to the organic matter it is formed from (Whiticar 1999). Methane can be released from the sediment into the lake water and further into the atmosphere by a range of processes such as diffusion, in-lake currents (e.g. vertical mixing currents during spring and autumn overturning), transport via vascular plants, or bubbles percolating through the sediment and water column (Kankaala et al. 2007b; Walter et al. 2007b; Bastviken et al. 2008). During transport from the sediment a considerable amount of this methane may be oxidized by chemical processes or methane oxidizing bacteria (MOB) (Frenzel et al. 1990; Bastviken et al. 2002; Kankaala et al. 2007b).

The distinct carbon isotopic composition of methane leads very low $\delta^{13}C$ values in the tissue of aquatic invertebrates feeding on MOB (Bastviken et al. 2003; Kankaala et al. 2007a; Jones et al. 2008). $\delta^{13}C$ of aquatic invertebrates can therefore provide insights into the relative importance of MOB in their diet and, indirectly, on whether methane-derived carbon is recycled into a lake's food web. Some invertebrates produce robust chitinous structures that preserve well in lake sediments and can be used to assess the importance of methane-derived carbon in lake ecosystems in the past (Chapter 1). In seven lakes in south-central Sweden

(Chapter 4) it was shown that the remains of chironomid larvae of the tribes Chironomini, Tanytarsini, and Tanypodinae as well as remains of the cladoceran genus *Daphnia* have lower $\delta^{13}C$ values at lakes and sites within lakes with higher methane production. A significant correlation was observed between $\delta^{13}C$ of Chironomini remains and diffusive methane fluxes (r = -0.90, p = 0.0062). It is unclear, however, to what extent this relationship also exists in lakes in other regions.

In this study, we examined ten lakes in Arctic Siberia. We measured diffusive methane fluxes on the lake surface and compared them to $\delta^{13}C$ values of chitinous invertebrate remains from surface sediments of these lakes. The aim of this study was to evaluate whether the relationship between the carbon isotopic composition of invertebrate remains and methane availability resembles patterns described from surface sediments in Swedish lakes and to corroborate the results produced in Chapter 4. In a second step we examined $\delta^{13}C$ of invertebrate remains in a sediment record from a Siberian thermokarst lake in order to assess whether changes in invertebrate $\delta^{13}C$ indicate periods of increased or reduced methane availability in the lake during the past ca. 1400 years.

Materials and methods

Site description

The study area (70°48′ N, 147°26′ E) is situated in the Kytalyk wildlife reserve, 30 km northwest of the town of Chokurdakh (Yakutia, Russia). Fig. 1 indicates the location of the ten shallow thermokarst lakes in the Arctic tundra that were examined in this study. Lakes N1 to N8 are located on the floodplain of the River Elon, a tributary of the River Indigirka. With the exception of Lake N2 these lakes are flooded by the river each year during snowmelt. Lakes S1 and S2 are located on higher tundra, outside the floodplain.
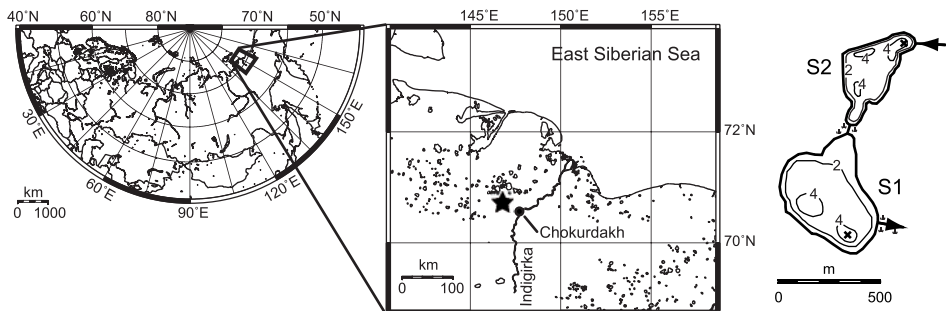

Fig. 1 Study area indicated with star, near the town Chokurdakh in Arctic Siberia. Lakes S1 and S2 are shown in right panel, including bathymetric maps and coring locations indicated by X.

Methane fluxes and water chemistry

Diffusive methane emission rates from the study lakes were measured using floating chambers (6.9 L volume, 0.071 m^2 area) constructed of dark PVC following Bastviken et al. (2004). Two to four replicate measurements were taken in 2007 on each lake along a transect from the shore to the deepest part. In 2009 two additional measurements were taken on six of the lakes only, the remaining four lakes could not be re-examined due to technical and logistical difficulties. Chambers were left floating on the lake surface during 15 min for each measurement during daytime. Gas samples were withdrawn at the start and after every 5 min through a rubber septum and transferred to evacuated infusion vials using a syringe. Concentrations were analyzed on a HP 5890A (Hewlett Packard) gas chromatograph. Methane fluxes were calculated by linear interpolation of the measurements and expressed per square meter and day. Flux measurements were rejected if the increase of methane was nonlinear, resulting e.g. from ebullition or sample loss.

Samples for water chemistry analyses were taken in the summer of 2007 and stored cold and dark in plastic bottles until processing upon return from the field. Water temperature and conductivity were measured *in situ* with a GMH 3410 conductivity probe (Greisinger Electronic GmbH) and pH with an Eijkelkamp 18.21 multi-meter (Eijkelkamp Agrisearch Equipment). Samples for dissolved organic carbon (DOC) concentrations were filtered through 0.2 µm glassfiber-filters and fixed with a few drops of concentrated HCl to keep pH below 2. DOC concentrations were analyzed by thermal oxidation on a Shimatsu TOC-5050A analyzer. Alkalinity was measured by potentiometric titration with HCl. Total phosphorus (TP) and total nitrogen (TN) were measured spectrophotometrically in unfiltered samples. TP was measured using a modified molybdate method (Murphy and Riley 1962) after oxidation with $K_2S_2O_8 + H_2SO_4$. TN was determined on nitrite-ions using Griss reagent after oxidation with $K_2S_2O_8 + NaOH$ and reduction of NO_3^- to NO_2^- in a Cu-Cd-reducer. Colour was determined by comparing the sample against colorimetric titration of a standard Pt-Co-solution in demineralized water.

Sediment characteristics and dating

The deepest part of each lake was located using echo-sounding and sediment cores were collected in the summer of 2007 with a gravity corer (UWITEC, Austria). Replicate surficial samples of 0-1, 1-2, and 2-3 cm sediment depth were taken in each lake. In Lake S2 a sediment core of 38 cm was retrieved and extruded on site at 0.5 cm resolution. All samples were stored in plastic zip-loc bags and kept cool and dark until freeze-drying upon arrival in the laboratory.

The freeze-dried sediment samples of Lake S2 were analysed for ^{210}Pb, [226]Ra, ^{137}Cs, and [241]Am by direct gamma assay. Radiometric dates and sedimentation rates were calculated using the 1963 peak in atmospheric ^{137}Cs deposition (Wright et al. 1999) and a constant rate of supply ^{210}Pb dating model
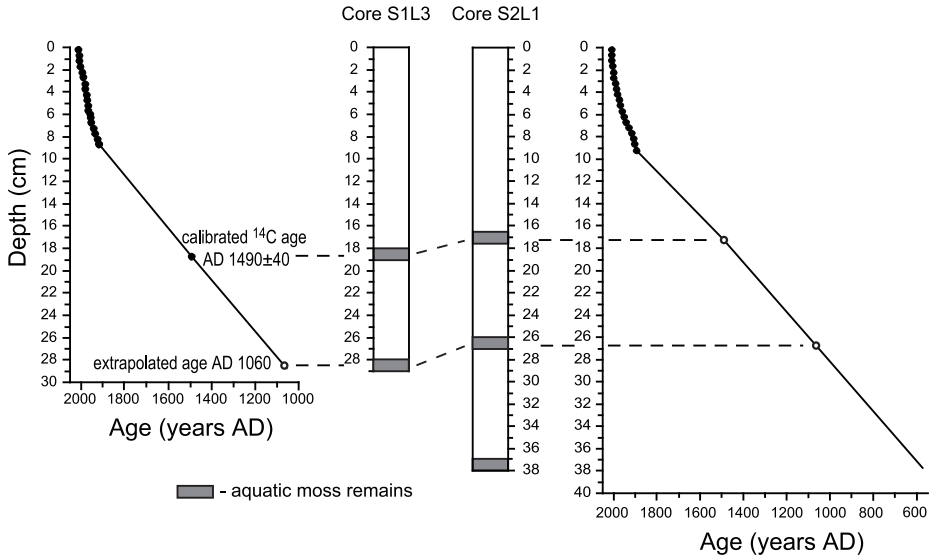
Fig. 2 Age-depth relationship for the sediment record from Lake S2. The record is correlated to the sediment core from adjacent and hydrologically connected Lake S1 based on distinct layers of aquatic mosses. The dating is based on a ²¹⁰Pb constant rate of supply model for the top 10 cm and the correlation of the upper moss layer at ca. 16.5-17.5 cm depth in Lake S2 with the upper moss layer in Lake S1 (18.0-19.0 cm depth), which has been ¹⁴C-dated based on terrestrial plant macrofossils.

(Appleby and Oldfield 1978) for the top section of the core. Based on distinct changes in the organic matter content and two conspicuous moss layers the core was correlated to a core retrieved from hydrologically connected Lake S1 (Fig. 1 and 2). Samples of bulk sediment, aquatic mosses, and one terrestrial plant remain in the Lake S1 core were AMS ¹⁴C-dated.

An aliquot of sediment was taken at 1 cm intervals from the sediment core obtained in Lake S2 for palaeoecological study of chironomid assemblages (B. Ilyashuk, in prep.). Another set of subsamples was taken for geochemical analysis of sediment organic matter at 1 cm intervals in the top 10 cm and at 2 cm intervals in the rest of the core. Organic content in the surface sediments was determined using loss-on-ignition at 550°C (LOI_{550}) and expressed as the percent weight loss after combustion at 550°C for 4 h (Heiri et al. 2001). Bulk sediment samples for stable carbon isotope analysis were soaked in 2.5% HCl for 15 minutes to remove carbonates, rinsed three times with demineralized water and centrifuged 4 min at 2000 rpm to remove excess water. C:N ratios, stable carbon isotopes of bulk organic matter in surface and down core samples were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS. The reference material used were secondary standards of known relation to international standards for VPDB (δ¹³C). Replicate sample measurements (n = 18) on internal standards gave analytical errors of ±0.03‰ (2 SD) for δ¹³C.

δ^{13}C of invertebrate remains

Sediment samples analyzed for invertebrate remains were combined for each 1 cm interval in the top 10 cm and for each 2 cm interval in the rest of the sediment record. The samples were deflocculated in 10% KOH for 2 h at room temperature and sieved through 200- and 100-µm sieves (Chapter 2). Sieve residues were soaked in 2.5% HCl for 15 min, rinsed three times, and stored in demineralized water in the dark. Remains were identified under a dissecting microscope at 40–100x magnification following Vanderkerkhove et al. (2004) for the resting stages of *Daphnia* and Brooks et al. (2007) for chironomid head capsules that were sorted into four groups: *Chironomus*, Chironomini (not belonging to the genus *Chironomus*), Orthocladiinae, Tanytarsini, and Tanypodinae. Furthermore, the chitinous linings of ostracod shells were collected. Remains were separated into different taxonomic groups and transferred directly into pre-weighed ultraclean tin cups with forceps. The tin cups were dried on a hotplate at 50°C for 24 h after which they were re-weighed and crimped for stable isotope analysis. Control samples of water from sieve residues were evaporated in tin cups and no carbon contamination was detected.

Samples were analyzed on a Fisons NA 1500 NCS Elemental Analyzer coupled to a Thermo Electron Delta plus IRMS. The reference material used was a secondary standard of known relation to the international standard of VPDB. Replicate sample measurements (n = 82) on this internal standard gave an analytical error of ±0.08‰ (2 SD). For sediment samples with enough material, replicates of individual taxa were analyzed; only average $\delta^{13}C$ values for these taxa are, however, reported here. Statistical analyses were performed using PAST v2.00.

Results

Methane fluxes and limnology of the study lakes

Diffusive methane fluxes ranged from 0.41 ±0.09 to 4.20 ±0.55 mmol m^{-2} d^{-1} (Table 1) in the studied thermokarst lakes. According to their water chemistry all lakes are oligo- to mesotrophic (Table 1). Conductivity in lakes N2 and S1 was higher than in other lakes, most likely caused by the influx of solutes from active thaw slumps at the margins of these lakes. All lakes were mixed and had an oxic water column. No significant correlation was observed between diffusive methane flux and any of the bulk sediment or water chemistry parameters listed in Table 1, except colour (r = 0.63, p = 0.049).

Table 1 Physical and chemical characteristics of the studied lakes and sediments. Diffusive methane fluxes are average values of replicate measurements in 2007 and 2009. Water chemistry values are based on average values of two water samples taken at 0.5 m depth and at 0.5 m from the bottom.

Lake	N1	N2	N3	N4	N5	N6	N7	N8	S1	S2
Coordinates	70°49'29" N 147°27'13" E	70°49'47" N 147°25'40" E	70°49'16" N 147°25'16" E	70°49'33" N 147°30'36" E	70°49'34" N 147°30'54" E	70°49'28"N 147°31'50"E	70°49'21" N 147°32'8" E	70°48'25" N 147°29'50 "E	70°44'26' N 147°34'53" E	70°44'47" N 147°35'8" E
Elevation (m a.s.l.)	5	5	5	5	5	5	5	5	7	7
Surface area (ha)	9.2	50	5	0.9	1.1	1.5	2.5	3.7	13.7	5.7
Maximum depth (m)	2.3	5.6	3.7	2.9	3.3	5.4	3.1	4.6	5.5	5.2
Secchi depth (m)	1.8	2.8	2.6	1	2.75	1.8	1.7	1.5	3.3	2.5
CH_4 flux (mmol $m^{-2}d^{-1}$)	1.16 ±0.44 (n=4)	0.41 ±0.09 (n=5)	n.a. 0.72 ±0.15 (n=5)	1.33 ±0.70 (n=4)	1.48 ±0.77 (n=5)	3.58 ±2.00 (n=6)	4.20 ±0.55 (n=4)	1.91 ±0.51 (n=6)	1.80 (n=2)	0.95 ±0.28 (n=6)
Conductivity (µS cm^{-1})	28.3	222	29.3	41	31.2	46.3	47.2	44.3	72.2	39.5
pH	6.6	7.8	6.3	6.3	6.3	6.4	6.4	6.3	6.8	6.6
Alkalinity (meq L^{-1})	0.19	1.88	0.22	0.31	0.22	0.39	0.39	0.38	0.62	0.31
DOC (mg L^{-1})	26.5	26.7	36.9	34.4	35.7	35.7	39.2	36.7	35.6	34.3
Color (Pt-Co deg)	94	11	94	141	76	135	153	129	19	27
TP (µg/L^{-1})	14	15	12	17	15	13	18	15	15	17
TN (mg/L^{-1})	0.45	0.40	0.41	0.46	0.38	0.36	0.54	0.40	0.41	0.43
LOI_{bulk} (%)	11.0	8.9	11.5	9.7	25.8	8.4	14.7	15.8	7.0	21.2
$δ^{13}C_{bulk}$ (‰ VPDB)	-29.3	-28.4	-30.6	-30.1	-31.8	-30.9	-32.5	-31.7	-28.4	-32.3
$C:N_{bulk}$	8.9	9.3	10.9	10.0	11.0	9.3	9.3	10.7	9.1	9.5

Table 2 Mean (±SD) δ¹³C values (‰ VPDB) for invertebrate remains from surface sediment samples from ten study lakes in arctic Siberia.

Lake	Chironomus	Chironomini	Tanytarsini	Orthocladiinae	Tanypodinae	Daphnia	Ostracoda
N1	-29.6	-28.8	-29.4 (n=2)	-27.7	-27.7		
N2		-33.3	-29.9	-29.1			-30.9
N3	-32.7 (n=2)	-32.1		-30.1 (n=2)	-31.6	-32.1 ±0.9 (n=3)	
N4	-33.9	-34.4	-31.0	-29.9	-30.7	-31.9 (n=2)	-36.9
N5	-35.9 ±0.6 (n=4)	-31.6	-32.9 (n=2)	-30.2 ±2.3 (n=8)	-33.6	-32.8 ±0.4 (n=3)	-32.9
N6	-32.3 (n=2)	-35.0	-35.1 (n=2)	-28.9 (n=2)	-31.5	-37.9 (n=2)	-33.7
N7	-34.3 ±0.8 (n=4)	-36.8	-32.3 ±1.8 (n=9)	-30.5 ±0.8 (n=4)	-32.0 (n=2)	-34.8 (n=2)	-34.8
N8	-33.3 ±0.8 (n=3)	-33.9	-34.8 (n=2)	-30.8	-33.2	-34.9 ±0.7 (n=3)	-35.0 ±0.4 (n=4)
S1	-33.6 ±0.8 (n=3)		-28.1	-27.1			-33.7 ±0.4 (n=5)
S2	-34.7	-33.6	-32.0	-29.4			-34.1

Invertebrate δ¹³C in surface sediments

The ranges of δ¹³C values vary between taxa (Table 2 and Fig. 3). *Chironomus* and ostracods have the lowest δ¹³C values, ranging from -35.9 to -29.6‰ and from -36.9 to -30.9‰, respectively. Orthocladiinae have the highest δ¹³C values of all taxa, ranging between -30.8 and -27.1‰. Chironomini show the largest range of δ¹³C values (-36.8 to -28.8‰).

 Several taxa, including *Chironomus*, Chironomini, Tanytarsini, Tanypodinae, *Daphnia*, and ostracods showed negative correlations between δ¹³C of their chitinous remains and diffusive methane fluxes (Fig. 3). Only for Chironomini and *Daphnia*, these correlations are statistically significant (r = -0.65, p = 0.040 and r = -0.66, p = 0.039, respectively). The lowest δ¹³C values in these taxa (-36.8 and -37.9‰, respectively) are found in Lakes N6 and N7, where the highest methane fluxes were measured.
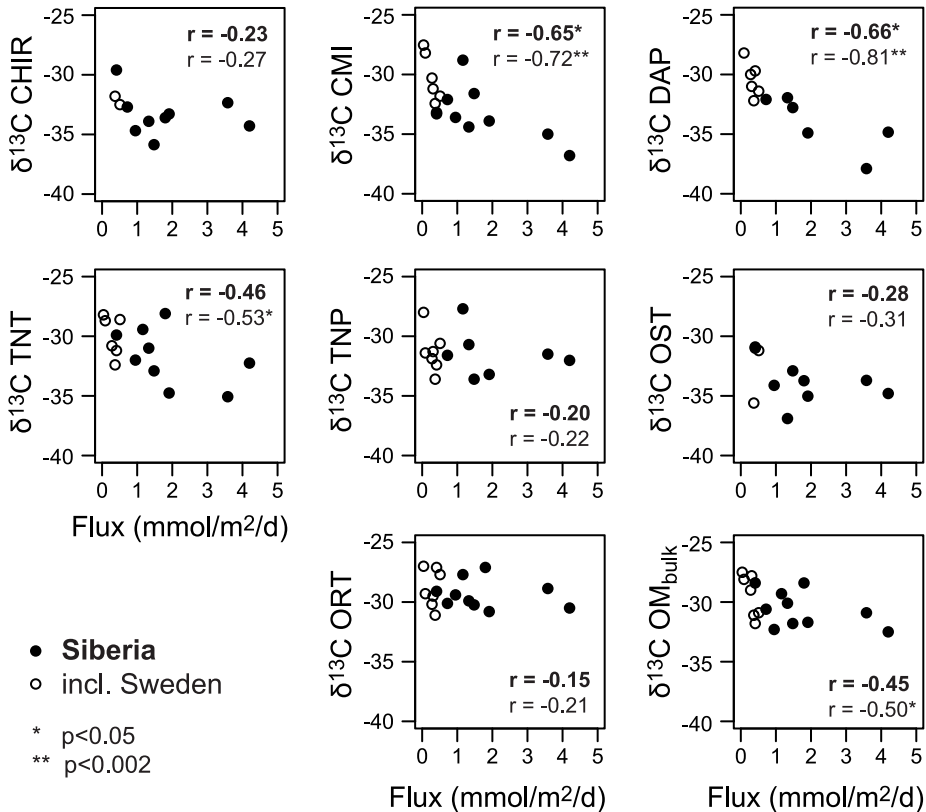
Fig. 3 Left three columns: scatter plots and correlations between methane fluxes (x-axis) and δ¹³C values (‰ VPDB) of invertebrate remains and bulk sediment (y-axis). CHIR = *Chironomus*, CMI = Chironomini excluding *Chironomus*, DAP = *Daphnia*, TNT = Tanytarsini, TNP = Tanypodinae, OST = Ostracoda, ORT = Orthocladiinae.

Replicate measurements of the same taxa indicate the natural variability of $\delta^{13}C$ that can be expected in surface sediments. For example, replicate samples for *Chironomus* indicate standard deviations of 0.6‰ (n = 4), 0.8‰ (n = 4), 0.8‰ (n = 3), and 1.4‰ (n = 3), with an average standard deviation of 0.9‰ in the different study lakes. Similar average standard deviations for the study lakes were observed in replicate samples of Tanytarsini (1.8‰), Orthocladiinae (1.6‰), *Daphnia* (2.1‰) and ostracods (0.5‰).

Sediment record

The 38 cm long sediment record obtained from Lake S2 consisted of soft, brown, organic-rich material that alternated with grey clay between 16-18 cm and below 32.5 cm. The age-depth model in the upper part of the record was constrained by a peak in ^{137}Cs activity corresponding to the 1963 bomb testing peak and the ^{210}Pb-inferred age model (Fig. 2). Due to the absence of suitable remains for ^{14}C-dating, the age of the lower part of the core was more difficult to constrain. ^{14}C-analyses of bulk sediment samples and aquatic mosses were inconsistent with the ^{137}Cs- and ^{210}Pb-data and clearly indicated that old carbon from surrounding peatlands and the slopes of the thermokarst lake affected ^{14}C-age of the record (B. Ilyashuk, unpublished results). Sediment cores of Lake S2 and adjacent, hydrologically connected Lake S1 could be correlated based on changes in the organic matter content and distinctive layers of aquatic mosses in the sediment cores of both lakes (Fig. 2). The upper moss layer in the sediment record of Lake S1 has been ^{14}C-dated to a calibrated age of AD 1490 ±40 using terrestrial plant remains in the same sample. This layer correlates to 17.0-17.5 cm depth in the sediment core of Lake S2. Assuming a constant sedimentation rate, the lower, second moss layer in Lake S1 dates to ca. AD 1060, which corresponds to a depth of 26.5-27.0 in the sediment core from Lake S2. Estimated ages for the lower part of the record from Lake S2 are based on a linear extrapolation of the age-depth relationship below 27.0 cm depth.

Three periods could be recognized in the sediment record based on the various parameters analyzed (Fig. 4). In the lowermost part of the record up to 25.5 cm C:N ratios are relatively low (between 11.0 and 12.0). Increasing organic matter content was observed (from 1.7 to 10.5%), while $\delta^{13}C$ values of bulk sediment and invertebrate remains decreased. Between 25.5 and 5 cm C:N ratios were slightly higher (between 12.2 and 13.1), except for the lower values in the clay-rich interval from 16-18 cm. The clay layer is also reflected by the lower organic matter content (1.7%) and higher $\delta^{13}C$ values of bulk sediment (-27.9‰) in this interval. $\delta^{13}C$ values of bulk sediment, Orthocladiinae, and ostracods were relatively stable in this period, whereas $\delta^{13}C$ of *Daphnia*, Chironomini, *Chironomus*, and Tanytarsini increased to values that were comparable to $\delta^{13}C$ of bulk sediment. In the top 5 cm of the core $\delta^{13}C$ values of bulk sediment and invertebrate remains decreased, especially for Chironomini and *Chironomus* that have low values of -36.5 and -37.5‰, respectively (Fig. 4).
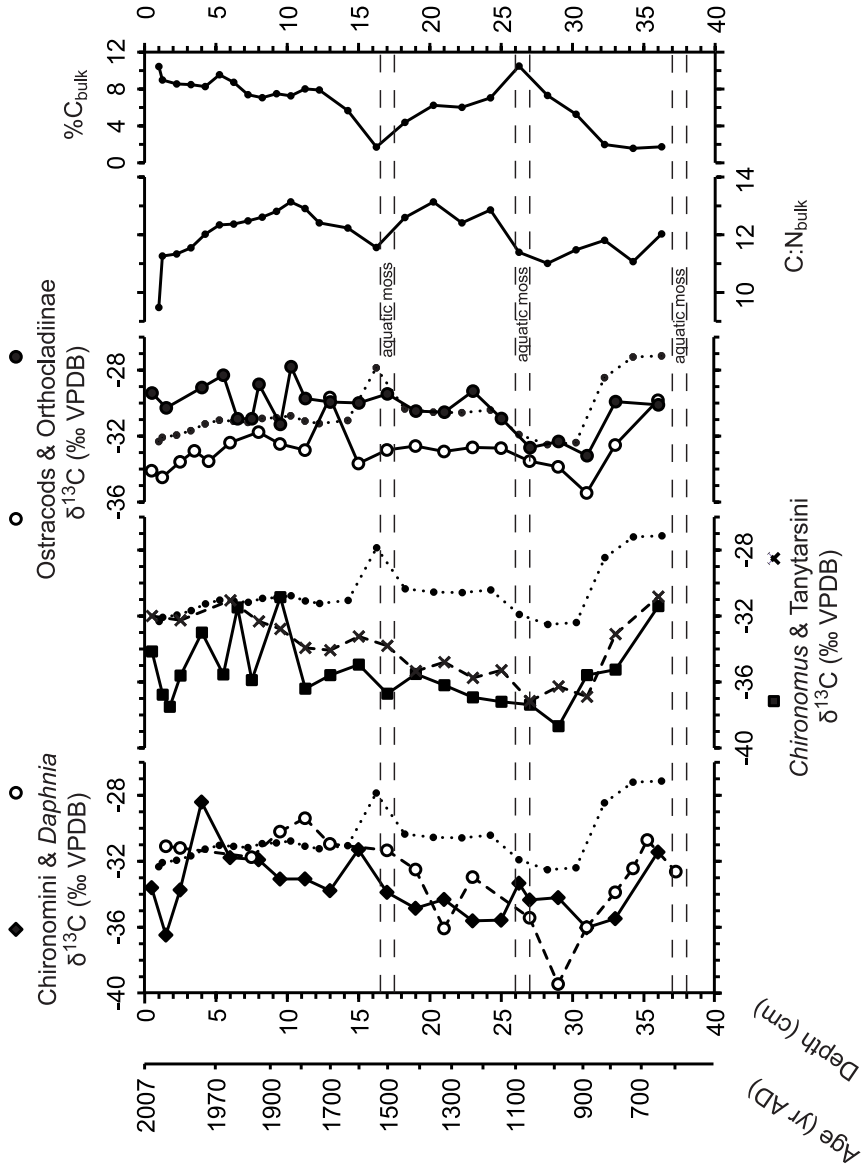
Fig. 4 Sediment record from Lake S2. $\delta^{13}C$ values (‰ VPDB) of invertebrate remains compared with bulk sediment $\delta^{13}C$ (dotted line plotted in three graphs with invertebrates), bulk sediment C:N-ratio, and organic matter content of bulk sediment. Dashed horizontal lines indicate layers with aquatic mosses.

Discussion

Methane fluxes and limnology of the study lakes

The diffusive methane fluxes measured in this study are at the high end of the range for tundra ponds and lakes that is reported in the literature (0.12-4.81 mmol m^{-2} d^{-1}; Bartlett et al. 1992; Kling et al. 1992; Zimov et al. 1997; Phelps et al. 1998; Reeburgh et al. 1998; Walter et al. 2006). Diffusive fluxes in this study were measured around mid-day and not corrected for diurnal variations in methane fluxes, which may have caused a slight overestimation of methane fluxes. However, thermokarst lakes such as our study sites are known to feature exceptionally high methane fluxes at the water surface. Also, lakes in this study are located on a river floodplain and nutrients enter the lake by annual flooding of the River Elon as well as by groundwater fluxes from the surrounding wet tundra, higher ridges, and permafrost slumps. Therefore, the study lakes are relatively productive and have elevated DOC concentrations compared to other Arctic lakes (Duff et al. 1999; Hamilton et al. 2001), both of which may also lead to relatively elevated methane fluxes.

Invertebrate δ¹³C in surface sediments

Chironomini in the surface sediments mainly consist of *Sergentia coracina*-type and *Stictochironomus*. Both taxa are mud-dwellers that build tubes on the lake bottom (Moller Pillot 2009). Recent studies have shown that these tubes can be colonized by methanotrophic bacteria which may form a considerable part of the diet of some chironomid larvae (Deines et al. 2007a). For *Sergentia*, δ¹³C values 10‰ lower than δ¹³C of the bulk sediment they live in have been reported (Jones et al. 2008) and for *Stictochironomus* δ¹³C values as low as -64‰ have been observed (Kiyashko et al. 2001), suggesting that both taxa can assimilate methane-derived carbon. The evidence for assimilation of methane-derived carbon in *Sergentia coracina*-type and *Stictochironomus* can explain the strong correlation between δ¹³C of Chironomini and methane fluxes. In Lake N1 δ¹³C of Chironomini are higher (-28.8‰) than in other lakes (Table 2) even though diffusive methane flux in Lake N1 is high compared to other lakes (1.16 ±0.44 mmol m^{-2} d^{-1}, see Table 1). The higher δ¹³C values can be explained by the relatively high abundances of *Cladopelma lateralis*-type and *Cryptochironomus* in Lake N1 because these taxa are likely to incorporate more plant- or animal-derived carbon. *Cladopelma* is described as grazer on diatoms and detritus feeder (Moog 2002; Moller Pillot 2009). Small larvae of *Cryptochironomus* feed on plant-detritus, whereas larger larvae are predating on small invertebrates (Merritt et al. 2008; Moller Pillot 2009).

Daphnia are filter feeders that can assimilate MOB, which can lead to low δ¹³C in their biomass (Bastviken et al. 2003; Taipale et al. 2008). This process may be most active when methane concentrations have built up in the lake water and become available in the oxic water column, e.g. after ice break-up in spring or at the onset of mixing in temporarily stratified lakes (Sundh et al. 2005; Kankaala et

al. 2007b). In our study *Daphnia* δ^{13}C significantly correlated with methane flux, suggesting that *Daphnia* could feed increasingly on biomass of methanogenic and methane-oxidizing bactera in lakes with a higher methane output (Fig. 3). The correlation between methane fluxes and δ^{13}C values of Chironomini and *Daphnia* becomes even stronger if data from the study lakes are combined with data of δ^{13}C in invertebrates in seven Swedish lakes that have been produced as described in Chapter 4. In the Swedish lakes the correlation between Chironomini and *Daphnia* and methane flux is also negative (r = -0.90 and -0.57, respectively) although only the relationship with Chironomini is statistically significant (p = 0.0062). If the two datasets are combined both correlations are strongly negative (r = -0.72 and -0.81 for Chironomini and *Daphnia*, respectively) and statistically significant (p = 0.0013 and p < 0.0001, respectively). This suggests that Chironomini and *Daphnia* are sensitive recorders of increased methane fluxes and that δ^{13}C values in the remains of Chironomini and *Daphnia* can be used to provide information about changes in methane fluxes in the past.

      Chironomus has a high tolerance to low oxygen conditions (Saether 1979) and most species in this genus build tubes in soft sediments, where they can access methane-derived carbon (Deines et al. 2007a), leading to specimens with δ^{13}C values as low as -72‰ (Jones et al. 2008). In our study lakes, *Chironomus anthracinus*-type was the dominant taxon. This morphotype consists of a number of species including *C. anthracinus* (*sensu stricto*) that has previously been reported to feed partly on methane-derived carbon and to have δ^{13}C values as low as -42‰ (Grey 2004; Jones et al. 2008). This can explain the relatively low δ^{13}C values in *Chironomus* compared with other remains in surface sediments, although the correlation between methane fluxes and δ^{13}C of *Chironomus* was weaker than between methane fluxes and δ^{13}C of Chironomini and *Daphnia* (Fig. 3).

      The chitinous linings of ostracod carapaces have low δ^{13}C values compared to other taxa, suggesting that ostracods may have assimilated methanogenic carbon. δ^{13}C values in the range of -39 to -31‰ are reported for this taxon in Chapter 4. A single very depleted sample with a δ^{13}C value of -39‰, suggests that part of the carbon of the chitinous ostracod cuticle of specimens included in this sample is derived from methane and that ostracods may therefore feed on MOB or other invertebrates living on them. Some benthic ostracods can burrow several cm into sediments (Griffiths and Martin 1993; Meisch 2000) and may therefore potentially assimilate methanogenic carbon originating from MOB or protozoans feeding on them. Methane-derived carbon in ostracod remains has also been suggested in studies of δ^{13}C in calcite of benthic ostracods (Curry et al. 1997; Schwalb 2003). However, only a weak negative correlation was found between methane fluxes and δ^{13}C of chitinous ostracod remains in the study lakes, similar to the correlation found for *Chironomus* (Fig. 3).

      Tanypodinae are mostly predators of Protozoa, small Crustacea, and Diptera larvae, but also ingest plant material (Vallenduuk and Moller Pillot 2007; Merritt et al. 2008). The most dominant taxon of this group in our lakes is *Procladius*. Jones et al. (2008) observed up to 10‰ lower δ^{13}C values in *Procladius* than in bulk sediment and Borderelle et al. (2008) found ^{13}C-depleted Tanypodinae in profundal sediments, suggesting that Tanypodinae could also

assimilate methane-derived carbon to some degree if they feed on depleted prey populations.

Most Tanytarsini are detritivores, although some are known to be tube-building filter feeders (Merritt et al. 2008), and could feed partly on methane-derived carbon similar to Chironomini, which may explain the negative correlation between δ¹³C of Tanytarsini remains and diffusive methane fluxes (Fig. 3). The dominant taxa in the surface sediments, *Tanytarsus lugens*-type, *Paratanytarsus*, and *Corynocera oliveri*-type, feed mainly on detritus in and on the sediment (Moog 2002).

Orthocladiinae are the most diverse group of chironomids in the studied lakes. The taxa found in the surface sediments are typical for littoral habitats and are reported to predominantly feed on algae and associated microorganisms growing on hard substrates such as rocks and macrophytes. The dietary preference for plant-derived material is also reflected by the relatively high δ¹³C values that are similar or up to 1.2 ±1.0‰ higher than δ¹³C of the bulk sediment in the surface sediments. δ¹³C of Orthocladiinae is similar to δ¹³C values reported for lacustrine algae (Meyers 1994). Based on their gut content and stable isotope analysis of larvae and various substrates Mihuc and Toetz (1994) estimated that 80-91% of the diet of the larvae of the orthocladiinae genus *Orthocladius* living in an alpine wetland consisted of diatoms and algae.

δ¹³C values of invertebrate remains vary substantially between the analyzed taxa and resemble the pattern found previously in the Swedish lakes studied in Chapter 4, with taxa such as ostracods characterized by the lowest δ¹³C values and groups such as Orthocladiinae with the highest. This suggests that variations in δ¹³C values between taxonomic groups could be explained by similar differences in habitats and feeding strategies as in Swedish study lakes, resulting in the assimilation of different carbon sources (Chapter 4). The remains of invertebrate taxa that assimilate mainly plant-derived carbon such as Orthocladiinae have elevated δ¹³C values, whereas taxa that assimilate more methane-derived carbon such as Chironomini, *Chironomus*, *Daphnia*, and ostracods have low δ¹³C values. δ¹³C values of the remains of chironomids and cladocerans are not as negative as reported for some living specimens that assimilate methane-derived carbon. Jones et al. (2008) suggest that it is only in lakes in which the late-summer oxygen concentration at the sediment–water interface decreases to <2-4 mg O_2 L^{-1} that markedly ¹³C-depleted specimens in the range of -70 to -40‰ can be expected. Invertebrate remains in our surface sediment samples can be expected to have been deposited over a period of several years. Therefore, these samples provide an averaged, integrated δ¹³C value of invertebrates that lived during this period, in which MOB concentrations can have varied. This may be the reason why they do not show as extremely ¹³C-depleted values as some of the living individuals reported in the literature that represent only a single season.

¹³C-depletion in zooplankton can also be caused by high production of heterotrophically respired dissolved inorganic carbon (DIC) in lakes. Such 'recycled' DIC may serve as ¹³C-depleted carbon source for phytoplankton and in turn for grazing *Daphnia*. This process is especially strong in dystrophic lakes with high DOC concentrations and in a number of regions decreasing δ¹³C values

in zooplankton have been observed with increasing DOC in lakes (Lennon et al. 2006; Karlsson 2007). Our lakes are characterized by high DOC concentrations of 26.5 to 39.2 mg L^{-1}. However, DOC concentrations were very similar in all the study lakes and could not explain the variability in *Daphnia* δ^{13}C since we did not observe a correlation between δ^{13}C of *Daphnia* and DOC (r = -0.06). Furthermore, recycled carbon would be expected to decrease the baseline δ^{13}C values of algal biomass in the lake, affecting not only δ^{13}C values of zooplankton, but also δ^{13}C values of bulk sediments and δ^{13}C values of invertebrates feeding on algae. A significant correlation was observed between DOC concentrations and δ^{13}C of bulk sediment (r = -0.66, p = 0.039), but not between DOC concentrations and δ^{13}C values of any invertebrate taxon. This suggests that recycled carbon could play a role in explaining ^{13}C-depleted carbon of the bulk sediment matrix. However, no evidence is available indicating that respired carbon from depleted organic matter was driving changes in invertebrate δ^{13}C and methane-derived carbon seems the more likely driver for δ^{13}C in taxa such as Chironomini and *Daphnia* in the studied lakes. This was also suggested in Chapter 4 for seven Swedish study lakes.

Higher δ^{13}C values of photoautotrophic biomass can occur in periods of increased productivity (Hollander et al. 1993). This is caused by the limitation of aqueous CO$_2$-availability and elevated growth rates that decrease discrimination against ^{12}C during photosynthesis, resulting in the synthesis of ^{13}C-enriched organic matter (Fogel and Cifuentes 1993). Hollander and Smith (2001) observed that the flux of photoautotrophic biomass from the epilimnion becomes sufficient to override the isotopic signal of methane-derived carbon above a critical threshold of 0.175 mg TP L^{-1}. However, with TP concentrations < 0.02 mg L^{-1} all our study lakes are well below this threshold, and no significant correlation was observed between nutrient concentrations and δ^{13}C values of bulk sediment or invertebrate remains. Therefore, we assume that differences in productivity between the study lakes did not have strong effects on δ^{13}C values of bulk sediment or invertebrate remains.

Sediment record

Two periods with relatively low δ^{13}C values in bulk sediment and invertebrate remains were observed between approximately AD 850 and 1150 (32-25.5 cm depth) and since the 1970s (5-0 cm depth). Based on observed patterns in the surface sediments from the lakes in this study and in seven Swedish lakes (Chapter 4), it seems likely that relatively low δ^{13}C values of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia*, and ostracods in these periods coincide with higher availability of methane-derived carbon in the lake. In the surface sediment samples that we examined, δ^{13}C values in Chironomini and *Daphnia* were most responsive to variations in the availability of diffusive methane fluxes (Fig. 3). Interestingly, periods with lower δ^{13}C values in Chironomini and *Daphnia* coincide with warmer periods that have been reconstructed based on a tree ring record from the region around the town of Chokurdakh (Sidorova

and Naurzbaev 2005; Fig. 5). A composite reconstruction based on 23 Arctic palaeoclimate records suggests that similar temperature variations affected the entire Arctic region (Kaufman et al. 2009).

Most striking in our taxon-specific δ¹³C data is the rapid decrease in δ¹³C values of Chironomini to -36.5‰ at 1-2 cm depth. This decrease is almost four times as large as the decrease in δ¹³C of bulk sediment. It is the lowest value for Chironomini in the record and comparable to the lowest δ¹³C value of Chironomini (-36.8‰) in the surface sediments, which is in the lake (Lake N7) with highest diffusive methane fluxes. A simultaneous decrease in δ¹³C values is observed in *Chironomus* and might have been caused by increased methane release from the sediments in this period as a response to rising temperatures. Unfortunately, *Daphnia* remains are absent from the uppermost sediments of Lake S2 and therefore can not provide insights on whether this change in δ¹³C was recorded by the zooplankton as well.
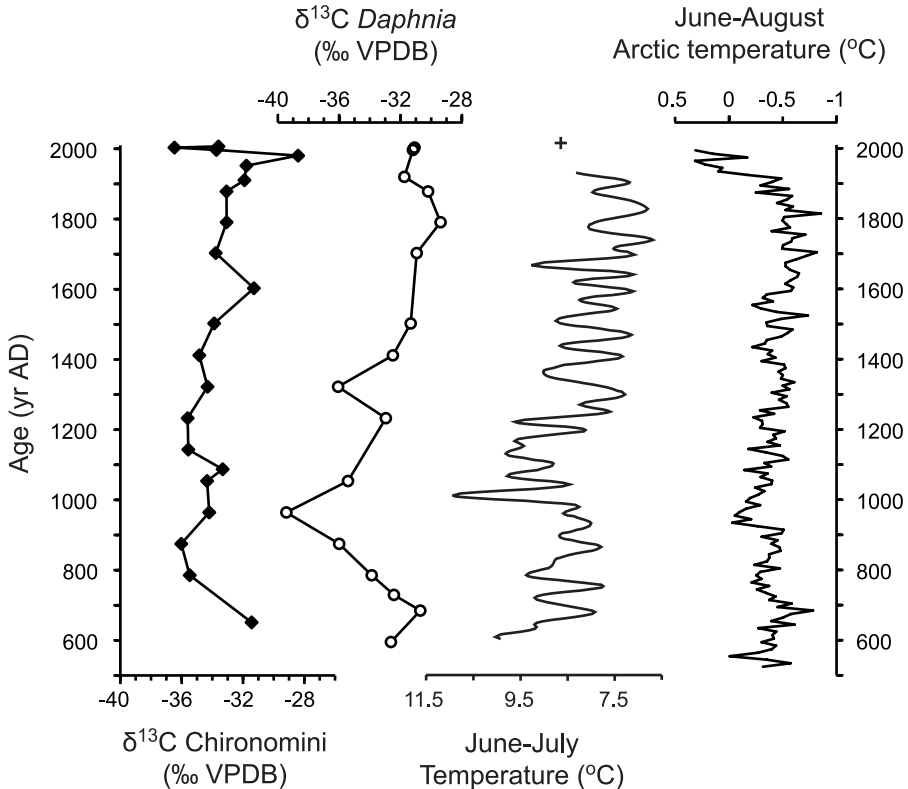
Fig. 5 From left to right: δ¹³C values (‰ VPDB) of remains of Chironomini and *Daphnia* compared with a June-July temperature reconstruction based on a tree-ring record from the region around the town of Chokurdakh, northern Yakutia (Sidorova and Naurzbaev 2005) with (+) indicating 1997-2006 average June-July temperatures in Chokurdakh, and reconstructed June-August temperatures based on a compilation of Arctic proxy-records (Kaufman et al. 2009, 2010).

105

Low δ^{13}C values between 25.5 and 32 cm depth and above 5 cm depth coincided with relatively low C:N ratios and a high organic matter content, which could indicate a long-term increase in autochtonous primary productivity (Meyers and Teranes 2001). In lakes with high DOC concentrations such as Lake S2, increased productivity could result in increased carbon recycling causing lower baseline δ^{13}C values in the lake (Lennon et al. 2006; Karlsson 2007). However, if carbon recycling was the main cause of lower δ^{13}C values, this could be expected to also affect δ^{13}C of bulk sediments and most invertebrates accordingly, which was not observed. Most sediment-dwelling chironomids (Chironomini, *Chironomus*, and Tanytarsini) and pelagic *Daphnia* in the sediment record had lower δ^{13}C values and larger shifts in δ^{13}C values compared with bulk sediment and Orthocladiinae (Fig. 4). This suggested that Chironomini, *Chironomus*, Tanytarsini, and *Daphnia* assimilated a ^{13}C-depleted carbon source that was neither recorded by bulk sediments nor by Orthocladiinae. Because of the relatively high δ^{13}C values of herbivorous Orthocladiinae it is unlikely that the ^{13}C-depleted carbon recorded by Chironomini, *Chironomus*, Tanytarsini, and *Daphnia* in these periods was derived from plants. Therefore, we suggest that the ^{13}C-depleted carbon source assimilated by Chironomini, *Chironomus*, Tanytarsini, and *Daphnia* was methane-derived, and that methane-derived carbon became more important in warmer periods in Lake S2.

Lakes have been largely ignored in models that simulate methane emissions from high latitude wetlands (van Huissteden 2004; Zhuang et al. 2004) because their methane emissions where considered marginal. However, recent studies have indicated that Arctic lakes, and especially thermokarst lakes such as the ones examined here, can contribute substantially to atmospheric methane (Walter et al. 2006). Our results suggest that methane availability varied considerably in Lake S2 during the past ca. 1400 years and that methane availability from this lake was larger during warmer periods than during cooler episodes. This is in line with models that suggest that during the previous glaciation Arctic wetlands produced more methane during the warmer interstadials than the cooler stadials (Velichko et al. 1998; Huber et al. 2006). If other high-latitude lakes responded similarly to past climatic changes as Lake S2 this would suggest that thermokarst lakes react dynamically in their methane output to environmental change.

Conclusions

Chitinous remains of several invertebrate taxa, including *Chironomus*, Chironomini, Tanytarsini, Tanypodinae, *Daphnia*, and ostracods have δ^{13}C values that are negatively correlated with diffusive methane fluxes from the lake surface and may incorporate methane-derived carbon. δ^{13}C of Chironomini and *Daphnia* remains appear to be especially sensitive to variations in methane availability, based on significant correlations between δ^{13}C of their remains and diffusive methane fluxes. In contrast, the relatively high δ^{13}C values of Orthocladiinae,

suggest that this taxon derives its carbon directly from photosynthetic primary producers.

In a ca. 1400-year sediment record obtained from Lake S2 δ¹³C values of chitinous remains of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* were lower and showed larger shifts than δ¹³C of Orthocladiinae and bulk sediment. δ¹³C of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* remains were lowest in sediment sections deposited between ca. AD 850 and 1150 and since ca. AD 1970. In these periods, higher temperatures are inferred in proxy-records from this and other Arctic regions. Based on the correlations between diffusive methane fluxes and δ¹³C in remains of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* in surface sediments, this suggest that methane availability varied considerably during the past ca. 1400 years and that methane availability in this lake was larger during warmer periods than during cooler episodes.

Clearly, more work is necessary to corroborate our results and confirm that methane availability in other Arctic lakes has shown similar variations as our results suggest for Lake S2. Also, our understanding of how physical and chemical characteristics affect the assimilation of methane-derived carbon in invertebrates needs to be improved. However, our initial results indicate that taxon-specific δ¹³C analyses of the chitinous remains of aquatic invertebrates have the potential to reconstruct past methane availability in lakes, based on lake sediment records.

Acknowledgements

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Samenvatting

Methaan is een belangrijk broeikasgas dat verantwoordelijk is voor ongeveer 20% van het broeikaseffect. Dit gas wordt onder andere gevormd wanneer organisch materiaal onder zuurstofloze omstandigheden wordt afgebroken, bijvoorbeeld onder water. Meren zijn daardoor één van de natuurlijke bronnen van methaan, maar de uitstoot van methaan uit meren is relatief weinig bestudeerd. Om een beter inzicht te krijgen in de huidige toename van methaan in de atmosfeer is het belangrijk om veranderingen in methaanuitstoot te bestuderen in relatie tot klimaatveranderingen in het verleden. Er zijn echter nauwelijks methodes om veranderingen in de methaanuitstoot van meren over langere tijdschalen te bestuderen. In dit proefschrift wordt een methode verkend waarmee dergelijke veranderingen onderzocht kunnen worden.

Deze methode is erop gebaseerd dat sommige ongewervelde dieren (invertebraten) die in meren leven, zoals de larven van dansmuggen en watervlooien, methaan oxiderende bacteriën kunnen gebruiken als voedselbron. Omdat de stabiele koolstofisotopenwaarden ($\delta^{13}C$) van methaan kenmerkend laag zijn, is dit lage $\delta^{13}C$-signaal terug te vinden in bacteriën, en daardoor ook in invertebraten. Een aantal van deze dieren maakt harde pantsers of rusteieren die erg stevig zijn en lange tijd bewaard blijven in meersedimenten. Door $\delta^{13}C$ van deze resten te meten kan bepaald worden of methaan oxiderende bacteriën een belangrijke voedingsbron zijn voor deze dieren.

In dit proefschrift is het verband bestudeerd tussen methaanuitstoot en $\delta^{13}C$ in de resten van invertebraten. Dit is gedaan aan de hand van laboratoriumexperimenten en veldonderzoek in Zweden en Siberië. Daarbij werd methaanuitstoot van meren vergeleken met de $\delta^{13}C$ van invertebratenresten uit het bovenste laagje (modern) meersediment. Vervolgens is $\delta^{13}C$ gemeten aan 'fossiele' resten van invertebraten uit sedimentkernen van een aantal meren om informatie te krijgen over veranderingen in de aanwezigheid van methaan door de tijd en onderzocht hoe dit zich verhoudt tot klimaatverandering.

Het eerste gedeelte van dit proefschrift beschrijft de methodes die gebruikt zijn voor het zeven en chemisch schoonmaken van sedimentmonsters om de resten van invertebraten te isoleren en de $\delta^{13}C$ ervan te meten. Verder werd onderzocht of de resten van invertebraten in sedimentmonsters representatief zijn voor de levende gemeenschap en of het gedeelte van invertebraten dat als rest overblijft een 'methaansigmaal' kan bevatten.

Hoofdstuk 1: Door middel van kweekexperimenten met dansmuglarven werd aangetoond dat het $\delta^{13}C$-signaal van methaan niet alleen terecht komt in het zachte weefsel van dansmuglarven dat doorgaans snel vergaat, maar ook in de hoofdjes die als fossielen in meersedimenten kunnen worden teruggevonden. Verder bleek dat de chemische methodes die gebruikt worden om hoofdjes en andere resten schoon uit het sediment te halen geen (of een verwaarloosbaar klein) effect hebben op de $\delta^{13}C$ van deze resten. Ook kon de $\delta^{13}C$-waarde van deze resten gemeten worden met een precisie van ±0.5‰, wat goed genoeg is

om onderscheid te kunnen maken tussen plantaardige en methaangerelateerde voedingsbronnen. Uit een serie metingen aan hoofdjes is ook gebleken dat de kleinst mogelijke hoeveelheid materiaal die gemeten kon worden ongeveer 20 microgram is.

Hoofdstuk 2: De snelheid van het uitsorteren van sedimentmonsters kan met 30-58% worden verhoogd door zeven te gebruiken met een maaswijdte van 200 micrometer ten opzichte van 100 micrometer die veel wordt gebruikt in palaeo-ecologische studies. Daardoor blijven vooral grotere, relatief zware en makkelijk op te pakken resten achter en kan het benodigde minimumgewicht voor δ^{13}C-metingen sneller worden verzameld.

Hoofdstuk 3: De resten van invertebraten (met name dansmuggen) in het sediment zijn representatief voor de levende gemeenschap in het meer De Waay (nabij Culemborg). Dit bleek door de gemeenschap van levende invertebraten te vergelijken met hun resten in de bovenste laag sediment. Slechts van een klein aantal zeldzamere soorten zijn geen resten gevonden. Van een aantal andere soorten werden wel de resten gevonden, maar niet de levende exemplaren, vermoedelijk omdat niet alle habitats in het meer bemonsterd zijn. Verder kwamen de meeste levende invertebraten voor in het ondiepe deel van het meer, in de buurt van de oever en van waterplanten. Slechts enkele soorten die zijn aangepast aan lage zuurstofconcentraties kwamen voor op grotere waterdiepte. De resten waren echter gelijkmatig verspreid over de meerbodem, wat erop duidt dat die resten van ondiepe naar diepe gedeeltes van De Waay zijn getransporteerd. Het bestuderen van de resten in een sedimentkern in het midden van een dergelijk meer kan dus een representatief overzicht geven van de soorten die erin voorkomen (en voorkwamen).

Het tweede gedeelte van dit proefschrift richt zich op het verband tussen methaanuitstoot uit meren en de δ^{13}C-waarden van resten van invertebraten in het bovenste laagje sediment. Door middel van gemeten δ^{13}C-waarden van 'fossiele' resten in sedimentkernen wordt vervolgens getracht om veranderingen in de aanwezigheid van methaan in meren af te leiden.

Hoofdstuk 4: In zeven Zweedse meren werd methaanuitstoot gemeten en vergeleken met δ^{13}C-waarden in de resten van een aantal groepen invertebraten (verschillende groepen dansmuggen, watervlooien, mosdiertjes, haften en mosselkreeftjes). Elk van deze groepen heeft een eigen voedselvoorkeur, leefwijze en habitat. Dat bleek ook uit hun δ^{13}C-waarden, die vergelijkbare verschillen toonden in alle onderzochte meren en ook op verschillende diepten binnen een meer. Larven van haften en van dansmuggen van de groep Orthocladiinae, bijvoorbeeld, hadden in elk meer relatief hoge δ^{13}C-waarden, wat duidt op plantaardige voeding. Mosselkreeftjes en sommige mosdiertjes hebben daarentegen lage δ^{13}C-waarden die duiden op een methaangerelateerde voedingsbron. Ook werden duidelijke relaties gevonden tussen methaanuitstoot en δ^{13}C-waarden van resten van een aantal invertebratengroepen: lagere δ^{13}C-waarden werden gemeten in meren waar grotere methaanuitstoot was. Ook werden lagere δ^{13}C-waarden gemeten op plekken binnen meren waar meer methaanuitstoot was. Deze relatie was met name sterk bij *Daphnia* (een groep

watervlooien) en Chironomini (een groep dansmuggen).

Hoofdstuk 5: In verschillende monsters van een sedimentkern uit het Zweedse Strandsjön werden δ^{13}C-waarden van invertebratenresten en geochemische eigenschappen gemeten. Het sediment is de afgelopen 140 jaar afgezet en bevat een aantal periodes waarin δ^{13}C-waarden van dansmuglarven gedaald zijn. Andere invertebraten die hun voeding uit het water filteren (watervlooien en mosdiertjes) hebben relatief constante δ^{13}C waarden, afgezien van een daling sinds ongeveer 1960. Het lijkt erop dat watervlooien en mosdiertjes een andere voedingsbron hebben dan dansmuglarven in dit meer. Opvallend is de enorme stijging in δ^{13}C-waarden van bulk sediment (maar niet van invertebratenresten) die dateert rond het eind van de negentiende eeuw. Mogelijk hangt dit samen met een kunstmatige verlaging van de waterspiegel van Strandsjön, die bekend is uit historische bronnen. Een andere mogelijkheid is een zeer grote productie van methaan oxiderende bacteriën in diepere lagen van het sediment.

Hoofdstuk 6: De relatie tussen methaanuitstoot en δ^{13}C van invertebratenresten werd ook bekeken in tien Siberische meren. Hogere δ^{13}C-waarden werden gevonden bij invertebratengroepen die voornamelijk plantaardig voedsel eten en lagere waarden in groepen die ook methaan oxiderende bacteriën opnemen. Net als in de Zweedse meren werden correlaties gevonden tussen methaanuitstoot en δ^{13}C waarden van een aantal invertebratengroepen, die het sterkst waren voor *Daphnia* en Chironomini. De correlaties tussen δ^{13}C waarden van invertebratenresten en andere chemische en fysische variabelen van de meren (diepte, waterchemie, δ^{13}C van bulk sediment) waren minder sterk. Dit suggereert dat de beschikbaarheid van methaan de δ^{13}C-waarden van bepaalde groepen invertebraten beïnvloedt (met name bij *Daphnia* en Chironomini).

Siberië is gevoelig voor klimaatverandering en hogere temperaturen zullen daar vermoedelijk zorgen voor het afsmelten van permafrost, waardoor meer natte gebieden ontstaan die zorgen voor een grotere methaanuitstoot. In één van de Siberische meren werd een sedimentkern geboord die ongeveer 1400 jaar beslaat en waaraan δ^{13}C-waarden werden gemeten van een aantal invertebratengroepen. Gedurende warmere periodes (tussen 850 en 1150 en vanaf 1970) zijn lagere δ^{13}C-waarden gevonden in de resten van onder andere Chironomini en *Daphnia*, de groepen die gevoelig zijn voor methaan oxiderende bacteriën in hun voeding. Larven van Orthocladiinae, die vooral plantaardig voedsel eten, laten dit patroon niet duidelijk zien. Het lijkt er daarom op dat er meer methaan aanwezig was in het onderzochte meer gedurende warmere periodes, wat overeenkomt met bestaande modellen en waarnemingen.

Samenvattend kan gesteld worden dat het meten van δ^{13}C-waarden aan diverse invertebratengroepen mogelijk is. Bepaalde groepen (met name Chironomini en *Daphnia*) lijken gevoelig te zijn voor veranderingen in de hoeveelheid methaan oxiderende bacteriën in hun voeding. Hun resten in sedimentkernen kunnen gebruikt worden om inzicht te krijgen in de methaandynamiek van meren onder veranderende (klimaat)omstandigheden. Dat is belangrijk om te kunnen inschatten in hoeverre de uitstoot van broeikasgas uit meren bijdraagt aan de huidige klimaatverandering.

Acknowledgements

This thesis is dedicated to Pim Tiggelman, who sadly will not be able to join me in celebrating its completion. Pim taught me the beauty of true commitment and friendship, which gives me strength when it is most needed. I will never forget.

I have been lucky with a number of very stimulating teachers, but would like to highlight Roger Wotton in particular. His enthusiasm for aquatic biology during lectures and discussions are as inspiring as his dedication to fundamental research. Ever since I was very little, I have been delighted by the little critters that I found in ponds and ditches at home and elsewhere, but it wasn't until meeting Roger Wotton that I seriously thought of a career in this field.

My promotor, Andy Lotter has challenged me ever since I've first met him. His critical eye has been unnerving at times, but always acted as a great stimulus to improve my work and I'm thankful for always experiencing Andy's support and freedom to expore new territories. Sufficiently expressing my gratitude towards Oliver Heiri, my co-promotor and daily supervisor, is nearly impossible. I have thoroughly enjoyed our intense discussion sessions, and learned a lot from his careful approach and thoughtful phrasing. Thanks for the great company (day and night) during our trips, and for the warm welcome in Bern, in the Reconmet project that inspired important parts of this thesis!

Furthermore, I would like to thank the members of the reading committee: Dr. David Bastviken, Prof.dr. Ellen van Donk, Prof.dr. Riks Laanbroek, and Prof. dr. Jack Middelburg. The Darwin Center for Biogeosciences and the Laboratory for Palaeobotany and Palynology are kindly thanked for financially supporting my research, and Staatsbosbeheer for access to Lake De Waay.

I am grateful for the opportunity to work with many wonderful colleagues and co-authors that I would like to thank for the stimulating discussions we had. I would especially like to name David Bastviken, Paul Bodelier, Steve Brooks, Jonathan Grey, Hanna Hartikainen, Roland Hall, Boris Ilyashuk, Emiliya Kirilova, Beth Okamura, Frans-Jan Parmentier, Angela Self, Frederike Verbruggen and Michiel Wilhelm.

Fellow PhD students at the Laboratory of Palaeobotany and Palynology (LPP) have made each day at university (and many evenings and nights out) a real treat! so many many thanks to Judith Barke, Peter Bijl, Nina Bonis, Sander Houben, Emiliya Kirilova, Emmy Lammertsma, Peter Spierenburg and Micha Ruhl. I want to say an especially warm 'thank you' to Frederike Verbruggen. We have spent a fair number of hours together in the same room, sharing not only the joy of picking chironomids, but also our stories about love, the universe, and everything.

I've been lucky with the extremely supportive environment at LPP and want to thank all (former) colleagues at LPP for this: Hans van Aken, Hanneke Bos, Henk Brinkhuis, Johan van der Burgh, Timme Donders, Ton van Druten, Walter Finsinger, Alejandra Goldenberg, Marjolein Hazekamp, Waldemar Herngreen, Han van Konijnenburg-van Cittert, Wolfram Kürschner, Roel Janssen, Jan de Leeuw, Gianluca Marino, Tammo Reichgelt, Francesca Sangiorgi, Jos Schilder,

Curriculum Vitae

Maarten van Hardenbroek was born 2 May 1982 in Utrecht, the Netherlands. After graduating from Christelijk Gymnasium Utrecht in 2000, he studied one year of Theater, Film and Television science at Utrecht University (UU) and was involved with a number of theater productions. In 2001 he did an internship at the Institute for Earth Education in Greenville, West Virginia after which he started a BSc. degree in Environmental Science at UU. In 2004 he participated in an Erasmus exchange programme with University College London, where his interest in palaeo-ecology was sparked. In 2005 he did an internship at the Laboratory for Palaeobotany and Palynology (UU) reconstructing environmental change using remains of cladocerans (water fleas). In 2005-2006 he did a MSc. degree in Quaternary Science at Royal Holloway, University of London with an internship at the Stable Isotope Research Laboratory, McMaster University. His MSc. thesis focused on stable hydrogen and oxygen isotopes in water beetle chitin and resulted in a graduation 'with distinction'.

In January 2007 he started as a PhD student at the Laboratory of Palaeobotany and Palynology (UU) within a multi-disciplinary project funded by the Darwin Center for Biogeosciences. In this project he explored the potential of stable carbon isotopes in invertebrate remains to reconstruct methane-derived carbon in lake ecosystems resulting in this thesis. From February 2011 he will work as post-doctoral researcher at the Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, Switzerland.

List of Publications

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