

Distinguishing trophic variation from seasonal and size-based isotopic ($\delta^{15}\text{N}$) variation of zooplankton

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Abstract: We measured the $\delta^{15}\text{N}$ of particulate organic matter (POM), *Daphnia pulex* (D), *Holopedium gibberum* (H), *Leptodiptomus tyrelli* (LT), *Epischura nevadensis* (E), and *Chaoborus trivittatus* (C) over an annual cycle in Council Lake, a pristine, oligotrophic, fishless lake. Annual averages of the $\delta^{15}\text{N}$ of plankton (C, LT, E > D, H > POM) matched expected differences in trophic position, but seasonal patterns differed among species. During midsummer, the $\delta^{15}\text{N}$ of D, H, and LT increased above the $\delta^{15}\text{N}$ of E and C, despite little temporal or spatial variation in the $\delta^{15}\text{N}$ of POM (<41 μm). Larger size fractions of POM (41–200 μm) helped explain some of the temporal variability of zooplankton $\delta^{15}\text{N}$. Body size of zooplankton was correlated with $\delta^{15}\text{N}$ for D, C, and E, which could indicate size-based, trophic-level variation; selective feeding; or physiological differences in ^{15}N enrichment. We propose that detailed time series of $\delta^{15}\text{N}$ for multiple zooplankton species can help determine the seasonal variability of trophic structure in zooplankton assemblages.

Résumé : Nous avons mesuré les $\delta^{15}\text{N}$ de la matière organique particulaire (POM), de *Daphnia pulex* (D), d'*Holopedium gibberum* (H), de *Leptodiptomus tyrelli* (LT), d'*Epischura nevadensis* (E) et de *Chaoborus trivittatus* (C) durant un cycle annuel au lac Council, un lac oligotrophe non perturbé et sans poissons. Les valeurs moyennes annuelles de $\delta^{15}\text{N}$ du plancton (C, LT, E > D, H > POM) correspondent aux différences attendues, compte tenu des positions trophiques, mais les patrons saisonniers varient d'une espèce à l'autre. Durant la mi-été, les $\delta^{15}\text{N}$ de D, H et LT dépassent les $\delta^{15}\text{N}$ de E et C, malgré le peu de variation temporelle ou spatiale du $\delta^{15}\text{N}$ de POM (<41 μm). Les fractions de plus grande taille de POM (41–200 μm) aident à expliquer une partie de la variabilité temporelle du $\delta^{15}\text{N}$ du plancton. Il y a une corrélation entre la taille corporelle du zooplancton et le $\delta^{15}\text{N}$ chez D, C et E, ce qui pourrait indiquer une variation du niveau trophique, une alimentation sélective ou des différences physiologiques dans l'enrichissement en $\delta^{15}\text{N}$ en fonction de la taille. Nous croyons que des séries chronologiques détaillées de $\delta^{15}\text{N}$ pour plusieurs espèces du zooplancton pourraient aider à déterminer la variabilité saisonnière de la structure trophique des peuplements de zooplancton.

[Traduit par la Rédaction]

Introduction

The trophic position of freshwater zooplankton varies among taxa, seasons, and different sizes (or life stages) of the same species (Moore et al. 1994; Sanders et al. 1996; Brandl 2005). Such trophic variation within zooplankton assemblages may arise from spatial and seasonal variation in the importance of algal and microbial food chains in pelagic food webs (Porter 1996). Depending on the species, calanoid and cyclopoid copepods can forage on algae, protists, rotifers, and small cladocerans (Sanders et al. 1996; Brandl 2005). Compared with the more herbivorous cladocerans, calanoids feed more readily on larger prey from the microbial food chain (Porter 1996; Burns and Schallenberg 2001), which could lead to a higher trophic position (sensu Vander

Zanden and Rasmussen 1996) of copepods compared with cladocerans (Matthews and Mazumder 2003; Karlsson et al. 2004). Intraspecific, trophic-level variation is also common in many zooplankton species (Moore et al. 1994; Brandl 2005). During their ontogeny, many invertebrate predators, such as *Leptodora*, *Chaoborus*, *Bythotrephes*, and *Mysis*, increase the average size and trophic position of prey in their diet (Moore et al. 1994; Branstrator 1998; Abrusan 2003). Naupliar stages of calanoid and cyclopoid copepods are mainly herbivorous, but can mature into herbivores, detritivores, omnivores, or predators (Chow-Fraser and Wong 1986; Hansen and Santer 1995; Brandl 2005).

Stable isotope analysis (using $\delta^{15}\text{N}$) is an intriguing way to reveal the complexities of inter- and intra-specific variation in the trophic position of zooplankton (Kling et al.

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1992; Matthews and Mazumder 2003; Karlsson et al. 2004). In particular, measuring the seasonal and size-based variation of zooplankton $\delta^{15}\text{N}$ may help detect seasonal changes in the trophic structure of plankton communities. Several studies have documented large seasonal variation in the $\delta^{15}\text{N}$ of individual zooplankton taxa (Graham 1997; Leggett et al. 2000; Matthews and Mazumder 2005). The $\delta^{15}\text{N}$ of *Daphnia* can change by >10‰ over a summer (Matthews and Mazumder 2005), even though differences in $\delta^{15}\text{N}$ between a consumer and its resource (trophic enrichment) are typically <3‰ (McCutchan et al. 2003; Vanderklift and Ponsard 2003). Most of the within-taxon temporal variation in the $\delta^{15}\text{N}$ of zooplankton is unrelated to trophic-level variation and can be explained by the large, seasonal, isotopic variability of primary producers (Leggett et al. 2000; Lehmann et al. 2004). The difference in $\delta^{15}\text{N}$ between zooplankton taxa (Δ_{Z1-Z2}) more likely reflects trophic-level differences (Kling et al. 1992; Matthews and Mazumder 2003; Karlsson et al. 2004). However, there is considerable uncertainty about the $\delta^{15}\text{N}$ of primary consumers (Vuorio et al. 2006) and about species-specific differences in ^{15}N enrichment (McCutchan et al. 2003; Vanderklift and Ponsard 2003). Nonetheless, seasonal variation in Δ_{Z1-Z2} could help reveal the dynamics of trophic structure in zooplankton assemblages, but no studies have reported detailed enough time series to explicitly test this hypothesis (Karlsson et al. 2004; Matthews and Mazumder 2005; Perga and Gerdeaux 2005).

Intraspecific variation in $\delta^{15}\text{N}$ is often correlated with consumer size (Fry et al. 1999; Genner et al. 2003), but few studies have explored such size-based isotopic variation in plankton (but see Branstrator et al. 2000, 2003). Several zooplankton taxa might exhibit positive relationships between body size and trophic position, based on ontogenetic changes in their diet (Branstrator 1998; Brandl 2005). Therefore, testing for relationships between zooplankton body size and $\delta^{15}\text{N}$ might reveal size-based diet variation within species. For example, as *Chaoborus* increases its body size, it may switch from feeding on small cladocerans (low $\delta^{15}\text{N}$) to larger copepods (high $\delta^{15}\text{N}$). Ultimately, using $\delta^{15}\text{N}$ to detect size-based, trophic-level variation of consumers depends on the relationship among $\delta^{15}\text{N}$, size, and the trophic position of prey. This is an ongoing challenge because relationships between size and $\delta^{15}\text{N}$ can also result from shifts in habitat use (Genner et al. 2003), temporal variation of food sources (Matthews and Mazumder 2005), or size-based variation in a consumer's trophic enrichment of ^{15}N (Overman and Parrish 2001; Trueman et al. 2005).

In this paper, we examine whether the seasonal patterns of zooplankton $\delta^{15}\text{N}$ reflect our expectations about trophic-level variation of different instars, sizes, and taxa of zooplankton. We sampled a pristine, fishless, oligotrophic lake (Council Lake) and measured the $\delta^{15}\text{N}$ of several common zooplankton species that have well-known dietary preferences, including *Daphnia pulex*, *Holopedium gibberum*, *Leptodiatomus tyrelli*, *Epischura nevadensis*, and *Chaoborus trivittatus*. These species can all potentially feed on multiple trophic levels (i.e., can all be omnivorous), but they vary in their propensity and ability to do so. Over their lifetime, *D. pulex* and *H. gibberum* tend to be more herbivorous than *L. tyrelli*, *E. nevadensis*, or *C. trivittatus*; however, the actual diet of a

Table 1. Limnological characteristics of Council Lake for the period May to August 2002.

Parameter	Mean (SE)
Surface area (ha)	17
Max depth (m)	17
Secchi depth (m)	7.8
TP ($\mu\text{g}\cdot\text{L}^{-1}$)	5.7
TN ($\mu\text{g}\cdot\text{L}^{-1}$)	77.0
DOC ($\mu\text{g}\cdot\text{L}^{-1}$)	2200
Chl <i>a</i> (<41 μm) ($\mu\text{g}\cdot\text{L}^{-1}$)	
E	0.50 (0.27)
M	0.69 (0.30)
H	0.89 (0.41)
Chl <i>a</i> (>0.7 μm) ($\mu\text{g}\cdot\text{L}^{-1}$)	
E	0.54 (0.16)
M	0.85 (0.37)
H	1.78 (0.48)

Note: Total phosphorus (TP), total nitrogen (TN), and dissolved organic carbon (DOC) are seasonal averages. Chlorophyll *a* < 41 μm was measured in the epilimnion (E), metalimnion (M), and hypolimnion (H) at the same depths as particulate organic matter (POM) < 41 μm . The standard errors (SE) are based on the number of sampling dates for each average.

zooplankton species will depend on the composition of the available resources (Anderson 1967; Folt and Byron 1989; Fedorenko and Swift 1972). Given the zooplankton assemblage in Council Lake (only a few species each with well-studied feeding behaviors), we expected covariation in the $\delta^{15}\text{N}$ of zooplankton over the season (as seen in Perga and Gerdeaux 2005). In addition, we tested for relationships between zooplankton body size and $\delta^{15}\text{N}$. We expected a positive relationship between body size and $\delta^{15}\text{N}$ for *C. trivittatus*, *E. nevadensis*, and *L. tyrelli*, but did not expect the same relationship for either *D. pulex* or *H. gibberum*.

Materials and methods

We studied the zooplankton community structure in Council Lake, which is situated on Vancouver Island (48°31'N, 123°41'W), in British Columbia, Canada. Council Lake is a warm, monomictic, oligotrophic lake that rarely freezes over. Detailed limnological data collection in Council Lake began in 2001, and we have compiled data for the summer of 2002 (May to September) from an ongoing research program investigating the water quality of lakes and reservoirs in Victoria, British Columbia (Table 1). Details about the limnological sampling procedures and sample analysis for these data are available elsewhere (Davies et al. 2004).

We sampled zooplankton and particulate organic matter (POM) <41 μm in Council Lake 28 times between 14 February 2002 and 17 March 2003. During lake stratification (May to November), we collected samples every 2 weeks, except in July when we sampled every 3–4 days. We collected daytime samples of zooplankton from the deepest site in the lake using a 50 cm diameter Wisconsin net (64 μm mesh) by vertical tows through the entire water column and horizontal tows through the epilimnion. Samples were

Table 2. Isotopic summary of main food web compartments.

	Mean $\delta^{15}\text{N}$ (SE)	25th and 75th percentiles	<i>n</i>
<i>Chaoborus trivittatus</i>	5.1 (0.1)	4.5, 5.6	28
<i>Epischura nevadensis</i>	4.9 (0.2)	4.4, 5.2	10
<i>Leptodiptomus tyrelli</i>	5.2 (0.3)	4.7, 6.0	15
<i>Holopedium gibberum</i>	2.9 (0.4)	1.9, 3.2	18
<i>Daphnia pulex</i>	2.4 (0.3)	1.7, 2.9	28
<i>D. pulex</i> (>1.8 mm)	2.9 (0.3)	2.0, 3.8	22
<i>D. pulex</i> (<1.8 mm)	1.8 (0.3)	1.4, 2.4	25
POM (<41 μm)	1.2 (0.2)	0.2, 1.5	27
E	0.8 (0.2)	0.2, 1.5	17
M	0.7 (0.2)	-0.1, 1.1	17
H	0.8 (0.2)	0.4, 1.3	17

Note: Averages of the zooplankton are for all measurements made during the entire sampling period 14 February 2002 to 17 March 2003. Particulate organic matter (POM) measurements in the epilimnion (E), metalimnion (M), and hypolimnion (H) are from sampling dates during the stratified period (5 May to 20 October).

frozen within 4 h and later sorted into five taxonomic groups (Table 2): *D. pulex*, *H. gibberum*, *L. tyrelli*, *E. nevadensis*, and *C. trivittatus*. Zooplankton individuals were hand-picked with fine-tip tweezers (Excelta) to reduce contamination from other planktonic material.

Prior to isotopic analysis, we measured the body size of all zooplankton individuals and the head length of *C. trivittatus* using a dissecting microscope, a digital camera, and zooplankton counting software (Z-Count). We separated larval instars of *C. trivittatus* on the basis of head length (Fedorenko and Swift 1972). For each cladoceran sample, we selected individuals of varying sizes, but for copepods we tended to exclude the smaller size classes (annual range of average size: *L. tyrelli*, 0.88 to 1.64 mm, $n = 15$; *E. nevadensis*, 1.14–2.19 mm, $n = 10$). *Chaoborus trivittatus* instars II and III only appeared in our samples from the middle of June to the end of July, whereas instar IV larvae were present all year.

In the spring of 2002, we used a Niskin bottle to collect lake water from three depths (2, 8, 16 m). Starting in May 2002, and for the rest of the study, we sampled six depths (~2, 6, 8, 10, 12, 14 m), except in July when we sampled nine depths (1, 3, 5, 7, 8, 9, 12, 14, 16 m). We chose these depths so that during the stratified period we could sample two to three discrete depths in the epilimnion, metalimnion, and hypolimnion. For each sample of POM < 41 μm , we filtered at least 1 L of lake water through a 41 μm Nitex mesh and onto precombusted (550 °C for 1 h) 25 mm GFC filters (Whatman).

Starting in July, we observed a bloom of *Synura lapponica* (a 100–250 μm colonial algae) that was restricted to the hypolimnion (Goldstein et al. 2005). In response, for seven dates in July we filtered >4 L for POM 41–200 μm (POM_{41–200}) from three depths (3, 9, and 14 m) and picked out clumps of POM from our zooplankton net samples that had large aggregations of *S. lapponica* (POM_{SL}), but that also included microzooplankton. We dried the POM filters overnight at 60 °C and packaged them in tin cups for isotopic analysis. We also analyzed chlorophyll *a* for replicate

POM₄₁ filters by extracting them in 95% ethanol at 4 °C overnight and analyzing the extracts on a spectrophotometer (Ultraspec® 2000, Amersham) using a 10 cm quartz cell (as in Davies et al. 2004). All stable isotope samples were analyzed on a Finnegan Delta Plus Advantage stable isotope ratio mass spectrometer at the Water and Watershed Research Laboratory at the University of Victoria. We included a powdered *Daphnia* internal standard ($\delta^{15}\text{N} = 1.7\text{‰}$) and found that its standard error was <0.2‰ for each run and among all runs. We used the percent carbon and nitrogen of POM to calculate particulate organic carbon concentrations and molar C/N ratios.

We used SYSTAT v. 10.2 (SYSTAT Software Inc., Point Richmond, California) for all our statistical analysis. Residuals from all analyses of variance (ANOVAs) and linear regressions were normally distributed, and Levene's tests indicated homogeneity of variance among groups. We used Pearson's *r* to test for relationships between variables, and in one case we used a nonparametric Spearman's rank correlation.

Results

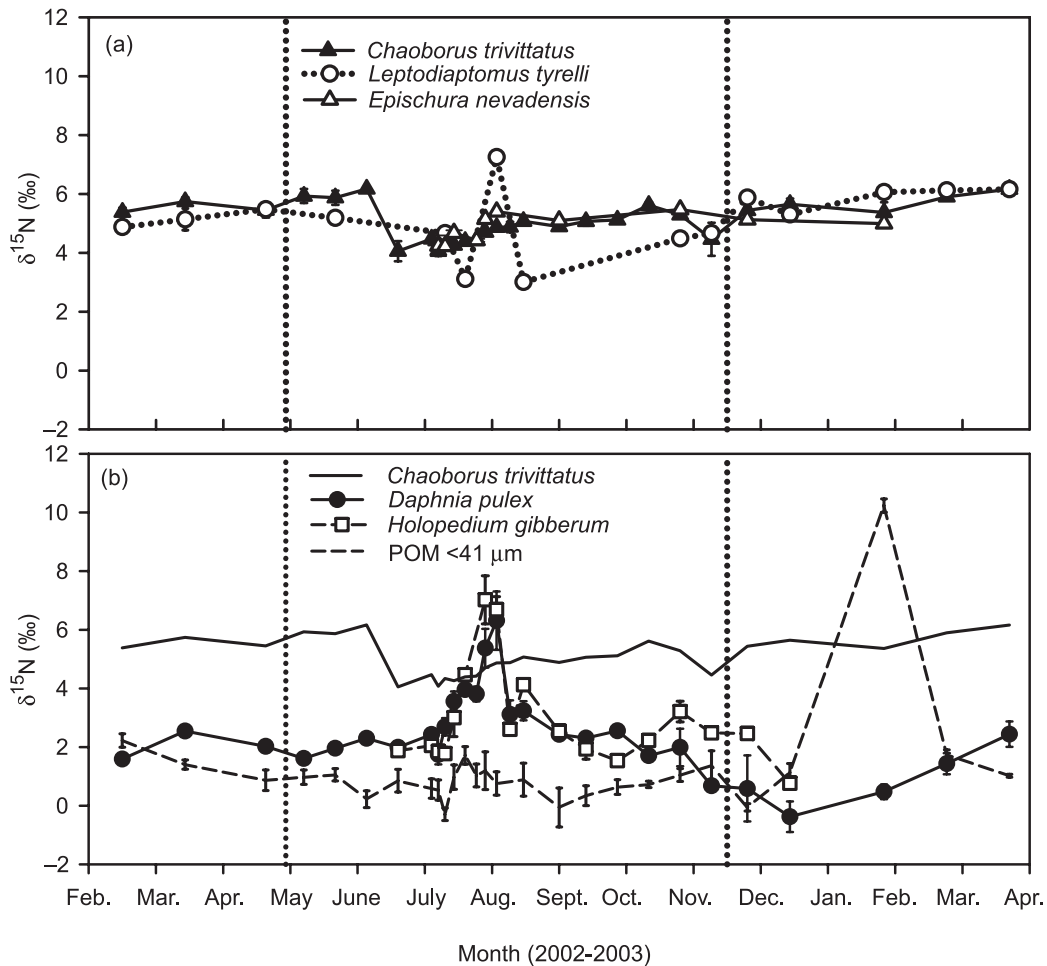
Variation in the $\delta^{15}\text{N}$ of zooplankton

Over the entire sampling season, the average $\delta^{15}\text{N}$ of *E. nevadensis* and *C. trivittatus* was about 2.4‰ higher than that of *D. pulex* and *H. gibberum* (Table 2). The seasonal average $\delta^{15}\text{N}$ of *L. tyrelli* was within 0.5‰ of *E. nevadensis* and *C. trivittatus*, but the difference depended on the time of year (Fig. 1). During July and August, the $\delta^{15}\text{N}$ of *D. pulex* and *H. gibberum* increased and surpassed the $\delta^{15}\text{N}$ of *E. nevadensis* and *C. trivittatus*. The $\delta^{15}\text{N}$ of *L. tyrelli* was similar to *E. nevadensis* and *C. trivittatus* during the unstratified period, but then reached a maximum at the end of July concurrently with the maximum $\delta^{15}\text{N}$ of *D. pulex* and *H. gibberum* (Figs. 1, 2).

Isotopic differences among zooplankton taxa depended primarily on the time of year (Fig. 1) and, for some taxa, secondarily on zooplankton body size (Figs. 3, 4, 5). The range of *D. pulex* $\delta^{15}\text{N}$ was 10.9‰ over the entire sampling period, and *D. pulex* body size accounted for 25% of this seasonal variability ($F_{[1,93]} = 31.4$, $r^2 = 0.25$, $p < 0.001$; Fig. 3a). Using nonparametric rank correlation (samples ranked for each sampling day), we found that larger *D. pulex* had a higher $\delta^{15}\text{N}$ than smaller *D. pulex* (Fig. 3b; Spearman's rank correlation: $r_s = 0.786$, $p < 0.001$). Isotopic differences between large (>1.8 mm) and medium-sized (<1.8 mm) *D. pulex* (categorized post hoc based on Fig. 4a) depended on the time of year (Fig. 4b). Only large *D. pulex* ever exceeded 5‰ (Fig. 4b) and on average were 1.1‰ (SD = 0.9, $n = 19$) higher than medium-sized *D. pulex*. Body size and $\delta^{15}\text{N}$ were uncorrelated in *H. gibberum* ($r = 0.01$, $p = 0.42$) over a narrow size range (0.8 to 1.2 mm), though the amplitude of variation in $\delta^{15}\text{N}$ of *H. gibberum* was similar to that of *D. pulex* (Fig. 1b).

In July and August, when instars II, III, and IV of *C. trivittatus* were all present in the water column, we found that $\delta^{15}\text{N}$ of *C. trivittatus* increased by about 0.5‰·mm⁻¹ of head length (Fig. 5). The slope of this positive relationship was not significantly different between months (analysis of

Fig. 1. (a and b) Temporal variability of plankton $\delta^{15}\text{N}$ in Council Lake in 2002 to 2003. Error bars are ± 1 SE, based on between one and five replicates per day. The period of thermal stratification is shown with broken vertical lines.



covariance, ANCOVA: $F_{[1,59]} = 0.004, p = 0.81$), but the intercept was slightly higher in August. The sharp drop in the $\delta^{15}\text{N}$ of *C. trivittatus* in the middle of June can partially be explained by the appearance of instar II and III larvae in our samples that had a lower $\delta^{15}\text{N}$. However, the $\delta^{15}\text{N}$ of instar IV larvae also declined by $\sim 1\text{‰}$ in June and then increased over the summer.

Seasonal variation in body size and $\delta^{15}\text{N}$ was positively correlated in *E. nevadensis* ($r = 0.55, p = 0.042$) but not significantly correlated in *L. tyrelli* ($r = 0.23, p = 0.33$). The maximum size of *L. tyrelli* (1.64 mm) coincided with its maximum $\delta^{15}\text{N}$ (7.3‰), but 2 weeks later a similar sized sample (1.56 mm) had a $\delta^{15}\text{N}$ of 3.0‰. In the current study, we only have a limited seasonal range of copepod body sizes, so we do not discuss potential ontogenetic trophic variation in these two copepod taxa.

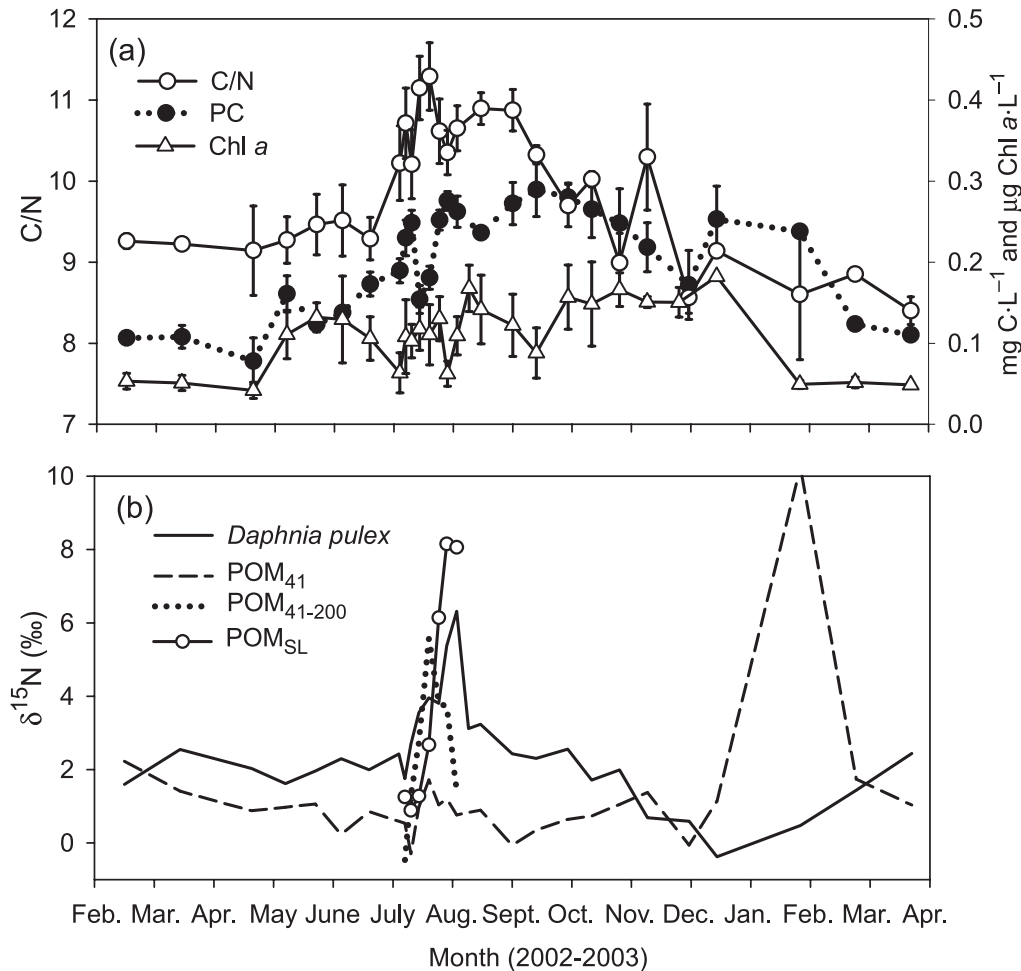
Temporal change in the composition of POM

The range in the $\delta^{15}\text{N}$ of $\text{POM}_{<41\ \mu\text{m}}$ was 12.5‰ over the entire study period (Fig. 2; Table 2). Excluding 20 January 2003, the total range of $\delta^{15}\text{N}$ for POM_{41} was 6.0‰ (minimum = -1.5‰ , maximum = 4.5‰). The average range in the $\delta^{15}\text{N}$ of POM_{41} over the entire water column was 2.3‰ (SE = 0.27, $n = 27$). During the strati-

fied period (May to October), the average POM_{41} $\delta^{15}\text{N}$ was not significantly different among strata (Table 2; ANOVA, $F_{[2,48]} = 0.06, p = 0.95$). During July and August, when the $\delta^{15}\text{N}$ of *D. pulex*, *H. gibberum*, and *L. tyrelli* reached their maxima, the $\delta^{15}\text{N}$ of POM_{41} did not increase substantially. As a result, the average isotopic difference between *D. pulex* and POM_{41} ($\Delta_{D-\text{POM}_{41}}$) was 3.1‰ during July and August (range: 2.2‰–5.6‰) and only 1.4‰ (range: 0.6‰–2.1‰) for the rest of stratification. The $\delta^{15}\text{N}$ of larger size fractions of POM (POM_{41-200} and POM_{SL}) increased rapidly prior to the peak $\delta^{15}\text{N}$ of *D. pulex*, *H. gibberum*, and *L. tyrelli* (Fig. 2b). The $\delta^{15}\text{N}$ of POM_{41-200} peaked on 17 July just prior to the peak $\delta^{15}\text{N}$ for *H. gibberum* (26 July) and *D. pulex* (31 July), whereas the $\delta^{15}\text{N}$ of POM_{SL} fraction increased to a 31 July maximum that was higher than that of either *D. pulex* or *H. gibberum*. We cannot explain the large isotopic excursion of POM_{41} on 20 January 2003. Lehmann et al. (2004) also found a high $\delta^{15}\text{N}$ for POM in the winter and attributed it to algal uptake of recycled nitrogen.

The C/N of POM_{41} was highest during thermal stratification and reached its two highest values on the 12th and 17th of July. On these two days, the average carbon concentration (PC) of POM in the water column ($0.17\ \text{mg C}\cdot\text{L}^{-1}$)

Fig. 2. (a) Temporal variability of average water column chlorophyll *a* (Chl *a*), particulate organic carbon (PC), and C/N ratio for particulate organic matter (POM) < 41 μm from Fig. 1. The right axis shows both the concentration of PC (in $\text{mg C}\cdot\text{L}^{-1}$) and one-sixth of the actual Chl *a* concentration (in $\mu\text{g}\cdot\text{L}^{-1}$). The seasonal average of Chl *a* in Council Lake for this period is $0.65 \mu\text{g}\cdot\text{L}^{-1}$. (b) Comparison of *Daphnia pulex* $\delta^{15}\text{N}$ with the $\delta^{15}\text{N}$ of other plankton size fractions (see text) and a fraction dominated by *Synura lapponica* (POM_{SL}).



was ~30% lower than during the rest of stratification (Fig. 2a). This week of low PC (low food quantity) with a high C/N (low food quality) occurred a week before the maximum $\delta^{15}\text{N}$ of *D. pulex*. However, Chl *a* (indicator of algal biomass) was not unusually low during this time period. The Chl *a* of POM₄₁ was typically <1 $\mu\text{g}\cdot\text{L}^{-1}$ and was lowest in the spring and at the beginning of July and August (Fig. 2a).

Vertical variation in the composition of POM

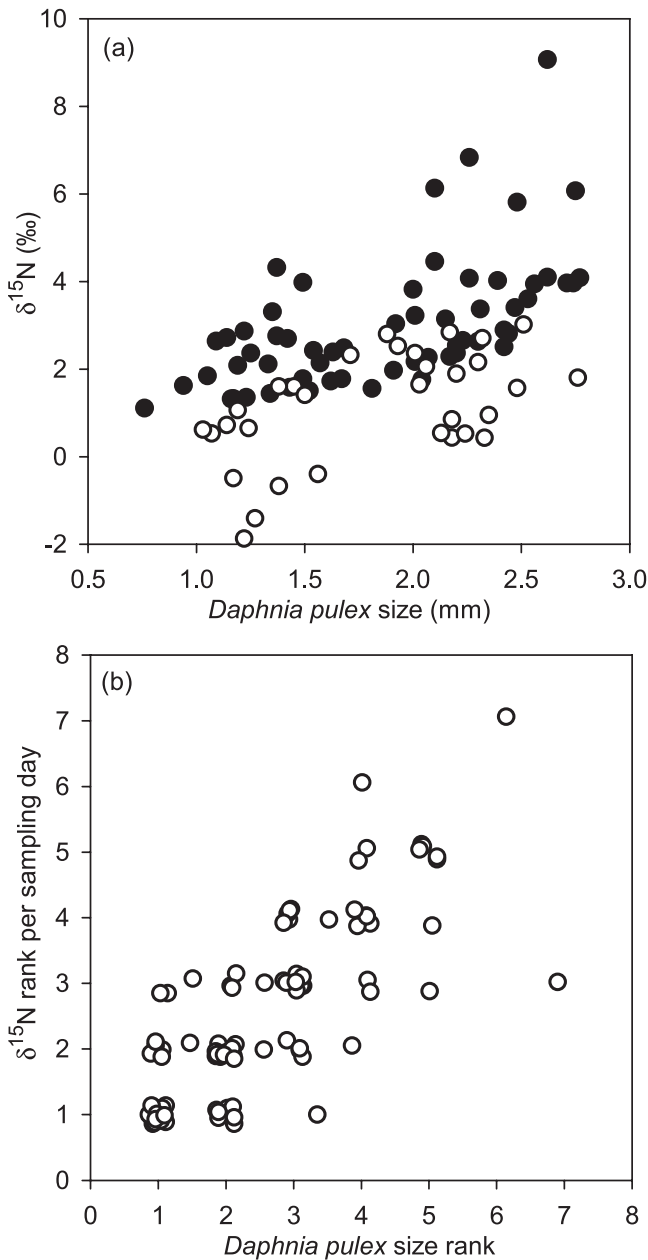
Previous zooplankton sampling in Council Lake (2001) revealed that *D. pulex* feed in the hypolimnion during the day and night in July (Matthews and Mazumder 2006). Therefore, in July 2002 we increased our sampling frequency and depth resolution of POM to test for potential effects of feeding depth on the $\delta^{15}\text{N}$ of zooplankton. We found that the $\delta^{15}\text{N}$ of POM₄₁ did not increase with depth and that the C/N was highest in the metalimnion and lowest in the hypolimnion (Fig. 6). Size-fractionated Chl *a* revealed that algal abundance was highest in the hypolimnion, particularly in larger size fractions. The average $\delta^{15}\text{N}$ of POM₄₁₋₂₀₀ in July was 2.6‰ (~2‰ higher than

POM₄₁) and did not vary among strata (ANOVA: $F_{[2,19]} = 2.59$, $p = 0.10$).

Discussion

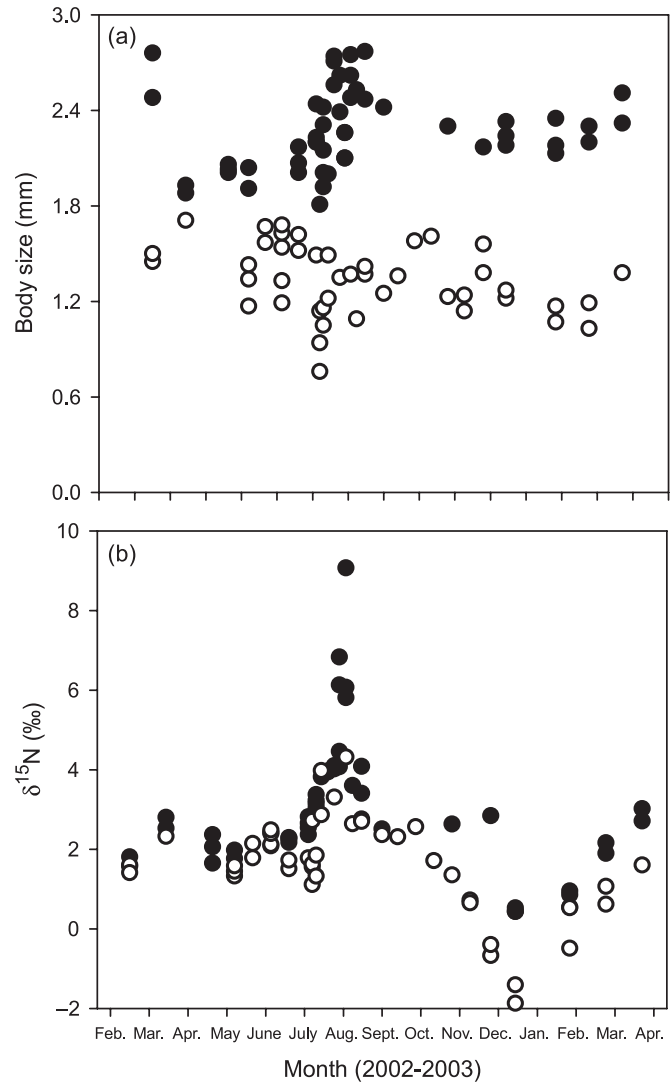
Studies that make repeated seasonal measurements of $\delta^{15}\text{N}$ for multiple taxa are rare in the literature, despite their potential for elucidating the dynamics of food web structure (Grey et al. 2001; Matthews and Mazumder 2005; Mooney and Tillberg 2005). A major challenge with this approach is to distinguish trophic-level variation from isotopic variation ($\delta^{15}\text{N}$). In eutrophic lakes, the $\delta^{15}\text{N}$ of POM can change by >10‰ over the summer because of the biogeochemical cycling of nitrogen or because of changes in the $\delta^{15}\text{N}$ of the lake's nitrogen source (Lehmann et al. 2004; Leavitt et al. 2006). In the current study, we found large seasonal variation in the $\delta^{15}\text{N}$ of zooplankton despite little seasonal variation in the $\delta^{15}\text{N}$ of POM. More importantly, we found that the differences in $\delta^{15}\text{N}$ between zooplankton taxa strongly depended on the time of year and that the $\delta^{15}\text{N}$ of the more herbivorous zooplankton surpassed the $\delta^{15}\text{N}$ of the more carnivorous zooplankton. We

Fig. 3. (a) Relationship between average *Daphnia pulex* body size and $\delta^{15}\text{N}$ for all samples picked in Council Lake over the entire study period. Each sample is a composite of >20 individuals. The solid circles are during thermal stratification of the lake, and the open circles are samples from the rest of the year. (b) Relationship between *D. pulex* size rank and the $\delta^{15}\text{N}$ rank for each sampling day with two or more samples (maximum seven). We have applied a random jitter to reduce overlapping points. Spearman's rank correlation is positive significant ($r_s = 0.786$, $p < 0.001$).



also found positive relationships between body size and $\delta^{15}\text{N}$ for both *D. pulex* and *C. trivittatus*. We conclude that both seasonal and size-based variation in the $\delta^{15}\text{N}$ of consumers should be considered when using $\delta^{15}\text{N}$ to detect trophic-level variation in natural communities. We believe

Fig. 4. (a) Average body size of *Daphnia pulex* individuals ($n = 30$ to >100 per sample) for each sampling event ($n = 1$ to 5 samples per day). In both panels, solid circles are large *D. pulex* (>1.8 mm), and open circles are medium-sized *D. pulex* (<1.8 mm). (b) $\delta^{15}\text{N}$ of each sample in panel (a) over the sampling season.

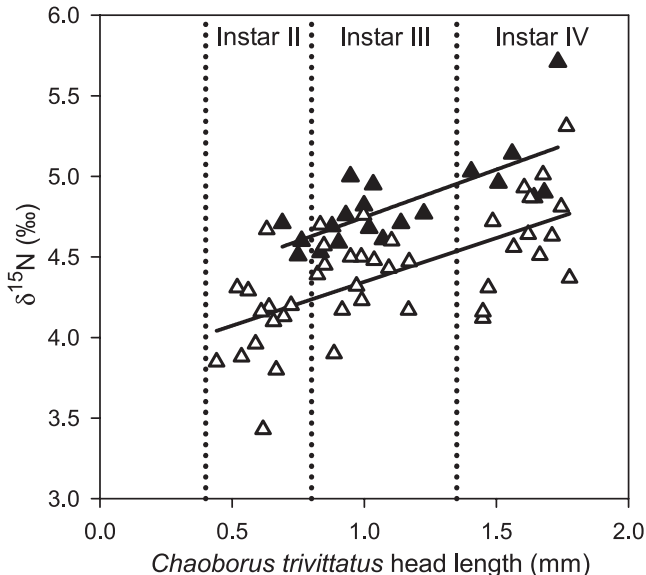


this conclusion is generally applicable to studies of trophic-level variation of consumers in diverse ecosystems.

Seasonal variation of $\delta^{15}\text{N}$

Ecological studies are increasingly using time series of $\delta^{15}\text{N}$ to detect omnivory in complex food webs (Lancaster et al. 2005; Mooney and Tillberg 2005). Our study has revealed additional complexities involved in detecting trophic-level variation using $\delta^{15}\text{N}$. The $\delta^{15}\text{N}$ of the more herbivorous zooplankton (*D. pulex*, *H. gibberum*) rapidly increased and surpassed the $\delta^{15}\text{N}$ of the more predacious zooplankton (*E. nevadensis*, *C. trivittatus*), despite little variation in the $\delta^{15}\text{N}$ of presumed basal food sources (POM₄₁). O'Reilly et al. (2002) argued that abrupt variation in the $\delta^{15}\text{N}$ at the base of a food chain can lead to a distortion in the isotopic representation of food web inter-

Fig. 5. Relationship between head length and $\delta^{15}\text{N}$ for *Chaoborus trivittatus* in July (open triangles) and August (solid triangles) in Council Lake. Vertical broken lines denote instar separation following Fedorenko and Swift (1972). Each data point is a composite of multiple individuals. The regression equation for July is $\delta^{15}\text{N} = 3.8$ (standard error, SE = 0.12) + 0.54 (SE = 0.10)·length ($F_{[1,41]} = 28.48$, $p < 0.001$, $r^2 = 0.41$), and for August is $\delta^{15}\text{N} = 4.2$ (SE = 0.16) + 0.59 (SE = 0.13)·length ($F_{[1,18]} = 26.6$, $p < 0.001$, $r^2 = 0.53$).



actions. This is unlikely the case in Council Lake, because the $\delta^{15}\text{N}$ of POM_{41} , which is a grazable size fraction for herbivorous zooplankton, did not increase rapidly prior to the peak $\delta^{15}\text{N}$ of zooplankton.

It is difficult to explain why the $\delta^{15}\text{N}$ of *D. pulex* and *H. gibberum* increased rapidly during midsummer in Council Lake. Larger size fractions of plankton (POM_{41-200} and POM_{SL}) also increased rapidly in early July and peaked just prior to the maximum $\delta^{15}\text{N}$ of large *D. pulex*. We visually confirmed, by looking at fecal pellets, that copepods can ingest colonies of *S. lapponica* (which makes up POM_{SL}), but we found no evidence of *S. lapponica* in the gut of large *D. pulex*. However, the $\delta^{13}\text{C}$ of both POM_{41-200} and POM_{SL} are similar to large *D. pulex* in Council Lake, suggesting that large *D. pulex* either directly consume these large size fractions or rely on a similar carbon source (Matthews 2006). The maximum particle size ingested by cladocerans increases with body size (Burns 1968), and it is possible that large *D. pulex* (>1.8 mm) can graze particles >41 μm . We are less certain that the high $\delta^{15}\text{N}$ of POM_{41-200} and POM_{SL} can explain the seasonal patterns of *H. gibberum* $\delta^{15}\text{N}$, because *H. gibberum* tend to graze a smaller size range of particles, particularly in oligotrophic lakes (Cyr 1998). Supporting this conclusion, the $\delta^{13}\text{C}$ of *H. gibberum* is more similar to the $\delta^{13}\text{C}$ of POM_{41} above the hypolimnion (Matthews and Mazumder 2006). Instead, we suspect that the $\delta^{15}\text{N}$ of POM_{41} masks the food source that *H. gibberum* assimilates. POM is a heterogeneous mixture of algae, detritus, and heterotrophs and may mask the $\delta^{15}\text{N}$ of any food source that makes up a small fraction of the total POM nitrogen (Vuorio

et al. 2006). It is possible that small algae within POM_{41} follow a similar $\delta^{15}\text{N}$ pattern as larger algae in POM_{41-200} provided they rely on the same source of dissolved inorganic nitrogen, and similarly discriminate against ^{15}N during uptake (but see Needoba et al. 2003). We believe that a combination of selective feeding by zooplankton within POM_{41} and direct feeding on larger size classes of POM can explain the large increase in $\delta^{15}\text{N}$ of *D. pulex*, *H. gibberum*, and *L. tyrelli* during midsummer in Council Lake.

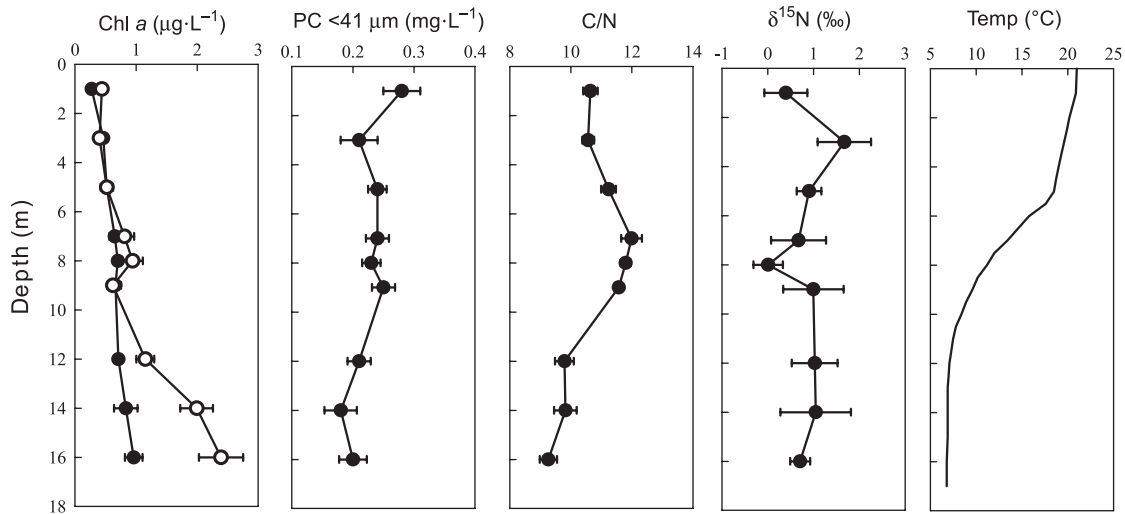
The vertical distribution of zooplankton food sources did not help resolve seasonal patterns of zooplankton $\delta^{15}\text{N}$ in Council Lake. Algal biomass and food quality (indicated by C/N) were both highest in the hypolimnion (>10 m). In a separate study, we found that zooplankton species in Council Lake differentially exploited deepwater algal resources (Matthews and Mazumder 2006). This led to divergent seasonal patterns in the $\delta^{13}\text{C}$ of *D. pulex* and *H. gibberum* that were associated with differences in feeding depth (Matthews and Mazumder 2006). Large *D. pulex* were rarely observed above the hypolimnion, and medium-sized *D. pulex* and *H. gibberum* were common in the epi- and meta-limnion. This vertical habitat segregation of zooplankton cannot directly explain the different seasonal $\delta^{15}\text{N}$ patterns among taxa, because there was little spatial variation with depth in the $\delta^{15}\text{N}$ of POM_{41} and POM_{41-200} .

Size-based intrapopulation variation of $\delta^{15}\text{N}$

It is a common difficulty to determine whether size-based variation of a consumer's $\delta^{15}\text{N}$ reflects trophic-level variation (Fry et al. 1999; Genner et al. 2003) or just size-based variation in the $\delta^{15}\text{N}$ of food sources that have the same trophic position. Our results from Council Lake provide some of the first evidence (see also Branstrator et al. 2003) that $\delta^{15}\text{N}$ can increase with body size in both a herbivore (*D. pulex*) and a predator (*C. trivittatus*). The positive relationship for *D. pulex* could occur if large individuals prey more on heterotrophs that have a higher $\delta^{15}\text{N}$ than algae. However, we are uncertain if the size-based variation in the $\delta^{15}\text{N}$ of POM is related to trophic-level differences among *D. pulex* diet items, so we cannot confirm that larger *D. pulex* are at a higher trophic position. In Council Lake, the situation is complicated by *S. lapponica*, which dominates the POM_{41-200} size fraction. Little is known about the ecology of *S. lapponica*, but it is a large colonial algae (100–250 μm) and may be mixotrophic (Pipes and Leedale 1992), which could explain its high $\delta^{15}\text{N}$ relative to POM_{41} . We can only speculate about a possible influence of mixotrophy on the $\delta^{15}\text{N}$ of zooplankton in Council Lake.

The positive relationship between $\delta^{15}\text{N}$ and body size of *C. trivittatus* is consistent with life history omnivory (sensu Branstrator et al. 2000), but we cannot exclude alternate hypotheses. *Chaoborus* is a size-selective predator whose gape width increases throughout its ontogeny, potentially leading to the positive relationship between *C. trivittatus* body size and $\delta^{15}\text{N}$. To attribute this relationship to trophic-level variation, we have to confirm that size-based variation in $\delta^{15}\text{N}$ of prey items also reflects trophic-level variation. If *C. trivittatus* switches from small cladocerans to copepods, then Fig. 5 could indicate trophic-level variation. Alternatively, the $\delta^{15}\text{N}$ of *C. trivittatus* may increase with body size because of a diet switch from small to large *D. pulex*. We

Fig. 6. Depth profiles of chlorophyll *a* (Chl *a*), particulate carbon (PC), molar C/N ratio, and $\delta^{15}\text{N}$ for particulate organic matter (POM) < 41 μm in July. Error bars are all ± 1 SE based on seven sampling dates in July. The temperature profile was taken on 12 July 2002 at the deepest site in Council Lake. Solid circles show samples taken from water filtered through a 41 μm mesh, whereas open circles are unfiltered.



cannot confirm size-based trophic-level variation in *C. trivittatus* (life history omnivory), because we could not confirm that the positive relationship between *D. pulex* body size and $\delta^{15}\text{N}$ reflects trophic-level differences. Species of *Chaoborus* are known to exhibit life history omnivory (Moore et al. 1994), and perhaps combining size-based isotopic analysis with gut content analysis would help quantify the trophic position of different larval instars (Fedorenko and Swift 1972).

Uncertainties in zooplankton trophic enrichment of ^{15}N

Uncertainties in trophic enrichment (i.e., the difference in $\delta^{15}\text{N}$ between consumers and their diet: Δ_N) currently challenge all our attempts at using $\delta^{15}\text{N}$ to detect seasonal differences in trophic position. Temporal variation in Δ_N of zooplankton relative to POM_{41} may partially explain the decoupling of $\delta^{15}\text{N}$ between zooplankton and POM_{41} .

The quantity, quality, and identity of zooplankton food are seasonally variable in Council Lake and may affect the Δ_N of herbivores (Spence and Rosenheim 2005). Adams and Sterner (2000) found that the $\delta^{15}\text{N}$ of *Daphnia magna* increased by $\sim 2.5\text{‰}$ concurrently with an increase in the C/N of an algal food source from 7.3 to 24.8. The C/N of POM in Council Lake only increased by ~ 1.4 following thermal stratification. Nonetheless, we found a positive relationship between the C/N of POM and the difference in $\delta^{15}\text{N}$ between *D. pulex* and POM_{41} for all sampling dates ($\Delta_{D-\text{POM}_{41}} = -15.4 + 1.7(\text{C/N})$, $p < 0.01$) and for all dates excluding 20 January 2003 ($\Delta_{D-\text{POM}_{41}} = -8.6 + 1.03(\text{C/N})$, $p < 0.01$). This result is consistent with the direction but not the magnitude of Adams and Sterner's (2000) experimental result that the Δ_N of *D. magna* increases with increasing food source C/N. However, we found no significant relationship between POM C/N and the difference in $\delta^{15}\text{N}$ between *H. gibberum* and POM_{41} ($\Delta_{H-\text{POM}_{41}} = -5.1 + 0.7(\text{C/N})$, $p = 0.17$). Given the rapid increase in the $\delta^{15}\text{N}$ of the large POM size fractions and the uncertainty about the zooplankton assimilation of POM_{41} , we believe it is unlikely that a 4‰ increase in the

$\delta^{15}\text{N}$ of *D. pulex* and *H. gibberum* is solely a result of an increase in the C/N of POM_{41} . Future studies would benefit from more detailed analysis of the composition of POM and from experiments that determine the sources of variation in zooplankton ^{15}N enrichment.

Positive relationships between a consumer's size (or age) and $\delta^{15}\text{N}$ may result from size-based variation in trophic enrichment (Δ_N), but the current research is inconclusive. Some studies suggest that Δ_N increases with organism age (Overman and Parrish 2001), whereas others have found no correlation between age and Δ_N (Minagawa and Wada 1984; Hobson and Clark 1992). Size-based variation in the nitrogen balance of *Daphnia* could lead to relationships between *Daphnia* size and $\delta^{15}\text{N}$. For example, larger *Daphnia* may have a higher trophic enrichment than smaller *Daphnia* if ingestion rate increases with body size and digestion efficiency decreases with body size (Power et al. 2003). Alternatively, if larger *Daphnia* are more susceptible to starvation then the $\delta^{15}\text{N}$ of *Daphnia* could increase with size (Adams and Sterner 2000). We believe this is unlikely in Council Lake because the summertime peak of *D. pulex* $\delta^{15}\text{N}$ coincided with a peak lipid content of large *D. pulex* (Matthews 2006). There are several physiological mechanisms that could lead to size-based variation in $\delta^{15}\text{N}$, but no studies have attempted to resolve these issues for *Daphnia* or any other zooplankton taxa.

Distinguishing trophic-level variation from isotopic variation

It is a general challenge for stable isotope studies to determine the source of temporal variation in the $\delta^{15}\text{N}$ of consumers and determine if it is related to trophic-level variation. A promising new approach involves coupling information about the rates of nitrogen flux among food web compartments with seasonal changes in the $\delta^{15}\text{N}$ of food web compartments (Rastetter et al. 2005). In our study, we can draw conclusions by comparing the relative seasonal patterns of each zooplankton species. The $\delta^{15}\text{N}$ of *L. tyrelli* generally

tracked the $\delta^{15}\text{N}$ of *C. trivittatus*, except for a few weeks in July and August when it followed the $\delta^{15}\text{N}$ of *D. pulex* and *H. gibberum*. This suggests that *L. tyrelli* switched its feeding behavior during this time period to more closely match the food sources of *D. pulex* and *H. gibberum*. This is consistent with a previous study that found the diet of *L. tyrelli* depended on the availability of food sources (Anderson 1967). The $\delta^{15}\text{N}$ of *E. nevadensis* was always within 0.5‰ of *C. trivittatus*, which is consistent with *E. nevadensis*'s more predatory style of feeding (Folt and Byron 1989).

Aquatic food webs consist of algal and microbial food chains that are intertwined (Porter 1996). Multiple pathways of nitrogen flow through a food web (i.e., multiple food chains) could explain the divergent seasonal $\delta^{15}\text{N}$ patterns of zooplankton in Council Lake. The $\delta^{15}\text{N}$ of a zooplankton species gives an indication of the temporal integration of the nitrogen cycle leading up to that species in the food chain (Robinson 2001). If a zooplankton community consists of a simple food chain, we would expect covarying seasonal patterns of $\delta^{15}\text{N}$ among taxa (with some time lags). In accordance with this expectation, Perga and Gerdeaux (2005) found seasonally correlated patterns of zooplankton $\delta^{15}\text{N}$ in Lake Geneva. Lake Geneva has many of the same zooplankton groupings (*Leptodora*, *Bythotrephes*, copepods, and *Daphnia*) as in Council Lake (*Chaoborus*, copepods, *Daphnia*), but in Council Lake, the divergent $\delta^{15}\text{N}$ patterns suggest a different food web organization. Therefore, we speculate that Council Lake has multiple food chains (i.e., algal and microbial) and that zooplankton species differentially rely on alternate pathways of nitrogen transfer from basal resources. This is consistent with the observation that the zooplankton species in Council Lake differentially rely on allochthonous carbon (Matthews and Mazumder 2006). We propose that time series of $\delta^{15}\text{N}$ for multiple consumer taxa may help detect alternate nitrogen pathways through complex food webs.

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