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Differential assimilation of methanotrophic and chemoautotrophic bacteria by lake chironomid larvae

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ABSTRACT: Bacteria play an important role in the nutrition of many lake-dwelling detritivorous macroinvertebrates, yet few studies have investigated the roles of differing groups of bacteria in freshwater systems. Recent stable carbon isotope analyses have revealed that methanotrophic bacteria help fuel lake food webs. We analysed individual larvae of co-existing *Chironomus plumosus* and *C. anthracinus* for stable sulphur isotopes as an alternative tracer for bacterial assimilation, and compared them with existing stable carbon isotope data. The combination of these 2 isotopes suggests that there are large inter- and intraspecific differences in the incorporation of bacteria rather than algae in the diet of both species. *C. anthracinus* appears to assimilate a greater proportion of chemoautotrophs relative to its congener, which is consistent with classical descriptions of *C. anthracinus* feeding mode relative to bacterial stratigraphy in the sediments. The higher intraspecific isotope variability of *C. plumosus* indicates variable proportions of methanotrophs, chemoautotrophs and phytoplankton to the diets of individuals.

KEY WORDS: Benthic food web · Carbon · Macroinvertebrate · Methane · Stable isotope · Sulphur

INTRODUCTION

Chironomid larvae often dominate the littoral and profundal benthos of lakes. As such, their ecology, and in particular their trophic role within food webs, has been intensively studied (see Berg 1995 and references therein). The food most commonly identified from gut content analyses of chironomids is detritus, typically constituting 50 to 90% (Johnson 1987). However, the nutritional value of detritus is questionable because much is refractory, and detritivorous macroinvertebrates typically lack the required enzymatic capacity to breakdown cellulose and other complex plant polysaccharides. Thus, the associated microbial flora is likely of greater nutritional importance, either directly as a food source, or via mineralisation of the detritus into more readily digestible forms (Johannsson & Beaver 1983). In fact, *Chironomus plumosus* and *C. riparius* are both capable of completing larval development on diets consisting solely of bacteria (Rodina 1971, Baker & Bradnam 1976).

The importance of bacteria in chironomid diets differs both between lakes of differing trophy, and seasonally within lakes. Goedkoop & Johnson (1992) found that in more productive lakes, bacterial carbon accounted for only 2% of total carbon demand, whereas this proportion increased to around 47% in oligotrophic systems. Seasonality and inter-annual variability of plankton primary productivity make such a basal resource rather unpredictable for benthic invertebrates (Johnson et al. 1989), whereas sediment bacterial biomass is relatively constant (Boström & Törnblom 1990, Brunberg & Boström 1992). Thus, *Chironomus plumosus* switched from a detrital-dominated diet in February to a more algal-biased diet, associated with the spring bloom, in Lake Erken (Johnson 1987). Conventional techniques such as gut

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content analyses reveal little information regarding the types of bacteria constituting the diet and, although specific fatty acid or amino acid biomarkers can be used to differentiate between bacterial groups (e.g. Boon et al. 1996), a chironomid dietary study that incorporated fatty acid analyses only differentiated between bacterial and algal components (Goedkoop et al. 1998). Molecular methods could provide information on the different phylotypes of the microbial community in the gut, but the effort and costs involved can be prohibitive. In comparison, rapid stable isotope analysis may provide additional insight into chironomid feeding behaviour.

Stable isotope analyses have recently indicated that species commonly assumed to be filter feeders of algal production in productive shallow lakes (e.g. *Chironomus plumosus*) might be heavily reliant upon methanotrophic bacterial biomass (Grey et al. 2004, Kelly et al. 2004). Biogenic methane exhibits a markedly ^{13}C-depleted isotopic signature which is distinct from the majority of other basal resources in freshwater systems (–35 to –20‰; Del Giorgio & France 1996), and thus can be used with confidence as a natural tracer. Chironomids and zooplankton, which exhibited $\delta^{13}C <$ –40‰, have been assumed to have had their isotopic signatures lowered by a contribution from methane, mediated by methane oxidising bacteria either in the plankton or in the sediment (Bastviken et al. 2003, Kelly et al. 2004).

However, rather than assimilating carbon partly from a methanotrophic source and partly from an algal or detrital source, as assumed in the mixing models used by Kelly et al. (2004), chironomids and zooplankton could exhibit isotopic signatures between –50 and –40‰ via consumption of chemoautotrophs that have assimilated respired carbon, as outlined by Kohzu et al. (2004). Briefly, this results either from the oxidation of methane (Hollander & Smith 2001) or indeed in high respiration dominated systems, where $\delta^{13}C$ values of dissolved inorganic carbon may approach –20‰, then further fractionation by chemoautotrophs such as sulphur oxidising bacteria results in bacterial $\delta^{13}C$ between –50 and –40‰ (e.g. Ruby et al. 1987). A further alternative is that lipid synthesis by chemoautotrophs under anoxic conditions can produce an isotopically light carbon pool (cf. Teece et al. 1999, Londry et al. 2004). These 2 alternatives are not mutually exclusive.

We hypothesised that analyses of sulphur stable isotopes could provide further insight into linkages between sediment microbial flora and macroinvertebrate consumers in freshwater lakes, as in marine systems (e.g. Brooks et al. 1987, Cary et al. 1989). Incorporation of elemental sulphur into chemoautotrophs results in lower $\delta^{34}S$ values, because ^{34}S-depleted sulphides are the likely source (Fry 1986). In addition, sulphur is an excellent source indicator because trophic fractionation is negligible (Peterson & Fry 1987). In previous isotopic studies of chironomid larvae, *Chironomus plumosus* typically had lower $\delta^{13}C$ than *C. anthracinus* and was assumed to be relatively more trophically reliant upon methanotrophic biomass. *C. anthracinus* more often exhibited $\delta^{13}C$ values within the –50 to –40‰ range (e.g. Grey et al. 2004). Allowing for a displacement of ~10‰ because of differing carbon sources between fresh and marine waters (Peterson & Fry 1987), this corresponds to the $\delta^{13}C$ range (–42 to –30‰) reported by Brooks et al. (1987) to represent use of sulphur-based energy sources, as opposed to more ^{13}C-depleted methane-based sources (<–40‰) in marine systems. Consequently, we expected *C. anthracinus* to exhibit lower $\delta^{34}S$ relative to its congener. We analysed the remaining material (when sufficient) used for the intraspecific stable carbon and nitrogen isotope study of Grey et al. (2004), to provide direct comparison between $\delta^{13}C$ and $\delta^{34}S$ data.

MATERIALS AND METHODS

In June 2002, sediment and associated chironomid larvae were collected by Ekman grab from 15 m depth in Esthwaite Water (54° 22′ N, 2° 59′ W), and 3 m depth in Wyresdale Park Lake, UK (53° 56′ N, 2° 44′ W). These 2 small (<100 ha), productive, dimictic lakes have been described by Kelly et al. (2004). Surficial sediment samples (50 ml) were removed directly from the grab; the remainder was sieved (2 mm mesh) *in situ*, and 4th instar chironomid larvae placed into containers with lake water. In the laboratory, larvae were placed into filtered water and left overnight for gut clearance (cf. Feuchtmayr & Grey 2003). Faecal material was removed periodically to prevent coprophagy. Individual larvae were placed into separate wells of cell culture plates and dried at 60°C for 24 h and stored in a dessicator. Further details of larval and sediment preparation procedures are outlined in Grey et al. (2004) and Kelly et al. (2004). Prior to analysis, elemental sulphur content of sediment samples (0.55 to 0.83%) was determined to calculate an appropriate sample weight. Sufficient sediment was weighed to yield approximately 40 µg of elemental sulphur. Whenever larval size allowed, 2 to 5 mg of chironomid tissue was weighed into tin cups. Sulphur sample preparation involved adding ~2× the sample weight of vanadium pentoxide catalyst to promote combustion. Stable sulphur isotopes were analysed by continuous flow isotope ratio mass spectrometry (ANCA-GSL elemental analyser coupled to a Geo20-20 isotope ratio

mass spectrometer, Europa Scientific) at Iso-Analytical, UK. Stable isotope ratios are expressed using the δ notation as per mil difference from the standard:

$$\delta^{34}S = ({}^{34}S/{}^{32}S_{sample}/{}^{34}S/{}^{32}S_{standard} - 1) \times 1000$$

The reference material against which all samples were measured was barium sulphate (NBS-127, $\delta^{34}S_{V\text{-}CDT}$ = +20.3‰) distributed by the International Atomic Energy Agency, Vienna. NBS-127, IAEA-S-1 (silver sulphide, $\delta^{34}S_{V\text{-}CDT}$ = −0.3‰) and Iso-Analytical OP-7 (barium sulphate, $\delta^{34}S_{V\text{-}CDT}$ = +11.0‰) were used for calibration and correction.

Stable isotope data were checked for normality using Lilliefors tests. The slope and intercept of $\delta^{13}C/\delta^{34}S$ relationships for each species in each lake were checked using least-squares linear regression. Since all but one of the latter relationships were non-significant, it was not possible to draw comparisons using ANCOVA, so we compared $\delta^{13}C$ and $\delta^{34}S$ between species and lakes using individual ANOVAs. All statistical analyses were conducted using SYSTAT v.8 (SPSS 1998).

RESULTS

Bulk sediment $\delta^{13}C$ values exhibited little variability between replicates within lakes, or between lakes (Table 1). Wyresdale Park Lake sediments were slightly ^{34}S-enriched (~1‰) relative to those from Esthwaite Water, again with low variability between replicates (max: 1.3‰, Esthwaite). Chironomid larvae of both species were consistently ^{13}C-depleted relative to bulk sediments in both lakes (Fig. 1); *Chironomus plumosus* tended to be more ^{13}C-depleted compared to its congener but only significantly so in Esthwaite ($F_{1,24}$ = 9.17, p = 0.006). Chironomid $\delta^{34}S$ ranged over 6‰ in both lakes, although species-specific ranges were typically smaller. There was overlap between *C. plumosus* $\delta^{34}S$ values and those of sediment in Wyresdale, both with maximum recorded $\delta^{34}S$ of 1.4‰. The remaining chironomid individuals were all ^{34}S-depleted relative to the corresponding sediment (by up to 8‰ in Esthwaite). *C. anthracinus* was typically ^{34}S-depleted compared to its congener: mean *C. plumosus* $\delta^{34}S$ (Table 1) was significantly heavier than that of *C. anthracinus* in both Wyresdale ($F_{1,29}$ = 37.51, p < 0.001) and Esthwaite ($F_{1,24}$ = 38.18, p < 0.001).

Interspecific isotope variability was marked, and exhibited a similar pattern in both lakes (Fig. 1). There were no significant differences in mean $\delta^{13}C$ for either species between lakes, or in mean $\delta^{34}S$ of *Chironomus anthracinus* between lakes. Only *C. plumosus* $\delta^{34}S$ varied significantly between lakes ($F_{1,38}$ = 18.18, p < 0.001).

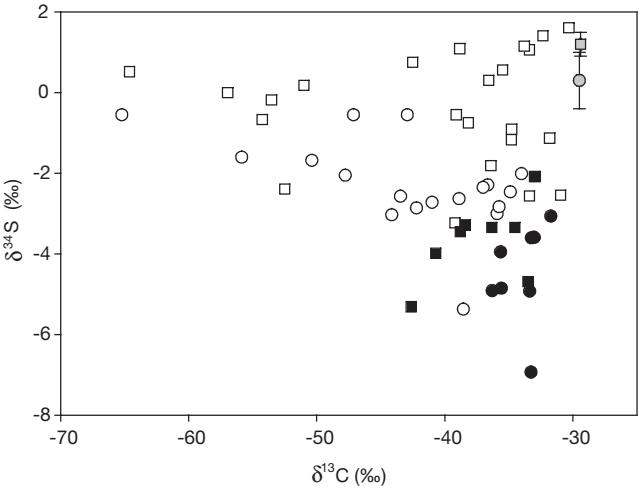

Fig. 1. Carbon and sulphur isotope signatures of chironomid larvae (each symbol corresponds to 1 individual larva) and bulk sediments (mean ± 1 SD) collected from 2 lakes in the UK. Squares: Wyresdale Park Lake; circles: Esthwaite Water; grey: sediment; white: *Chironomus plumosus*; black: *C. anthracinus*

Table 1. Stable carbon and sulfur isotope data for 2 congeneric tubicolous chironomid species (*Chironomus plumosus* and *C. anthracinus*) and the bulk sediment, from Esthwaite Water and Wyresdale Park Lake, UK

Species/sediment	n	$\delta^{13}C$ (‰)			$\delta^{34}S$ (‰)		
		Mean ± SD	Max.	Min.	Mean ± SD	Max.	Min.
Esthwaite Water							
C. plumosus	17	−41.4 ± 7.7	−33.2	−65.2	−2.1 ± 0.9	−0.6	−3.0
C. anthracinus	9	−34.6 ± 2.0	−31.7	−38.6	−4.6 ± 1.2	−3.1	−6.9
Sediment	6	−29.5 ± 0.2	−29.0	−29.7	0.3 ± 0.7	1.1	−0.2
Wyresdale Park Lake							
C. plumosus	23	−40.7 ± 9.5	−30.3	−64.6	−0.5 ± 1.4	1.4	−3.2
C. anthracinus	8	−38.2 ± 3.1	−32.8	−44.0	−3.7 ± 1.0	−2.1	−5.3
Sediment	3	−29.4 ± 0.1	−29.4	−29.5	1.2 ± 0.3	1.4	1.0

DISCUSSION

Grey et al. (2004) previously found that chironomid larvae exhibited considerable (~30‰) intraspecific variability in $\delta^{13}C$. The present study showed that these same chironomid larvae collected at a single location within a lake on a specific date also showed large variability in $\delta^{34}S$ values (up to 4.6‰ for *Chironomus plumosus* in Wyresdale Park Lake). Together, these isotopic data suggest alternative dietary intake between individual larvae collected from the same environs. Larvae assimilating settling phytoplankton should exhibit relatively ^{13}C- and ^{34}S-enriched signatures, reflecting phytoplankton use of carbon dioxide, bicarbonate and sulphate in the water column (Fry 1986). Conversely, larvae using primarily methanotrophic biomass in the sediments should exhibit $\delta^{13}C$ <–50‰ (Grey et al. 2004) and, although there are no data available for the $\delta^{34}S$ values of methanotrophs per se, they are more likely to reflect sulphide rather than sulphate use and exhibit $\delta^{34}S$ tending to 0‰ or lower (Fry 1986). Thus, there should be a positive relationship between larval $\delta^{13}C$ and $\delta^{34}S$ if larvae are assimilating differing proportions from these 2 dietary sources. However, we found no significant, positive relationship in either species from either lake. Indeed, the degree of ^{34}S-depletion exhibited by both species indicates primarily bacterial, rather than algal, sources, despite there being considerable primary productivity (June chlorophyll *a* values: Esthwaite, 29.0 µg l^{-1}; Wyresdale, 60.5 µg l^{-1}; Kelly et al. 2004) available in the water column. This is at odds with the findings of Goedkoop & Johnson (1992), who suggested that the percentage contribution derived from bacterial carbon to larval carbon demand is generally low in productive lakes.

Moreover, in accordance with our expectation, *Chironomus anthracinus* was typically ^{34}S-depleted relative to its congener in both lakes, and thus in each lake, the combination of stable carbon and sulphur isotopes qualitatively separated the 2 species (Fig. 1). The stable sulphur isotope data suggest that *C. anthracinus* may actually be rather reliant on chemoautotrophic bacteria, reflected in the lighter $\delta^{34}S$ values exhibited by this species. In that case, light $\delta^{13}C$ values may not represent such an important dietary contribution from methanotrophic bacterial biomass, as previously suggested by Kelly et al. (2004), but simply reflect assimilation of respired carbon (sensu Kohzu et al. 2004). The isotope data do not preclude assimilation from methanotrophs, rather, that relative to its congener, *C. anthracinus* assimilates a greater dietary proportion from chemoautotrophs. *C. plumosus* in Wyresdale Park Lake exhibited the greatest intraspecific isotopic variability, in both carbon and sulphur, and the greatest range in $\delta^{34}S$ (4.6‰) corresponding to $\delta^{13}C$ values between –40 and –30‰. This increased variability suggests that some *C. plumosus* individuals utilise more methanotrophic bacterial biomass, some more chemoautotrophic bacterial biomass, and others, a relatively larger phytoplankton diet proportion.

The 2 species found in our study lakes are classically described as having different modes of feeding. *Chironomus plumosus* is typically a filter feeder within its tube, although it can switch to deposit feeding under periods of low plankton availability; *C. anthracinus* is a deposit feeder of detritus around its tube mouth. The diagrams of tube morphology and feeding mode, elegantly presented in Jónasson (2003), suggest that the chironomid larvae may be exploiting different communities of chemoautotrophic bacteria within the sediment strata. *C. anthracinus* sweep the sediment surface surrounding their tubes using the anterior part of their body, and generally feed down to the depth between oxidised and reduced sediment. These bioturbated sediment strata contain microsites with high concentrations of sulphur-oxidising chemoautotrophs compared to non-bioturbated sediments (Goni-Urriza et al. 1999), which we suppose are likely to be ingested by foraging larvae. Indeed, when Jónasson & Thorhauge conducted feeding experiments with *C. anthracinus* from Lake Esrom in aquaria, removal of the top 2 cm of the Lake Esrom mud (i.e. that layer with highest percentage organic matter and associated bacteria) resulted in larval starvation, indicating its nutritional importance (P. M. Jónasson pers. comm.). *C. plumosus* in Esthwaite was significantly ^{34}S-depleted, and by inference more reliant upon bacterial sources than in Wyresdale, which may reflect the deeper water column in Esthwaite (15 m compared to 3 m) and associated recycling of nutrients therein.

Microbial chemosynthesis has been implicated as the primary source of reduced carbon for organisms inhabiting the environs of marine hydrocarbon vents and seeps (e.g. Rau 1981, MacAvoy et al. 2002). It may occur via symbiotic relationships, such as those between lucinid bivalves or marine nematodes and sulphur-oxidising bacteria, in sub-oxic, anoxic and sulphidic benthic habitats (Spiro et al. 1986, Dando et al. 1994, Ott et al. 2004). Multiple trophic resources for marine chemoautotrophic communities have been demonstrated using multiple stable isotopes (Brooks et al. 1987, Cary et al. 1989) but comparatively little work has been conducted regarding the importance of these alternative trophic pathways in freshwater systems. Perhaps this is because of the perceived lack of basal resource (<1% elemental sulphur in our freshwater sediments). Cary et al. (1989) described a cold water brine seep that was dominated by 2 macrofauna: an

undescribed mytilid mussel reliant on methane oxidation; and a vestimentiferan worm, *Escarpia laminata*, reliant on sulphide oxidation for growth. The result of our stable isotope study of the 2 congeneric chironomids is similar to that of Cary et al. (1989), with *Chironomus plumosus* appearing more reliant upon methanotrophic biomass and *C. anthracinus* more reliant upon chemoautotrophic biomass. However, the intraspecific isotope variability indicates that they are not as exclusively reliant upon each source as animals in the marine system. Thus, we have shown that complementing stable carbon with stable sulphur isotope analyses can potentially identify such trophic pathways in freshwaters.

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