Use of deep autochthonous resources by zooplankton: Results of a metalimnetic addition of ^{13}C to a small lake

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Abstract

  Resources in lakes are vertically partitioned due to stratification and trophic interactions. Metalimnetic phytoplankton could be an important resource for zooplankton that either reside in the metalimnion or migrate through this layer. However, it is difficult to estimate metalimnetic resource use, especially using isotope approaches, because surface and deep phytoplankton often have similar isotopic compositions. To overcome this limitation, we experimentally enriched the metalimnetic dissolved inorganic carbon (DIC) ^{13}C pool in Peter Lake to enhance the isotopic separation between metalimnetic phytoplankton and other resources. Metalimnetic δ^{13}C-DIC peaked at 73.2‰ after the isotope addition and maintained an average enrichment of 34.5‰ above epilimnetic δ^{13}C-DIC for 62 d. Combining hydrogen and carbon stable isotope values, we estimated the epilimnetic, metalimnetic, and terrestrial resource use by zooplankton, using a Bayesian mixing model that accounted for uncertainties in both consumers and sources. We also measured diel vertical migration and net ecosystem production with in situ bottle incubations over the course of the experiment. Metalimnetic resource use was minor (0–8%) for zooplankton that either resided in the epilimnion of the lake during the day or migrated there at night. For consumers that resided in the metalimnion, metalimnetic phytoplankton accounted for 18–21% of zooplankton isotope composition. The most important resource for all zooplankton was terrestrial organic matter (56–73% of consumer mass), regardless of habitat. This experiment indicates that, in lakes like Peter Lake, metalimnetic autochthonous resources are of minor importance to zooplankton relative to epilimnetic autochthonous and allochthonous resources.

In lake ecosystems, consumers have varying quantities and qualities of autochthonous (aquatically derived) resources and allochthonous (terrestrially derived) subsidies available for consumption (Marcarelli et al. 2011; Wilkinson et al. 2013*a*). In the pelagic zone of lakes, autochthonous resources can be further divided based on strong vertical gradients that determine the distribution of aquatic resources and consumers. These gradients are both abiotic (light, temperature, dissolved oxygen) and biotic (resource quantity and quality, predation threat). Metalimnetic primary production (production in the thermocline in stratified lakes) has been suggested to be a significant pelagic resource exploited by aquatic consumers, which augments resources from epilimnetic production (Matthews and Mazumder 2006; Francis et al. 2011; Batt et al. 2012). Though light limited, phytoplankton growing in the metalimnion have access to the large pool of recycled nutrients that are unavailable to epilimnetic phytoplankton (Sawatzky et al. 2006), generally increasing their resource quality (Elser and George 1993). Metalimnetic resources are available to consumers residing in that thermal layer as well as to zooplankton that migrate vertically and inhabit the metalimnion only part of the time (Gliwicz 1986; Lampert and Grey 2003).

Patterns of diel vertical migration in zooplankton are the result of predator avoidance, competition for resources, and thermal requirements for growth and reproduction (Lampert 1989). In lakes with primary production in the metalimnion, zooplankton that migrate deep in the water column during the day can potentially exploit resources that can be rich in phosphorus (Sterner and Schwalbach 2001), thereby aiding growth (DeMott et al. 2004) while avoiding visual predators such as fish (Beklioglu et al. 2008). However, the cold water within the thermocline can significantly slow zooplankton growth and reproduction rates (Loose and Dawidowicz 1994). It is also unclear whether the resources available in the metalimnion are ideal for zooplankton growth and reproduction, especially at metalimnetic temperatures (Williamson et al. 1996; Cole et al. 2002; Winder et al. 2004). The trade-off between predator avoidance and food quality and quantity among systems leads to varying strengths of diel vertical migration and metalimnetic resource exploitation by consumers (Loose and Dawidowicz 1994; Brosseau et al. 2012). Whereas there have been numerous mesocosm studies (Williamson et al. 1996; Kessler and Lampert 2004) investigating how migration trade-offs affect metalimnetic resource consumption by zooplankton, there have been few studies that have explicitly quantified metalimnetic, epilimnetic, and terrestrial resource exploitation by migrating pelagic consumers in natural lakes (Armengol et al. 2012).

Stable isotopes are useful for quantifying consumer resources. Stable hydrogen isotopes (^{2}H and ^{1}H) have recently been used to trace aquatic consumer resource use (Doucett et al. 2007; Finlay et al. 2010; Karlsson et al. 2012) because the separation in isotopic composition between terrestrial and aquatic resources is larger for hydrogen than for those of carbon. Although the separation

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between pelagic resources (epilimnetic and metalimnetic phytoplankton) is also greater for hydrogen isotopes than carbon isotopes, there is still overlap in many lakes, making it difficult to estimate metalimnetic resource use (Wilkinson et al. 2013a). Based on ambient carbon and nitrogen stable isotope techniques, metalimnetic resources are important to migrating consumers in some lakes (Matthews and Mazumder 2006; Francis et al. 2011; Batt et al. 2012). However, other investigators have not been able to separate epilimnetic and metalimnetic resources in their lakes using ambient stable isotopes (Cole et al. 2011; Wilkinson et al. 2013a).

One way to parse out consumer epilimnetic, metalimnetic, and terrestrial resource use is to artificially increase the isotopic value of one of the aquatic end members (Carpenter et al. 2005; Taipale et al. 2008; Armengol et al. 2012) in combination with ambient hydrogen stable isotope sampling to determine terrestrial contribution. During the summer stratification period, the metalimnia of strongly stratified lakes are cut off from exchange with the atmosphere, and there is little movement of solutes across the thermocline (Hutchinson 1957; Cole and Pace 1998; Houser 2001). Conceptually, adding inorganic ^{13}C to a discrete layer below the thermocline where metalimnetic phytoplankton are abundant would create an enriched inorganic carbon pool available for fixation by metalimnetic primary producers but unavailable to epilimnetic primary producers. Phytoplankton growing in the metalimnion would become isotopically labeled by the addition, as would zooplankton that consume resources while in the metalimnion.

We performed an addition of ^{13}C-DIC to the entire metalimnion of Peter Lake during the summer of 2012 in order to enrich metalimnetic primary production isotopically. Previous studies demonstrated that terrestrial material is approximately 50% of the particulate organic matter pool (POM) in Peter Lake and that 35–75% of zooplankton biomass is derived from terrestrial resources (Carpenter et al. 2005; Cole et al. 2006; Solomon et al. 2011). However, the ambient epilimnetic and metalimnetic isotopic end members are not well separated in this system (Wilkinson et al. 2013a). We combined ambient hydrogen isotopes with the ^{13}C addition to investigate metalimnetic, epilimnetic, and terrestrial resource use by zooplankton. Peter Lake has been used in a number of food-web manipulations, which have resulted in different zooplankton communities over time. Most recently, a switch to piscivorous fish caused a shift from small-bodied zooplankton, generally poor migrators, to large-bodied cladocerans, which are often strong migrators (Carpenter et al. 2011; Farrell and Hodgson 2012; Pace et al. 2013). We hypothesized that, in its present food-web configuration, there would be extensive use of metalimnetic resources by migrating zooplankton.

Methods

Study site—Peter Lake (89°50′W, 46°25′N) is a small, circular lake, with circumneutral pH, located at the University of Notre Dame Environmental Research Center. It has been described extensively elsewhere (Carpenter and Kitchell 1993). An oxygen maximum was centered vertically at 5 m in Peter Lake during the summer stratification period in 2012, but extended from 4 to 6 m. The depth of 5% light ($\pm$ standard deviation [SD]) was 4.4 ± 0.4 m during 2012, and the depth of 1% light was 6.4 ± 0.5 m. The current zooplankton community is dominated by cyclopoid copepods, *Daphnia* spp., and *Chaoborus* spp. (Carpenter et al. 2011; Pace et al. 2013).

Metalimnion isotope addition—After the thermal profile of the lake was vertically stable during early summer and the thermocline reached an average depth of 4 m, we initiated the ^{13}C addition. The label was added starting on day of year (DOY) 164 in the form of 500 g of 99% pure $NaH^{13}CO_3$ (Isotech) dissolved in 50 liters of filtered lake water. Vacuum-sealed isotope labeling devices were fabricated for the purpose of holding the isotope label at depth for thermal equilibration and then allowing the solution to be gently released into the metalimnetic layer for horizontal mixing (Fig. 1A). These were 2.5 liter polyvinyl chloride (PVC) tubes with gasket caps. Twenty of these devices were filled with solution, vacuum sealed, and deployed to a depth of 5 m. The devices were attached to two transect lines at the surface of the lake to create a broad area of isotope addition (Fig. 1B). The devices were left unopened for 24 h to allow the temperature of the water inside to equilibrate with metalimnion water in order to limit thermal mixing upon release of the isotope solution. The vacuum seal was released on DOY 165 at the surface, opening the labeling devices, which were hanging at 5 m. The isotope labeling devices were removed from the lake on DOY 170. The surface transect lines remained in the lake for the duration of the experiment.

Isotope sampling—Sampling for dissolved inorganic carbon (DIC), POM, and dissolved organic matter (DOM) occurred over the deepest point in the lake (where the two transect lines intersect; Fig. 1B) at frequent intervals both before and after the addition. DIC, POM, and DOM profiles were taken weekly ($n = 11$) from 1 m to 6 m at 1 m intervals using a Van Dorn sampler. For all other sampling events ($n = 11$), depths of 1–3 m and 4–6 m were pooled as epilimnion and metalimnion samples for DIC, POM, and DOM. Depths of 4–6 m were selected because they were directly below the thermocline (considered the top of the metalimnion), within the peak of the oxygen maximum, and directly above the depth of 1% light (considered the bottom of the photic zone). Both POM and DOM samples were analyzed for carbon and hydrogen isotopes. Filtered ^{13}C-DIC samples were analyzed for only carbon isotopes, and filtered water samples from the pooled sampling events were analyzed for hydrogen isotopes.

Spatial profile sampling for ^{13}C-DIC also occurred on two occasions in the four quadrants of the pelagic zone. Profiles extended to a depth of 8 m. The sampling locations for the spatial sampling were equidistant from the two transect lines forming two sides of each quadrant and 50 m from where the transect lines crossed (Fig. 1B). These
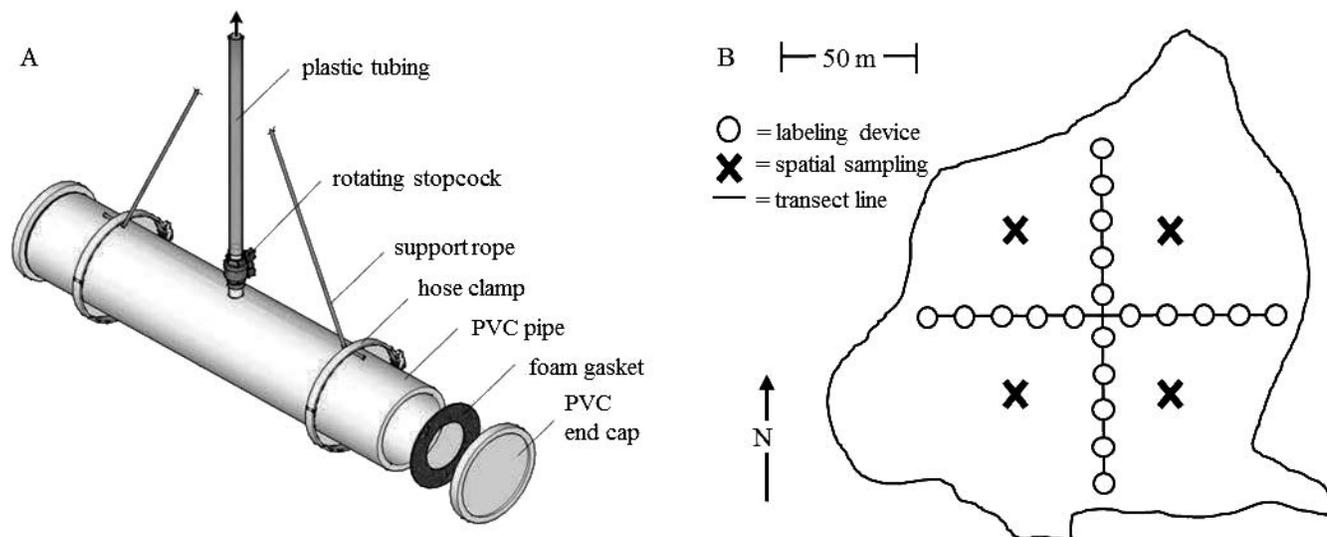
Fig. 1. The isotope addition device and the deployment arrangement in the lake. (A) The isotope labeling device that was used to deploy label in the metalimnion. (B) The devices were deployed attached to buoys on two transect lines in the pelagic zone of Peter Lake (the outline of the surface of the lake is shown).

locations were chosen because they were the farthest point from where the isotope label had been added.

Water samples for ^{13}C-DIC were stored in amber borosilicate vials with no air, capped with rubber butyl septa, preserved with sodium azide, and stored at 4°C until analysis. Water samples for δ^2H_2O analysis were taken biweekly, filtered using Whatman 25 mm GF/F filters, and stored without air at 4°C. Samples for POM were filtered onto 47 mm MicronSep Cellulosic filters (nominal pore size = 0.8 μm), back-rinsed, and dried at 60°C. A liter of filtrate was acidified with 1 mL of a 1 mol L^{-1} HCl solution and was evaporated at 60°C for DOM. Both POM and DOM samples were ground to a fine powder and were stored in borosilicate vials until analysis.

Zooplankton were sampled in both the epilimnion (0–3 m) and the metalimnion (5–6 m) during the day. At night, zooplankton were sampled in the epilimnion. Epilimnetic zooplankton were sampled via oblique tows in each of the four quadrants, using a 153 μm mesh conical net. Samples from the quadrants were combined into one sample, from which individual taxa were separated. The taxa separated in the epilimnion samples were *Daphnia pulex* (the dominant cladoceran in the system), *Chaoborus* (only at night, biomass dominated by *C. trivittatus*), *Mesocyclops edax*, and combined cyclopoids and calanoids (hereafter small copepods). Zooplankton from the metalimnion were sampled using an open diaphragm bilge pump, with the ouput filtered using a 153 μm mesh net held in a 75 gallon holding tank (Pace 1986). A second bilge pump was used to pump the ^{13}C-labeled water in the holding tank to a depth of 10 m in order not to contaminate the epilimnetic DIC pool. *Daphnia* and *Chaoborus* were separated from the bulk metalimnetic zooplankton sample for isotope analysis. Zooplankton samples were separated by taxa under a dissecting microscope, using fine point tweezers. All zooplankton samples were dried at 60°C and were ground to a fine powder.

Assessment of diel vertical migration—Zooplankton samples were taken both day and night from each meter of the lake (0–10 m) using a 12 liter Schindler-Patalas trap. The oxycline in Peter Lake was at 7 m. Day and night sampling events occurred six times over the course of the isotope labeling experiment. On average, samples were taken 14 d apart. Zooplankton were identified to order (small copepods) or genus (*Chaoborus*, *Daphnia*, and *Mesocyclops*) and were counted using a dissecting microscope. Zooplankton abundance was converted to percent abundance because the total number of individuals sampled was much higher at night than during the day, likely due to some diel horizontal migration of taxa such as *Daphnia* (L. M. Anderson pers. comm.). The percent abundance at each depth was averaged over all six sampling events.

Limnological sampling—A number of water column characteristics were monitored weekly during the experiment. These included chlorophyll *a* (Chl *a*) concentration, water color (absorbance at 440 nm), dissolved organic carbon (DOC) concentration, and pH, using standard methods as described in Wilkinson et al. (2013*b*).

Weekly estimates of epilimnetic and metalimnetic metabolism were made by performing in situ light and dark bottle incubations and measuring the change in O_2 concentration. The bottle incubation method was used to isolate pelagic metabolism processes without the influence of benthic metabolism, which significantly influences diel oxygen fluxes for Peter Lake (Van de Bogert et al. 2012). Glass bottles with ground glass stoppers were filled and capped with no headspace. Sets of light and dark bottles ($n = 5$) were hung in both the epilimnion, at a depth of 1 m, and in the metalimnion, at a depth of 5 m. The bottles were incubated in situ for 10–12 h during daylight. Initial O_2 concentration samples were taken by filling bottles with water from both depths ($n = 5$) and immediately

transferring subsamples to 12 mL borosilicate vials that were gas tight with no headspace and were preserved with 0.2 mL of a saturated solution of $HgCl_2$. Upon the termination of the incubation, samples were preserved as described above and were stored at $4°C$ until analysis.

The samples were analyzed using a membrane inlet mass spectrometer, following the procedure of Kana et al. (1994). The change in O_2 concentration was calculated using the ratio of O_2: Ar, which is precise (coefficient of variation for sample replicates ranged from 0.001 to 0.43% across all weeks). The estimates of net ecosystem production (NEP) from the weekly light bottle incubations (calculated as the difference between the final and initial oxygen concentration) were used to calculate mean NEP (μmol L^{-1} d^{-1}) for the season at each depth as well as volumetrically weighted estimates of NEP (mol d^{-1}) calculated using the volume of each water layer. Additionally, gross primary production (GPP) was calculated as NEP + respiration (calculated as the difference between the initial and final oxygen in the dark bottle) and weighted by depth.

Isotope analysis—All isotope analyses were performed at the Colorado Plateau Stable Isotope Laboratory (CPSIL). Solid ^{13}C and ^{13}C-DIC samples were analyzed following the standard procedures of CPSIL. For obtaining the isotopic value of the non-exchangeable H fraction in solid material, methods for analysis followed those of Doucett et al. (2007). CPSIL uses a benchtop equilibration procedure to correct for the exchange of H atoms between a set of standards, including ground algal material and ambient water vapor. The analytical precision for dried organic matter replicate samples at CPSIL is 2‰ for δ^2H and 0.1‰ for $\delta^{13}C$ (M. Caron pers. comm.). Full details of the δ^2H methods are published in Hondula et al. (2014).

Bayesian mixing model—A three-source, two-isotope Bayesian mixing model was used to determine the proportion of each resource contribution to consumer biomass (available from the author). The mixing model is based on the published model by Solomon et al. (2011), as adapted by Batt et al. (2012) and Wilkinson et al. (2013a). Uncertainties are accounted for in several parts of the mixing model: uncertainty in the isotopic values of the source end members (epilimnetic algae, metalimnetic algae, and terrestrial material), uncertainty in the isotopic values of consumers, and uncertainty in the value of dietary water contribution to consumer tissue. The model estimates the probability distribution of the fractional contribution of each end member (ϕ_E for epilimnetic algae, ϕ_M for metalimnetic algae, and ϕ_T for terrestrial material) to the consumer isotopes over the 22 sampling dates of the experiment.

The terrestrial hydrogen and carbon end member was estimated from live leaf samples taken from vegetation in the Peter Lake watershed (δ^2H, $n = 67$; $\delta^{13}C$, $n = 12$). The mean and SD of terrestrial δ^2H and $\delta^{13}C$ were -132.2 ± 15.4‰ and -29.0 ± 1.3‰, respectively (Solomon et al. 2011). The epilimnetic and metalimnetic algal hydrogen and carbon end member was estimated for each sampling

date using the measured isotope value of the inorganic pool (δ^2H_2O and $\delta^{13}CO_2$) and adding the discrimination values ε_H (-160.9 ± 17.0‰) and ε_C (-14.4 ± 2.6‰), respectively. $\delta^{13}CO_2$ was calculated from $\delta^{13}C$-DIC using pH and water temperature (Zhang et al. 1995). The mean and SD of ε_C and ε_H are based on field measurements from 10 lakes in the region in which it was possible to obtain particulate samples almost completely composed of phytoplankton (Yang et al. in press). Additional values of ε_H calculated from algal regrowth experiments of algae from lakes including and near Peter Lake (Solomon et al. 2011) were added to the pool of ε_H values for $n = 14$ estimates. Consumer (Cons) isotopic variance was estimated from replicate samples from previous years (Carpenter et al. 2005). Consumer δ^2H was corrected for dietary water (ω) based on trophic position (ω_{tot}, Solomon et al. 2009) with assigned trophic positions of 1 for *Daphnia*, 2 for *Chaoborus*, and 1.5 for small copepods and *Mesocyclops*. The value of ω used in the analysis was 0.13 ± 0.03 from Wilkinson et al. (2013a).

The mixing model for hydrogen and carbon for a given consumer on each sampling date (t) is

$$\delta^2H_{Cons(t)} = (1 - \omega_{tot})$$
$$\times \left[(\phi_E \times \delta^2H_{E(t)}) + (\phi_M \times \delta^2H_{M(t)}) + (\phi_T \times \delta^2H_T) \right]$$
$$+ (\omega_{tot} \times \delta^2H_2O_{(t)})$$
$$\delta^{13}C_{Cons(t)} = (\phi_E \times \delta^{13}C_{E(t)}) + (\phi_M \times \delta^{13}C_{M(t)}) + (\phi_T \times \delta^{13}C_T)$$
$$1 = \phi_E + \phi_M + \phi_T$$

where ϕ_E, ϕ_M, and ϕ_T are the fractional proportion of consumer biomass from epilimnetic phytoplankton, metalimnetic phytoplankton, and terrestrial material, respectively. On each sampling date, we measured distributions of δ^2H and $\delta^{13}C$ of consumers and epilimnion and metalimnion end members, and δ^2H_2O of lake water, as designated by the time (t) subscripts of these values in the mixing equations. We assumed that terrestrial end member isotope distributions and the distributions of the dietary water fraction (ω) were constant over the duration of the experiment. The distributions of ϕ_E, ϕ_M, and ϕ_T from each sampling day were pooled to obtain distributions of ϕ_E, ϕ_M, and ϕ_T for the entire experiment. All calculations were done using JAGS (Just Another Gibbs Sampler implementing Monte Carlo Markov Chain sampling; MCMC JAGS Project 2012) and R (R Development Core Team 2012).

Results

The sharp thermocline in Peter Lake creates very distinct epilimnetic and metalimnetic zones with some differences in variables such as Chl *a*, DOC, and DIC concentrations (Table 1). The water column was strongly stratified before the isotope addition and remained stratified for the duration of the sampling period (Fig. 2). The label, added at 5 m, remained in the metalimnion and did not mix above 4 m or below 7 m. The DIC pool in the metalimnion

Table 1. Mean values and standard deviations (SDs) of water column characteristics in the epilimnion and metalimnion of Peter Lake during the time of the ^{13}C addition (DOY 156–227).

Variable	Mean	SD
Epilimnion (0–3 m)		
Chl *a* (μg L^{-1})	3.13	0.69
Color (m^{-1})	1.15	0.004
DIC (mg C L^{-1})	1.46	0.1
DOC (mg C L^{-1})	5.36	0.15
pH	6.75	0.08
Secchi depth (m)	4.84	0.93
Metalimnion (4–6 m)		
Chl *a* (μg L^{-1})	5.76	2.62
Color (m^{-1})	1.38	0.01
DIC (mg C L^{-1})	1.87	0.39
DOC (mg C L^{-1})	5.11	0.29

remained enriched in ^{13}C after the isotope addition (Fig. 3A). Spatial sampling in each of the quadrants indicated that the isotope label had spread throughout the metalimnion. The mean of the samples at 5 m among quadrants 16 d after the addition was 63.84 ± 4.9‰, compared to 62.36‰ at 5 m in the center of the lake. The peak $\delta^{13}C$-DIC value (73.2‰) in the metalimnion occurred at a depth of 5 m on DOY 174, 9 d after the isotope addition.

Similarly, $\delta^{13}C$-POC increased in the metalimnion after the addition (Fig. 3B). However, the POC pool did not become as enriched in ^{13}C as the DIC pool. The metalimnion DOC pool was also enriched in ^{13}C after the addition (Fig. 3C), although the enrichment was minimal compared to the POC and DIC pools.

Metabolism estimates—The epilimnion was net autotrophic (NEP was positive) 6 out of the 11 weeks of in situ light and dark bottle incubations (Fig. 4). The metalimnion was net autotrophic 2 out of 10 weeks. NEP on DOY 168,
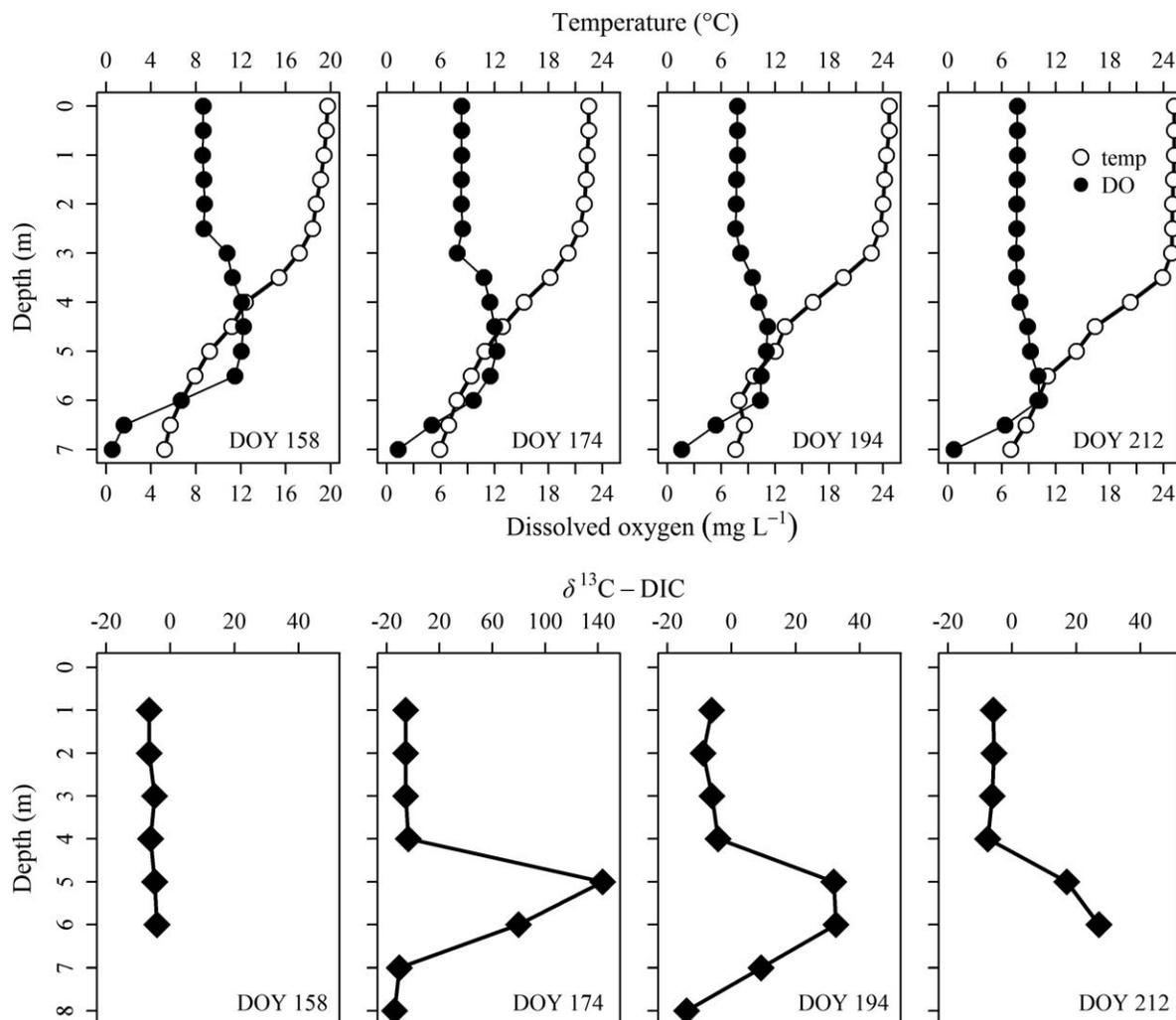

Fig. 2. Temperature (temp), dissolved oxygen (DO), and $\delta^{13}C$-DIC profiles from four sampling dates during the course of the experiment. The profiles taken on DOY 158 were before the isotope addition. On two of the sampling dates, $\delta^{13}C$-DIC profiles were extended to 8 m.
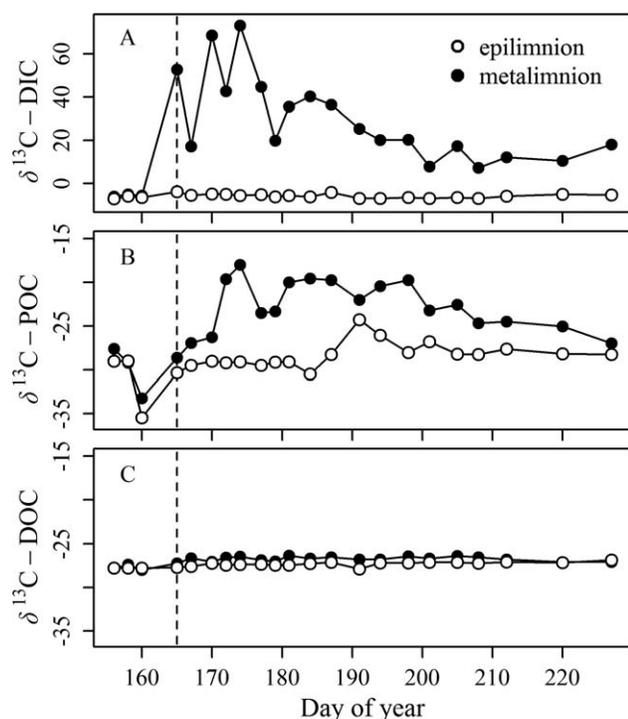
Fig. 3. $\delta^{13}C$ time series. The label was added on DOY 165, indicated by the vertical dashed line. (A) DIC; (B) POM; (C) DOM.

3 d after the isotope addition, was positive in both layers. The volume-weighted epilimnetic ($\pm$ SD) NEP for the lake over the season was 12.6 $\pm$ 49.8 mol O_2 d^{-1}. The volume-weighted metalimnetic mean ($\pm$ SD) NEP was $-26.6 \pm$ 30.0 mol O_2 d^{-1}. Mean ($\pm$ SD) GPP in the epilimnion was 171.6 $\pm$ 32 mol O_2 d^{-1} and was 44.7 $\pm$ 22.5 mol O_2 d^{-1} in the metalimnion.

Zooplankton vertical migration—Larger-bodied zooplankton taxa migrated vertically in 2012 in Peter Lake, whereas the smaller-bodied forms did not. *Daphnia* underwent diel vertical migration throughout the course of the experiment, with daytime peak abundance at 5 m and nighttime peak at 1 m (Fig. 5A). *Chaoborus* also migrated vertically and had the largest difference in peak daytime and nighttime depths. The daytime peak abundance for *Chaoborus* was at 10 m, and the nighttime peak abundance was at 4 m (Fig. 5B). Small copepods did not migrate between the metalimnion and epilimnion because the daytime peak abundance was at 2 m and the nighttime peak abundance was 1 m (Fig. 5C). The large copepod, *Mesocyclops*, migrated vertically, with a daytime peak abundance at 7 m and a nighttime peak at 1 m (Fig. 5D).

Zooplankton ^{13}C and model results—*Daphnia* ^{13}C ($\delta^{13}C_{Daphnia}$) in the epilimnion during the day, during the night, and in the metalimnion increased after the isotope addition (Fig. 5E). Similarly, $\delta^{13}C_{Chaoborus}$ in the metalimnion during the day increased after the addition, whereas $\delta^{13}C_{Chaoborus}$ in the epilimnion at night remained largely unchanged (Fig. 5F). In contrast to *Daphnia* and *Chaoborus*,
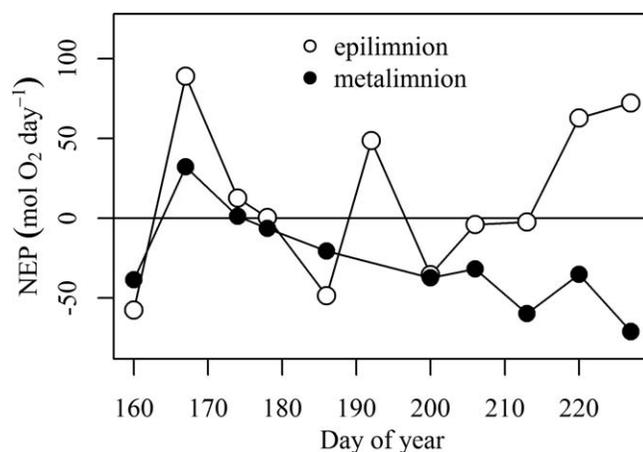

Fig. 4. Net ecosystem production for the entire metalimnion and epilimnion, weighted by the volume at each depth. Standard errors of the mean (or standard deviations or 95% confidence intervals) are smaller than the size of the data points.

$\delta^{13}C_{Copepod}$ values in the epilimnion during the day and at night remained largely indistinguishable from each other after the enrichment (Fig. 5G). $\delta^{13}C_{Mesocyclops}$ in the epilimnetic daytime samples increased after the isotope addition (Fig. 5H). For every sampling date, consumer isotope values fell within the bounds of the end members determined for that date. For the most part, consumer isotope values were also within the bounds of the end members averaged over time (Fig. 5I–L), and all values are in bounds if end member uncertainty is considered.

Terrestrial material (ϕ_T) provided the greatest contribution to consumer biomass for all taxa, ranging from 0.56 to 0.73 terrestrial among all consumers. Metalimnetic phytoplankton (ϕ_M) was not the largest contributing source fraction for any consumer, except *Chaoborus*, in the metalimnion at any depth or time of day during the course of the experiment. *Daphnia* in the metalimnion during the day had the highest median ϕ_M of 0.21 (Fig. 6A). *Daphnia* in the epilimnion at night had a ϕ_M of 0.08, similar to the ϕ_M of 0.06 for *Daphnia* in the epilimnion during the day. For *Daphnia* sampled in the epilimnion both day and night, ϕ_E was greater than ϕ_M. *Chaoborus* in the metalimnion during the day had the second highest ϕ_M of 0.18 (Fig. 6B). *Chaoborus* in the epilimnion were not supported at all by metalimnetic phytoplankton (ϕ_M = 0.0). The small copepod median ϕ_M of 0.08 was the same for epilimnion samples both day and night (Fig. 6C). *Mesocyclops* median ϕ_M was similar to *Daphnia* and small copepod ϕ_M values, 0.08 and 0.06 for the epilimnion day- and nighttime, respectively (Fig. 6D).

Discussion

The ^{13}C isotope label was successfully added to the metalimnetic layer in Peter Lake, effectively elevating metalimnetic relative to epilimnetic $\delta^{13}C$-DIC. Profile sampling after the isotope addition revealed that the label remained at the intended depth with minimal vertical mixing, yet nearly uniform horizontal dispersion. Even
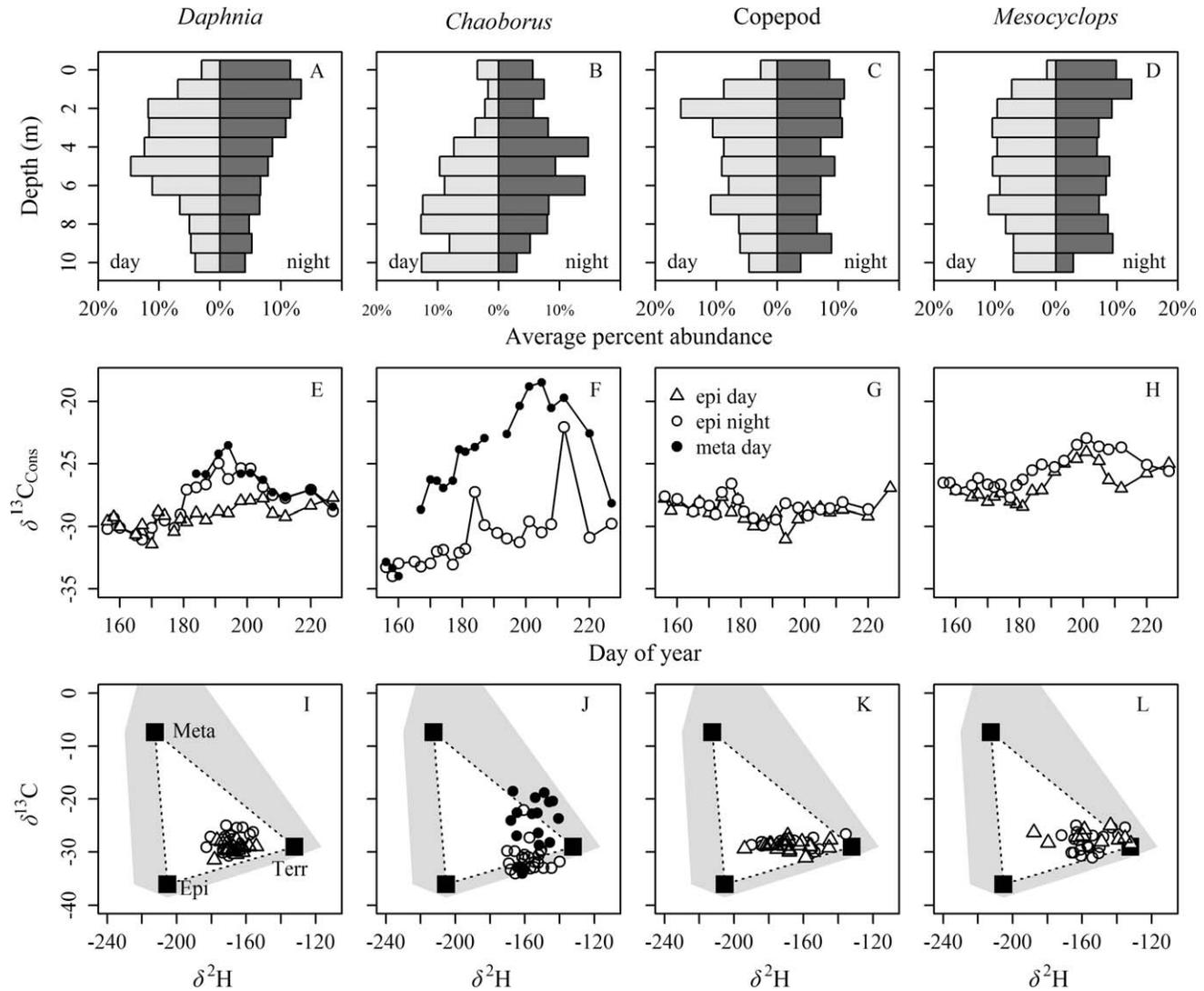
Fig. 5.   Consumer isotope values and vertical distributions throughout the course of the experiment. Consumers are organized by columns: (A, E, I) *Daphnia*; (B, F, J) *Chaoborus*; (C, G, K) small copepod; and (D, H, L) *Mesocyclops*. (A–D) The mean percent abundance of zooplankton over depth both day and night during the course of the experiment. (E–H) $\delta^{13}C$ isotope time series for all consumers (cons). (I–L) Consumer isotope values, corrected for dietary water, used in the Bayesian mixing model. The filled squares are the means for the end members (terr = terrestrial organic matter) from the 22 sampling dates for reference. The gray region represents the uncertainty (SD) around the end members for the 22 sampling dates combined. Note that the SD of the metalimnetic $\delta^{13}C$ end member is large because that end member was manipulated, and the SD shown is that of the entire sampling period. All consumers fall within the bounds of the end members (epi = epilimnion phytoplankton, meta = metalimnion phytoplankton) if uncertainty in both the end member and consumer values is considered.

though there was a decline in the degree of labeling over the course of the addition (due to phytoplankton uptake and some loss to deeper depths), the difference in epilimnetic and metalimnetic $\delta^{13}C$-DIC was never less than 30‰, substantially above nominal values.

Although the isotope label persisted in the metalimnion over the course of the experiment, very little of the isotope label was incorporated into primary producer or consumer biomass. If the POC pool in the metalimnion was composed entirely of phytoplankton produced in that layer, POC enrichment would be expected to mirror DIC enrichment with a small reduction (10–30‰) due to phytoplankton isotope discrimination (Pace et al. 2004;

Carpenter et al. 2005; Taipale et al. 2008). However, metalimnetic $\delta^{13}C$-POC was much lower than metalimnetic $\delta^{13}C$-DIC (mean difference was 53‰). The low GPP in the metalimnion of Peter Lake compared to the epilimnion partially accounts for the lack of enrichment of the metalimnetic POC. Additionally, the dilution of labeled metalimnetic phytoplankton by terrestrial material (Wilkinson et al. 2013b) and by sinking, unlabeled, epilimnetic phytoplankton could account for the low level of enrichment in metalimnetic $\delta^{13}C$-POC. A minor enrichment of metalimnetic DOC was also observed. DOC in Peter Lake is dominated by terrestrial material (Bade et al. 2007; Wilkinson et al. 2013b). However, extracellular release of
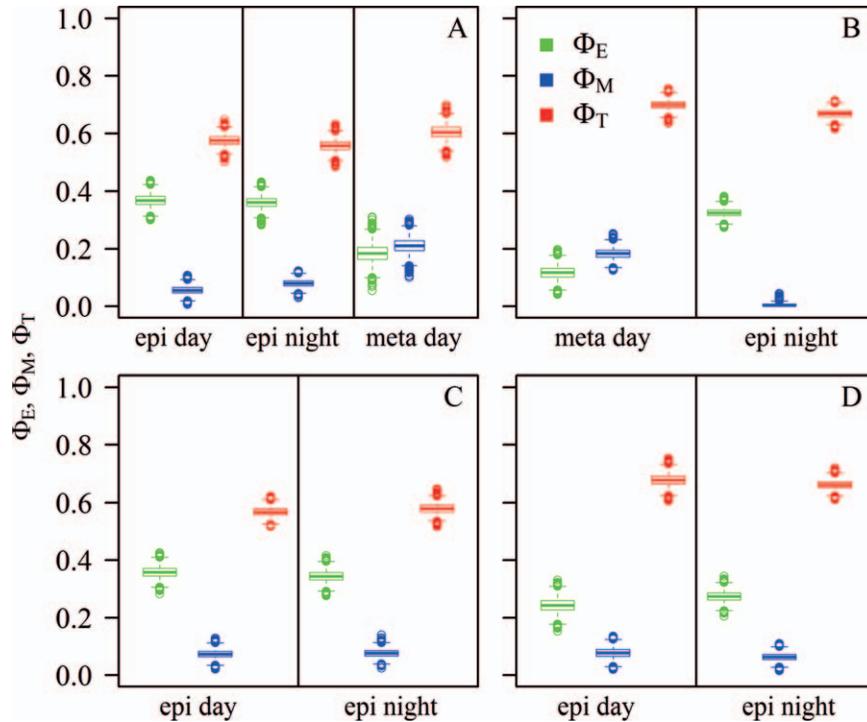
Fig. 6. The posterior distributions of each resource contribution to consumer biomass. Box plots grouped by habitat, sampling time, and consumer: (A) *Daphnia*, (B) *Chaoborus*, (C) small copepods, (D) *Mesocyclops*.

labeled organic carbon by algae (Baines and Pace 1991) could account for the observed labeling of DOC in the metalimnion.

Metalimnetic support of consumers—Metalimnetic support (ϕ_M) of consumers sampled in the epilimnion either during the day or night did not exceed 8% in Peter Lake. Consumers that resided in the metalimnion during the day (*Daphnia* and *Chaoborus*) were more reliant on metalimnetic resources, but metalimnetic contributions to these consumers' biomass did not exceed 21%. Because primary production was almost four times higher in the epilimnion than the metalimnion, the low metalimnetic support of consumers is not surprising. Using an ambient isotope approach, Batt et al. (2012) found a similar degree of metalimnetic support of the calanoid copepod *Skistodiaptomus oregonensis* and *Chaoborus* (26–28%) in a nearby, naturally productive lake. Our findings are also consistent with the results of another metalimnetic isotope addition. Armengol et al. (2012) added an inorganic ^{15}N label to the metalimnion of a small karst lake and observed minimal consumer labeling, even though the metalimnetic seston was highly enriched with [15]N. Both of these whole-lake labeling estimates of the use of metalimnetic phytoplankton are much lower than the subsurface resource use estimated by Matthews and Mazumder (2006) and Francis et al. (2011) for lakes in the Pacific Northwest. The lakes studied by the aforementioned authors are deeper, highly oligotrophic systems with high transparency, which may facilitate increased algal production and, hence, metalimnetic support of zooplankton.

Diel vertical migration patterns of consumers were consistent with metalimnetic resource use. The negligible use of metalimnetic phytoplankton by small copepods and *Mesocyclops* in the epilimnion both day and night is corroborated by lack of observed diel vertical migration by these consumers. *Daphnia* were the most abundant crustacean zooplankton in the metalimnion during the day, and a portion of the population migrated to the epilimnion at night. The evidence of diel vertical migration in *Daphnia* supports the estimates for *Daphnia* ϕ_M among habitats and time. *Daphnia* in the metalimnion during the day had the largest degree of metalimnetic support (ϕ_M = 21%). The lowest ϕ_M of 6% for *Daphnia* was the population sampled in the epilimnion during the day. *Daphnia* sampled in the epilimnion at night had an intermediate ϕ_M of 8% because the population in the epilimnion at night was a mixture of labeled, vertically migrating individuals and nonmigrating, unlabeled individuals.

Chaoborus residing in the metalimnion during the day had the second highest metalimnetic support; however, the peak abundance of *Chaoborus* was not in the metalimnion during the day. The δ^{15}N data (not shown) indicate that *Chaoborus* in the metalimnion during the day are feeding at a lower trophic level than *Chaoborus* in the epilimnion at night (mean difference = 3.1‰). *Chaoborus* sampled in the epilimnion at night were entirely unsupported by metalimnetic phytoplankton (ϕ_M = 0%). Given the low ϕ_M of the other zooplankton consumers that serve as prey for *Chaoborus* in the epilimnion at night, it is feasible that *Chaoborus* moving through the metalimnion during vertical

migration fed in the epilimnion and were not affected by the ^{13}C enrichment, whereas the population sampled in the metalimnion did not migrate. It is also possible that *Chaoborus* are consuming resources even deeper in the water column, such as bacteria and detritus that were not isotopically labeled or that were otherwise unaccounted for in the analysis. Resources such as methanotrophic bacteria are generally more depleted in ^{2}H and ^{13}C than phytoplankton (Jones and Grey 2011); however, we did not observe any extremely depleted consumers based on their δ^2H or δ^{13}C values. Because the consumers all fell within the end member values (exclusive of any methanotrophic end members) on each sampling date, it is possible, but unlikely, that a significant resource was unaccounted for in the analysis.

The metalimnion is characterized as a zone of high vertical heterogeneity. Water samples from 4, 5, and 6 m depths were pooled to integrate across the metalimnion; however, the ^{13}C-DIC pool at depth 4 m remained largely unlabeled throughout the course of the experiment. By including an unlabeled depth in the pooled sample, the measured metalimnion δ^{13}C-DIC value used in the model was likely less labeled than a sample from just 5 m and 6 m pooled would have been. In turn, this would lead to a slight overestimation of metalimnetic resource use because the metalimnion end member value based on the pooled sample was closer to the consumer isotope value than it would have been if the 4 m water were omitted from the pooled sample. Conversely, if consumers were exclusively using metalimnetic primary production from 4 m, the estimate of metalimnion resource use generated from this experiment would be low. This is an unlikely scenario, however, especially considering that the oxygen maximum, which is often considered to be the product of deep primary production, occurred below 4 m and Chl *a* concentrations were highest at 6 m among the depths sampled.

In all cases, the metalimnetic resource use of consumers was either less than or equivalent to their epilimnetic resource use. This is consistent with the literature demonstrating that, although the metalimnion is rich in resources (Elser and George 1993) with potentially high quality (Sterner and Schwalbach 2001), migrating zooplankton are not predominately exploiting this resource (Cole et al. 2002). Mesocosm studies have revealed that the consumers migrate as a trade-off between predator avoidance and the warm waters of the epilimnion most suitable for growth and reproduction (Williamson et al. 1996). Even in the absence of fish predation, *Daphnia* graze less below the thermocline, even if there is a large disparity in food quantity between the epilimnion and metalimnion (Kessler and Lampert 2004).

Terrestrial support of consumers—Terrestrial organic matter comprised the largest proportion of consumer biomass, ranging from 56 to 73% among consumers. Previous studies of zooplankton resource use in Peter Lake have estimated terrestrial support of pelagic consumers in the range of 34–70% (Carpenter et al. 2005; Cole et al. 2006; Solomon et al. 2011). Concern has been raised in the literature (Brett et al. 2009; Francis et al. 2011) that

previous epilimnetic ^{13}C additions to Peter Lake and other similar lakes overestimated terrestrial resource use by pelagic consumers because migrating consumers were potentially utilizing unlabeled metalimnetic resources that were unaccounted for in the analysis. Estimates from studies using ambient hydrogen isotopes supported the original estimates based on the epilimnion ^{13}C enrichment experiments (Cole et al. 2011; Solomon et al. 2011; Wilkinson et al. 2013*a*), as do the results of this study. In the previous epilimnion ^{13}C additions, zooplankton were sampled in the epilimnion at night (Carpenter et al. 2005). The average of the median ϕ_M values for consumers sampled in the epilimnion at night in this study is 5.5%. Assuming a 5.5% reduction in terrestrial resource use to account for metalimnetic phytoplankton does not drastically change the estimate of terrestrial resource use by consumers in the previous ^{13}C addition studies (Carpenter et al. 2005), especially since the zooplankton body size was much smaller at the time.

Our results provide direct estimates of the relative proportions of resources supporting consumers. Phytoplankton produced in the metalimnion is a minor resource for zooplankton in lakes such as Peter Lake, except for consumers that spend a significant amount of time in the metalimnion of the lake. However, even for those consumers that do reside in the metalimnion, metalimnetic phytoplankton was not the most important resource, leading us to conclude that metalimnetic phytoplankton provide only a small fraction of the organic matter that supports the food web of lakes like Peter Lake.

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