High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae

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Abstract

Stable isotope analyses of chironomid trophic interactions have recently indicated the potential importance of isotopically light biogenic methane as a carbon source. Mass balance of isotope ratios suggests that small proportional differences in ingestion of such an isotopically distinct basal resource by individual consumers can result in considerable intraspecific variability. To test this, we collected individual larvae of two closely related chironomid species (*Chironomus anthracinus* and *Chironomus plumosus*) from six lakes and analyzed their $\delta^{13}C$ and $\delta^{15}N$. Intraspecific variability in larval $\delta^{13}C$ and $\delta^{15}N$ values was greater in lakes where chironomids were more ^{13}C depleted. *C. plumosus* exhibited higher intraspecific variability relative to *C. anthracinus*. In two lakes, individual *C. plumosus* exhibited a range of 35‰ for $\delta^{13}C$ and 16‰ for $\delta^{15}N$ (equivalent to five trophic levels). There was a strong positive relationship between larval $\delta^{13}C$ and $\delta^{15}N$, both between individuals from the same lake and also between lakes, suggesting that the underlying causative mechanisms are similar. Furthermore, larvae from deeper sites, which are more susceptible to prolonged anoxia, exhibited greater intraspecific variability, and larger larvae were significantly ^{13}C depleted. Such high intraspecific variability can confound the interpretation of benthic food web stable isotope values. We advocate the reporting of more intraspecific isotopic variability as a means to further examine niche breadth and feeding behavior.

Particulate organic matter (POM) in freshwater food webs typically exhibits carbon stable isotope values ($\delta^{13}C$) in the range -35‰ to -20‰ (del Giorgio and France 1996). In systems with high respiration, $\delta^{13}C$ values for dissolved inorganic carbon can approach -20‰ and via fractionation during photosynthesis could in principle result in phytoplankton of -45‰ (Peterson and Fry 1987), although this may be rare in practice (Jones et al. 2001; Cole et al. 2002). However, recent studies have revealed very depleted $\delta^{13}C$ values in some lake systems, with chironomid species as low as -64‰ (Bunn and Boon 1993; Kiyashko et al. 2001; Grey 2002; Jones and Grey in press). Bunn and Boon (1993) pos-

tulated that this phenomenon was caused by incorporation of isotopically light biogenic methane produced in anoxic sediments. Comparable relationships have been reported from the marine environment, particularly in relation to seeps (Levin and Michener 2002).

Chironomid larvae feeding exclusively on an isotopically light carbon source should express individual larval $\delta^{13}C$ values tightly clustered around that of the dietary source. Yet published studies of *Chironomus* diet rarely indicate complete exclusivity to a particular source (see review by Berg 1995 and refs. therein). Much of the research into chironomid trophic behavior using gut content analysis (Johnson 1985, 1987) or fatty acid composition (Goedkoop et al. 1998) has demonstrated that most species are omnivorous. Stable isotope ratios of individual larvae are then likely to reflect a variable mix of basal resources. Even a small contribution from a basal resource characterized by an extremely light isotopic value might be expected to have a relatively large influence on the stable isotope ratio of the consumer. Small differences in the proportional contribution from an isotopically light basal resource ingested by individual larvae will be amplified and result in considerable intraspecific variability. Thus, populations of chironomid larvae with greater potential to include an isotopically light basal resource in their diet are more likely to show high intraspecific variability in $\delta^{13}C$ values. The aim of the current study was to test the following specific hypotheses:

1. Assuming that chironomid larvae consume more than one basal resource, then the larvae of those species showing greater propensity for ^{13}C depletion from partial incor-

Table 1. Environmental characteristics recorded from the six study lakes. Relative depth was calculated according to Wetzel (1983). n.a., not available.

Characteristic	Gr. Binnensee	Schöhsee	Whitemere	Esthwaite	Wyresdale	Plußsee
Surface area (ha)	483	78	25.5	98	16	14
Mean and (in parentheses) maximum depth (m)	2.0 (2.5)	10.9 (28.0)	n.a. (17.0)	6.4 (15.5)	3.0 (5.5)	9.4 (28.0)
Relative depth (%)	0.1	2.9	3.0	1.4	1.2	6.6
Depth sampled (m) and (in parentheses) duration [O_2] < 1 mg L^{-1} (days)	1.0 (0)	10.0 (25) 15.0 (60)	7.0 (n.a.)	10.0 (50) 15.0 (130)	3.0 (60) 4.0 (115)	4.5 (90)
[Total phosphorus] (μg L^{-1})	n.a.	21.0	780.0*	44.0*	97.0*	129.0
[Chlorophyll *a*] (μg L^{-1})	47.3	18.0	91.0	29.0	60.5	20.7

* Source of data, Grey et al. (2000).

poration of a distinct, isotopically light basal resource should also exhibit greater intraspecific variability.

2. If biogenic methane produced in anoxic sediments is the principal ^{13}C-depleted basal resource, chironomid larvae from sites most susceptible to prolonged anoxia will be expected to exhibit greater intraspecific variability in δ^{13}C values.

3. Because chironomid burrows are sites of enhanced microbial activity (cf. Kajan and Frenzel 1999), larger larvae, with a greater girth and digging deeper burrows, will exhibit more pronounced ^{13}C depletion.

Methods

Samples were collected from six lakes during summer 2002: Whitemere, Wyresdale Park Lake, and Esthwaite Water in the U.K. and Großer Binnensee, Plußsee, and Schöhsee in Germany. Some environmental characteristics of the lakes are given in Table 1. The relative depth (the ratio of the maximum depth as a percentage of the mean diameter of the lake at the surface, expressed as a percentage; Wetzel 1983) was calculated as a surrogate measure of water-column stability. Water samples were collected and analyzed for total phosphorus and chlorophyll *a* using standard limnological techniques (cf. Grey et al. 2000). Vertical profiles for oxygen concentration were determined using a Yellow Springs Instruments probe at weekly or monthly intervals in each lake except Whitemere, as part of concurrent research. The approximate duration that each chironomid sampling depth was exposed to oxygen concentrations <1 mg L^{-1} was determined from these profiles. The deepest point or the maximal depth of chironomid colonization in each lake was selected as a sampling location (Table 1). Sediment samples were collected by Ekman grab, sieved through 2-mm mesh in situ and chironomid larvae (of both *Chironomus plumosus* and *Chironomus anthracinus* when possible) were picked out and retained in ambient lake water until return to the laboratory. To test for effects of water-column depth and associated variable degree of anoxia, further collections of larvae were made from both 10-m and 15-m depths in both Esthwaite and Schöhsee on separate occasions. The deeper sites in these two lakes are subjected to greater duration of anoxia (approximately 2 and 1 month for Esthwaite and Schöhsee, respectively; Table 1). Individuals were identified and fourth-instar larvae were separated by species into vessels

containing filtered lake water, where they were maintained alive to allow gut clearance. Gut contents of chironomid larvae are known to be sufficient to distort isotope ratios if whole animals are analyzed (H. Feuchtmayr and J. Grey unpubl. data). Excess fecal material was removed periodically to prevent coprophagy. When no more fecal material was evident (usually after 24 h), individual fourth-instar larvae were picked out and placed into preweighed aluminium boats in cell culture plates and oven dried at 55°C for 24 h. After cooling in a dessicator, the aluminium boats were reweighed and larval dry mass (milligrams) calculated by difference. Individual larvae were then homogenized in an agate pestle and mortar and stored in the dessicator.

For carbon and nitrogen stable isotope analyses, duplicate samples from each homogenized fourth-instar larva were weighed (~0.7 mg) into tin cups prior to combustion in a Carlo-Erba NA1500 elemental analyzer coupled to a Micromass Isoprime continuous flow isotope ratio mass spectrometer. Where duplicate analyses yielded standard deviation >0.2‰, a third subsample was analyzed and the outlier rejected. Means of the duplicate analyses are given using the δ notation expressed in units per mil (‰). The reference materials used were secondary standards of known relation to the international standard of Vienna Pee Dee belemnite for carbon and atmospheric N$_2$ for nitrogen. Repeat analyses of an internal standard ($n = 50$) resulted in typical precision of ±0.1‰ for carbon and ±0.3‰ for nitrogen.

Variation in δ^{13}C was analyzed with an ANCOVA model (SAS GLM), in which δ^{15}N was included as a covariate and species and lakes as class variables. All nonsignificant interactions were discarded during the procedure. Differences between larval isotope signatures from different water-column depths were analyzed by independent samples *t*-test. Relationships between larval dry mass and δ^{13}C were characterized using linear regression analysis (SAS GLM).

Results

For both *Chironomus* species, intraspecific variability in δ^{13}C and δ^{15}N differed between lakes (Fig. 1). Gr. Binnensee allopatric *C. plumosus* exhibited the highest δ^{13}C and δ^{15}N values, although individual variability was low (Table 2). Likewise, the isotope values of allopatric *C. anthracinus* from Schöhsee were tightly clustered and the range of δ^{13}C and δ^{15}N values was <2‰ for larvae from 10 m and <4‰
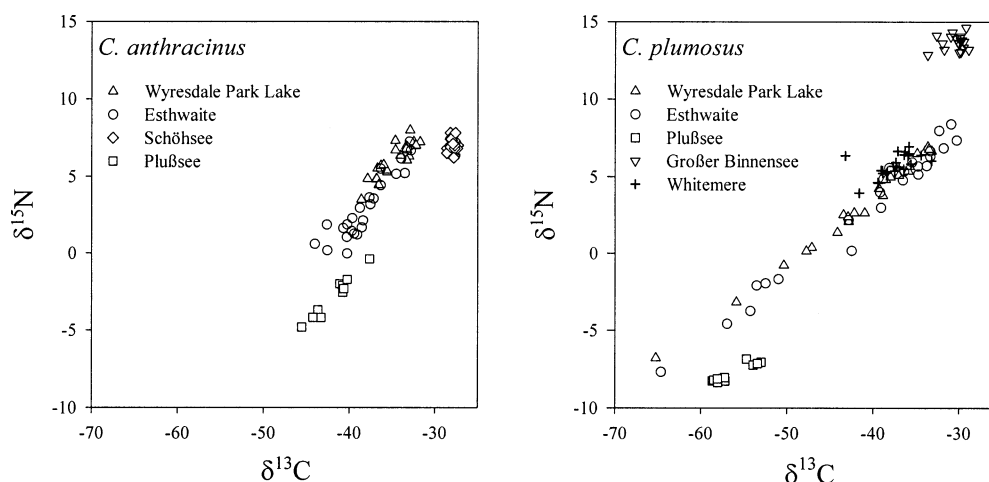
Fig. 1.   Relationship between $\delta^{15}N$ and $\delta^{13}C$ values (‰) plotted for individual *Chironomus anthracinus* and *C. plumosus* from different lakes.

for larvae from 15 m. In contrast, allopatric *C. plumosus* from Whitemere exhibited greater variability in both $\delta^{13}C$ and $\delta^{15}N$. Larvae from the three lakes with sympatric populations exhibited the greatest intraspecific variability. Data from Esthwaite and Wyresdale were remarkably consistent and overlapped those from Whitemere, whereas those of Plußsee exhibited the lowest $\delta^{15}N$. In general, the degree of depletion and range of $\delta^{13}C$ and $\delta^{15}N$ was greater in *C. plumosus* than in *C. anthracinus*. In the three lakes with sympatric populations, there was a strong positive correlation between $\delta^{13}C$ and $\delta^{15}N$ ($F_{1,91} = 815.6$, $P < 0.001$) and a significant interaction between $\delta^{15}N$ and species ($F_{1,91} = 24.9$, $P < 0.001$). Because $\delta^{15}N$ was included as a covariate in the analysis of $\delta^{13}C$ variability, the analysis indicates that the relationship between $\delta^{13}C$ and $\delta^{15}N$ for *C. plumosus* was consistently different from that exhibited by *C. anthracinus*.

Individuals of both chironomid species collected from Esthwaite on the same date but from different depths revealed further intraspecific differences (Table 2, Fig. 2). Isotope values of larvae from 10 m were tightly clustered, although *C. plumosus* was clearly more ^{13}C depleted than *C. anthracinus*. At 15 m, both species showed greater individual variability in both $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2), with *C. plumosus* again exhibiting more pronounced isotopic depletion. Both *C. plumosus* and *C. anthracinus* individuals differed significantly between depths (*t*-tests: $P < 0.001$ for both isotopes and both species). In Schöhsee (Table 2), *C. anthracinus* larvae from 15 m (mean ± 1 SD: $\delta^{13}C$ −32.7 ± 1.1‰, $\delta^{15}N$ 5.0 ± 0.33‰) were significantly depleted in both ^{13}C and ^{15}N (*t*-tests: $P < 0.001$) relative to those from 10 m ($\delta^{13}C$ −27.9 ± 0.34‰, $\delta^{15}N$ 7.0 ± 0.47‰). However, both isotope depletion and intraspecific variability were less extensive than in Esthwaite.

C. plumosus fourth-instar individuals from both Esthwaite

Table 2.   The range of carbon and nitrogen stable isotope values from individual chironomid larvae collected from the indicated depths within six lakes.

| Lake	Species	n	Depth sampled (m)	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
				Min	Max	Min	Max
Großer Binnensee	*C. plumosus*	20	1.0	−33.7	−28.9	12.9	14.6
Schöhsee	*C. anthracinus*	24	10.0	−28.6	−27.3	6.2	7.8
Whitemere	*C. plumosus*	25	7.0	−43.2	−33.2	3.9	6.9
Esthwaite	*C. plumosus*	24	15.0	−65.2	−33.2	−6.8	6.9
	C. anthracinus	24	15.0	−38.6	−31.7	3.5	8.0
Wyresdale Park Lake	*C. plumosus*	24	3.0	−64.6	−30.3	−7.7	8.4
	C. anthracinus	24	3.0	−44.0	−32.8	0.0	7.2
Plußsee	*C. plumosus*	10	4.5	−58.7	−53.0	−8.4	−6.9
	C. anthracinus	10	4.5	−45.6	−37.6	−4.8	−0.4
Depth comparison							
Schöhsee	*C. anthracinus*	15	10.0	−28.5	−27.3	6.4	7.7
		15	15.0	−34.3	−30.4	4.5	5.5
Esthwaite	*C. plumosus*	15	10.0	−39.3	−33.3	8.0	10.3
		12	15.0	−58.9	−44.0	−6.0	1.8
	C. anthracinus	15	10.0	−34.5	−30.5	7.3	9.5
		12	15.0	−43.6	−32.7	1.8	7.8
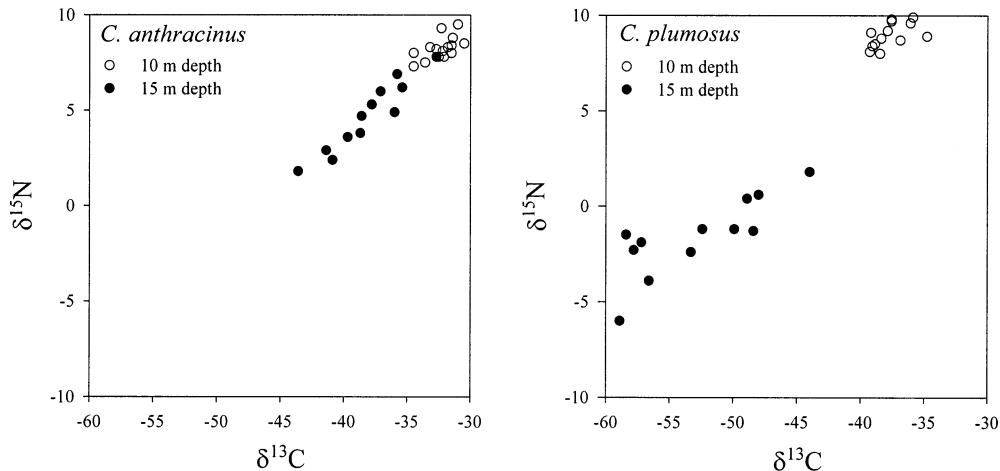
Fig. 2. Relationship between $\delta^{15}N$ and $\delta^{13}C$ values (‰) plotted for individual fourth-instar *Chironomus anthracinus* and *C. plumosus* from Esthwaite. Individuals collected from either 10-m or 15-m depth.

and Wyresdale exhibited considerable differences in body size. The relationships between larval $\delta^{13}C$ and dry mass (Fig. 3) showed significant, negative slopes from both lakes (Esthwaite: slope = −6.91, t = −6.37, degrees of freedom = 1; P < 0.001; Wyresdale: slope = −6.29, t = −2.69, degrees of freedom = 1, P = 0.014).

Discussion

We found that *C. plumosus* was consistently depleted in ^{13}C relative to *C. anthracinus*. Assuming this reflects different feeding behaviors that make *C. plumosus* larvae more likely to include an isotopically light basal resource in their diet, we hypothesized that *C. plumosus* should therefore also express greater intraspecific variability in such lakes. In general, greater intraspecific variability in larval stable isotope signatures was indeed found in lakes where chironomids were more ^{13}C depleted (Fig. 1). Intraspecific variability was less marked in Plußsee, but this may simply reflect the reduced number of individuals collected (Table 2). Moreover, in Esthwaite, both species showed greater intraspecific variability in $\delta^{13}C$ values in individuals from 15-m depth (where oxygen depletion is more acute, Table 1) than from 10-m depth (Fig. 2). *C. anthracinu* exhibited a similar difference in Schöhsee (Table 2). These data are consistent with our second hypothesis. Our data further suggest that extensive intraspecific variability is more likely to occur when *C. plumosus* and *C. anthracinus* are sympatric. However, this phenomenon could be purely coincidental if greater availability of methane-derived resources occurs in Esthwaite, Wyresdale, and Plußsee, where the two species coexist, so data from a wider range of allopatric and sympatric populations are needed to test the robustness of our suggestion.

Differences in the degree of ^{13}C depletion expressed by different instars of allopatric *C. plumosus* from Gr. Binnensee have been found previously (J. Grey unpubl. data). Such variation may be induced by ontogenetic changes in diet as the larvae mature (Berg 1995). However, we analyzed only fourth-instar larvae and thus should have excluded variabil-

ity due to ontogeny. We suggest that the body size of the larva in conjunction with the dimensions of its tube makes an important contribution to the observed individual isotopic variability. Larger larvae have greater girths and create longer tubes (van de Bund and Groenendijk 1994). Consequently, the tube surface area available as a site for microbial colonization is also greater. Because tube walls have been shown to act as microsites of intense methanogen methanotroph activity (Kajan and Frenzel 1999) and tubicolous *Chironomus* sp. forage mostly in the region immediately surrounding the tube and within the tube itself (Berg 1995), larger larvae are more likely to ingest greater amounts of ^{13}C-depleted microbes. Our analysis of intrainstar variability from both Esthwaite and Wyresdale support this hypothesis: larger fourth-instar *C. plumosus* exhibited significantly greater ^{13}C depletion (Fig. 3). However, we cannot exclude the possibility that the isotopically light basal resource produc-
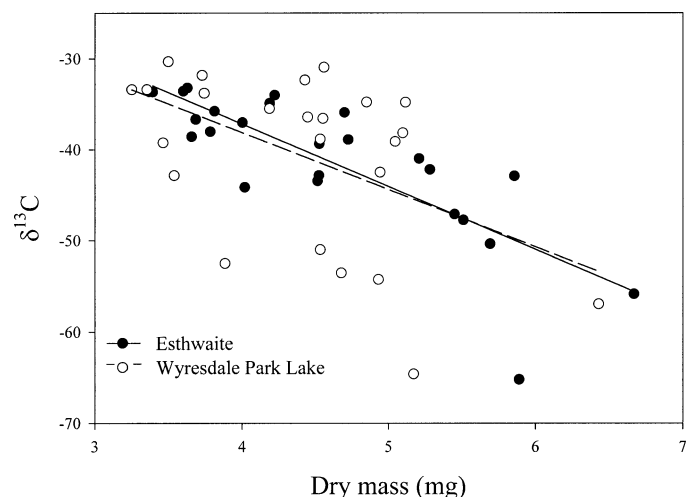

Fig. 3. Relationship between fourth-instar *Chironomus plumosus* larval $\delta^{13}C$ values (‰) and larval dry mass (mg) in two lakes. Esthwaite, y = −6.91x − 9.54, r^2 = 0.65; Wyresdale Park Lake, y = −6.29x − 12.95, r^2 = 0.25.

ing the low larval $\delta^{13}C$ values offers a more nutritious dietary component, resulting in faster growth and higher weights at the time of sampling.

In Esthwaite, Wyresdale, and Plußsee, larvae were found to exhibit $\delta^{15}N$ values down to $-8‰$, which is rare for animal material. Previously, only microorganisms consuming dissolved ammonium from acid hot springs (Estep 1983) or dissolved nitrate from saline lakes (Wada et al. 1981) have been reported below $-8.0‰$. Moreover, low chironomid larval $\delta^{15}N$ values were clearly correlated with low $\delta^{13}C$ values (Figs. 1, 2), a relationship previously shown only with limited data from the littoral of Lake Biwa (Kiyashko et al. 2001) or between small Finnish boreal lakes (Jones and Grey in press). Chironomids excrete nitrogen in the form of ammonium directly into the tube and overlying water (Devine and Vanni 2002). Elevated ammonium concentrations provide an ideal N substrate for bacterial metabolism and via essential fractionation of ammonia can result in a ^{15}N-depleted microbial community (Macko et al. 1987; Macko and Ostrom 1994). Methanotrophic bacteria are also capable of oxidizing ammonium (Lee and Childress 1994) and thus are likely to exhibit correspondingly light $\delta^{15}N$ values. Hence, nitrogen within the tube may be continuously cycled between the larva and microbial consortia. The strength of the positive relationship between the larval $\delta^{13}C$ and $\delta^{15}N$ is indicative that the underlying causative mechanisms are similar.

In addition to the insights our results provide into variability in chironomid larval feeding, our findings have wider implications for the interpretation of stable isotope data from benthic food webs subjected to anoxia. Assuming the typical ^{15}N relationship between a consumer and its diet to be approximately 3.4‰ (Minagawa and Wada 1984), different *C. plumosus* individuals from one discrete sampling location in a lake such as Esthwaite can exhibit a range of values equivalent to five trophic levels (Table 2). These findings caution against indiscriminate use of chironomid larvae as baseline indicators, a concept whereby the $\delta^{15}N$ (and occasionally $\delta^{13}C$, where appropriate) of primary consumers is used to represent the isotopic base of the food web in preference to the isotope ratios of the actual basal resources (Cabana and Rasmussen 1996). In principle, the isotope ratios of a good baseline indicator organism should provide a better time-integrated isotopic perspective and be unaffected by small fluctuations in basal resource values. Thus, in a recent study to elucidate macroinvertebrate food webs in saline wetlands, Hart and Lovvorn (2002) used Chironominae and Orthocladinae as baseline indicator organisms because they were assumed to integrate the most available algal and detrital foods. This assumption would certainly not be valid if a particular chironomid species was selected to represent the base of the Esthwaite food web, and the problem would be exacerbated if no distinction was made between species. Moreover, high intraspecific variability means that large numbers of individuals of potential baseline indicator species need to be pooled to ensure a statistically reliable sample (cf. Lancaster and Waldron 2001). Selection of three individuals (cf. Hart and Lovvorn 2002) from the Esthwaite sample potentially results in $\delta^{13}C$ of $-48.6 \pm 29.6‰$ and $\delta^{15}N$ of $0.2 \pm 7.6‰$ (by mass balance of the minimum, maximum,

and closest to median value for *C. plumosus* larvae). Such standard deviations would negate their use as a satisfactory baseline. Hence, choice of species and sample number are critical when considering the confidence one can place on data derived from pooled baseline indicator organisms (see Post 2002).

Ecologists have tended to view stable isotope analyses as convenient means of integrating diverse processes within food webs. Although this may be appropriate to achieve certain goals, we suggest that intraspecific isotopic variation can actually provide valuable insights into feeding behavior and niche breadth of species, certainly of lake chironomid larvae and probably of other organisms. We strongly encourage more reporting of interindividual isotopic variability.

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