

## Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production

*A. E. Hershey<sup>1</sup> and S. Beaty*

Department of Biology, 312 Eberhart Building, University of North Carolina at Greensboro, Greensboro, North Carolina 27402

*K. Fortino*

Department of Environmental Sciences and Engineering, Rosenau Hall, University of North Carolina at Chapel Hill, North Carolina 27599-7431

*S. Kelly and M. Keyse*

Department of Biology, 312 Eberhart Building, University of North Carolina at Greensboro, Greensboro, North Carolina 27402

*C. Luecke*

Department of Aquatic, Watershed, and Earth Resources, College of Natural Resources, Utah State University, 5210 Old Main Hill, Logan, Utah 84322-5210

*W. J. O'Brien*

Department of Biology, 312 Eberhart Building, University of North Carolina at Greensboro, Greensboro, North Carolina 27402

*S. C. Whalen*

Department of Environmental Sciences and Engineering, Rosenau Hall, University of North Carolina at Chapel Hill, North Carolina 27599-7431

### *Abstract*

We tested the hypotheses that benthic macroinvertebrates, especially chironomid larvae in the Tribe Chironomini, from small oligotrophic arctic lakes are only weakly linked to pelagic food sources, and that they are trophically linked to biogenic methane. All offshore benthic macroinvertebrates sampled from 20 lakes were depleted in  $^{13}\text{C}$  compared with nearshore benthic macroinvertebrates. Nearshore macroinvertebrates had  $\delta^{13}\text{C}$  consistent with feeding on periphyton and/or terrestrial detritus. However,  $\delta^{13}\text{C}$  of offshore Chironomini, the dominant macroinvertebrate group, and oligochaetes, was more depleted than that of other offshore macroinvertebrates to a degree that could only be explained by a diet that included carbon derived from biogenic methane. Seston  $\delta^{13}\text{C}$  showed a small shift toward greater depletion in deeper lakes, but  $\delta^{13}\text{C}$  of Chironomini, oligochaetes, and predatory chironomids varied as a function of dissolved oxygen concentration (DO) at the sediment–water interface, which was generally lower in shallow lakes that were deep enough to stratify. In lakes with lower DO in bottom waters, the importance of methane-based carbon to Chironomini was greater. Experimental  $^{15}\text{N}$  enrichment of phytoplankton in small- and mid-sized arctic lakes resulted in limited  $^{15}\text{N}$  enrichment of most benthic macroinvertebrates, indicating that their productivity was not closely tied to pelagic production. It is likely that aerobic benthic metabolism of dissolved organic carbon (DOC) also is an important energy source for benthic macroinvertebrates in sediments because the link between benthic macroinvertebrate production and contemporary pelagic primary production was weak regardless of dependence on biogenic methane.

Coupling of benthic and pelagic food webs historically has been neglected in limnological studies, which have fo-

cused primarily on pelagic processes (see Vadeboncoeur et al. 2002). A general paradigm has been that benthic secondary production is strongly linked to pelagic primary production. This view is supported by observations that benthic secondary production is high in eutrophic lakes (see Jónsson 1972) and increases when lakes are experimentally fertilized (see Davies 1980; Welch et al. 1988). These studies and others show clearly that benthic secondary production is strongly linked to pelagic production in eutrophic lakes. However, in small oligotrophic lakes, which are very abundant in northern regions, a link between benthic and pelagic

<sup>1</sup> Corresponding author (aehershe@uncg.edu).

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production is less clear. In this article, we examine the relative importance of sediment methanotrophy and seston as energy sources for benthic macroinvertebrates, especially members of the Tribe Chironomini (Diptera: Chironomidae), which are often dominant macrobenthos in small oligotrophic arctic lakes.

Considerable advances in the understanding of aquatic food webs have been made recently through the use of stable isotopes, and these advances have led to a new appreciation of the role of terrestrial and benthic production in lake food webs (see France 1995; Vander Zanden and Rasmussen 1999; Pace et al. 2004). Natural abundances of stable carbon isotopes often serve as effective tracers of food sources in aquatic ecosystems, whereas natural abundances of stable nitrogen isotopes often serve as tracers of trophic structure (Peterson and Fry 1987). However, especially in cases in which there is no clear distinction between the isotopic signatures of autochthonous and allochthonous food sources, stable isotope enrichment studies can provide important information about food sources, organic matter processing, and trophic pathways (see Peterson et al. 2001).

Source materials for aquatic consumers include pelagic and benthic algae and autochthonous and allochthonous detritus. Hecky and Hesslein (1995) reviewed stable isotope studies from a broad range of lakes, concluding that benthic algae and phytoplankton made nearly equal contributions to diets of aquatic consumers. Rau (1980) estimated that 38% of insect biomass emerging from a small lake in Washington was derived from terrestrial detritus. In Canadian Shield lakes, France (1995) showed that littoral communities had  $\delta^{13}\text{C}$  signatures that reflected utilization of a mixture of benthic algae and terrestrial carbon. Pelagic invertebrates were isotopically depleted in  $^{13}\text{C}$  compared with littoral consumers and particulate organic matter (POM), consistent with selective feeding on algae from pelagic POM (France 1995). In Orinoco River floodplain lakes, stable isotope data indicated that microalgae (which were considerably depleted in  $^{13}\text{C}$  compared with bulk POM), rather than detritus, were the major food source for macroinvertebrates (Hamilton et al. 1992). Bunn and Boon (1993), studying Australian billabongs, also found that many consumers were very depleted in  $^{13}\text{C}$  compared with bulk seston or terrestrial detritus. They hypothesized that the depleted signature could either be caused by selective feeding on  $^{13}\text{C}$ -depleted algae (which were not separated from bulk seston in their samples) or could be attributed to assimilation of methanotrophic bacteria, which are consistently highly depleted in  $^{13}\text{C}$  compared with all primary producer food sources (Bunn and Boon 1993).

Methane oxidation is an important mechanism for recycling carbon in lakes (see Rudd and Hamilton 1978). Methanotrophs at the sediment–water interface are believed to consume most of the methane produced in the sediments (Kuivila and Murray 1984; Oremland and Culbertson 1992), although considerable methane is also released to the atmosphere from northern lakes, especially at ice melt (Striegl and Michmerheizen 1998). Recent studies have shown that larval chironomids, frequently the dominant macroinvertebrate group in lake sediments, often utilize methanotrophic bacteria (see Bunn and Boon 1993; Kiyashko et al. 2001;

Grey et al. 2004a). Such utilization is evident because methane is highly depleted in  $^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{methane}}$  range reported from  $-80$  to  $-52$ ) (Bunn and Boon 1993), whereas most other sources are near that of  $\text{C}_3$  or  $\text{C}_4$  plants ( $\delta^{13}\text{C} \approx -28\%$  and  $-13\%$ , respectively) (see Peterson and Fry 1987). The extent of such utilization of methane-derived carbon appears to vary with microhabitat conditions (Kiyashko et al. 2001), seasonality (Grey et al. 2004b), and chironomid species (Grey et al. 2004a,b), but has been reported for several eutrophic lakes. However, recent results from a small, oligotrophic arctic lake showed that 17–37% of *Chironomus* diet also is derived from methane-based carbon but that differences among taxa are owing to environmental conditions within lake sediments rather than to species-specific trophic differences (Hershey et al. 2005).

Many Chironomini construct tubes that they irrigate by undulating their bodies, replenishing  $\text{O}_2$  while removing wastes (Walshe 1951). Such tubes have higher methane oxidation rates and higher densities of methanotrophic bacteria than do surrounding bulk or surficial sediments (Kajan and Frenzel 1999). Thus, the tube-dwelling behavior may facilitate utilization of methanotrophs.

A recent  $^{13}\text{C}$  dissolved inorganic carbon, whole-lake enrichment experiment has shown that much of pelagic bacterial production is supported by allochthonous carbon (Pace et al. 2004), and that much of that subsidy is dissolved organic carbon (DOC) (Kritzberg et al. 2004). DOC in a lake is derived from both allochthonous and autochthonous sources, but usually  $>90\%$  of DOC in oligotrophic lakes is allochthonous and much of the decomposition of DOC occurs within the sediments (Wetzel 2001). Thus, DOC metabolism within sediments is a potentially important mechanism for subsidizing lake food webs with organic matter delivered from the terrestrial environment. Methanogenesis and subsequent methane oxidation at the sediment–water interface represents a portion of DOC metabolism that can be traced readily into macroconsumers owing to the  $^{13}\text{C}$ -depleted signature of methane, whereas the remaining DOC is isotopically similar to the particulate sources from which it is derived, either allochthonous or autochthonous.

In this article we test the hypotheses that in small oligotrophic arctic lakes (1) offshore benthic macroinvertebrates are not strongly linked to pelagic food sources, and (2) Chironomini and oligochaetes are trophically linked to biogenic methane. To test these hypotheses, we (1) evaluate the role of pelagic primary production in the diet of Chironomini by using data from two lakes in which phytoplankton were enriched in  $^{15}\text{N}$ ; (2) compare the spatial distribution of the  $\delta^{13}\text{C}$  values of Chironomini and several other invertebrate taxa in nearshore (shallow) and offshore (4.5–5.0 m depth) areas of arctic lakes; (3) examine  $\delta^{13}\text{C}$  values of Chironomini, oligochaetes, and predatory chironomids (Tanypodinae) across lakes as a function of dissolved oxygen concentration (DO) and depth; and (4) compare Chironomini  $\delta^{13}\text{C}$  values across lakes with results from a reciprocal transplant experiment to examine short-term (1-month) changes in  $\delta^{13}\text{C}$  Chironomini under different lake sediment conditions.

Arctic lakes are ideal for testing our hypotheses because they are abundant on the landscape; are oligotrophic or ultraoligotrophic (Whalen and Cornwell 1985; Levine and

Whalen 2001); have high DOC loading (Whalen and Cornwell 1985); are relatively uninfluenced by anthropogenic disturbances such as watershed disturbances, nutrient enrichments, or exotic species introductions; and have well-understood food webs (see Hershey et al. 1999).

### Study area

The study was conducted in the vicinity of Toolik Lake Field Station (68°38'N, 149°38'W) on the North Slope of the Brooks Range in arctic Alaska. The vegetation is rolling tundra underlain by continuous permafrost. The abundant lakes of the region are ultraoligotrophic or oligotrophic (see Levine and Whalen 2001), relatively shallow, and ice-covered for ~9 months. Lake GTH 112, Toolik Lake, Lake E-5, and Lake E-6 were sampled most intensively, but a total of 26 lakes were sampled for the present study that ranged in maximum depth ( $Z_{\max}$ ) from 1.6–25 m. Lake GTH 112 (68°40'N, 149°14'W) has a surface area of 0.025 km<sup>2</sup>,  $Z_{\max}$  of 5.6 m, and  $Z_{\text{mean}}$  of ~2.1 m. Toolik Lake has a surface area of 1.49 km<sup>2</sup>,  $Z_{\max}$  of 25 m, and  $Z_{\text{mean}}$  of ~7.1 m. Lake E-5 (68°38'N, 149°37'W) has a surface area of 0.11 km<sup>2</sup>,  $Z_{\max}$  of 12.0 m, and  $Z_{\text{mean}}$  of ~5.2 m. Lake E-6 (68°38'N, 149°37'W) has a surface area of 0.02 km<sup>2</sup>,  $Z_{\max}$  of 3.0 m, and  $Z_{\text{mean}}$  of ~2 m. Lake E-5 and Lake E-6 were Long Term Ecological Research (LTER) experimental lakes fertilized with H<sub>3</sub>PO<sub>4</sub> and NH<sub>4</sub>NO<sub>3</sub> to achieve a 4× inorganic nitrogen and phosphorus loading rate based on Toolik data from Whalen and Cornwell (1985), which still resulted in chlorophyll *a* values within the range of those observed in area lakes (Levine and Whalen 2001; Whalen et al. in press; <http://ecosystems.mbl.edu/arc/data.doc/lakes/lakedefault.htm>). Evaluation of fertilization effects are beyond the scope of this article. The epilimnia of Lakes E-5 and E-6 also were enriched with <sup>15</sup>N by dripping a solution of <sup>15</sup>NH<sub>4</sub>Cl into surface waters from 02 July to 10 August 2001–2004 to achieve a target  $\delta^{15}\text{N}$  of the phytoplankton of 100 per mil.

Benthic invertebrates in area lakes are dominated by larval Chironomidae, especially *Stictochironomus rosenschoeldi* and *Chironomus* spp. of the Tribe Chironomini, and a few species of molluscs (see Hershey et al. 1999).

### Methods

Benthic macroinvertebrates, including Chironomini (*Chironomus* and *Stictochironomus*), *Grensia* (Trichoptera: Limnophilidae), *Lymnaea* (Gastropoda), *Valvata* (Gastropoda), fingernail clams (Sphaeriidae), and Oligochaeta, were collected for stable isotope analyses from nearshore (via wading or standing onshore) and offshore (by use of an Eckman grab) areas of 20 lakes during the open water season of 2003. However, not all taxa were collected from all lakes, and taxa collected from one habitat could not always be found in sufficient mass for isotope analyses in the other habitat within the same lake. Nearshore samples were collected by use of a D-net, and offshore samples were collected with an Eckman grab from depths of 4.5–5.0 m or from  $Z_{\max}$  for the two lakes that were shallower than 4.5 m. Animals were

sorted from samples while alive, placed in filtered lake water overnight to clear guts, and then dried and assayed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Colorado Plateau Stable Isotope Facility). We also measured DO at 0.25-m intervals throughout the water column on at least one visit for each lake (all during the open water season).

We re-examined data from a reciprocal transplant experiment ( $\delta^{13}\text{C}$  results reported in Hershey et al. 2005) to place our  $\delta^{13}\text{C}$  data in the context of DO and Chironomini  $\delta^{13}\text{C}$  data from other lakes in the area. Differences in  $\delta^{13}\text{C}$  of *Chironomus* sp. and *Stictochironomus* larvae from Lake GTH 112 and Toolik Lake, respectively, were owing to differences in conditions within the lake sediments rather than to trophic differences between species (Hershey et al. 2005). The methods for this experiment are described in Hershey et al. (2005), but they are briefly reiterated here. Four sediment cores each were collected from 4.5 m in Toolik Lake and Lake GTH 112 and were incubated intact in a water bath at Toolik Lake epilimnetic temperature. Ten *Chironomus* larvae from GTH 112 were introduced into each core from Toolik Lake (where *Chironomus* was not present), and 10 *Stictochironomus* larvae were introduced into each core from GTH 112 (where *Stictochironomus* was not present). These taxa are morphologically distinct without the aid of microscopy (*Stictochironomus* lacks anal tubules). Larvae were also collected from each lake and analyzed for initial isotope values. After a 1-month incubation period, larvae were recovered and prepared for stable isotope analyses.

To evaluate the extent that Chironomini and other macroinvertebrates were utilizing planktonic resources, we sampled sediment trap seston, pelagic seston, and benthic components for isotope analyses from Lake E-5 and Lake E-6, where phytoplankton were experimentally enriched in <sup>15</sup>N as part of the Arctic Tundra LTER project. Because the phytoplankton were enriched with <sup>15</sup>N, the  $\delta^{15}\text{N}$  of the invertebrates should approach that enrichment over time to the extent that animals utilized phytoplankton or phytoplankton detritus as a food source. Invertebrate samples were collected on 02 July, 19 July, and 06 August 2003. Surficial sediments were collected on the same dates from the surface of Eckman grab samples after inspection to ensure that the surface appeared to be undisturbed. Sedimented seston was collected in sediment traps suspended above the lake bottoms from 16 July 2003–12 August 2003. Pelagic seston was collected on 08 July, 15 July, and 27 July 2002, but pelagic seston data are not available for 2003. Samples were prepared for stable isotope analyses as described previously.

We evaluated the importance of <sup>15</sup>N-labeled phytoplankton in diets of macroinvertebrates in Lake E-5 and Lake E-6 by using multiple approaches. First, we estimated the contribution of <sup>15</sup>N-enriched phytoplankton as seston to macroinvertebrate diets by using a two-source mixing model with the assumptions that organisms were at isotopic equilibrium with their diets when sampled (phytoplankton had experienced two summer seasons of <sup>15</sup>N enrichment), and that their  $\delta^{15}\text{N}$  in the absence of <sup>15</sup>N enrichment would have been similar to that in nearby unenriched lakes. For this model, the  $\delta^{15}\text{N}$  of sediment trap seston, corrected for an expected trophic shift of 3.4 per mil (Vander Zanden and Rasmussen 1999), was used as one end member, and the  $\delta^{15}\text{N}$  of the



respective organism in unenriched lakes was used as the other end member:

$$\delta^{15}\text{N}_{\text{taxon E-5,E6}} = s \cdot (\delta^{15}\text{N}_{\text{sediment traps}} + 3.4) + (1 - s) \cdot \delta^{15}\text{N}_{\text{taxon from unenriched lakes}} \quad (1)$$

where  $s$  is the proportion of diet from sediment trap seston in the enriched lake.

In a second approach, we performed a mixing model to estimate the level of enrichment that would be expected if *Stictochironomus* fed primarily on seston, was unenriched at the beginning of the 2003 season (i.e., did not carry over a signal from the previous season), and turned over a portion of its biomass, based on its instantaneous growth rate. As one end member, biomass turnover of *Stictochironomus* was multiplied by the  $\delta^{15}\text{N}$  of sediment trap seston, corrected for an expected trophic shift of 3.4 per mil. Biomass turnover (0.58) was estimated assuming exponential growth ( $m_t = m_0 e^{kt}$ ) in conjunction with the observed change in individual mass of larvae collected from Lake E-5 on 06 July and 19 July 2001 (Hershey unpubl. data). The value determined for  $k$  was then substituted in the same equation to estimate *Stictochironomus* biomass turnover for the 17-day  $^{15}\text{N}$ -enrichment interval experienced by *Stictochironomus* before being sampled on 19 July 2003 and assuming the same final individual mass as observed on 19 July 2001. The mean  $\delta^{15}\text{N}$  of *Stictochironomus* from unenriched lakes was the other end member of the mixing model.

$$\delta^{15}\text{N}_{\text{hypothetical Sticto}} = 0.58 \cdot (\delta^{15}\text{N}_{\text{sediment trap}} + 3.4) + 0.42 \cdot \delta^{15}\text{N}_{\text{Sticto other lakes}} \quad (2)$$

We also used the mean  $\delta^{15}\text{N}$  of pelagic seston from 29 June 2002–15 July 2002 (40.6‰) as an end member instead of the mean 2003 sediment trap  $\delta^{15}\text{N}$  in a variation of the Eq. 2 mixing model. The rationale for this variation was that sediment traps were in the lake from 16 July 2003–12 August 2003, whereas *Stictochironomus* was sampled on 19 July 2003. Thus, although the sediment traps should integrate the seston  $\delta^{15}\text{N}$  signal over the season (plus any fractionation in the traps), they may have failed to represent the early season period before the seston reached equilibrium with the added  $^{15}\text{N}$ . The estimates of *Stictochironomus* hypothetical  $\delta^{15}\text{N}$  could then be compared with the observed value and corrected for background  $\delta^{15}\text{N}$  from unenriched lakes, using a simple back-calculation to determine the percentage of enriched seston in the *Stictochironomus* diet under the same assumptions:

$$\begin{aligned} \text{\% enriched seston in diet} \\ = (\delta^{15}\text{N}_{\text{Sticto observed}} - \delta^{15}\text{N}_{\text{Sticto other lakes}}) \cdot 100\% \\ \times (\delta^{15}\text{N}_{\text{hypothetical Sticto}})^{-1} \end{aligned} \quad (3)$$

The exponential growth coefficient used in the mixing model ( $k = 0.027 \text{ d}^{-1}$ ) is within the range measured by Beaty (2004), who also worked at the Toolik Lake site, for *Chironomus* (0.017–0.035  $\text{d}^{-1}$ ) in microcosms under various sediment conditions. We did not make similar estimates of seston contribution to *Chironomus* growth in Lake E-6 because *Chironomus* was sampled at the beginning of the 2003

$^{15}\text{N}$  addition, and thus any enrichment in their tissue would be owing to feeding on seston produced in the previous season. Because the observed *Chironomus*  $\delta^{15}\text{N}$  was within the range of seasonal variability in *Chironomus*  $\delta^{15}\text{N}$  observed in other lakes (see Results), we did not feel we could use this value as a meaningful estimate of *Chironomus* ingestion of seston. This method also could not be applied to other taxa because we lacked estimates of growth for those taxa.

Finally, we sampled macroinvertebrates from the lake outlets as an additional approach to document the level of enrichment that might occur in benthic macroinvertebrates feeding heavily on seston. Larval black flies (Lake E-6) and limnephilid caddisflies (Lake E-5) from the lake outlets were collected on 31 July and 02 August 2003 and similarly processed for stable isotopes. Note that black fly larvae from lake outlets are filter feeders utilizing lake seston (see Merritt and Cummins 1996). Limnephilids are generally classified as collectors or shredders (Merritt and Cummins 1996) but were sampled from the E-5 outlet because larval black flies or other filter feeders were absent.

To evaluate the degree to which surficial sediment (sampled on 02 August 2003) in Lakes E-5 and E-6 accumulated a  $^{15}\text{N}$  signature over the 2003 season, we performed an additional mixing model that used the mean  $\delta^{15}\text{N}$  values of sediment trap seston and the initial surficial sediment as end members:

$$\delta^{15}\text{N}_{\text{final surficial sediments}} = s \cdot (\delta^{15}\text{N}_{\text{sediment traps}}) + (1 - s) \cdot \delta^{15}\text{N}_{\text{initial surficial sediments}} \quad (4)$$

We tested whether offshore macroinvertebrate  $\delta^{13}\text{C}$  values were more depleted than those from nearshore by means of a paired  $t$ -test, in which the values for nearshore versus offshore samples were pairs for each taxon. Because not all taxa were collected from the same lakes, we also performed a paired  $t$ -test to evaluate the hypothesis that offshore taxa were more depleted than were nearshore taxa by use of the 15 pairs of samples in which a taxon was collected nearshore and offshore in the same lake (one *Chironomus*, one *Stictochironomus*, one Tanypodinae, one Tanytarsini, two *Grensia*, three *Lymnaea*, four *Valvata*, and two *Pisidium*). The relationship between  $\delta^{13}\text{C}$  versus DO and  $Z_{\text{max}}$  for Chironomini collected from lakes of different depths, and the relationship between DO and depth were evaluated with linear regression. Regressions were examined for outliers that might overly influence the significance of a relationship. This approach resulted in dropping a point from the regression of  $\delta^{13}\text{C}$  versus DO. We examined the relationship between phytoplankton (as seston; six lakes) and surficial sediments (11 lakes)  $\delta^{13}\text{C}$  and Chironomini  $\delta^{13}\text{C}$  by use of a linear regression for lakes where both values were measured. If Chironomini were relying primarily on a diet of seston or surficial sediments, then their  $\delta^{13}\text{C}$  signature should be significantly correlated with those food sources across lakes. We also used linear regression to determine the relationship between  $\delta^{13}\text{C}$  of offshore consumers in Lakes E-5 and E-6 and the estimated percentage of seston in the diets of those consumers determined with the mixing model of Eq. 1. This analysis tested the hypothesis that consumers utilizing more seston-derived algae or periphyton would be more depleted

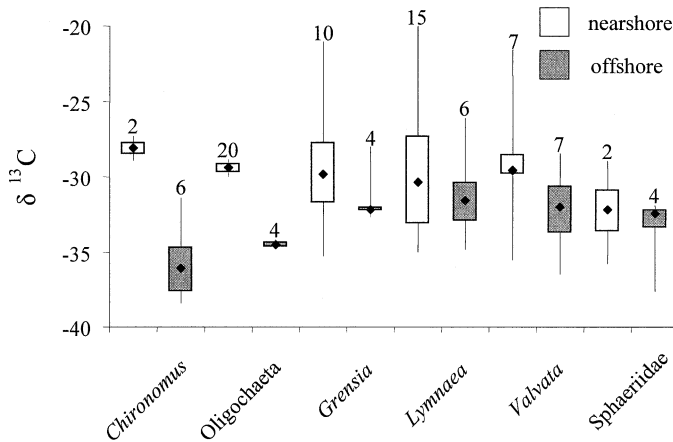


Fig. 1. Benthic macroinvertebrate  $\delta^{13}\text{C}$  (median, interquartile ranges, minima, and maxima) in nearshore (open bars) and offshore (shaded bars) areas of up to 20 lakes illustrating that offshore invertebrates are generally depleted in  $^{13}\text{C}$  compared with nearshore invertebrates. The number of lakes represented by each bar is indicated on the bars.

in  $^{13}\text{C}$  than would other consumers if the algae were more depleted in  $^{13}\text{C}$  than bulk seston. We used ANOVA to evaluate the effect of sampling date on  $\delta^{13}\text{C}$  of *Chironomus* collected from Lake GTH 112 on six dates during 2002–2003. We also used ANOVA to evaluate time (before vs. after incubation), species effects, and the time  $\times$  species interaction for the reciprocal transplant experiment. A priori comparisons were made by using *t*-tests to evaluate the effects of time on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each species and to evaluate initial differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between species used in the experiment. All analyses were performed by using SAS.

## Results

**Spatial distribution of  $\delta^{13}\text{C}$** —Dominant macroinvertebrates collected from offshore areas of 20 lakes were significantly depleted in  $^{13}\text{C}$  relative to the same taxa collected from nearshore (paired *t*-test using mean values for six taxa across all lakes:  $t = 3.35$ ,  $p = 0.01$ ,  $df = 5$ ; paired *t*-test using 15 pairs matched by lake:  $t = 2.69$ ,  $p < 0.01$ ,  $df =$

14). The magnitude of the difference between nearshore and offshore values varied among taxa but was greater for *Chironomus* and *Oligochaeta*, which are tube dwellers, than for other taxa (Fig. 1).

Chironomini (*Chironomus* and *Stictochironomus*)  $\delta^{13}\text{C}$  values were positively associated with DO at the sediment–water interface ( $R^2 = 0.60$ ,  $p < 0.0001$ ) in offshore areas; Chironomini from lakes with lower DO were depleted in  $^{13}\text{C}$  compared with those from lakes with higher DO (Table 1). A linear regression model weakly explained Chironomini  $\delta^{13}\text{C}$  as a function of lake  $Z_{\text{max}}$  ( $R^2 = 0.07$ ,  $p < 0.03$ ), but stepwise multiple regression revealed that  $Z_{\text{max}}$  was not significant when DO was included in the model. However,  $Z_{\text{max}}$  did explain some of the variation in DO at the sediment–water interface, such that deeper lakes tended to be well oxygenated, whereas shallower lakes (but deep enough to stratify,  $Z_{\text{max}} > \sim 3$  m) often had low DO at the sediment–water interface ( $R^2 = 0.25$ ,  $p < 0.001$ ; Table 1).  $\delta^{13}\text{C}$  of Tanypodinae, which are free-living predatory chironomids, also increased significantly with DO ( $R^2 = 0.85$ ,  $p < 0.0001$ ; Table 1), as did oligochaete  $\delta^{13}\text{C}$  ( $R^2 = 0.91$ ,  $p < 0.03$ ; Table 1). However, oligochaetes, which were far less abundant than chironomids, were collected for stable isotope analyses from offshore areas of only four lakes.

Seston  $\delta^{13}\text{C}$  decreased slightly, but significantly, with lake depth ( $p < 0.0001$ ; Table 1) for 18 lakes sampled, thus the pattern of chironomid and oligochaete  $\delta^{13}\text{C}$  is opposite that of the  $\delta^{13}\text{C}$  pattern in the seston.  $\delta^{13}\text{C}$  of offshore surficial sediments ( $-29.6 \pm 0.3\%$ ) was slightly but not significantly ( $t = 0.11$ ,  $df = 25$ ) higher than that of seston ( $-30.3 \pm 0.4\%$ ).  $\delta^{13}\text{C}$  of Chironomini was not correlated with  $\delta^{13}\text{C}$  of either seston or surficial sediments (Table 1).

*Chironomus*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from Lake GTH 112 differed among the dates sampled ( $p < 0.05$  and  $p = 0.002$ , respectively), demonstrating that the isotope signatures change with conditions in the lake (Table 2). However, for the limited seasonal ranges that we studied, there was no consistent seasonal pattern to these changes for either isotope (Table 2). In addition, there was no significant relationship between the values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for GTH 112 *Chironomus* ( $R^2 = 0.004$ ,  $p > 0.8$ ).

**Reciprocal transplant experiment**—*Chironomus* and *Stictochironomus* both showed significant changes in  $\delta^{13}\text{C}$  dur-

Table 1. Parameters for linear regression analyses of relationships between isotope ratios of Chironomini and Oligochaeta, potential source materials, and physical characteristics of lakes.

Response variable	$x$ variable	Slope	Intercept	$p$ value of slope	$R^2$	df	No. lakes
$\delta^{13}\text{C}$ Chironomini	DO*	0.63	-37.2	<0.0001	0.60	50	19
$\delta^{13}\text{C}$ Chironomini	$Z_{\text{max}}$ *	0.17	-36.1	<0.005	0.07	50	20
$\delta^{13}\text{C}$ Oligochaeta	DO	0.76	-38.0	<0.03	0.91	3	4
$\delta^{13}\text{C}$ Tanypodinae	DO	1.13	-40.4	<0.0001	0.85	14	13
DO	$Z_{\text{max}}$	0.31	2.4	<0.001	0.25	50	19
$\delta^{13}\text{C}$ Seston	$Z_{\text{max}}$	-0.13	-29.3	<0.001	0.35	57	18
$\delta^{13}\text{C}$ Chironomini	$\delta^{13}\text{C}$ seston	n. s.	n. s.	0.25	0.15	5	6
$\delta^{13}\text{C}$ Chironomini	$\delta^{13}\text{C}$ surficial sediments	n. s.	n. s.	0.99	0.11	10	11
% seston in offshore consumer diets (Eq. 1)	$\delta^{13}\text{C}$ offshore E-5 and E-6 consumers	n. s.	-32.7	0.69	0.03	5	2

\* DO, dissolved oxygen concentration;  $Z_{\text{max}}$ , maximum depth.

Table 2.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *Chironomus* collected on various dates from Lake GTH 112. When  $n \geq 3$  samples, values are given as means  $\pm$  SE.  $p$  Values from one-way ANOVA indicated significant differences between dates for each isotope  $\delta$  value. Note, however, that the direction of change was not consistent between sampling years for either isotope.

Date	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
27 Jun 02	-34.9	6.0
	-35.9	5.7
16 Jul 02	-37.2	3.9
31 Jul 02	-37.5	4.2
27 Jul 03	-37.7	3.6
30 Jun 03	-35.7 $\pm$ 0.48	4.3 $\pm$ 0.27
08 Jul 03	-36.9 $\pm$ 0.50	9.8 $\pm$ 2.6
$p$ Value	0.047	0.002

ing the course of the incubation experiment. The direction of change in  $\delta^{13}\text{C}$  was taxon specific (significant taxon  $\times$  date interaction,  $p < 0.001$ ). *Stictochironomus* from Toolik Lake had higher  $\delta^{13}\text{C}$  values initially than did *Chironomus* from GTH 112 ( $p < 0.0001$ ; Table 3), but *Stictochironomus*  $\delta^{13}\text{C}$  decreased significantly ( $p < 0.001$ ) whereas *Chironomus*  $\delta^{13}\text{C}$  increased significantly ( $p < 0.02$ ).  $\delta^{15}\text{N}$  of both taxa declined during the experiment (Table 3).

Table 3. Results from a reciprocal transplant experiment showing that both *Stictochironomus* and *Chironomus* became more depleted in  $^{15}\text{N}$  during incubation. *Stictochironomus* from Toolik Lake became more depleted in  $^{13}\text{C}$  when incubated in sediment cores from Lake GTH 112 and *Chironomus* became more enriched in  $^{13}\text{C}$  following incubation in sediment cores from Toolik Lake. Values are mean  $\pm$  SE ‰ ( $\delta^{13}\text{C}$  data after Hershey et al. 2005)

	Before	After
<i>Stictochironomus</i> $\delta^{13}\text{C}$	-33.2 $\pm$ 0.15	-36.3 $\pm$ 0.37
<i>Chironomus</i> $\delta^{13}\text{C}$	-36.9 $\pm$ 0.50	-34.6 $\pm$ 0.55
<i>Stictochironomus</i> $\delta^{15}\text{N}$	6.7 $\pm$ 0.36	3.2 $\pm$ 0.23
<i>Chironomus</i> $\delta^{15}\text{N}$	9.8 $\pm$ 1.3	5.25 $\pm$ 0.12

*Whole-lake  $^{15}\text{N}$  enrichment studies*—Pelagic seston and sediment trap seston from Lake E-5 were highly enriched in  $^{15}\text{N}$  (Table 4). Offshore surficial sediment  $\delta^{15}\text{N}$  in Lake E-5 in 2003 was 3.8‰ on 02 July, 4.5‰ on 19 July, and 11.2‰ on 02 August. Thus, offshore surficial sediments in Lake E-5 were slightly enriched in  $^{15}\text{N}$  on average ( $\sim$ 4‰) compared with those from other lakes but increased during the season. *Stictochironomus* larvae from offshore of Lake E-5 had a  $\delta^{15}\text{N}$  of 8.5‰ and were enriched by  $\sim$ 6‰ in  $^{15}\text{N}$  compared with surficial sediment (periphyton) and sediment trap seston from other lakes. However, they were only enriched by

Table 4.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean  $\pm$  SE) and mixing model estimates for food web components in two lakes in which  $^{15}\text{N}$  tracer was experimentally added during July 2002 and 2003 at similar rates, and for similar food web components in unenriched lakes (No. lakes). Mixing model estimates of percent contribution of sediment trap seston to consumer diets are derived from Eq. 1, expressed as a percentage.

Component	E-5 and -6 dates	$\delta^{13}\text{C}$	$\delta^{15}\text{N}_{\text{observed}}$	$\delta^{15}\text{N}_{\text{other lakes}}$	% seston
Lake E-5					
Pelagic seston	2002 mean	-31.7 $\pm$ 0.3	57.1 $\pm$ 7.0	21 $\pm$ 0.2 (17)	—
Sediment traps	2003 mean	-31.6, -31.9	32.7, 34.7	2.9 $\pm$ 0.3 (4)	—
Surficial sediment OM offshore*	2003 mean	-30.7 $\pm$ 1.4	6.5 $\pm$ 2.3	2.4 $\pm$ 0.3 (24)	—
<i>Stictochironomus</i> (Chironomidae) offshore	19 Jul 2003	-31.6	8.5	7.1 $\pm$ 0.4 (8)	4.7
<i>Mondiamesa</i> (Chironomidae) offshore	02 Jul, 19 Jul 2003	-33.5, -31.6	8.9, 11.2	11.9	0
Orthocladinae (Chironomidae) offshore	02 Jul 2003	-32.7	16.6	5.6	34.9
Fingernail clams nearshore	02 Jul 2003	-25.9	5.2	3.8 $\pm$ 0.2 (16)	5.1
<i>Grensia</i> (Limnephilidae) nearshore	19 Jul, 02 Aug 2003	-29.6 $\pm$ 0.3	23.5 $\pm$ 4.7	5.7 $\pm$ 0.4 (22)	56.7
<i>Valvata</i> (Gastropoda) nearshore	02 Jul 2003	-27.9	21.6	3.6 $\pm$ 0.2 (11)	53.7
Lake E-5 outlet Limnephilidae	31 Jul 2003	-33.2 $\pm$ 0.5	34.3 $\pm$ 1.9	5.7	91.0
Lake E-5 outlet periphyton	31 Jul 2003	-27.7 $\pm$ 0.5	14.9 $\pm$ 1.6	—	—
Lake E-6					
Pelagic seston	2002 mean	-29.5 $\pm$ 0.1	13.9	2.1 $\pm$ 0.2	—
Sediment traps	2003 mean	-30.1, -30.2	19.3, 19.9	2.9 $\pm$ 0.3 (4)	—
Sediment surface OM offshore*	2003 mean	-31.7 $\pm$ 0.4	2.1 $\pm$ 0.2	2.4 $\pm$ 0.3 (24)	—
<i>Chironomus</i> (Chironomidae) offshore	02 Jul 2003	-31.6 $\pm$ 0.2	9.4 $\pm$ 1.2	5.8 $\pm$ 0.7 (8)	31.3
Tanytopodinae (Chironomidae) offshore	2003 mean	-32.8 $\pm$ 0.6	6.6 $\pm$ 0.1	3.8 $\pm$ 0.6 (11)	16.6
Tanytarsini (Chironomidae) offshore	19 Jul 2003	-36.2	6.2	3.7 $\pm$ 1.5 (3)	14.7
Fingernail clams offshore	02 Jul, 02 Aug 2003	-34.7, -33.4	6.9, 7.2	4.2 $\pm$ 0.7 (8)	25.7
Fingernail clams nearshore	02 Aug 2003	-30.9 $\pm$ 0.7	14.8 $\pm$ 2.0	3.8 $\pm$ 0.2 (16)	81.5
Oligochaeta offshore	02 Jul 2003	-32.0	7.4	5.2 $\pm$ 1.0 (3)	18.2
Oligochaeta nearshore	02 Aug 2003	-28.4	6.0	3.8 $\pm$ 0.4 (5)	16.3
<i>Grensia</i> (Limnephilidae) nearshore	19 Jul, 02 Aug 2003	-29.1 $\pm$ 0.2	8.1 $\pm$ 0.7	5.7 $\pm$ 0.4 (22)	20.7
<i>Valvata</i> (Gastropoda) nearshore	19 Jul, 02 Aug 2003	-26.6 $\pm$ 0.7	5.6 $\pm$ 0.1	3.6 $\pm$ 0.2 (11)	14.6
Lake E-6 outlet black fly larvae	02 Aug 2003	-34.0 $\pm$ 0.5	31.0 $\pm$ 0.7	3.8 $\dagger$	215

\* OM, organic matter.

$\dagger$   $\delta^{15}\text{N}$  of black fly larvae obtained from nearby Kuperuk River (Peterson et al. 1993).



~1.4‰ compared with *Stictochironomus* from other lakes (Table 4). Thus, the  $\delta^{15}\text{N}$  of *Stictochironomus* suggests little incorporation of autochthonous  $^{15}\text{N}$ . Similarly, *Monodiamesa bathyphila* (Chironomidae: Diamesinae), a free-living omnivorous chironomid, was not enriched in  $^{15}\text{N}$  above that observed in a nearby lake. Orthoclad (Chironomidae: Orthoclaadiinae) chironomids, generally known as grazers, were considerably enriched in  $^{15}\text{N}$  ( $\delta^{15}\text{N} = 16.6\text{‰}$ ) compared with another lake. Fingernail clams from nearshore were ~1.4‰ enriched compared with clams in other lakes. In contrast, nearshore samples of the surficial feeding snail *Valvata* ( $\delta^{15}\text{N} = 21.6\text{‰}$ ) and limnephilid caddisfly *Grensia* ( $\delta^{15}\text{N} = 23.5 \pm 4.7\text{‰}$ ) were both sufficiently enriched in  $^{15}\text{N}$  to indicate that much of their diet was derived from autochthonous sources (Table 4). However, limnephilid caddisflies from the lake outlet had a much stronger  $^{15}\text{N}$  signature ( $\delta^{15}\text{N} = 34.3 \pm 1.9\text{‰}$ ) than did those collected from the lake sediments.

Pelagic seston ( $\delta^{15}\text{N} = 13.9 \pm 3.1\text{‰}$ ) and sediment trap seston ( $\delta^{15}\text{N} = 19.6\text{‰}$ ) in shallow Lake E-5 were less enriched than those in Lake E-5 (Table 4). Offshore surficial sediment  $\delta^{15}\text{N}$  in Lake E-6 in 2003 was 2.5‰ on 02 July, 1.8‰ on 19 July, and 2.1‰ on 02 August. Thus E-6 surficial sediments were not  $^{15}\text{N}$  enriched compared with 2.4‰ observed in other lakes (Table 4). *Chironomus* from offshore in Lake E-6 ( $\delta^{15}\text{N} = 9.4 \pm 1.2\text{‰}$ ) had a similar to slightly more enriched  $\delta^{15}\text{N}$  signature as *Stictochironomus* does in Lake E-5, but was ~3.6‰ enriched compared with the mean *Chironomus* from other lakes (Table 4). However, as noted above, *Chironomus*  $\delta^{15}\text{N}$  was highly variable within Lake GTH 112 (Table 2), where it was measured repeatedly, and the observed  $\delta^{15}\text{N}$  of Lake E-6 *Chironomus* was within the range of that observed in GTH 112. Thus, we cannot evaluate whether *Chironomus*  $\delta^{15}\text{N}$  reflected an enrichment above background variation. *Valvata*, Tanypodinae, Tanytarsini (Chironomidae), oligochaete, *Grensia*, and offshore fingernail clam  $\delta^{15}\text{N}$  values were slightly higher than were  $\delta^{15}\text{N}$  for those taxa in other lakes (Table 4). Nearshore fingernail clams were the only Lake E-6 taxon that was highly enriched in  $^{15}\text{N}$ . Black fly larvae collected from the lake outlet also had high  $\delta^{15}\text{N}$  values (Table 4).

$\delta^{13}\text{C}$  values for Lake E-5 and Lake E-6 consumers were more enriched for nearshore than for offshore samples (Table 4), consistent with the lake surveys (Fig. 1). Pelagic seston, sediment trap seston, and surficial sediments generally had similar  $\delta^{13}\text{C}$  values, thus  $\delta^{13}\text{C}$  was not informative in evaluating source materials for offshore taxa.  $\delta^{13}\text{C}$  values of offshore taxa were more variable than were the potential source materials measured. *Stictochironomus* and *Chironomus* had similar  $\delta^{13}\text{C}$  to surficial sediment and seston, and other offshore taxa were more depleted in  $^{13}\text{C}$  than any of the measured source materials. Both Lake E-5 and Lake E-6 were well oxygenated at the sediment–water interface where offshore samples were taken (5 m and 2 m, respectively), and the  $\delta^{13}\text{C}$  values for Chironomina, Tanypodinae, and Oligochaeta were relatively enriched compared with those in lakes with lower DO (Table 1).

Mixing models from Eq. 1, which assumed that components were sampled when at isotopic equilibrium with sediment trap seston, indicated that the percentage utilization of  $^{15}\text{N}$ -enriched seston varied from 5–82% and differed between

Lakes E-5 and E-6 and between nearshore and offshore areas (Table 4). In Lake E-5, utilization of autochthonous sources was greatest for surficial feeding taxa collected from nearshore areas, including *Valvata* (54%) and *Grensia* (57%), and for offshore Orthoclaadiinae (35%). The nearshore taxa may have been relying on  $^{15}\text{N}$ -labeled nearshore periphyton (which we did not sample) as well as seston, and Orthoclaadiinae were likely feeding on deposited seston, which did accumulate during the 2003 season. *Stictochironomus* from offshore appeared to utilize only ~5% of seston production and *Monodiamesa* did not utilize seston. In lake E-6, the same mixing model approach indicated that macroinvertebrates showed less variation between nearshore and offshore taxa, except that nearshore fingernail clams appeared to utilize ~82% seston. Among offshore taxa, *Chironomus* appeared to have the highest level of utilization of seston. However, this estimate is problematic for *Chironomus* because its  $\delta^{15}\text{N}$  values ranged from 3.6–9.8‰ in Lake GTH 112 from 27 June 2003–08 July 2003, thus the observed  $\delta^{15}\text{N}$  value of 9.4 is within the range of natural variability of *Chironomus*.  $\delta^{13}\text{C}$  of offshore consumers in Lakes E-5 and E-6 was not significantly correlated to the percentage of seston estimated from Eq. 1 mixing models (Table 1).

By use of the mixing model wherein *Stictochironomus* in Lake E-5 fed entirely on material from sediment traps and turned over 58% of its biomass during the 2003  $^{15}\text{N}$ -enrichment period, we estimated that its  $\delta^{15}\text{N}$  value would have been ~26‰ or 30‰ (from Eq. 2), depending on the assumed  $\delta^{15}\text{N}$  of its pelagic food resource (Table 5). Both of these values are considerably more enriched than was the observed value of 8.5‰, and they support the hypothesis that seston is not the primary food source for *Stictochironomus* in Lake E-5. Back-calculation of percentage of enriched seston utilization using the same assumptions of biomass turnover and 2003 initial unenriched condition indicates that *Stictochironomus* acquired 4.7–5.4% (depending on the assumed  $\delta^{15}\text{N}$  of seston utilized) of its nitrogen from seston. These values are in close agreement with those from the equilibrium model (4.7%; Table 4).

A mixing model using sediment trap seston and surficial sediment from 03 July 2003 (Eq. 4) suggested that offshore surficial sediments comprised 24% autochthonous material in Lake E-5 on 02 August 2003, whereas Lake E-6 sediments showed no evidence of  $^{15}\text{N}$  enrichment (Table 5).

## Discussion

A general paradigm in limnology is that benthic secondary production is closely linked to pelagic primary production. This view is supported by observations of high benthic production or emergence rates from eutrophic lakes and from ecosystem-scale fertilization studies (Jónasson 1972; Davies 1980; Welch et al. 1988). Below, we argue that the weight of evidence presented in this article supports our hypothesis that the linkage between pelagic primary production and benthic secondary production is not strong for small oligotrophic lakes, where terrestrial inputs may be relatively important compared with pelagic primary production. Furthermore, our data also support the hypothesis that in some lakes

Table 5. Mixing models used to test hypotheses about the potential for Lake E-5 *Stictochironomus* to acquire a seston  $^{15}\text{N}$  signal from the pelagia. Models use observed growth rates to evaluate the contribution of seston to the observed  $\delta^{15}\text{N}$  value of surficial sediments in Lakes E-5 and E-6.

Hypothesis	Mixing model	Parameters
<i>Stictochironomus</i> turns over 58% of its biomass during the 17 d of $^{15}\text{N}$ enrichment prior to sampling using sediment trap seston ( $\delta^{15} = 33.7\text{‰}$ ).	$\delta^{15}\text{N}_{\text{hypothetical } Stic} = 0.58 \times (\delta^{15}\text{N}_{\text{sediment trap}} + 3.4) + 0.42 \times \delta^{15}\text{N}_{Stic \text{ other lakes}}$	$\delta^{15}\text{N}_{\text{hypothetical } Stic} = 25.9\text{‰}$
<i>Stictochironomus</i> turns over 58% of its biomass during the 17 d of $^{15}\text{N}$ enrichment prior to sampling using food with a $\delta^{15}\text{N}$ value of mean pelagic seston from 29 Jun 2002–15 Jul 2002 (40.6‰).	$\delta^{15}\text{N}_{\text{hypothetical } Stic} = 0.58 \times (\delta^{15}\text{N}_{\text{pelagic seston}} + 3.4) + 0.42 \times \delta^{15}\text{N}_{Stic \text{ other lakes}}$	$\delta^{15}\text{N}_{\text{hypothetical } Stic} = 29.9\text{‰}$
Surficial sediment from Lake E-5 on 02 Aug 2003 has a $\delta^{15}\text{N}$ value that reflects surficial sediments on 02 Jul 2003 and input from sediment trap seston (33.7‰).	$\delta^{15}\text{N}_{\text{final surficial}} = s \times (\delta^{15}\text{N}_{\text{sediment traps}}) + (1 - s) \times \delta^{15}\text{N}_{\text{initial sediments}}$	$s = 0.24$
Surficial sediment from Lake E-6 on 02 Aug 2003 has a $\delta^{15}\text{N}$ value that reflects surficial sediments on 02 Jul 2003 and input from sediment trap seston (19.6‰).	$\delta^{15}\text{N}_{\text{final surficial}} = s \times (\delta^{15}\text{N}_{\text{sediment traps}}) + (1 - s) \times \delta^{15}\text{N}_{\text{initial sediments}}$	Does not converge; final sediments are more depleted than either hypothesized source

a significant portion of the benthic secondary production is based on biogenic methane, via macroinvertebrate feeding on methanotrophic bacteria.

*Limited coupling of benthic consumers to the pelagia*—We considered whether a diet primarily of phytoplankton and phytodetritus could account for the observed  $^{13}\text{C}$  depletion seen in Chironomini, Tanypodinae, and oligochaetes, and we concluded that such a mechanism is unlikely. The range of  $\delta^{13}\text{C}$  of Chironomini, Tanypodinae, and oligochaetes among lakes was much greater than the range of  $\delta^{13}\text{C}$  of sediment trap seston or pelagic seston, and the pattern of more depleted seston in deep lakes was not consistent with the pattern of lower  $\delta^{13}\text{C}$  in these macrobenthic taxa in lakes with low DO, which were relatively shallow. Furthermore, DO at the sediment–water interface accounted for much of the  $\delta^{13}\text{C}$  variability in Chironomini, Tanypodinae, and oligochaetes, and  $\delta^{13}\text{C}$  of seston accounted for none.  $^{13}\text{C}$ -depleted periphyton is also an unlikely explanation because Chironomini sampled from GTH 112, which were among the more  $^{13}\text{C}$ -depleted of those sampled, were taken from below the photic zone, as GTH 112 is a relatively turbid lake owing to resuspension of glacial silt deposits (Whalen et al. in press). Tanypodinae are generally considered predatory and thus could have acquired a depleted  $\delta^{13}\text{C}$  from feeding on  $^{13}\text{C}$ -depleted prey. However, our data show that Tanypodinae were generally slightly more depleted than were Chironomini and oligochaetes and thus must also have been ingesting a more depleted food source than those measured. Hamilton et al. (1992) separated the algal component in the seston in the Orinoco River floodplain and found algae to be more  $^{13}\text{C}$  depleted than was bulk seston or other source materials for the food web. Thus, seston  $\delta^{13}\text{C}$  may not reflect the  $\delta^{13}\text{C}$  of algae, and if consumers assimilate primarily the algal component, they could have a very different  $\delta^{13}\text{C}$  than the  $\delta^{13}\text{C}$  of bulk seston would suggest.

For feeding on  $^{13}\text{C}$ -depleted algae to account for the difference we observed among lakes in Chironomini, Tanypodinae, and oligochaete  $\delta^{13}\text{C}$ , the following conditions would need to apply: (1) photosynthetic fractionation differed among lakes such that greater fractionation of  $^{13}\text{C}$  by phytoplankton occurred in low DO lakes compared to well-oxygenated lakes; (2) the consumers selectively fed on or selectively assimilated phytoplankton; and (3) consumers that assimilated more seston-derived algae were depleted in  $^{13}\text{C}$  compared with those that assimilated less.

We cannot directly evaluate the fractionation issue with our data. However, it is unlikely that greater algal  $^{13}\text{C}$  fractionation occurred in low DO lakes because seston in deeper lakes had slightly lower  $\delta^{13}\text{C}$ , suggesting that if there was a difference in  $^{13}\text{C}$  fractionation among lakes, greater fractionation occurred in the deeper lakes. With regard to the second condition, selective feeding on algae has been reported for some profundal Chironomini in eutrophic lakes (Johnson et al. 1989), although the importance of algae, bacteria, and detritus in the diets of Chironomini varies considerably (Rasmussen 1985; Johnson 1987; Goedkoop and Johnson 1996). However, if the consumers had been either selectively ingesting or selectively assimilating sedimenting algae, these organisms in Lakes E-5 and E-6 would have become en-



riched in  $^{15}\text{N}$  to levels comparable to their food, as suggested by the mixing model of Eq. 1, and they were not. Assimilation of  $^{15}\text{N}$ -enriched food varied across taxa, but it was not the major food source for most taxa studied and was not the major food source for any offshore taxon (Table 4). Finally, the  $\delta^{13}\text{C}$  values of offshore consumers were not correlated with the estimated percentage of seston in their diets. Thus, the depleted  $\delta^{13}\text{C}$  values seen in macrobenthic consumers in lakes with low DO are not consistent with a diet primarily of  $^{13}\text{C}$ -depleted phytoplankton.

Both *Stictochironomus* and *Chironomus* from Lake E-5 and Lake E-6, respectively, had  $\delta^{15}\text{N}$  values that were more similar to the same taxa from unenriched lakes than they were to  $^{15}\text{N}$ -enriched seston. This was most evident for *Stictochironomus* in Lake E-5, indicating that it was not strongly coupled to the pelagia. Furthermore, mixing model scenarios indicated that the contribution of autochthonous material to the *Stictochironomus* diet was relatively low. The mixing model of Eq. 2 further illustrates that lack of  $^{15}\text{N}$ -enrichment in *Stictochironomus* cannot be attributed to slow biomass turnover time, and that this turnover-based mixing model gives nearly identical results as does the equilibrium model of Eq. 1. Given that the equilibrium model works well for *Stictochironomus*, which is the longest lived taxon studied (4-yr life cycle in Toolik Lake) (Hershey 1985), we feel that the equilibrium models must also be reasonable approximations for the other taxa. It is also clear from the reciprocal transplant experiment that both *Stictochironomus* and *Chironomus* larvae rapidly shifted in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in response to changing sediment conditions.

It is more difficult to evaluate the relationship of *Chironomus* to seston in Lake E-6 than of *Stictochironomus* to seston in Lake E-5. Mixing model 1 indicates that *Chironomus* in Lake E-6 may be more strongly linked to autochthonous production than is *Stictochironomus* in Lake E-5. However, the high background variability in *Chironomus*  $\delta^{15}\text{N}$  observed in GTH 112 (Table 2) raises the possibility that the level of enrichment of *Chironomus* in Lake E-6 may be unrelated to the  $^{15}\text{N}$ -enriched producers in the lake.

Orthoclaadiinae were more closely tied to the pelagia in Lake E-5 than were other offshore macrobenthos. Offshore surficial sediments in Lake E-5 were taken from near the 1% light limit, and thus, the level of  $^{15}\text{N}$  enrichment likely reflected primarily seston deposition rather than periphyton. Orthoclaadiinae typically feed by grazing or gathering outside of their tubes at the sediment surface and thus should be expected to be more closely tied to pelagic production than are deposit feeders. Tanypodinae appeared to utilize 16.6% seston in Lake E-6. *Procladius* (Tanypodinae) in Toolik Lake consume a variety of foods but feed on a disproportionate number of orthoclads (Hershey 1986). Thus, Tanypodinae may have acquired an enriched  $^{15}\text{N}$  signature from orthoclad prey.

Mixing models and  $\delta^{15}\text{N}$  values for nearshore non-Chironomini taxa suggested a wide range of utilization of  $^{15}\text{N}$ -labeled sources. By use of sediment trap seston as representative of a pelagic end member, nearshore taxa in Lake E-5 (*Grensia* and *Valvata*) appeared to be utilizing >50% pelagic sources. However, the  $^{15}\text{N}$  label also could have been derived from periphyton, which, unfortunately, we did not

sample nearshore. Thus, we cannot distinguish between the alternative hypotheses that nearshore macroinvertebrates were more closely linked to the pelagia than were offshore macroinvertebrates, or whether nearshore macroinvertebrates derived their  $^{15}\text{N}$  signature from periphyton. However, these results do show that autochthonous production is relatively more important to nearshore compared with offshore macroinvertebrate production.

Lake E-6 is uniformly shallow, so we might expect that much of the autochthonous production would be benthic, as is true in shallow lakes elsewhere (Vadeboncoeur et al. 2002). However, offshore sediments in Lake E-6 were not enriched in  $^{15}\text{N}$ , thus it is likely that periphyton were relying on the sediment rather than the pelagic pool of DIN, and the enrichment observed in both nearshore and offshore macroinvertebrates reflected a closer coupling to pelagic resources in Lake E-6 than in Lake E-5. However, in both lakes the  $^{15}\text{N}$  signature was too weak to suggest that pelagic production was the primary food source for the offshore macrobenthic community. Our results suggest that in Lake E-5, allochthonous organic matter sources, rather than seston from the pelagia, were relatively more important as the food base for the offshore macroinvertebrate community. In Lake E-6, either unlabeled periphyton or allochthonous sources could account for the observed  $^{15}\text{N}$  signatures in offshore macroinvertebrates, but in either case, coupling to the pelagia was weaker than was reliance on nonpelagic sources.

Both limnephilid caddisflies from the E-5 outlet and black flies from the E-6 outlet had considerably higher  $\delta^{15}\text{N}$  values than did any of the macroinvertebrates in either lake. Limnephilids in the E-5 outlet appear to have a 3-yr life cycle, similar to brachycentrid caddisflies in nearby Kuparuk River (Hershey and Hiltner 1988), and had a  $\delta^{15}\text{N}$  value that was very similar to sediment trap seston, suggesting that they were near isotopic equilibrium with this food source. This conclusion is also consistent with mixing model calculations (Table 4). The black fly larvae in the Lake E-6 outlet were considerably more enriched than was sediment trap seston. Enrichment above basal resources previously has been attributed to either selective grazing or selective assimilation (Tank et al. 2003).

For the  $^{15}\text{N}$  addition experiments in lakes E-5 and E-6, one alternative interpretation of the weak  $^{15}\text{N}$  enrichment of offshore macroinvertebrates is that bioturbation or turbulence at the sediment–water interface served to mix the  $^{15}\text{N}$ -enriched settling algae with older unenriched sediments, diluting the overall  $\delta^{15}\text{N}$  of the food supply in bulk sediments. Although this mechanism may well explain part of the response, it is less likely to be the major mechanism contributing to the weak  $^{15}\text{N}$  signature than is the hypothesis of weak coupling of most macrobenthic consumers to the pelagia. Chironomini studied here construct rambling tubes at or near the sediment–water interface. Even with tubes at or near the surface, Chironomini larvae still remained little enriched in  $^{15}\text{N}$  compared with the seston in Lakes E-5 and E-6. In Lake E-6, the sediment surface itself was unenriched, suggesting either that little seston was being deposited or that it was being consumed by grazers, such as snails and orthoclads. Orthoclads did appear to be much more closely tied to the seston than were Chironomini in Lake E-5, sug-

gesting that grazing of seston as it was being deposited was important. The grazing snail *Lymnaea* in nearby Lake N-2 was previously shown to be food limited in offshore areas (Hershey 1992), which is consistent with the argument that the deposited seston could have been consumed by grazers. In Lake E-5, surficial sediments at 5-m depth became sufficiently enriched by 02 August 2003 to indicate that their composition was comprised of 24% seston from 2003 alone (Table 4). However, a similar level of deposition should have occurred during 2002, but surficial sediments were only slightly enriched at the beginning of 2003 in Lake E-5 and not at all enriched in Lake E-6, consistent with the argument that it is being consumed.

In addition to consumption by grazers, newly deposited Lake E-5 seston could have been focused into profundal areas (Lake E-6 has no profundal zone), which we did not sample. Focusing has been shown to redistribute the majority of newly deposited seston in small lakes on seasonal to annual time scales (Stephenson et al. 1995) and thus could easily account for the limited residual  $^{15}\text{N}$  enrichment of surficial sediments at the beginning of the 2003 season in Lake E-5.

*Utilization of biogenic methane*—The depleted  $\delta^{13}\text{C}$  values of larval Chironomina and oligochaetes in offshore areas of low DO lakes is best explained by utilization of methane-based bacterial production (see Kajan and Frenzel 1999; Kiyashko et al. 2001; Grey et al. 2004b). All groups of macroinvertebrates studied were at least slightly  $^{13}\text{C}$  depleted in offshore samples compared with nearshore samples (Fig. 1), which is consistent with the observations of France (1995) for littoral versus pelagic invertebrates. Offshore macroinvertebrates were also  $^{13}\text{C}$  depleted compared with pelagic seston. However, Chironomina and oligochaetes, the tube-dwelling forms, were considerably more  $^{13}\text{C}$  depleted than were other taxa (*Grensia* has a case but feeds surficially with its head and legs extended from the case). These results are consistent with the hypothesis that Chironomina and oligochaetes were relying partially on methanotrophs, which Kajan and Frenzel (1999) found to be more abundant in association with chironomid tubes than in bulk or surficial sediments. The slight depletion in surficial feeding snails (*Valvata* and *Lymnaea*) and *Grensia* compared with pelagic seston is consistent with assimilation of sediment organic matter, including smaller quantities of methanotrophs compared with methanotroph assimilation by tube-dwellers, selective feeding on algal components of seston that may be more  $^{13}\text{C}$  depleted than bulk seston, or both of these mechanisms.

Recent stable isotope studies provide strong evidence that chironomid feeding on methanotrophs is widespread, but variable between and within lakes (see Grey et al. 2004a,b). The variability in chironomid  $\delta^{13}\text{C}$  values could be caused by several factors. Grey et al. (2004b) found that in a shallow well-oxygenated lake,  $\delta^{13}\text{C}$  of chironomids was relatively constant throughout the year and was not depleted in  $^{13}\text{C}$  compared with particulate food sources. However, chironomids in deeper lakes with anoxic sediment–water interfaces had seasonally variable but  $^{13}\text{C}$ -depleted  $\delta$  values (Grey et al. 2004b). These data suggest that feeding on methano-

trophs is limited by the spatial distribution of that food resource.

Our lake survey illustrates that the pattern of utilization of methane-based bacteria is closely related to hypolimnetic DO, which is correlated with  $Z_{\text{max}}$ . Relatively deep lakes in our study region have well-oxygenated bottom waters throughout the year, reflecting their low productivity and large hypolimnetic volume to support under-ice respiration (see Whalen and Cornwell 1985). Very shallow (<3 m) lakes do not stratify and thus are well oxygenated, but shallow lakes >3 m have lower oxygen in bottom waters during summer stratification owing to their small hypolimnetic volume and are thus more suitable conditions for methanogenesis at or near the sediment–water interface; we also would expect that they are hypoxic under ice. We do not have specific information on the internal environment of Chironomina tubes in our study lakes. However, Chironomina are known to irrigate their tubes, enhancing DO supply. They can extract DO even at very low concentrations (Walshe 1951), thereby creating microsites for methanotrophic activity in close proximity to anaerobic microsites suitable for methanogens. Kajan and Frenzel (1999) noted that chironomid tubes also may extend from oxygenated surficial sediments into anoxic sediments, and they suggested that the tubes function as microsites of intense microbial activity and coupled methanogenesis and methanotrophy. In contrast, in lakes with high DO at the sediment–water interface, anoxic microsites suitable for methanogens would be expected to be less common in the surficial sediments. The variability that we observed in  $\delta^{13}\text{C}$  of Chironomina among lakes, its correlations with DO at the sediment–water interface, and the results of our reciprocal transplant experiment are consistent with the hypothesis that in shallow lakes with hypoxic bottom water, microhabitat conditions associated with Chironomina tubes facilitate methanotrophs, which subsequently serve as an important C source for the chironomids.

*General discussion and conclusions*—Bacterial production in small oligotrophic lakes is supported primarily by DOC, which is largely of terrestrial origin (Kritzberg et al. 2004), and much of this DOC is metabolized in lake sediments (see Wetzel 2001). In our study region, DOC loading from the landscape is very high (Whalen and Cornwell 1985). The depleted  $\delta^{13}\text{C}$  values of Chironomina provides a means to estimate the proportion of the DOC metabolism that is based on methane-derived carbon, which Hershey et al. (2005) estimated to be 17–37% for *Chironomus* in Lake GTH 112 and 10–22% for *Stictochironomus* in well-oxygenated Toolik Lake. These represent lower-bound estimates of DOC-derived macroinvertebrate production because considerable DOC-based production also may be supported by non-methane-based bacterial production in the sediments. This conclusion gains support from the fact that only ~4–35% of offshore macroinvertebrate production appears to be derived from pelagic primary production over 2 yr. However, the  $\delta^{13}\text{C}$  of terrestrial DOC is indistinguishable from particulate sources from which it is derived (see Kritzberg et al. 2004). Allochthonous POC is probably not very important. It is far less abundant than DOC in this region (Whalen and Cornwell 1985). In addition, POC would have been sampled

as part of the seston in Lakes E-5 and E-6, and because little  $^{15}\text{N}$ -labeled seston was assimilated by the macroinvertebrate community, allochthonous POC could not have been very important in macroinvertebrate diets either. Pace et al. (2004) showed that in a small north temperate lake, 22–50% of zooplankton production was derived from allochthonous carbon, and Kritzberg et al. (2004) estimated that 35–70% of pelagic bacterial biomass was derived from terrestrial DOC, indicating that terrestrial DOC is an important subsidy to oligotrophic lake food webs. Our data are consistent with their results but extend them to suggest that DOC utilization in the sediments also supports much benthic invertebrate production. We suggest that this also may be a feature of most small oligotrophic lakes worldwide.

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