

## Use of allochthonous and autochthonous carbon sources by *Chironomus* in arctic lakes

Anne E. Hershey, Elizabeth Binkley, Kenneth Fortino, Matthew D. Keyse, Cassandra Medvedeff and Robert M. Northington

### Introduction

Larval *Chironomus* commonly dominate the zoobenthos of lakes and serve as an important food resource for fish. Stable isotope studies that trace carbon (C) through lake food webs have shown that *Chironomus*, as well as other consumers, are often depleted in  $^{13}\text{C}$  relative to pelagic seston and detritus (BUNN & BOON 1993). Two general mechanisms for the isotopic depletion relative to basal resources have been proposed: (1) consumers may be selectively feeding on algae that may have a more negative  $\delta^{13}\text{C}$  than bulk seston (HAMILTON et al. 1992, LENNON et al. 2006); (2) consumers may be feeding on significant quantities of methane oxidizing bacteria (MOB; KIYASKO et al. 2001, GREY et al. 2004, JONES & GREY 2004, ELLER et al. 2005, HERSHEY et al. 2005, 2006), which also have a more negative  $\delta^{13}\text{C}$  than pelagic seston, detritus, or bulk sediments.

Allochthonous inputs can support benthic or pelagic components of lake food webs, especially in small lakes (FRANCE 1995, HECKY & HESSLEIN 1995, KRITZBERG et al. 2004). Furthermore, MOB may be an especially important food source in lakes with high dissolved organic carbon (DOC). Hypolimnetic  $\text{CH}_4$  accumulation is correlated positively with epilimnetic DOC (HOUSER et al. 2003). Pelagic MOB production was estimated to be of similar magnitude to production by other heterotrophic microbes in humic Lake Kjelssapputten (HESSEN & NYGAARD 1992). Within the sediments, tubes produced by larval chironomids have higher  $\text{CH}_4$  oxidation rates and higher densities of MOB than surrounding sediments (KAJAN & FRENZEL 1999).

Previous research in arctic Alaskan lakes suggests that phytoplankton-derived food sources are of low importance and MOB are of greater importance in the nutrition of larval *Chironomus* (HERSHEY et al. 2006). Here we used intact sediment cores as mesocosms to experimentally evaluate the role of seston and DOC as basal carbon resources supporting *Chironomus* production.

**Key words:** arctic lakes,  $^{13}\text{C}$ -labeled seston, *Chironomus*, dissolved organic carbon (DOC), methane, methane-oxidizing bacteria (MOB)

### Study area

The study was conducted at Toolik Lake Field Station (68°38'N; 149°38'W) sited in rolling tundra on the North Slope of the Brooks Range in arctic Alaska. The abundant lakes in the region are ultraoligotrophic (e.g., LEVINE & WHALEN 2001) and generally shallow. Sediment core samples, food web components, and/or animals used in this study were collected from relatively shallow nearby lakes: Lake GTH 112 and Lake GTH 114, with maximum depths of 6.0 m and 6.6 m, respectively. The lakes stratify during calm summer days but are polymictic.

### Methods

We sampled Lake GTH 112 and GTH 114 in 2005 to assess  $\delta^{13}\text{C}$  of *Chironomus* larvae and potential organic matter source materials (surficial sediments, seston, and DOC). We also collected samples to assess  $\delta^{13}\text{C}$ - $\text{CH}_4$  from Lake GTH 114 on 19 July 2006.

We conducted a 4-wk experiment in which larval *Chironomus* were incubated in intact sediment cores treated with various organic matter supplements, and measured *Chironomus*  $\delta^{13}\text{C}$  as an indicator of carbon assimilation. *Chironomus* larvae and sediment cores were collected from approximately 5 m depth in Lake GTH 112. Ten Instar III *Chironomus* larvae were introduced into each of 7 intact sediment cores. Instar III larvae were selected because several larvae recovered from one core would provide sufficient biomass for isotope analyses, yet were unlikely to emerge during the 4-week incubation period. Overlying water was siphoned from each core and replaced with either hypolimnetic water from Lake GTH 112 (control), water from a mountain spring known to have very low DOC concentration (DOC removal), or  $^{13}\text{C}$ -labeled seston. The  $^{13}\text{C}$ -labeled seston was prepared by collecting water from Lake NE-9B, which was known to support a deep chlorophyll layer. We incubated the water in 4-L cubitainers inoculated with  $^{13}\text{C}$  labeled bicarbonate. Incubated water was filtered through membrane filters, which were then backwashed into hypolimnetic water from GTH 112.

**Table 1.**  $^{13}\text{C}$  values and concentrations of potential C source materials for *Chironomus* from Lakes GTH 112 and GTH 114. Data for Lake GTH 114 are presented because comparable data were not available for all food web components from Lake GTH 112, where experimental cores were collected. Lake GTH 114 is approximately 1 km from Lake GTH 112 and is of similar size and depth.

C source	DOC/TOC (mg/L)	$^{13}\text{C}$ source
Lake GTH 112 $\text{CH}_4$	29.2	—
Lake GTH 114 $\text{CH}_4$	$35.0 \pm 7.3$	$-60.1 \pm 2.0$
Lake GTH 112 seston (hypolimnion)	—	$-31.8 \pm 0.1$
Lake GTH 114 seston (hypolimnion)	—	$-31.2 \pm 0.1$
Lake GTH 114 DOC (hypolimnion)	$11.9 \pm 1.8$	$-25.7 \pm 2.3$
Lake GTH 112 TOC (hypolimnion)	$17.0 \pm 0.1$	$-28.9 \pm 0.2$
Lake GTH 114 TOC (hypolimnion)	—	$-27.1 \pm 0.1$
Lake GTH 112 surficial sediments	—	-29.3
Lake GTH 112 <i>Chironomus</i> (2005)	—	$-40.3 \pm 0.2$
Lake GTH 114 <i>Chironomus</i> (2005)	—	$-37.8 \pm 0.1$

**Table 2.**  $\delta^{13}\text{C}$  values and concentration of potential C source materials for *Chironomus* from the core experiments.

C source	DOC/TOC (mg/L)	$\delta^{13}\text{C}$ source
Concentrated terrestrial DOC	15.8	-27.35
Control DOC	7.8	-25.38
DOC removal	1.8	-27.84
$\delta^{13}\text{C}$ enriched seston TOC	5.5	92.28

Cores were incubated in a water bath at Toolik Lake epilimnetic temperature for 4 weeks. Larvae were then recovered, dried at 65 °C for 2 days, ground to a powder, and analyzed for  $\delta^{13}\text{C}$  at the Northern Arizona Stable Isotope Laboratory. Initial samples of *Chironomus* were similarly prepared for isotope analyses. Values of  $\delta^{13}\text{C}$  were compared among treatments to evaluate effects of treatment water on carbon assimilation using one-way ANOVA. Treatment means were compared with Tukey's test.

For larvae collected from Lakes GTH 112 and GTH 114, 2-source mixing models were used to estimate the contribution of  $\text{CH}_4$ -derived carbon to *Chironomus* production (HERSHEY et al. 2005).

## Results

Seston  $\delta^{13}\text{C}$ , TOC, and surficial sediments ranged from approximately -25‰ to -32‰ in lakes GTH 112 and GTH 114 (Table 1). However, for these same lakes, *Chironomus*  $\delta^{13}\text{C}$  was 6–15‰ lower than those sources. The only carbon source with a  $\delta^{13}\text{C}$  value lower than that of *Chironomus* was  $\text{CH}_4$  (Table 1).

Manipulation of DOC in experimental cores provided treatments with overlying water that either elevated DOC concentration by approximately 2-fold (terrestrial DOC enrichment) or provided a DOC concentration that was <25% that of control cores (DOC removal). The  $\delta^{13}\text{C}$  DOC of both DOC treatments was approximately 2‰

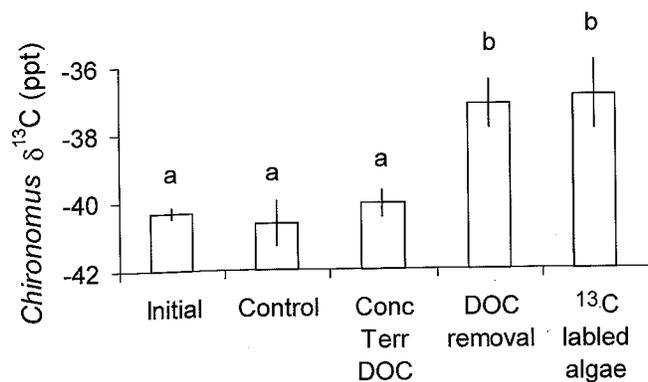
lower than DOC in control cores. The  $^{13}\text{C}$ -labeled seston TOC was approximately 118‰ enriched compared to DOC of control cores (Table 2).

*Chironomus* incubated in DOC removal cores showed significant  $^{13}\text{C}$  enrichment compared to controls or compared to those where allochthonous DOC was experimentally elevated with concentrated terrestrial DOC (Fig. 1). Concentrated terrestrial DOC did not affect *Chironomus*  $\delta^{13}\text{C}$  relative to controls.

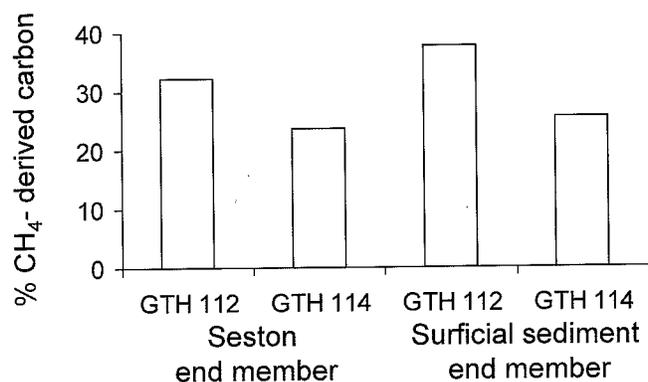
*Chironomus* from cores with high concentrations of  $^{13}\text{C}$ -labeled seston also showed significant  $^{13}\text{C}$  enrichment compared to controls (Fig. 1). However, the increase in  $\delta^{13}\text{C}$  *Chironomus* was relatively small (+3.8‰) compared to the  $\delta^{13}\text{C}$  value of the source material (+92.3), suggesting that direct incorporation of seston-derived C was negligible.

## Discussion

*Chironomus*  $\delta^{13}\text{C}$  was lower than that of DOC and particulate C source materials, consistent with published reports from a wide variety of lentic ecosystems (KAJAN & FRENZEL 1999, KIYASHKO et al. 2001, GREY et al. 2004). By manipulating DOC concentration and introducing  $^{13}\text{C}$ -labeled seston, then measuring *Chironomus*  $\delta^{13}\text{C}$ , we experimentally examined the linkage of *Chironomus* to either seston or DOC as a carbon source. Dis-



**Fig. 1.** *Chironomus* δ<sup>13</sup>C from intact sediment cores incubated for 4 weeks. Overlying water was removed and replaced with either hypolimnetic water (control) from Lake GTH 112 where cores were collected, a concentrated solution of terrestrial DOC (Conc terr DOC), water with very low DOC (DOC removal), or δ<sup>13</sup>C labeled algae.



**Fig. 2.** Estimates of the contribution of CH<sub>4</sub>-derived carbon to carbon assimilation of *Chironomus* from Lakes GTH 114 and GTH 112 based on 2-source mixing models using as end members Lake GTH 114 δ<sup>13</sup>C-CH<sub>4</sub> and either δ<sup>13</sup>C seston or δ<sup>13</sup>C surficial sediment from the respective lake where *Chironomus* were collected.

solved organic carbon can serve as a basal resource for *Chironomus* and other consumers via a variety of aerobic and anaerobic microbial pathways; however, low consumer δ<sup>13</sup>C compared to DOC can be explained by consumer assimilation of MOB, but not by consumer assimilation of aerobic bacteria. Thus, our experiment provided an indirect means of assessing the sensitivity of *Chironomus* assimilation of MOB to DOC availability.

The δ<sup>13</sup>C-DOC of lake hypolimnetic water was slightly enriched compared to δ<sup>13</sup>C of both concentrated terrestrial DOC and that of the DOC removal treatment water. However, the difference was small (2‰) compared to the range of potential source materials, especially <sup>13</sup>C-

enriched seston (Table 2), and still much higher than the δ<sup>13</sup>C of *Chironomus* (Table 1).

The response of δ<sup>13</sup>C *Chironomus* after 4 weeks of DOC removal shows that *Chironomus* is linked to water column DOC over a relatively short time scale. Furthermore, the increase in *Chironomus* δ<sup>13</sup>C following DOC removal is consistent with loss in importance of methanogenesis and methane oxidation pathways for rendering DOC available for assimilation by *Chironomus*. Biogenic CH<sub>4</sub> is more negative than DOC; thus, loss of MOB as a food source must result in an increase in *Chironomus* δ<sup>13</sup>C.

*Chironomus* δ<sup>13</sup>C increased by virtually the same amount when exposed to δ<sup>13</sup>C enriched seston as it did in the DOC removal treatment. We estimated the exponential growth coefficient ( $k = 0.027/d$ ) for *Chironomus* (HERSHEY et al. 2006) based on a growth experiment with *Chironomus* conducted at Toolik Lake (BEATY et al. 2006). This growth rate would permit Instar III *Chironomus* to more than turn over its biomass in 28 days (HERSHEY et al. 2006). The shift in *Chironomus* δ<sup>13</sup>C in response to DOC removal shows that the time scale was adequate for a *Chironomus* response to a water column carbon source. Thus, if *Chironomus* selectively fed on algal components of particulate organic matter, they would have become highly enriched in <sup>13</sup>C. Under natural conditions, consumers can become depleted in <sup>13</sup>C by selectively assimilating algal carbon, even if δ<sup>13</sup>C of bulk POM is more positive than the consumers (LENNON et al. 2006). Although that pathway may, in fact, occur for some consumers, our results provide strong evidence that selective assimilation of algae is not the mechanism contributing to low *Chironomus* δ<sup>13</sup>C. Furthermore, if *Chironomus* were as linked to seston as to DOC, regardless of whether feeding was selective on algal components, they would have shown a proportionally larger shift in δ<sup>13</sup>C because the δ<sup>13</sup>C shift between *Chironomus* and seston was much larger than the shift between *Chironomus* and CH<sub>4</sub> or DOC. Thus, although *Chironomus* δ<sup>13</sup>C did increase in the <sup>13</sup>C enriched treatment, the magnitude of the change was very small compared to the level of <sup>13</sup>C enrichment.

In previous studies we used 2-source mixing models to compute a range of percent methane-based carbon assimilated by *Chironomus* (HERSHEY et al. 2005, 2006). Iso-source has also been used to place limits on the ranges of possible contributions of multiple basal sources to consumer diets (e.g., PHILLIPS & GREGG 2003). The values we observed for *Chironomus* δ<sup>13</sup>C are consistent with those of our previous studies in the same lakes. A similar application of a 2-source mixing model using the δ<sup>13</sup>C-CH<sub>4</sub> observed in GTH 114 as an end member suggests that *Chironomus* from GTH 114 and GTH 112 used 24–38% CH<sub>4</sub>-derived carbon, assuming either seston or sur-

ficial sediments as one end member, and  $\delta^{13}\text{C}$   $\text{CH}_4$  from GTH 114 as the alternate end member (Fig. 2). Because seston and surficial sediments have very similar  $\delta^{13}\text{C}$  values, the method is not very sensitive to choice of the non- $\text{CH}_4$  end member. The method is very sensitive to  $\delta^{13}\text{C}$ - $\text{CH}_4$ , however, because it is so negative relative to the particulate end member and can vary both spatially and temporally in lakes. The  $\delta^{13}\text{C}$ - $\text{CH}_4$  is known to reflect temperature-dependent and concentration-dependent fractionation of acetate by acetoclastic methanogens (KRZYCKI et al. 1987). Because we manipulated DOC experimentally and incubated cores at Toolik Lake epilimnetic temperature, it would not be appropriate to apply the  $\delta^{13}\text{C}$ - $\text{CH}_4$  value from GTH 114 in a mixing model for the experimental cores. However, even without the benefit of a mixing model, the  $\delta^{13}\text{C}$  *Chironomus* response in the DOC removal treatment relative to the controls clearly indicates that MOB became less important when DOC was lower.

In conclusion, our results provide strong support for the hypothesis that  $\text{CH}_4$ -derived carbon is trophically important for *Chironomus* larvae in arctic lakes, that *Chironomus* do not selectively assimilate pelagic algae in these lakes, and that algal-derived carbon cannot account for depleted  $^{13}\text{C}$  values often observed for *Chironomus* larvae. Furthermore, response of  $\delta^{13}\text{C}$  *Chironomus* to DOC removal shows that carbon assimilation is temporally closely linked to water column basal resources. These results illustrate that DOC metabolism is important to higher trophic levels in arctic lakes. We note that the role of DOC in fueling higher trophic levels has been underemphasized relative to the importance of algal-derived carbon and suggest that more focus on DOC is essential for understanding lake trophic interactions.

## Acknowledgements

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