Relationship between $\delta^{13}C$ of chironomid remains and methane flux in Swedish lakes

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SUMMARY

1. Methanogenic carbon can be incorporated by methane-oxidising bacteria, leading to a ^{13}C-depleted stable carbon isotopic composition ($\delta^{13}C$) of chironomids that feed on these microorganisms. This has been shown for the chironomid tribe Chironomini, but very little information is available about the $\delta^{13}C$ of other abundant chironomid groups and the relationship between chironomid $\delta^{13}C$ and methane production in lakes.

2. Methane flux was measured at the water surface of seven lakes in Sweden. Furthermore, fluxes from the sediments to the water column were measured in transects in two of the lakes. Methane fluxes were then compared with $\delta^{13}C$ of chitinous chironomid remains isolated from the lake surface sediments. Several different chironomid groups were examined (Chironomini, Orthocladiinae, Tanypodinae and Tanytarsini).

3. Remains of Orthocladiinae in the seven study lakes had the highest $\delta^{13}C$ values (−31.3 to −27.0‰), most likely reflecting $\delta^{13}C$ of algae and other plant-derived organic matter. Remains of Chironomini and Tanypodinae had lower $\delta^{13}C$ values (−33.2 to −27.6‰ and −33.6 to −28.0‰, respectively). A significant negative correlation was observed between methane fluxes at the lake surface and $\delta^{13}C$ of Chironomini ($r = -0.90$, $P = 0.006$). Methane release from the sediments was also negatively correlated with $\delta^{13}C$ of Chironomini ($r = -0.67$, $P = 0.025$) in the transect samples obtained from two of the lakes. The remains of other chironomid taxa were only weakly or not correlated with methane fluxes measured in our study lakes ($P > 0.05$).

4. Selective incorporation of methane-derived carbon can explain the observed correlations between methane fluxes and $\delta^{13}C$ values of Chironomini. Remains of this group might therefore have the potential to provide information about past changes in methane availability in lakes using sediment records. However, differences in productivity, algal $\delta^{13}C$ composition and the importance of allochthonous organic matter input between the studied lakes may also have influenced Chironomini $\delta^{13}C$. More detailed studies with a higher number of analysed samples and detailed measurement of $\delta^{13}C$ of different ecosystem components (e.g. methane, dissolved inorganic carbon) will be necessary to further resolve the relative contribution of different carbon sources to $\delta^{13}C$ of chironomid remains.

Keywords: chironomids, lake sediment, methane, methane-oxidising bacteria, stable carbon isotopes

Introduction

Organic matter derived from the oxidation of methane by methane-oxidising bacteria is a potential carbon and energy source for lentic invertebrates in a range of lake types and circumstances (Bastviken *et al.*, 2003; Jones & Grey, 2011). The carbon isotopic value ^{13}C-depleted stable carbon isotopic composition ($\delta^{13}C$) of methane and of

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methane-oxidising bacteria growing on this carbon source is distinctly lower (−100 to −50‰) (Jahnke *et al.*, 1999) than $\delta^{13}C$ of organic matter produced by algae and higher plants (−40 to −8‰) (France, 1995; Meyers & Teranes, 2001; Bade *et al.*, 2006). Therefore, carbon isotopic analyses can provide insights into the importance of methanogenic carbon in different compartments of lacustrine foodwebs (Bastviken *et al.*, 2003; Grey & Deines, 2005). Carbon isotopic analyses have indicated that the lake-living larvae of at least some groups of chironomid midges are exceptionally prone to ingesting and incorporating methanogenic carbon into their biomass (Jones & Grey, 2011). For example, larvae of the chironomid genera *Chironomus* and *Stictochironomus* from a number of lakes in the U.K., Finland, Germany, Japan and Sweden have been reported with $\delta^{13}C$ as low as −72 and −64‰, respectively (Kiyashko, Narita & Wada, 2001; Jones *et al.*, 2008), values clearly lower than those representative of organic matter produced by higher plants and algae. Methanogenic carbon may form a more important carbon source for chironomids than for other invertebrates living in lakes because the habitat of burrowing species is often at or close to the oxycline in lake sediments. As a consequence, methane-oxidising bacteria that need access to both methane and oxygen for growth are often found at very high abundances in chironomid tubes or in the sediments within which the chironomid larvae are burrowing (Jones & Grey, 2011).

van Hardenbroek *et al.* (2010a) have recently demonstrated that the carbon isotopic signature of methanogenic carbon is recorded in the fossilising exoskeleton structures of chironomid larvae (the larval head capsules). This raises the possibility of analysing $\delta^{13}C$ of chitinous remains of chironomids to provide insights into past changes in methane production and oxidation in lakes (Heiri *et al.*, 2009). The $\delta^{13}C$ of larvae of only some chironomid groups, most of them belonging to the tribe Chironomini, have been studied in detail in lakes (Jones *et al.*, 2008). In contrast, very little information is available about $\delta^{13}C$ of other abundant chironomid groups, such as the tribe Tanytarsini or the subfamilies Orthocladiinae and Tanypodinae. Furthermore, since fossil chironomid assemblages in lake sediments can incorporate remains produced in different parts of the lake basin (van Hardenbroek *et al.*, 2011), it is presently unclear whether the relationship between methane concentrations and carbon isotopic composition described for some groups of modern chironomid larvae (Deines & Grey, 2006) is apparent in fossil assemblages as well.

Here we present a study of the stable carbon isotopic composition of chironomid remains in surface sediments sampled from seven lakes in south central Sweden. The study sites were selected based on their different levels of methane production. We present measurements of $\delta^{13}C$ of subfossil remains of different chironomid groups in different lakes together with estimates of methane flux from the water surface to the atmosphere. Our study provides a more extensive assessment of whether chironomid remains deposited in natural lacustrine environments have the potential to be used as palaeoecological indicators of past methane availability in lakes than the initial laboratory experiments of van Hardenbroek, Heiri & Lotter (2010b). In addition to examining the relationship between $\delta^{13}C$ of chironomids and methane output between the seven lakes, we also explore whether similar patterns can be found when comparing chironomid $\delta^{13}C$ with methane flux from sediments to the water column in transects within two of the examined lake basins.

Methods

Study sites

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

Methane fluxes and water chemistry

Methane emissions from the water surface to the 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 to 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 both estimates of diffusive flux and total flux (diffusive flux and ebullition combined). Three to five chambers were deployed in each of the following depth zones: 0–1, 1–2, 2–4 and >4 m (14 chambers in total). Gas in the chambers was sampled after 24 h, and CH_4 concentrations were measured in the laboratory by gas chromatography using a flame ionisation detector (Shimadzu 8A) with a Porapack Q column. Depth profiles of oxygen concentrations and temperature in the water column were measured in Strandsjön, Lötsjön

Table 1 Physical characteristics (surface area, maximum depth), water chemistry (diffusive methane flux, pH, DOC, TP, TN), and bulk sediment characteristics (loss-on-ignition at 550 °C, $\delta^{13}C$ and C : N ratio) of the study lakes. Methane fluxes are average values of replicate measurements in the measuring period in June to 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
Maximum depth (m)	10	5	11.2	20	5	4	7
Diffusive flux (mmol m^{-2} day^{-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 ($n = 31$)
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

DOC, dissolved organic carbon; LOI, loss-on-ignition; TN, total nitrogen; TP, total phosphorus.

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, dissolved organic carbon (DOC), total nitrogen (TN) and total phosphorus (TP) were measured in surface water samples of all lakes. pH was analysed using a Hach-Lange HQD 40D pH meter with an Intellical gel-filled pH electrode. Concentrations of DOC and TP were measured following Pace & Cole (2002).

In Långsjön and Strandsjön, within-lake variability of methane fluxes from the sediments to the water column was also assessed. Sediment cores collected along the transects were first used to quantify methane release before the surface sediment layers were sampled for chironomid remains. Triplicate cores were retrieved at 5–6 locations with increasing water depth, transported to the laboratory and kept at approximately *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 sediment layers. Methane release from the sediments was measured after 12 h in the dark following Conrad & Rothfuss (1991) using a carbon dioxide and methane analyzer (Los Gatos Research Inc., Mountain View, CA, U.S.A.). The cores were capped with rubber stoppers that were pierced by two needles and connected by PVC tubing to the inlet and outlet of the gas analyzer to create a closed circuit. Methane concentrations in the core headspace were monitored for 3–15 min in each core and methane fluxes calculated based on the linear increase in methane

concentrations (measurements repeated in three replicate cores per water depth and reported ± standard error).

Sedimentological analyses

The top 0–2 cm of sediments in each of the gravity cores was sampled. Lakes in the area typically incorporate particles deposited over 2–5 years in the uppermost 2 cm of sediments (Gaillard *et al.*, 1991; Routh *et al.*, 2007), providing temporally integrated samples of the fossilising chironomids of the studied lake ecosystems. An aliquot was used to determine the concentration of sedimentary organic matter using loss-on-ignition (Heiri, Lotter & Lemcke, 2001). Subsamples for analysing $\delta^{13}C$ of bulk organic matter were rinsed in a 2.5% HCl solution for 15 min to remove carbonates, rinsed three times with demineralised water, centrifuged for 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 then analysed 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 ($\delta^{13}C$) were used as references. Replicate sample measurements on standards gave analytical errors (2σ) of ±0.05‰ ($n = 43$).

Sediment samples for the analysis of chironomid remains were deflocculated in 10% KOH solution for 2 h at room temperature and sieved using 200- and 100-μm sieves (van Hardenbroek *et al.*, 2010b). Sieve residues were exposed to 2.5% HCl solution for 15 min, rinsed three times and stored in demineralised water in the dark. Residues were sorted under a dissecting microscope at

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

40–100x magnification, and remains of the different chironomid subgroups *Chironomus* spp., Chironomini (excluding *Chironomus*), Tanytarsini, Tanypodinae and Orthocladiinae were transferred by forceps directly into pre-weighed ultraclean tin cups. These 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.

Chironomid samples were analysed on a Fisons NA 1500 NCS Elemental Analyzer (Fisons Instruments, Beverly, MA, U.S.A.) interfaced to a Thermo Electron Delta plus IRMS (ThermoScientific, Bremen, Germany). 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‰ (2σ). In sediment samples with enough material, replicates of individual taxa were analysed and average $\delta^{13}C$ values weighted by sample mass are reported here. Typically, 10–100 individual remains ($\sim$20 µg) were required for one stable carbon isotope analysis. Therefore, not enough material could be collected for all taxa in each sediment sample. For example, some remains of *Chironomus plumosus* type were encountered in the sediments but only at low abundances. Therefore, only few samples could be measured for $\delta^{13}C$, and *Chironomus* is not further analysed within this study. Statistical analyses were performed using PAST v2.00 (Hammer, Harper & Ryan, 2001).

Results

Methane fluxes

Average values of diffusive methane fluxes at the lake surface ranged from 0.04 ± 0.01 to 1.33 ± 1.38 mmol m^{-2} day^{-1} in the seven studied lakes (Table 1). A significant negative correlation between diffusive fluxes and $\delta^{13}C$ of bulk organic carbon of the lake sediment was detected ($r = -0.81$, $P = 0.027$), but no significant correlations with other environmental parameters were apparent (Table 3).

Within Strandsjön and Långsjön, the highest methane releases were measured in sediments from the shallowest sampling stations (Fig. 2). However, the measurements at 1.3 m water depth in Strandsjön were very variable. These sediments consisted of coarse plant debris (mainly stems of *Phragmites*), and there were observations during fieldwork of the sediment matrix being disturbed during the coring process. This may have led to significantly higher methane release in the laboratory than might have occurred within

Table 3 Linear correlations (*r*-values) between δ13C values, surface area, diffusive methane flux, pH, dissolved organic carbon (DOC), total phosphorous (TP), loss-on-ignition at 550 °C and C : N ratio of sedimentary organic matter in the seven study lakes (*P < 0.05, **P < 0.01).

	Area	Flux	LOI_{550}	$\delta^{13}C_{bulk}$	$C:N_{bulk}$	DOC	TP	pH	CMI	TNT	ORT	TNP
Area		0.61	−0.77*	−0.91**	−0.91**	−0.08	0.95**	0.61	−0.74	−0.50	0.30	−0.41
Flux			−0.63	−0.81*	−0.64	0.20	0.68	0.48	−0.90**	−0.46	−0.05	−0.54
LOI_{550}				0.81*	0.95**	0.48	−0.73	−0.86*	0.62	0.49	−0.02	0.32
$\delta^{13}C_{bulk}$					0.87*	0.04	−0.91**	−0.61	0.84*	0.58	−0.04	0.62
$C:N_{bulk}$						0.38	−0.84*	−0.80*	0.68	0.48	−0.22	0.30
DOC							0.09	−0.54	−0.37	−0.29	−0.30	−0.50
TP								0.63	−0.81*	−0.56	0.10	−0.57
pH									−0.37	−0.11	0.17	−0.02
CMI										0.74	0.15	0.73
TNT											0.58	0.86*
ORT												0.66

$\delta^{13}C_{bulk}$, δ13C of bulk sediment; CMI, Chironomini; TNT, Tanytarsini; ORT, Orthocladiinae; TNP, Tanypodinae; LOI, loss-on-ignition.
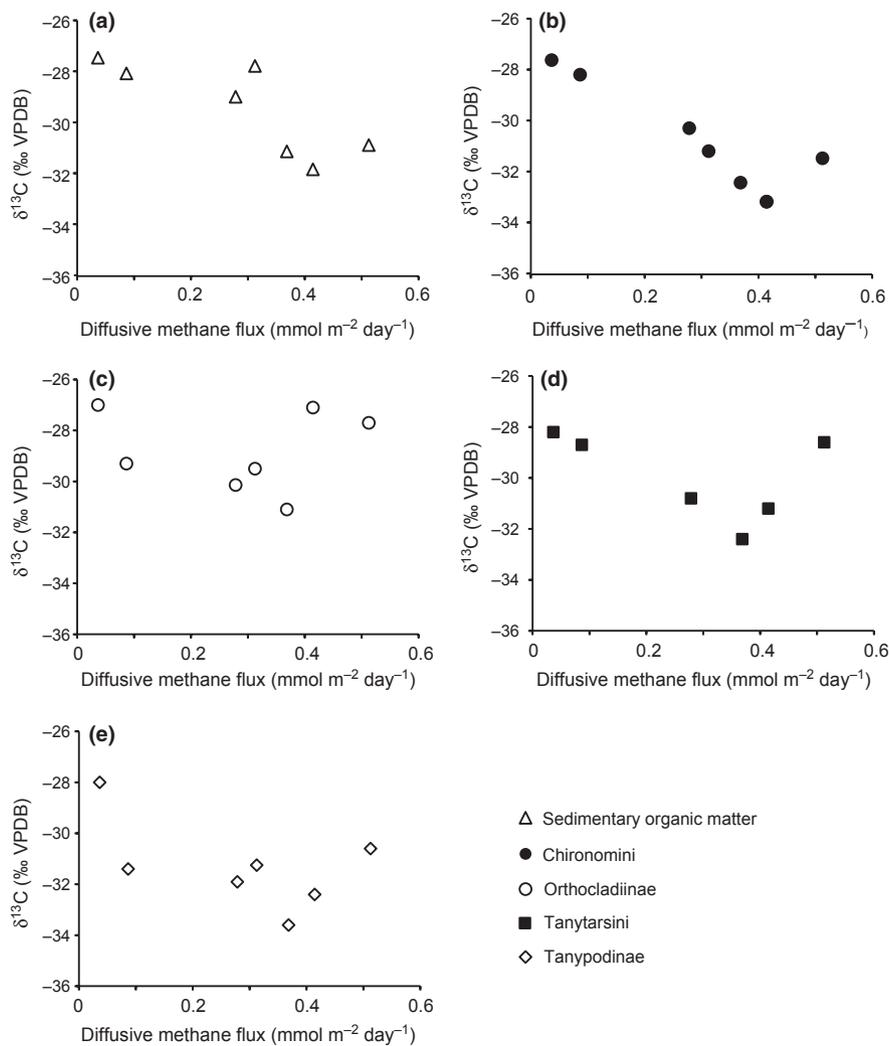
Fig. 1 δ13C values of bulk sediment organic matter and chironomid remains in surface sediments sampled in the deepest part of the seven study lakes plotted against mean diffusive methane fluxes at the water surface. (a) Bulk sediment, (b) Chironomini, (c) Orthocladiinae, (d) Tanytarsini, (e) Tanypodinae.
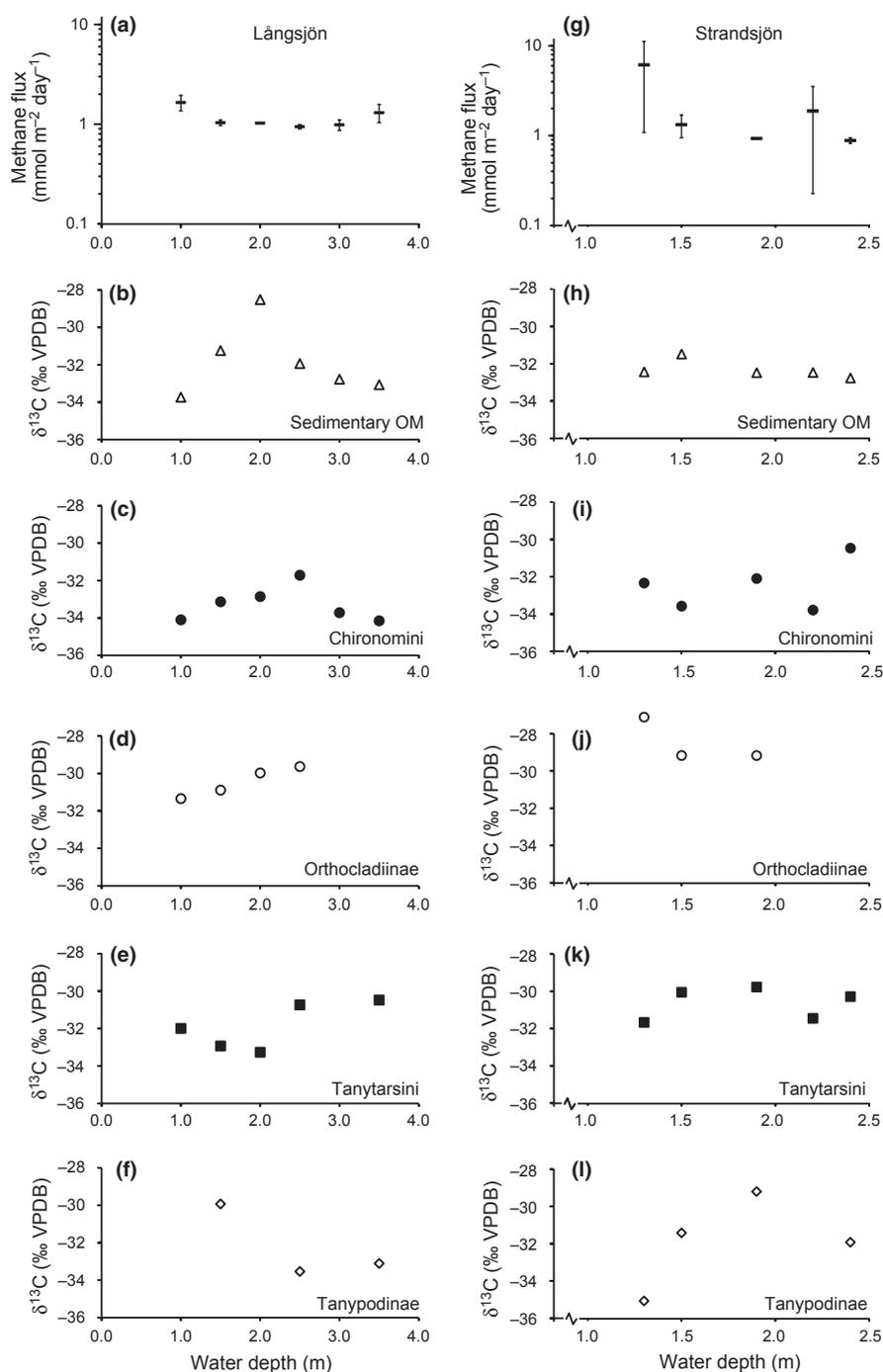
Fig. 2 Relationship between methane flux from the sediments and $\delta^{13}C$ of bulk organic matter and chironomid remains in lake surface sediments within Långsjön (a–f) and Strandsjön (g–l). (a,g) Methane release from sediments (mean ± SE in three replicate cores) indicated by vertical bars. (b–f, h–l) Mean $\delta^{13}C$ values of bulk sedimentary organic matter and chironomid remains: (b,h) bulk sediment, (c,i) Chironomini, (d,j) Orthocladiinae, (e,k) Tanytarsini, (f,l) Tanypodinae.

the lake. Therefore, methane flux measurements in Strandsjön at this water depth were considered unreliable and are excluded from further analysis. High methane release was also detected from cores taken in the deepest part of Långsjön (Fig. 2), whereas methane release was relatively low in cores from intermediate water depth.

Chironomid $\delta^{13}C$ variability

$\delta^{13}C$ of chironomid remains in sediments from the seven study lakes ranged from −33.6 to −27.0‰ (Fig. 1, Table S1). Highest $\delta^{13}C$ values were observed in Orthocladiinae (−31.1 to −27.0‰) and lowest values in Chiro-

nomini (−33.2 to −27.6‰) and Tanypodinae (−33.6 to −28.0‰). $\delta^{13}C$ of Chironomini was strongly negatively correlated with diffusive methane flux measured at the lake surface ($r = -0.90$, $P = 0.006$) and TP concentrations ($r = -0.81$, $P = 0.03$), and positively correlated with bulk sediment $\delta^{13}C$ ($r = 0.84$, $P = 0.02$). $\delta^{13}C$ of other groups showed only weak-to-moderate correlations (all non-significant) with diffusive methane flux and other environmental parameters (Table 3).

$\delta^{13}C$ of chironomid remains in lake sediments from transects in Strandsjön and Långsjön ranged from −35.1 to −27.2‰ and −35.0 to −29.6‰, respectively (Fig. 2). In both lakes, the highest $\delta^{13}C$ values were again observed in Orthocladiinae (−33.1 to −27.2‰) and lowest values in Chironomini (−34.1 to −30.5‰) and Tanypodinae (−35.1 to 29.2‰). The small number of data points and the small amplitude of changes in $\delta^{13}C$ make the detection of significant relationships between carbon isotope values and other parameters difficult in the two studied transects. No significant correlations between $\delta^{13}C$ of chironomids and water depth or $\delta^{13}C$ of bulk sediment were observed in Strandsjön and Långsjön for any of the chironomid taxa (Fig. 3, Table 4). However, if the rejected methane concentration measurements from the shallowest sample in Strandsjön (1.3 m water depth) are eliminated and the data from the two transects are combined, a significant negative correlation was observed between methane release and $\delta^{13}C$ in Chironomini ($r = -0.67$, $P = 0.025$).

Discussion

Between-lake variability of chironomid $\delta^{13}C$

Several studies of $\delta^{13}C$ of chironomid larvae in lakes are available (Bunn & Boon, 1993; Grey, Kelly & Jones, 2004; Borderelle *et al.*, 2008; Jones *et al.*, 2008; Deines, Wooller & Grey, 2009). Most of these focus on the genera *Chironomus*, *Stictochironomus*, *Sergentia* (all Chironomini) and *Procladius* (Tanypodinae). A number of studies revealed exceptionally low $\delta^{13}C$ values in the range of −72 to −40‰ in some Chironomini, indicating that these specimens had fed on methane-derived carbon.

Chironomini larvae are largely mud dwellers, making tubes on the lake bottom and feeding either as filterers or as deposit feeders on organic matter sinking from the water column (Moller Pillot, 2009). Several species have high tolerance to low-oxygen conditions (Saether, 1979; Quinlan & Smol, 2001), and the tubes of at least some Chironomini can be an ideal microhabitat for methane-oxidising bacteria (Deines, Bodelier & Eller, 2007a; Deines
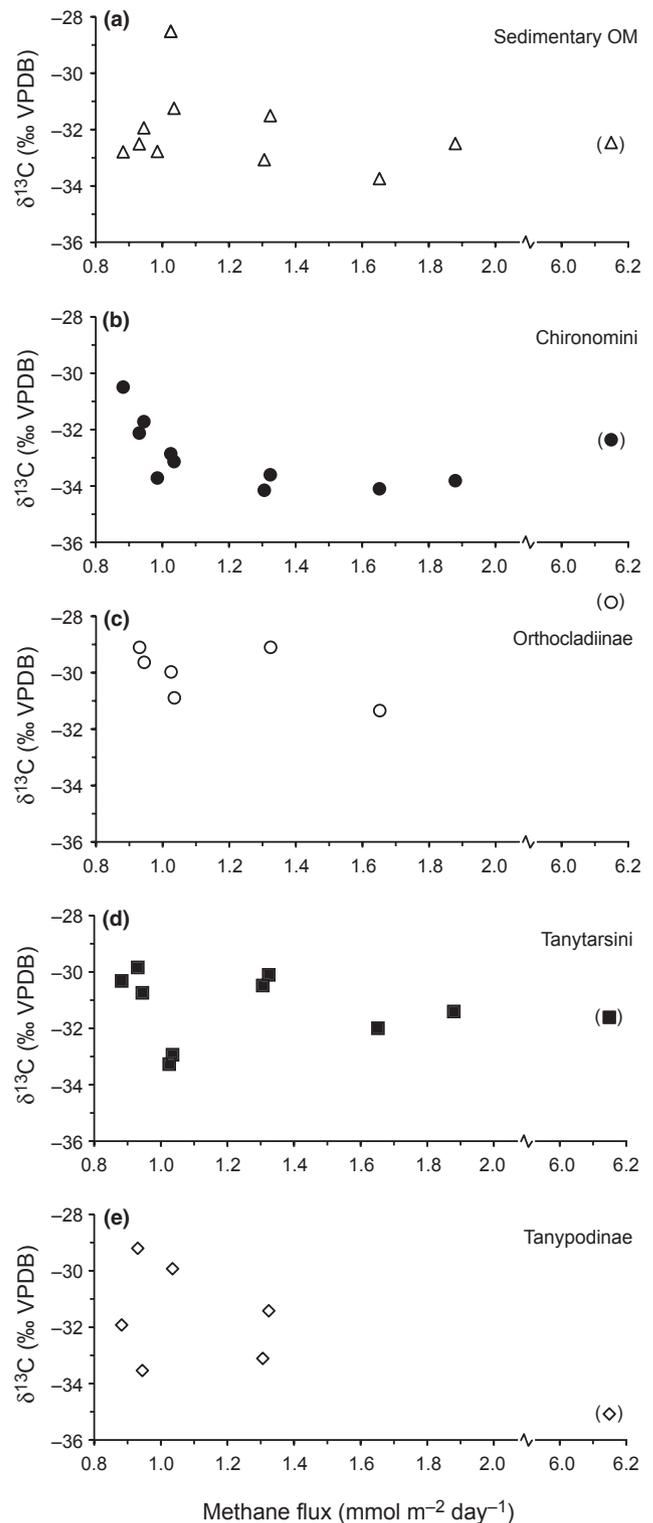

Fig. 3 $\delta^{13}C$ values of bulk organic matter and chironomid remains from surface sediment transects in Långsjön and Strandsjön plotted against mean methane release from the sediments. Note discontinuous x-axis. The data point based on a potentially unreliable methane measurement from the lake reed belt is in brackets. (a) Bulk sediment, (b) Chironomini, (c) Orthocladiinae, (d) Tanytarsini, (e) Tanypodinae.

Table 4 Linear correlations between $\delta^{13}C$ of chironomid remains and diffusive methane fluxes in the seven study lakes. In the transect data, the samples from Långsjön and Strandsjön were pooled and the data point from 1.3 m depth from Strandsjön was treated as outlier (see text for details)

	Seven study lakes			Transects		
	r	P	n	r	P	n
Chironomini	−0.90	0.0062	7	−0.67	0.033	10
Tanytarsini	−0.46	0.30	6	−0.11	0.76	9
Orthocladiinae	−0.05	0.92	7	−0.33	0.36	6
Tanypodinae	−0.54	0.21	7	−0.10	0.78	6

et al., 2007b). The strong and statistically significant negative correlation observed between Chironomini $\delta^{13}C$ and methane flux agrees with the expectation that Chironomini larvae are the most susceptible to incorporating methanogenic carbon in lakes with a high abundance and availability of methane.

Remains of Orthocladiinae were characterised by the highest $\delta^{13}C$ values. The larvae of many orthoclads, especially those occurring in mesotrophic and eutrophic lakes, are littoral dwellers feeding on available plant detritus and algae (Mihuc & Toetz, 1994; Vander Zanden & Rasmussen, 1999; Merritt, Cummins & Berg, 2008). As expected, based on their habitat and feeding preferences, no clear relationship was apparent between methane flux and Orthocladiinae $\delta^{13}C$. The scatter of $\delta^{13}C$ in Orthocladiinae between the different study sites may be a consequence of the larvae feeding on different types of organic material in the different lakes. For example, diatoms are characterised by lower $\delta^{13}C$ than other algal groups (Bade et al., 2006; Vuorio, Meili & Sarvala, 2006), and periphyton is often characterised by relatively high values in a range of lake types (France, 1995). High availability of diatoms in the lake plankton or periphyton may have affected Orthocladiinae $\delta^{13}C$ values. Regardless of the exact composition of the diet, the Orthocladiinae are the chironomid group expected to be mostly dependent on a diet of algae and other plant-derived matter, and their carbon isotopic composition can be considered to provide an indication of the $\delta^{13}C$ values of material available for algivorous and phytophagous benthic invertebrates in the littoral regions of the study lakes.

Tanytarsini are predominantly collector–gatherers feeding on particles sedimenting to the lake bottom, although the group also includes a few algivorous taxa (Merritt et al., 2008). The $\delta^{13}C$ of Tanytarsini was also negatively correlated with diffusive methane flux but not to the same extent as the Chironomini, and the relationship for Tanytarsini was not statistically significant. Since Tanytarsini are generally smaller than Chironomini, it can be expected that they are more limited in their ability to

burrow deeply into the sediments than the latter taxon. Similarly, although at least some Tanytarsini do contain haemoglobin (Panis, Goddeeris & Verheyen, 1996), the ability to survive periods of hypoxia and anoxia is less pronounced than in the Chironomini.

The Tanypodinae are predominantly predators and, in contrast to the other chironomid groups discussed here, more mobile than the generally sedentary larvae of the Chironomini, Orthocladiinae and Tanytarsini (Vallenduuk & Moller Pillot, 2007; Merritt et al., 2008). $\delta^{13}C$ of Tanypodinae remains in the different study lakes was very variable, even more so than for the Chironomini. Tanypodinae also showed a negative correlation with methane flux, but the relationship was clearly much weaker than for the Chironomini and not statistically significant. Since the carbon isotopic composition of the Tanypodinae will mainly be dependent on the diet of their food sources and since these may include a range of invertebrate groups with different feeding modes, such as chydorids, oligochaetes or other chironomids, variations in $\delta^{13}C$ of the Tanypodinae are more difficult to interpret than the carbon isotopic composition of the other chironomid groups.

Chironomid $\delta^{13}C$ values measured in our study range from −35 to −27‰. This encompasses the range of values generally found in lacustrine algae of −40 to −25‰ (Yoshioka, Wada & Hayashi, 1994; France, 1995; Grey & Jones, 1999; Bade et al., 2006; Vuorio et al., 2006) and in aquatic macrophytes and allochthonous plant remains of −33 and −8‰ (France, 1996; Meyers & Teranes, 2001). Therefore, the absolute values measured in our samples do not by themselves provide evidence that chironomid larvae in our study lakes ingested methane-oxidising bacteria or other organisms feeding on them. However, the strong and statistically significant negative correlation observed between Chironomini $\delta^{13}C$ and methane flux does suggest that methane-derived carbon is incorporated by Chironomini in some of the study lakes and that this food source became progressively more important in lakes with higher methane production. Exceptionally, low

δ^{13}C values below $-40‰$ in the literature have been restricted to a few chironomid genera (mainly *Chironomus* and *Stictochironomus*). It may be that these chironomids are more selective in their diet than other Chironomini leading to more negative δ^{13}C values in the larvae than in other groups. Low abundance or absence of *Chironomus* and *Stictochironomus* in our study lakes may therefore explain the absence of markedly depleted δ^{13}C values below $-40‰$ in the analysed Chironomini.

Lower δ^{13}C values in certain chironomid taxa may also reflect the ingestion of relatively ^{13}C-depleted phytoplankton. δ^{13}C of phytoplankton is strongly related to the δ^{13}C values of dissolved inorganic carbon (DIC) in lake water, a parameter that can vary between lakes depending on catchment geology, phytoplankton productivity and heterotrophic respiration (Bade *et al.*, 2004, 2006). Catchment geology is very similar in all our study lakes, and we do not expect a strong effect of catchment geology on DIC δ^{13}C. Productivity affects δ^{13}C of phytoplankton because of the preferential uptake of ^{12}C by phytoplankton during photosynthesis (Farquhar, Ehleringer & Hubick, 1989). In unproductive lakes, this typically leads to low δ^{13}C values of planktonic algae (Hollander & Smith, 2001). In more productive lake types, this may lead to enrichment in ^{13}C of the inorganic carbon pool and to correspondingly more positive algal δ^{13}C values. In exceptional cases, δ^{13}C of algal biomass can be as low as $-41‰$ in oligotrophic lakes (Kankaala *et al.*, 2010). However, in our sites, we observed lowest δ^{13}C values in bulk sediment organic matter and chironomid taxa in the most productive and nutrient-rich lakes (Strandsjön, Långsjön and Lötsjön), which suggests that productivity-related δ^{13}C variations in autochthonous algal production are not the main mechanism responsible for ^{13}C-depleted carbon isotopic values in the chironomid remains.

Allochthonous organic matter sources generally have higher C : N ratio and higher δ^{13}C than phytoplankton (France, 1996; Meyers & Teranes, 2001). Therefore, if the relative importance of allochthonous sources is greater in small lakes compared with large lakes, lake size could also affect the δ^{13}C and C : N of bulk sediment and chironomids feeding on it. Indeed, lake size was significantly correlated with bulk sediment δ^{13}C and C : N in the study lakes. However, no significant correlations were observed between δ^{13}C of chironomid remains and lake size or bulk sediment C : N. Therefore, it seems unlikely that lake size could be responsible for the observed variations in chironomid δ^{13}C or the correlation between Chironomini δ^{13}C and methane flux in our data set. More detailed δ^{13}C analyses of various allochthonous and autochthonous carbon sources would be required to assess the exact nature of the relationship between lake size, allochthonous organic matter input and chironomid δ^{13}C in our study lakes.

Within-lake δ^{13}C variations

In Långsjön and Strandsjön, we compared chironomid δ^{13}C with methane release from the sediments in different parts of the lake basins. δ^{13}C of Chironomini showed a negative correlation with methane release from sediments in the two examined transects when data from both lakes were combined. Deines & Grey (2006) found a similar relationship between methane production and δ^{13}C values in larvae of *Chironomus plumosus* and *Chironomus anthracinus* at increasing depths in Esthwaite Water (U.K.) and recorded δ^{13}C values as low as $-58.8 \pm 4.3‰$ at 15 m depth. Vander Zanden *et al.* (2006) measured decreasing δ^{13}C values in chironomid larvae and oligochaetes with increasing depth in Castle Lake in California, and found δ^{13}C values as low as $-49‰$ in samples from the greatest depth. Such markedly low δ^{13}C values provide strong evidence for the incorporation of methane-derived carbon. Långsjön and Strandsjön do not have very extensive anoxic zones in their deepest parts that would allow high methane concentrations in the water column and surface sediments, which may partly explain why the absolute δ^{13}C values measured in Chironomini and other chironomid taxa did not reach values lower than δ^{13}C values that can be produced by planktonic algae in oligotrophic lake systems (Jones & Grey, 2011). However, the significant correlation between methane release from the sediments and Chironomini δ^{13}C in the lake transect data suggests that methanogenic carbon is ingested and incorporated by Chironomini where methane is abundant in the studied lakes.

Heterotrophically respired carbon, which is more abundant in deeper waters of stratified lakes (Rau, 1978) and in lakes with high DOC concentrations, has been put forward as an alternative ^{13}C-depleted carbon source which could explain low δ^{13}C values in lacustrine foodwebs and aquatic invertebrates, particularly zooplankton (Lennon *et al.*, 2006). Concentrations and δ^{13}C of DOC and DIC are not available for our transects, but in the seven study lakes, no significant correlations were found between DOC concentration and δ^{13}C of chironomid remains. If low δ^{13}C values measured in chironomid remains were reflecting the ingestion of algal material produced from respired carbon, we would expect that δ^{13}C of most chironomid remains and bulk sediment δ^{13}C should all be affected by this process and follow similar patterns. In Strandsjön, no similar patterns in bulk sediment δ^{13}C and chironomid δ^{13}C were

observed with depth. In Långsjön, $\delta^{13}C$ of bulk sediment, Chironomini and Orthocladiinae was lower in the shallowest and deepest sediments compared with the sediments from intermediate depths. However, Tanytarsini and Tanypodinae did not show this pattern. Since it could be expected that both Orthocladiinae and Tanytarsini also ingest algal material produced in the water column, it seems unlikely that depleted carbon isotopic values in planktonic primary producers related to high concentrations of respired carbon caused the observed patterns.

Our study of chironomid remains in lake surface sediments in Swedish lakes has revealed differences in the relationship between $\delta^{13}C$ and methane availability in different chironomid groups. Whereas $\delta^{13}C$ of several chironomid taxa (e.g. Tanypodinae, Tanytarsini) showed only a weak negative correlation with methane flux, $\delta^{13}C$ of Chironomini, the group reported to be most susceptible to incorporating methanogenic carbon into its biomass, showed a strong, statistically significant and negative relationship with methane availability both among and within lakes. A major confounding factor of our study is the relatively low number of sites and samples we were able to analyse. $\delta^{13}C$ measurements on a larger number of samples will be necessary to corroborate our results. Furthermore, it would be desirable that in future studies $\delta^{13}C$ of DIC and methane in the lake water would be analysed as well. This would reveal the extent to which variations in chironomid $\delta^{13}C$ are related to differences in baseline $\delta^{13}C$ of algal or methane-oxidising bacteria primary production.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. $\delta^{13}C$ values of all measured samples in this study.

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