

Stable isotope analysis of chironomid larvae from some Finnish forest lakes indicates dietary contribution from biogenic methane

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Larval chironomids (mostly *Chironomus tenuistylus*) were collected from the sediments of five small forest lakes in southern Finland during summer 2002. Carbon and nitrogen isotope ratios of larvae were determined by isotope ratio mass spectrometry. Isotope ratio variability among 23 individuals from one lake was appreciable, with $\delta^{13}\text{C}$ showing a range spanning 1.6‰ and $\delta^{15}\text{N}$ values a range spanning 5.6‰. Between the lakes, larvae showed considerable variation in $\delta^{13}\text{C}$ with values as low as -55‰ . $\delta^{15}\text{N}$ values were strongly correlated with $\delta^{13}\text{C}$ values. The ^{13}C -depletion found in the benthic chironomid larvae correlated with that found previously in zooplankton from the same lakes. It is argued that the low $\delta^{13}\text{C}$ values are most likely attributable to incorporation of carbon from biogenic methane, and that methane production can therefore make an important contribution to benthic as well as to pelagic food webs in these small forest lakes.

Introduction

Chironomid larvae are keystone members of lake ecosystems. In soft sediments they often dominate the profundal community together with oligochaete worms. Most profundal lake chironomid larvae are collector-gatherers or collector-filterers (Berg 1995), exploiting the rain of fine organic detritus sinking through the water column to the sediment surface. In many lakes this detritus is largely comprised of phytoplankton cells, but other forms of detritus, including allochthonous material, can also be important. Chironomid larvae, or the pupae as they ascend to the lake surface, are a valuable food for fish, so chironomids play a critical role in “recycling”

organic matter that would otherwise be lost in the sediments. It is widely assumed that chironomid larvae directly utilise the sedimented detrital material, although it is known that bacteria colonising the detritus contribute to chironomid diets (Goedkoop and Johnson 1992). Dietary studies of chironomid species to date have demonstrated a degree of selective foraging, and have attempted to evaluate the relative contributions of algae and bacteria via gut content or fatty acid analysis (Johnson *et al.* 1989, Gullberg *et al.* 1997, Goedkoop *et al.* 1998). Stable isotope analyses are now being used with increasing frequency and effect to elucidate food web structure and function in aquatic ecosystems. The technique is reliant upon the determination of

distinct and robust basal resource isotope ratios, or signatures. In the case of carbon, the tissues of a consumer organism generally reflect the dietary isotope signature in a dependable manner — typically showing an enrichment of ^{13}C by around 1‰, although this can vary from -3‰ to +3‰ (De Niro and Epstein 1978). Therefore, in the case of profundal chironomid larvae feeding on sedimenting organic matter, we would expect their $\delta^{13}\text{C}$ to be very close (within 3‰) to that of their putative food material, either the bulk particulate organic matter (POM) or more specifically the phytoplankton.

In fact, a number of recent studies have reported ^{13}C -depleted values from lake chironomid larvae. Bunn and Boon (1993) measured Chironominae with $\delta^{13}\text{C}$ values as low as -38‰ in shallow Australian billabongs where the POM had values around -25‰ to -30‰. Kiyashko *et al.* (2001) measured a value of -64‰ from a single sample of *Stictochironomus* sp. from the profundal zone of Lake Biwa where POM $\delta^{13}\text{C}$ was not below -30‰. Grey (2002) reported values of -41‰ for *Sergentia* spp. from Loch Ness and -53.5‰ for *Chironomus* spp. from Esthwaite Water, again far below the respective values for POM. These reports have generated speculation that the unexpectedly low $\delta^{13}\text{C}$ values exhibited by some chironomid larvae might be a result of consumption of bacteria involved in the methane cycle in lakes, since biogenic methane is known to be extremely ^{13}C -depleted (Woltemate *et al.* 1984, Whiticar 1996).

Methane cycling in freshwaters has been extensively documented (*see* review by Rudd and Taylor 1980), but the possible contribution of methane production to higher trophic levels has not yet been well quantified. Small, oligotrophic, humic forest lakes with steep thermal stratification, prolonged hypolimnetic anoxia and heavy loading of organic matter should provide ideal environments for high relative importance of methane production. Previously we reported a trend of declining carbon stable isotope signatures of zooplankton with increasing water colour (a surrogate for humic carbon) from 12 small forest lakes in southern Finland (Jones *et al.* 1999). We hypothesised that this trend might reflect increasing usage of methanotrophic bacteria by zooplankton in the more humic lakes

and we suggested that the contribution of methanogenesis and methanotrophy to planktonic food webs in such lakes might have been underestimated (*see* also Bastviken *et al.* 2003). Here we report on a return visit to the same small forest lakes in which we sampled benthic chironomid larvae and analysed their carbon and nitrogen stable isotope signatures. We hypothesised that in lakes exhibiting a greater apparent contribution of methane cycling to planktonic food webs we should find a corresponding enhanced contribution to the benthic food web, and that this would be evident from lower $\delta^{13}\text{C}$ values from their chironomid larvae.

Methods

Twelve small forest lakes in the Evo forest area, close to Lammi Biological Station in southern Finland, were each visited once between 30 September and 2 October 2002. Details of the lakes are given by Jones *et al.* (1999). Sediment was collected using an Ekman grab and was sieved through a metal sieve (mesh size 1 mm) from which retained chironomid larvae were picked manually. No attempt was made to quantify the larval density; repeated sediment samples were collected and sieved from various locations around each lake until *either* around 20–50 individuals had been collected *or* at least one hour of sampling effort had been expended.

Animals were returned to the laboratory at Lammi Biological Station where they were sorted by species and size (instar), placed into clean water and left overnight to allow gut evacuation. A sub-sample of individuals from each lake was preserved in 70% ethanol for later confirmation of identification. The remaining individuals from each lake were pooled into a single sample (Table 1), oven-dried at 60 °C and homogenised prior to isotope analysis. However, from one lake, Valkea-Kotinen, 23 larvae were dried and analysed individually to provide an indication of inter-individual variability. Isotope analyses were undertaken using a Micro-mass Isoprime continuous flow isotope ratio mass spectrometer interfaced with a Carlo Erba elemental analyser. Isotope ratios are expressed conventionally using the δ notation expressed

in units of parts per thousand (‰), where $\delta = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The reference materials used were secondary standards of known relation to the international standards, Pee Dee belemnite for carbon and atmospheric air for nitrogen. Typical precision for a single analysis was $\pm 0.1\text{‰}$ for carbon and $\pm 0.3\text{‰}$ for nitrogen.

Relations between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and between chironomid and zooplankton $\delta^{13}\text{C}$ values were analysed with linear regression and Pearson's product-moment correlation, respectively.

Results

Although all 12 lakes sampled during our previous survey of zooplankton were revisited, we managed to obtain samples of chironomid larvae from only five of the lakes (Table 1), and even from some of these the number of individuals obtained was small. Almost all collected individuals were identified as *Chironomus tenuistylus* (Brundin). On the basis of their size, most were thought to be 4th instar stages, although a few were classed as third instar. One individual from Halsjärvi appeared to be a fourth instar larva of *Chironomus plumosus* (Linnaeus). After sorting individuals by size (instar) and bulking small individuals to provide a sufficient sample mass for analysis, only in some cases was it possible to carry out replicate analyses. From

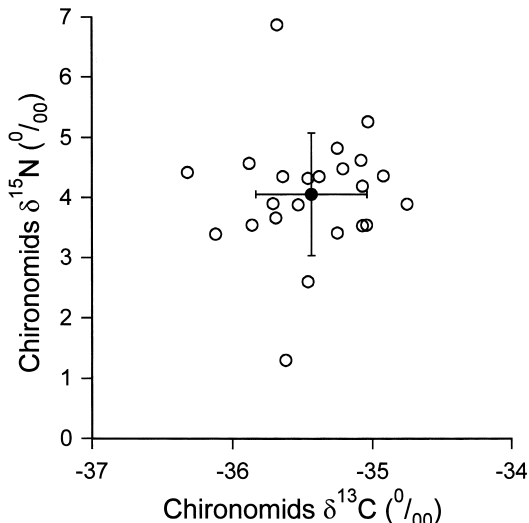


Fig. 1. Carbon and nitrogen isotope values of 23 individual 4th instar larvae of *Chironomus tenuistylus* from the lake Valkea-Kotinen. Solid point shows the mean value and bars indicate ± 1 standard deviation for each isotope.

one lake, Valkea-Kotinen, we were able to collect sufficient individuals of adequate size (4th instar) to carry out analysis of 23 individual animals and hence obtain a measure of individual variability (Fig. 1). Most individuals were quite tightly clustered with respect to both carbon and nitrogen isotope values. However, the full range of values from individuals was rather high, with $\delta^{13}\text{C}$ spanning 1.6‰ and $\delta^{15}\text{N}$ 5.6‰ .

Table 1. Carbon and nitrogen stable isotope values ($\delta\text{‰}$) of chironomid larvae from five Finnish forest lakes. The lakes are some of those previously sampled and described by Jones *et al.* (1999) from where the water colour values have been taken. Species are *Chironomus tenuistylus* (C.t.) and *C. plumosus* (C.p.) and instars were designated from larval size (*see text*). For Valkea-Kotinen, replicate analyses represent 23 individual larvae. For each of the other lakes, one or two replicate analyses were made from a single pooled sample comprising (*n*) individual larvae.

| Lake | Water colour (mg Pt l ⁻¹) | Species and instar (by size) | Number of replicate analyses | $\delta^{13}\text{C}$ (‰) | S.D. | $\delta^{15}\text{N}$ (‰) | S.D. |
|----------------|--|------------------------------------|------------------------------------|------------------------------|------|------------------------------|------|
| Valkea-Kotinen | 117 | C.t. 4th | 23 | -35.4 | 0.4 | 4.1 | 1.0 |
| Tavilampi | 121 | C.t. 3rd | 1 (5) | -38.4 | | -0.2 | |
| Halsjärvi | 79 | C.t. 4th | 1 (3) | -42.4 | | 0.8 | |
| | | C.p. 4th | 1 (1) | -55.3 | | -3.3 | |
| Iso-Valkjärvi | 25 | C.t. 4th | 2 (15) | -30.5 | 0.1 | 3.0 | 0.1 |
| Syrjälanunen | 12 | C.t. 4th | 2 (3) | -44.3 | 0.3 | -0.4 | 0.7 |
| | | C.t. 3rd | 2 (6) | -44.1 | 0.7 | -0.2 | 0.1 |

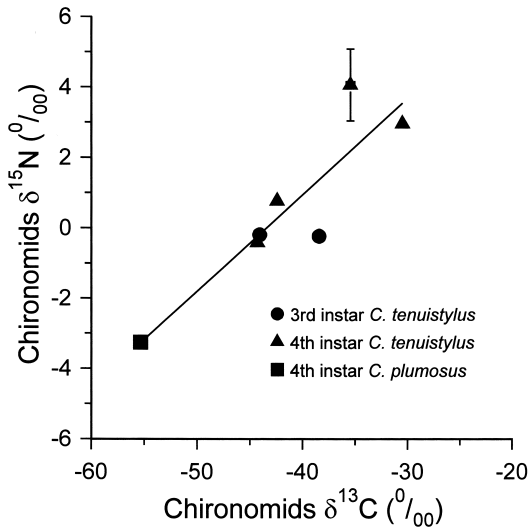


Fig. 2. Relation between nitrogen and carbon stable isotope values for chironomid larvae from five Evo lakes. Vertical and horizontal bars on one data point indicate ± 1 standard deviation for nitrogen and carbon values respectively from the 23 individual larvae from Valkea-Kotinen (see Fig. 1). Regression shown is $\delta^{15}\text{N} = 11.88 + 0.274 \cdot \delta^{13}\text{C}$ ($r = 0.899$, $n = 7$, $p < 0.01$).

Between lakes, the range in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was much greater (Fig. 2). There was a strong correlation ($r = 0.899$, $p < 0.01$) between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, indicating that the source of the between-lake variation in isotope values influenced both carbon and nitrogen isotope signatures in a co-ordinated manner. There was no

clear pattern in the variation of isotope signatures between third and fourth instar larvae, with values overlapping. The fourth instar larva of *C. plumosus* appeared to be more depleted in both ^{13}C and ^{15}N , but these values were from a single individual from Halsjärvi.

Values for particulate organic matter $\delta^{13}\text{C}$ obtained from the lakes in 1997 (Jones *et al.* 1999) showed a rather limited range (Table 2). Comparison of the $\delta^{13}\text{C}$ values for the chironomid larva with those for zooplankton obtained previously from the same lakes (Fig. 3) showed that both groups of organisms were ^{13}C -depleted relative to the mean $\delta^{13}\text{C}$ values for POM. For third and fourth instar larvae of *C. tenuistylus* there was a strong correlation ($r = 0.917$, $p < 0.02$) between their $\delta^{13}\text{C}$ values and those of zooplankton from the corresponding lake. However, the single value for fourth instar *C. plumosus* from Halsjärvi fell outside this correlation.

Discussion

We managed to obtain chironomid larvae from only five of the twelve lakes we visited, despite vigorous sampling at all lakes. Those five lakes were amongst the least coloured of the twelve (see Jones *et al.* 1999). Regular measurements since March 2000 of methane concentrations near the bottom of the water column in Valkea-

Table 2. Estimated percent contribution of biogenic methane to carbon content of chironomid larvae from five Finnish forest lakes. Estimates are derived from a simple two-source mixing model assuming alternative dietary carbon sources of sedimented POM and methanotrophic bacteria. Chironomid $\delta^{13}\text{C}$ values correspond to those in Table 1, with the two values from Halsjärvi being respectively for *C. tenuistylus* and *C. plumosus*. Values for $\delta^{13}\text{C}$ of POM are from Jones *et al.* (1999). Two alternative values for $\delta^{13}\text{C}$ of methanotrophic bacteria are used based on the typical range of $\delta^{13}\text{C}$ for biogenic methane of -50‰ to -80‰ (Whiticar 1996) and assuming a carbon isotope fractionation of -16‰ during methanotroph assimilation of methane (Summons *et al.* 1994). See text for further details.

| Lake | Chironomid $\delta^{13}\text{C}$ (‰) | POM $\delta^{13}\text{C}$ (‰) | Contribution of biogenic methane to chironomid carbon (%) | |
|----------------|---|----------------------------------|--|--|
| | | | Methane $\delta^{13}\text{C}$ -50‰ | Methane $\delta^{13}\text{C}$ -80‰ |
| Valkea-Kotinen | -35.5 | -26.7 | 25 | 14 |
| Tavilampi | -38.4 | -30.4 | 25 | 14 |
| Halsjärvi | -42.4 | -28.1 | 40 | 23 |
| | -55.3 | -28.1 | 74 | 42 |
| Iso-Valkjärvi | -30.5 | -30.6 | 3 | 1 |
| Syrjänalunen | -44.3 | -29.4 | 43 | 24 |

Kotinen have shown maximum concentrations of 250 nmol l⁻¹ in September prior to autumnal turnover and minimum concentrations of < 10 nmol l⁻¹ after autumnal turnover (P. Kankaala unpubl. data). Recently, higher methane concentrations have been measured in some more humic lakes in the Evo region (P. Kankaala, unpubl. data). These concentrations are comparable with published values from the water column of other lakes (e.g. Utsumi *et al.* 1998), although much higher concentrations occur in anoxic lake sediments (e.g. Nüsslein and Conrad 2000). Therefore, the limited available data indicate that methane production probably is important in the sampled lakes, and especially in the more coloured lakes with a higher loading of dissolved organic matter.

The larvae we obtained were almost all identified as *Chironomus tenuistylus*, a species considered characteristic of dystrophic, polyhumic lakes (Saether 1975, Wülker 1991). Although no specific information appears to be available on the feeding mode of *C. tenuistylus*, the species is included in the *C. plumosus* type. Such larvae build tubes of various shapes in the profundal sediments of lakes and feed using a combination of filter feeding and ingestion of sediment so that the gut content of larvae often shows a composition similar to the composition of the sediment (Frank 1982, Berg 1995). The small, sheltered forest lakes we sampled develop a strong thermal and oxygen stratification with pronounced deoxygenation of the hypolimnion; indeed, many are at least partially meromictic (Salonen *et al.* 1984). Hypolimnetic deoxygenation tends to be more acute in the more coloured lakes because of their greater oxygen consumption and the more pronounced thermal stratification. Therefore, oxygen conditions in the profundal regions of the most coloured lakes may be simply too severe even for chironomid larvae that are tolerant of oxygen depletion, in which case our failure to collect animals from some lakes probably reflected a genuine absence of chironomids from those lakes. Moreover, many of these forest lakes lack a true littoral zone, being fringed by a floating *Sphagnum* raft, so if profundal conditions are unfavourable no other sediment habitat is available. With animals from only five lakes, our data set is limited and con-

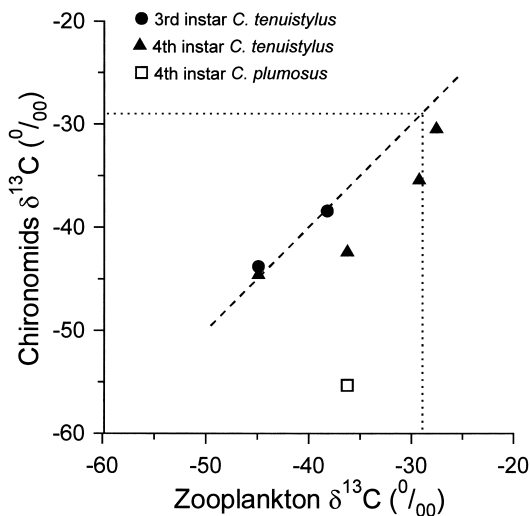


Fig. 3. Relationship between carbon stable isotope values for chironomid larva from five Evo lakes and values for zooplankton from the corresponding lakes obtained previously (Jones *et al.* 1999). The correlation between *C. tenuistylus* (both 3rd and 4th instars) and zooplankton is $r = 0.917$ ($n = 6$, $p = 0.01$). The dashed line indicates equivalent $\delta^{13}\text{C}$ values for *C. tenuistylus* larvae and zooplankton. Dotted lines show the mean carbon isotope value for POM for the five lakes taken from Jones *et al.* (1999).

strains our ability to draw general conclusions. Nevertheless, some important points emerge from our data.

Since the number of animals analysed from most lakes was small, it is important to assess the magnitude of variation in isotope ratios that can be expected between individuals from a single lake. The analysis of 23 third instar individuals from Valkea-Kotinen (Fig. 1) revealed a standard deviation around the mean of only 0.4‰ for $\delta^{13}\text{C}$ and 1.0‰ for $\delta^{15}\text{N}$. These results give us confidence that the observed inter-lake variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values probably represent genuine differences in the isotope ratios of chironomid larvae from the different lakes. Nevertheless, the range of values amongst the 23 individuals from Valkea-Kotinen does suggest appreciable dietary variation amongst individual chironomid larvae within a single lake habitat.

The close correlation between values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 2) was not something we had predicted. However, Kiyashko *et al.* (2001) also found that littoral *Stictochironomus* larvae from

Lake Biwa showed a correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Depletion of ^{13}C in chironomids could be explained by a food web link to biogenic methane, but it is not clear why this should also be reflected as ^{15}N depletion. Methane-oxidising bacteria generally assimilate nitrogen as ammonium and there is evidence that microbial uptake of ammonium can result in nitrogen fractionation of -20% (Macko and Ostrom 1994), so it is possible that a higher proportion of methanotrophic bacteria in the diets of some chironomid larvae results in a corresponding depletion in both ^{15}N and ^{13}C . Another possibility is that the observed correlation derives from the physiology of the chironomid larvae. Animals are generally enriched in ^{15}N relative to their diets due to preferential excretion of lighter ^{14}N (De Niro and Epstein 1981). However, if organic matter produced via the methane cycle in lakes has a relatively high C:N ratio, chironomids using such organic matter could be expected to conserve nitrogen more efficiently and hence to be less enriched in ^{15}N .

A striking result from our study was the correlation between $\delta^{13}\text{C}$ of *Chironomus tenuistylus* and that of zooplankton from the equivalent lakes (Fig. 3), in line with our original hypothesis. Zooplankton $\delta^{13}\text{C}$ values used for each lake were averages of cladoceran and copepod values presented in Jones *et al.* (1999), since from any one lake differences between the values for cladocerans and copepods were very slight. In fact, *C. tenuistylus* larvae and zooplankton from the same lake exhibited almost equivalent ^{13}C -depletion, in some cases extending way below the isotopic values for POM. This suggests that an isotopically light carbon source, probably biogenic methane, contributes to the diets of both planktonic and benthic organisms in these lakes and to an approximately equivalent extent. The single analysis of fourth instar *Chironomus plumosus* from Halsjärvi did not follow quite the same relationship, being more markedly depleted in ^{13}C . This could reflect a greater contribution of methanotrophic bacteria in the diet of this species which is particularly tolerant of low oxygen conditions. During a wide ranging survey of European lakes we have found *C. plumosus* larvae to exhibit more strongly ^{13}C -depleted values than *C. anthracinus* from the same lakes

(RIJ and JG, unpubl. data). It is unfortunate that we were not able to obtain chironomid larvae for analysis from the more coloured lakes we visited. Previously (Jones *et al.* 1999) we had found that zooplankton $\delta^{13}\text{C}$ in the lakes showed a trend of decreasing as water colour increased. If methane production and utilisation is indeed more important in the more humic lakes, as the earlier zooplankton data suggested, we would have expected this trend to apply equally to the benthic food web. However, the more humic of these small forest lakes may generate environmental conditions in deeper water that simply exclude development of a macroinvertebrate benthic food web.

Some indication of the potential importance of biogenic methane in these foodwebs can be obtained by applying a simple two-source mixing model (e.g. Bunn and Boon 1993). Here we assume dietary fractionation of carbon by chironomid larvae of $+1\%$ (De Niro and Epstein 1978). We also assume that sedimented POM represents one alternative dietary component and use the $\delta^{13}\text{C}$ values (Table 2) determined previously for each lake (Jones *et al.* 1999). Finally, we assume a range for $\delta^{13}\text{C}$ of biogenic methane to be from -50% to -80% (Whiticar 1996) and a discrimination against the heavier carbon isotope of -16% by methanotrophic bacteria (Summons *et al.* 1994). Then the contribution of methane carbon to chironomid larvae in the lakes (Table 2) could range from near zero in Iso-Valkjärvi to as much as 74% in Halsjärvi with the less ^{13}C -depleted methane value. Of course, these estimates have a high uncertainty. Nevertheless, the results from this stable isotope study of chironomid larvae do support our previous contention that a carbon flow pathway from allochthonous organic matter via methane production and utilisation is likely to be an important part of the carbon budget of the numerous small forest lakes in the boreal region.

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