

Temporal variation in body composition (C : N) helps explain seasonal patterns of zooplankton $\delta^{13}\text{C}$

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SUMMARY

1. The stable carbon isotope ratio $\delta^{13}\text{C}$ is a useful tracer of energy flow in lake food webs, and the zooplankton signature is commonly used to establish a baseline for the pelagic habitat. However, sources of temporal variability in the $\delta^{13}\text{C}$ of different zooplankton taxa are rarely considered.
2. Here, we investigate to what extent temporal variation in the $\delta^{13}\text{C}$ of particulate organic matter (POM) (<41 μm) and the C : N of zooplankton can explain the temporal variability in $\delta^{13}\text{C}$ of freshwater zooplankton. We compare temporal patterns of $\delta^{13}\text{C}$ and C : N for *Daphnia*, *Hesperodiaptomus franciscanus* and *Leptodiaptomus tyrelli* over a 6-month period at four sites in two oligotrophic lakes.
3. In all three taxa, seasonal variation in zooplankton C : N explained more of the variation in zooplankton $\delta^{13}\text{C}$ than did the $\delta^{13}\text{C}$ of POM. This suggests that variation in the lipid content of zooplankton can strongly influence temporal variation of $\delta^{13}\text{C}$ in zooplankton.
4. Using these data, we evaluate procedures that estimate the $\delta^{13}\text{C}$ of only the non-lipid component of zooplankton. If zooplankton lipids are primarily dietary in origin, then extracting lipids or 'normalising' $\delta^{13}\text{C}$ based on C : N will exclude a major dietary source, and therefore may be inappropriate.
5. We conclude that temporal variation in body composition (C : N) of zooplankton can significantly influence the temporal variation of zooplankton $\delta^{13}\text{C}$ signatures.

Keywords: carbon, lipid, stable isotopes, stoichiometry, zooplankton

Introduction

The stable carbon isotope ^{13}C is commonly used to quantify energy flow in lake food webs (Vander Zanden & Rasmussen, 1999; Post, Pace & Hairston, 2000; Bastviken *et al.*, 2003). Primary consumers, such as mussels or herbivorous zooplankton provide a useful baseline to compare stable isotope signatures among and within lakes because they integrate the $\delta^{13}\text{C}$ of pelagic resources, and are less temporally variable than primary producers (Kling, Fry & O'Brien, 1992; Cabana & Rasmussen, 1996; Post,

2002; Matthews & Mazumder, 2003). Understanding the sources of temporal variability in $\delta^{13}\text{C}$ signatures of zooplankton is critical for stable isotope analysis of consumers at higher trophic levels (Leggett *et al.*, 1999; Schmidt *et al.*, 2003). Among lakes, numerous factors influence the $\delta^{13}\text{C}$ of the food of zooplankton, including lake productivity and respiration (France, del Giorgio & Westcott, 1997), lake trophic status (Grey, Jones & Sleep, 2000), and sources of allochthonous dissolved inorganic carbon (DIC) (Aravena *et al.*, 1992). Within a lake, seasonal variation in the $\delta^{13}\text{C}$ of the food of zooplankton food sources is influenced by seasonal variations in the $\delta^{13}\text{C}$ signature of DIC (Quay *et al.*, 1986; Leggett *et al.*, 1999; Jonsson *et al.*, 2001), the composition of particulate organic matter (POM) (Grey, Jones & Sleep, 2001), algal productivity (Hollander & McKenzie, 1991), algal species composition

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(Zohary *et al.*, 1994) and algal lipid synthesis (van Dongen, Schouten & Damste, 2002).

The amplitude of temporal variation in the $\delta^{13}\text{C}$ of bulk plankton varies from $<3\text{‰}$ to 20‰ among lakes (Zohary *et al.*, 1994). Some of this variability is because of changes in species composition, as different zooplankton taxa can have different $\delta^{13}\text{C}$ signatures (Matthews & Mazumder, 2003; Pel, Hoogveld & Floris, 2003). However, individual zooplankton species can also exhibit large seasonal changes in their $\delta^{13}\text{C}$ signature (Leggett *et al.*, 1999; Pel *et al.*, 2003), and few studies have explored the potential sources of this temporal variability (but see Gu, Alexander & Schell, 1999). It is challenging to determine the sources of variability in zooplankton $\delta^{13}\text{C}$, because it is difficult to isolate the $\delta^{13}\text{C}$ of distinct food sources (Grey *et al.*, 2001). Temporal change in POM $\delta^{13}\text{C}$ is the most obvious source of temporal variability in zooplankton $\delta^{13}\text{C}$ signatures, but to our knowledge no study has tested this directly. The $\delta^{13}\text{C}$ of bulk zooplankton is typically lower than various size fractions of POM (typically $<30\ \mu\text{m}$) (del Giorgio & France, 1996), particularly in oligotrophic lakes (Grey *et al.*, 2000). Selective feeding, lipid accumulation, and habitat selection by zooplankton can all contribute to discrepancies between zooplankton and POM $\delta^{13}\text{C}$ signatures (del Giorgio & France, 1996). In addition, isotopic heterogeneity within plankton communities may confound the relationship between the $\delta^{13}\text{C}$ of zooplankton and POM (Matthews & Mazumder, 2003; Pel *et al.*, 2003).

Seasonal changes in zooplankton body composition (C : N) may account for some temporal variability in the $\delta^{13}\text{C}$ of individual species. C : N is positively related to lipid content (McConnaughey, 1978; Lesage, 1999; Schmidt *et al.*, 2003), and lipids are depleted in ^{13}C relative to proteins and carbohydrates (Parker, 1964; Tieszen *et al.*, 1983). The $\delta^{13}\text{C}$ of synthesised lipids in freshwater and marine algae are on average 12.9‰ (range $5.5\text{--}15.8\text{‰}$) lower than monosaccharides (van Dongen *et al.*, 2002), and zooplankton can store large volumes of dietary lipids, depending on the time of year (Arts, 1998) and the ambient food concentration (Goulden *et al.*, 1998). In addition, zooplankton can selectively assimilate and allocate different components of an algal diet (Cowie & Hedges, 1996).

There are two general approaches to determine if zooplankton lipids affect zooplankton $\delta^{13}\text{C}$ signa-

tures. First, we could either measure the $\delta^{13}\text{C}$ of zooplankton fatty acids (FA) directly (Pel *et al.*, 2003), or measure the $\delta^{13}\text{C}$ of zooplankton before and after lipid extraction (Kling *et al.*, 1992). It is labour intensive to measure temporal patterns in the $\delta^{13}\text{C}$ of individual FA for several species of zooplankton, and only recent methodological advances make this type of approach feasible (Pel *et al.*, 2003). A more common approach is to extract lipids from organisms prior to $\delta^{13}\text{C}$ analysis (Kelly, 2000), under the rationale that synthesised lipids do not reflect the $\delta^{13}\text{C}$ of the organism's food source (Tieszen *et al.*, 1983). However, lipids in freshwater zooplankton are typically dietary in origin (Goulden & Place, 1990; Arts, 1998), so both the $\delta^{13}\text{C}$ of lipids and lipid-extracted tissue are valuable for analyses of the diet of zooplankton. Removing lipids prior to isotope analysis, or 'normalising' the $\delta^{13}\text{C}$ of zooplankton based on their C : N ratio (*sensu* McConnaughey & McRoy, 1979; Leggett, 1998), might be contrary to the goals of a dietary analysis using $\delta^{13}\text{C}$.

A second approach, and the one used here, is to compare variation in zooplankton C : N (as an indicator of lipid content) with variation in zooplankton $\delta^{13}\text{C}$ among or within lakes. As C : N is positively correlated with lipid content (McConnaughey, 1978; Lesage, 1999; Schmidt *et al.*, 2003), and the $\delta^{13}\text{C}$ of lipid is lower than other body constituents (Parker, 1964; Tieszen *et al.*, 1983), we might expect a negative relationship between C : N and $\delta^{13}\text{C}$ in freshwater zooplankton. To test this hypothesis we could measure the C : N and $\delta^{13}\text{C}$ of consumers in a sample of lakes and look for a correlation among taxa (*sensu* France, 1995). However, this relationship may be confounded by feeding diversity (France, 1995), variable tissue turnover rates among consumers (Schmidt *et al.*, 2003), or lake specific differences in baseline $\delta^{13}\text{C}$ signatures (Post, 2002; Matthews & Mazumder, 2003). An alternative is to look for a relationship between C : N and $\delta^{13}\text{C}$ in an individual species found at a given lake site.

Detecting a negative relationship between C : N and $\delta^{13}\text{C}$ for a single zooplankton species may be challenging because the range of zooplankton C : N is often small at any one time. However, if we found large variation in $\delta^{13}\text{C}$ concurrently with low variability in C : N, this would indicate a limited impact of lipids on $\delta^{13}\text{C}$ signatures. In an attempt to increase the range of observed C : N (see Villar-Argaiz,

Medina-Sanchez & Carrillo, 2002), we collected zooplankton over a 6-month period. The drawback of this approach is that temporal variability in the $\delta^{13}\text{C}$ of food sources may mask a relationship between C : N and $\delta^{13}\text{C}$. To test for a relationship between C : N and $\delta^{13}\text{C}$ for different zooplankton species, we selected two oligotrophic lakes that are morphologically similar, occur in adjacent catchments, and experience similar climatic conditions. We also sampled a deep and shallow basin in each lake, because the stability of stratification may influence the baseline variation of pelagic $\delta^{13}\text{C}$ signatures (Quay *et al.*, 1986).

The goals of this study were (i) to partition the temporal variability of zooplankton $\delta^{13}\text{C}$ between variation in the $\delta^{13}\text{C}$ of POM and variation in the C : N ratio of zooplankton, and (ii) to evaluate the applicability of the widely used lipid normalisation technique (McConnaughey & McRoy, 1979) for interpreting variability of zooplankton $\delta^{13}\text{C}$ signatures (Kline, 1999; Schmidt *et al.*, 2003). To accomplish these goals, we compare seasonal changes in $\delta^{13}\text{C}$ of *Leptodiaptomus tyrelli* Poppe, a calanoid copepod with a seasonally variable C : N, with seasonal changes in $\delta^{13}\text{C}$ of *Hesperodiaptomus franciscanus* Lilljeborg and *Daphnia* spp., which both have a smaller seasonal range of C : N. We compare these patterns with temporal variability in the $\delta^{13}\text{C}$ of POM (<41 μm) collected in the epilimnion and metalimnion over the same time period.

Methods

We collected zooplankton samples every 2 or 3 weeks from June to November 2001 from two coastal oligotrophic lakes on Vancouver Island, Canada, Sooke Lake Reservoir (SOL; 48°33'N, 123°41'W) and Shawnigan Lake (SHL; 48°33'N, 123°38'W). In each lake we sampled at a deep basin [SOL-D (70 m) and SHL-D (53 m)] and a shallow basin [SOL-S (22 m) and SHL-S (27 m)]. The lakes are morphologically similar and are in adjacent catchments. They are temperate, warm monomictic and rarely have permanent ice cover. Mean summer epilimnetic chlorophyll *a* for both lakes is typically <2 $\mu\text{g L}^{-1}$ (Davies, Nowlin & Mazumder, 2004). SOL was historically a natural lake, and is now the main source of drinking water for the city of Victoria, British Columbia. Low summer rainfall and high consumer water demand leads to a large water level drawdown (>5 m) over the summer

and autumn, which results in a higher flushing rate at SOL-S compared with the other three sites (Nowlin *et al.*, 2004).

We collected POM from the epilimnion with a 6 m section of Tygon tubing. We collected metalimnetic samples from the middle of the thermocline using a vertically oriented Niskin sampler. We filtered at least 1 L of lake water through a 41 μm Nitex mesh, onto precombusted (550 °C for 1 h) 25 mm GF-C filters (Whatman, Florham Park, NJ, U.S.A.). Filters were dried overnight at 60 °C and packed in tin cups for isotopic analysis. We collected zooplankton with a 64 μm mesh, 50 cm diameter Wisconsin net from the entire water column, or to a maximum depth of 30 m. Within 24 h of collection we sorted live zooplankton into different categories. From both sites in SHL we picked out *Daphnia pulicaria* Forbes and the dominant calanoid species *Hesperodiaptomus franciscanus*. *Leptodiaptomus tyrelli* was also present in SHL but was not abundant enough in our samples for isotopic analysis. Both sites of SOL had *L. tyrelli* and *H. franciscanus*, although we only sorted *H. franciscanus* at the shallow basin (SOL-S) where they were numerous. We picked samples of *Daphnia rosea* Sars from both sites in SOL.

Our goal for isotopic analysis was to get approximately 1 mg of zooplankton tissue for each zooplankton sample, and two samples per date. Depending on the size of the zooplankton, samples consisted of 40–80 *Daphnia* and 80–150 calanoid copepodids. Samples were analysed at the University of Waterloo Environmental Isotope Laboratory (Waterloo, Ontario, Canada) on a VG Micromass isotope ratio mass spectrometer (precision < 0.1‰; Mississauga, ON, Canada). Samples were analysed for $\delta^{13}\text{C}$, percent carbon and percent nitrogen. Herein, C : N is expressed as a molar ratio.

Predicting temporal change in zooplankton $\delta^{13}\text{C}$

We used multiple regression to determine how much temporal variation in zooplankton $\delta^{13}\text{C}$ signatures could be accounted for by variation in the C : N ratio of zooplankton and the $\delta^{13}\text{C}$ of POM (<41 μm). For each sampling date, we took the average $\delta^{13}\text{C}$ and C : N sample for each taxon at each site, and the average $\delta^{13}\text{C}$ of POM (<41 μm) in the epilimnion and metalimnion. This simplification was justified because the average difference between the $\delta^{13}\text{C}$ of epilimnetic

and metalimnetic POM was $<0.5\%$. For the analysis in Table 1 and Fig. 4, we standardised both predictor variables and the response variable for each species at each site, so that we could compare the partial regression slopes on a common scale (Quinn & Keough, 2002). Adding a 2-week lag to the $\delta^{13}\text{C}$ of POM did not improve the ability of POM to predict temporal change in the $\delta^{13}\text{C}$ of zooplankton, therefore we used a zero lag for all regression models.

Evaluating the process of $\delta^{13}\text{C}$ normalisation based on C : N

In this paper, we consider the lipid normalisation techniques of McConnaughey & McRoy (1979) and Leggett (1998) as attempts to estimate the $\delta^{13}\text{C}$ of the non-lipid fraction of a consumer. The ideal way to evaluate these normalisation procedures would be to create and validate a stoichiometric model that related lipid concentration to organism C : N, and concurrently measure the $\delta^{13}\text{C}$ of lipids and lipid extracted tissue. This is beyond the scope of this paper, and here we only evaluate the sensitivity of the normalisation procedure in general, and its applicability for freshwater zooplankton.

McConnaughey & McRoy (1979) used an empirical relationship between C : N and percent lipid for a collection of marine fishes and crustaceans (see McConnaughey, 1978), to parameterise the lipid factor (L) as shown in eqn 1.

$$L = 93/[1 + 1/(0.246 \times C/N - 0.775)] \quad (1)$$

They then used L to correct $\delta^{13}\text{C}$ values as shown in eqn 2:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + D[-0.2068 + 3.90/(1 + 287.1/L)] \quad (2)$$

where $\delta^{13}\text{C}'$ is the corrected value of the observed $\delta^{13}\text{C}$, and D is defined as the average difference between the $\delta^{13}\text{C}$ of lipids and proteins. McConnaughey (1978) models lipid extracted tissue as proteins and carbohydrates, and assumes that the $\delta^{13}\text{C}$ of proteins and carbohydrates are the same. Therefore, D can be estimated as the difference in $\delta^{13}\text{C}$ between lipid and lipid extracted tissue.

Leggett (1998) developed an alternate approach that involved lipid extraction and regression analysis, which Johannsson *et al.* (2001) then used to infer temporal variability in the feeding behaviour of *Mysis* in Lake Ontario. However, Leggett (1998) noted that his normalisation procedure may not be applicable to cases other than *Mysis* in Lake Ontario, and hypothesised that the relationship between C : N and $\delta^{13}\text{C}$ was species specific in freshwater zooplankton.

To determine the sensitivity to the parameter D of McConnaughey & McRoy's (1979) lipid normalisation, we normalised our isotopic data from SOL-D using eqn 2, with values of D equal to 0, 4, 6, and 12.9% . We consider the latter value as an upper limit, because it is an average difference between monosaccharides and lipids in freshwater and marine algae (mean \pm SD = $12.9 \pm 3.8\%$, $n = 6$; van Dongen *et al.*, 2002).

Table 1 Multiple regression analysis of C : N and the $\delta^{13}\text{C}$ of POM used to predict the $\delta^{13}\text{C}$ of zooplankton for each taxa at each site

Taxa/lake/site	$\beta_{C:N}$ (SE)	$\beta_{\text{POM}\delta^{13}\text{C}}$ (SE)	$\beta_{C:N \times \text{POM}\delta^{13}\text{C}}$ (SE)	F-stat	P-value	R ²
<i>Daphnia</i>						
SOL-S	0.34 (0.43)	0.73 (0.43)	NS	$F_{(1,9)} = 1.70$	0.236	0.27
SOL-D	-0.77 (0.09)*	0.60 (0.10)*	-0.35 (0.09)*	$F_{(1,8)} = 40.1$	<0.001	0.94
SHL-S	-0.11 (0.46)	0.44 (0.46)	NS	$F_{(1,9)} = 0.71$	0.559	0.14
SHL-D	-0.79 (0.21)*	0.41 (0.21)	NS	$F_{(1,9)} = 7.48$	0.01	0.62
<i>H. franciscanus</i>						
SOL-S	-0.33 (0.32)	-0.30 (0.32)	NS	$F_{(1,8)} = 1.19$	0.354	0.23
SHL-S	-0.79 (0.22)*	0.48 (0.22)	NS	$F_{(1,9)} = 7.01$	0.015	0.61
SHL-D	-0.57 (0.35)	0.22 (0.35)	NS	$F_{(1,9)} = 1.40$	0.296	0.24
<i>L. tyrelli</i>						
SOL-S	-0.83 (0.18)*	0.27 (0.18)	NS	$F_{(1,8)} = 13.7$	0.006	0.82
SOL-D	-1.22 (0.09)*	0.51 (0.09)*	NS	$F_{(1,9)} = 103.2$	<0.001	0.96

$\beta_{C:N}$ is the multiple regression coefficient that represents the C : N of zooplankton, and $\beta_{\text{POM}\delta^{13}\text{C}}$ represents the $\delta^{13}\text{C}$ of POM. NS indicates that the interaction term was not significant and so was dropped from the final model. An asterisk denotes significant regression coefficients ($P < 0.05$), and SE is the standard error of the estimate.

Results

Among all sites, the range of variation in C : N for *Daphnia* (5.1–5.9) and *H. franciscanus* (4.8–6.0) was

small compared with *L. tyrelli* (6.7–14.7), and was small compared with interspecific variation in the C : N of 24 zooplankton genera reviewed by Elser *et al.* (2000) (Fig. 1a). The seasonal pattern in the C : N

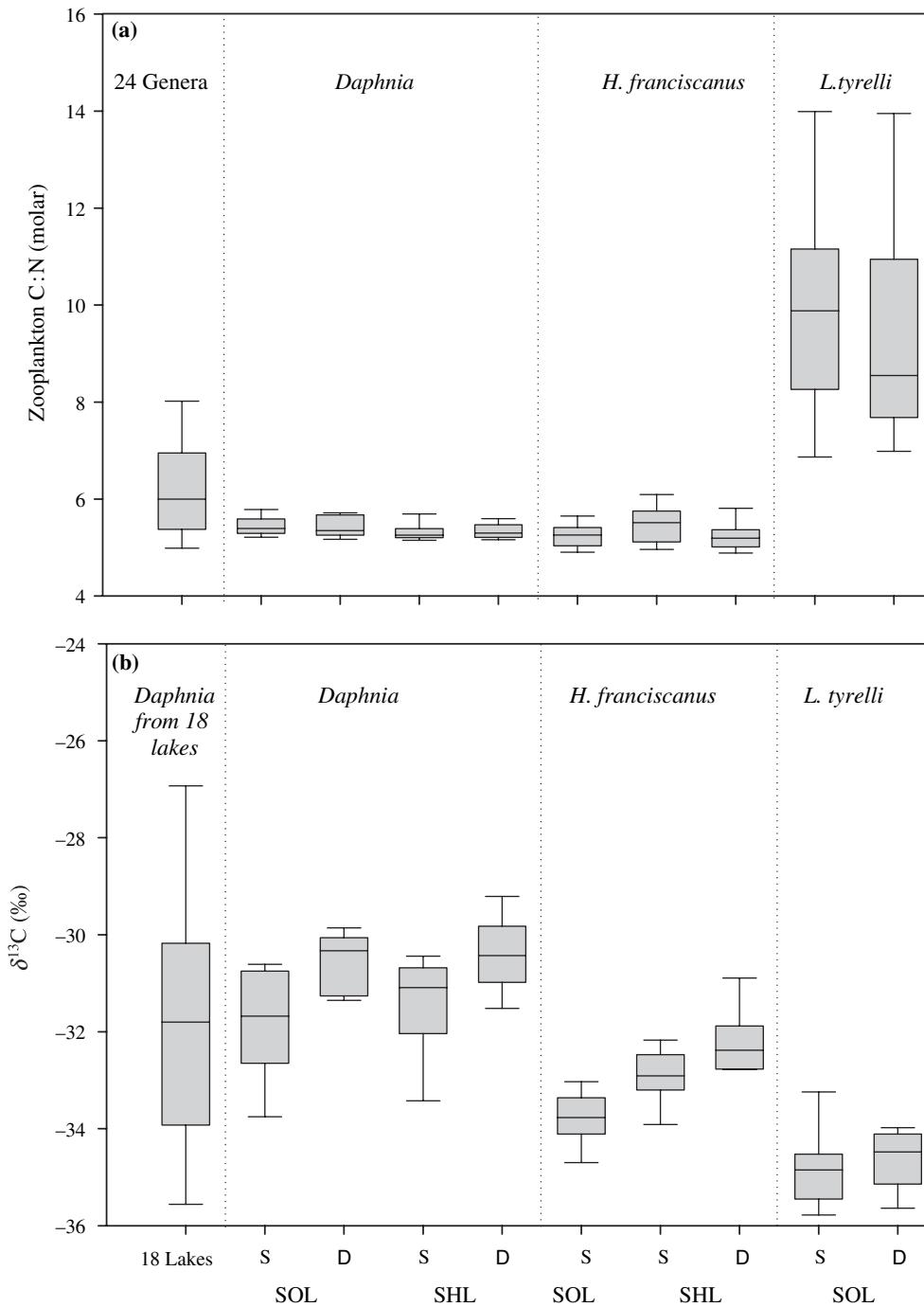


Fig. 1 A comparison of temporal variability of (a) C : N and (b) $\delta^{13}\text{C}$ of *Daphnia*, *H. franciscanus*, and *L. tyrelli* with the variability of C : N for 24 genera (a) from Elser *et al.* (2000), and *Daphnia* $\delta^{13}\text{C}$ from 18 lakes (b) (Hansson *et al.*, 1997; Grey & Jones, 1999; Campbell *et al.*, 2000; Matthews & Mazumder, 2003). Boxplots show the quantiles of the data distribution, and data outside this range were omitted from the graph.

of zooplankton depended on the taxa, but was similar among sites for the same taxa (Fig. 2). *Daphnia* and *H. franciscanus* typically had a higher C : N in early June and late October. In comparison, the C : N ratio of *L. tyrelli* increased throughout the sampling period at both sites in SOL. This was consistent with our observations of a seasonal increase in lipid droplet concentration in the body of *L. tyrelli*, and the seasonal increase in the amount of carbon per unit body length in individuals of *L. tyrelli*, which continually increased from 2.72 (June) to 9.6 $\mu\text{gC individual}^{-1} \text{mm}^{-1}$ (October).

The $\delta^{13}\text{C}$ of POM (<41 μm) was similar in the epilimnion (0–6 m) and metalimnion (8–14 m), based on paired *t*-tests (SOL-S: $\delta^{13}\text{C}_{\text{epi-meta}} = 0.71\text{‰}$, $t_9 = 2.39$, $P = 0.041$; SOL-D: $\delta^{13}\text{C}_{\text{epi-meta}} = 0.37\text{‰}$, $t_8 = 1.46$, $P = 0.182$; SHL-S: $\delta^{13}\text{C}_{\text{epi-meta}} = 0.04\text{‰}$, $t_7 = 0.172$, $P = 0.87$; SHL-D: $\delta^{13}\text{C}_{\text{epi-meta}} = 0.35\text{‰}$, $t_6 = 2.26$, $P = 0.07$), and overall, the average difference was <0.5‰ ($\delta^{13}\text{C}_{\text{epi-meta}} = 0.39\text{‰}$, $t_{33} = 2.99$, $P = 0.005$). Considering all sites, zooplankton had a lower $\delta^{13}\text{C}$ than the average $\delta^{13}\text{C}$ of POM (on the same sampling day) by 0.51‰ for *Daphnia* ($n = 44$), 2.8‰ for *H. franciscanus* ($n = 32$), and 4.3‰ for *L. tyrelli* ($n = 19$). The $\delta^{13}\text{C}$ of *Daphnia* was only significantly different from the average $\delta^{13}\text{C}$ of POM at SOL-S ($\delta^{13}\text{C}_{\text{POM-Daphnia}} = 1.67\text{‰}$, $t_{10} = 4.711$, $P < 0.001$). At all sites, both calanoid species always had a lower $\delta^{13}\text{C}$ signature than either *Daphnia* or average POM (Fig. 3).

Seasonal variability of zooplankton $\delta^{13}\text{C}$ was generally small in our lakes (1.75–4.7‰), compared with the range of *Daphnia* $\delta^{13}\text{C}$ in 18 lakes gathered from the literature (20.6‰) (Fig. 1b). The pattern of temporal variation in $\delta^{13}\text{C}$ depended on the site and species (Fig. 3). In all four basins the $\delta^{13}\text{C}$ of *Daphnia* was highest in midsummer, but the range and coefficient of variation was higher in the both shallow basins (SOL-S: range 3.97‰, CV 3.6%; SOL-D: range 2.01‰, CV 1.8%; SHL-S: range 4.70‰, CV 3.3%; SHL-D: range 2.58‰, CV 2.6%). The $\delta^{13}\text{C}$ of *L. tyrelli* declined throughout the sampling period of 2001 at both sites in SOL concurrently with seasonal increases in C : N ratio (Figs 2 & 3).

Predicting the temporal change in zooplankton $\delta^{13}\text{C}$

Multiple regression analysis revealed that the temporal change in the $\delta^{13}\text{C}$ of zooplankton was better predicted by the changes in C : N ($\beta_{\text{C:N}}$) than the

$\delta^{13}\text{C}$ of POM ($\beta_{\text{POM}\delta^{13}\text{C}}$). Five of the nine regression models found $\beta_{\text{C:N}}$ significant, compared with two that found $\beta_{\text{POM}\delta^{13}\text{C}}$ significant (Table 1). In the five significant regression models, $\beta_{\text{C:N}}$ was larger than $\beta_{\text{POM}\delta^{13}\text{C}}$ and had the opposite sign (Table 1). As expected, changes in the $\delta^{13}\text{C}$ of zooplankton were generally positively related to the $\delta^{13}\text{C}$ of POM ($\beta_{\text{POM}\delta^{13}\text{C}} > 0$) when the C : N of zooplankton was held constant (Fig. 4d–f), and negatively related to the C : N of zooplankton ($\beta_{\text{C:N}} < 0$) when the $\delta^{13}\text{C}$ of POM was held constant (Fig. 4a–c). The larger $\beta_{\text{C:N}}$ compared with $\beta_{\text{POM}\delta^{13}\text{C}}$, suggests that observed changes in zooplankton C : N had a larger impact on the $\delta^{13}\text{C}$ of zooplankton than did changes in the $\delta^{13}\text{C}$ of POM. The empirical relationship between C : N and $\delta^{13}\text{C}$ depends on the taxa considered, but in general the slope of the relationship is steeper for *Daphnia* than copepods (Fig. 5).

Evaluating the process of $\delta^{13}\text{C}$ normalisation based on C : N

Changing the parameters of the lipid normalisation technique developed by McConnaughey & McRoy (1979) changed the seasonal average $\delta^{13}\text{C}$ of each zooplankton species, the average difference between zooplankton species and, in some cases, the temporal pattern of $\delta^{13}\text{C}$ (Fig. 6). For example, in SOL-D the seasonal average difference in $\delta^{13}\text{C}$ between *Daphnia* and *L. tyrelli* was 4.0‰ ($\delta^{13}\text{C}_{\text{D-L}}$). At intermediate levels of *D* (6‰; McConnaughey & McRoy, 1979), $\delta^{13}\text{C}_{\text{D-L}}$ decreased to 2.7‰ following normalisation. At high levels of *D* (12.9‰; van Dongen *et al.*, 2002) $\delta^{13}\text{C}_{\text{D-L}}$ declined to 1.2‰, and the temporal trend in $\delta^{13}\text{C}$ of *L. tyrelli* actually reversed, and converged with the $\delta^{13}\text{C}$ of *Daphnia* by the end of the 2001 sampling season (Fig. 6).

Discussion

Temporal variability in the C : N of zooplankton and changes in the $\delta^{13}\text{C}$ of their food sources, both affect the interpretation of seasonal patterns in the $\delta^{13}\text{C}$ of zooplankton (Zohary *et al.*, 1994; Thompson *et al.*, 2000). Among lakes, the $\delta^{13}\text{C}$ of zooplankton is predictable from the $\delta^{13}\text{C}$ of POM, if we account for an average depletion of zooplankton $\delta^{13}\text{C}$ from POM (del Giorgio & France, 1996). Within a lake, large seasonal changes in the $\delta^{13}\text{C}$ of POM will certainly

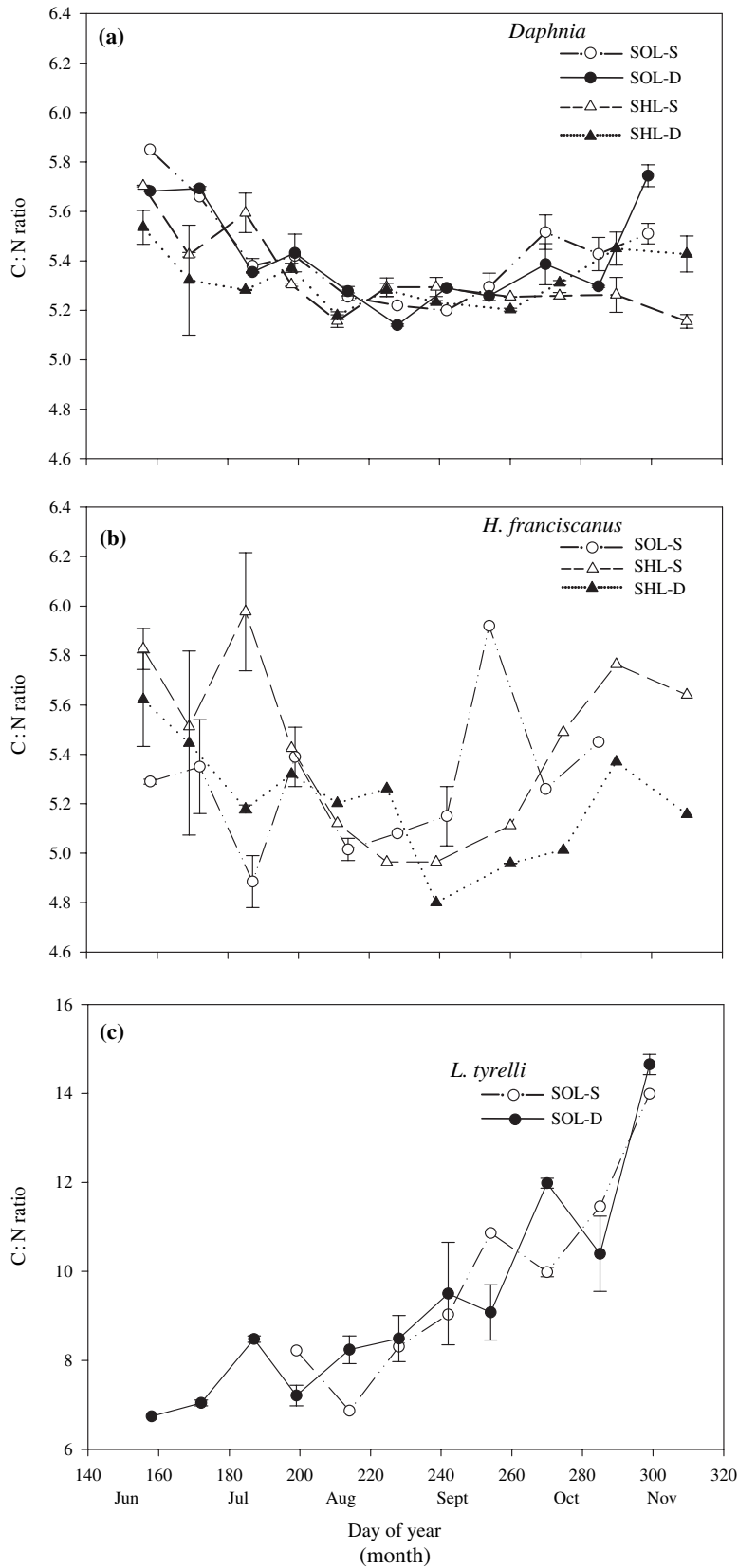


Fig. 2 Temporal pattern of C : N ratio for three zooplankton taxa, (a) *Daphnia*, (b) *H. franciscanus* and (c) *L. tyrelli*, at the sites where they occur in Sooke Lake Reservoir (SOL) and Shawnigan Lake (SHL). SOL-D and SHL-D are sites in the deep basins, whereas SHL-S and SOL-S are sites in the shallow basins (see Methods).

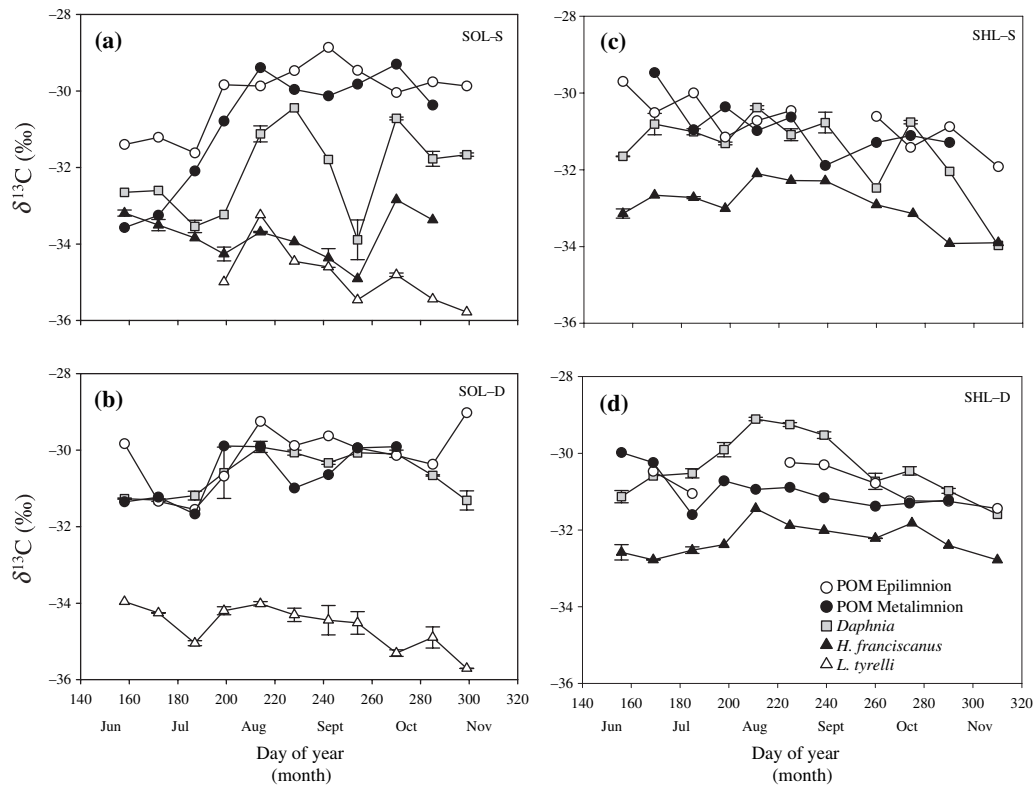


Fig. 3 Temporal pattern of mean (± 1 SE) $\delta^{13}\text{C}$ for zooplankton at four sites; (a) SOL-S, (b) SOL-D, (c) SHL-S and (d) SHL-D. Where error bars are not present, only a single sample was taken for that sampling date.

influence the seasonal pattern of zooplankton $\delta^{13}\text{C}$, provided that zooplankton assimilate that POM. In our study lakes, which have small seasonal changes in the $\delta^{13}\text{C}$ of POM, temporal variation of zooplankton body composition (C : N) accounted for most of the temporal variation in zooplankton $\delta^{13}\text{C}$ (Fig. 4). There are several reasons why changes in the $\delta^{13}\text{C}$ of zooplankton might not respond to seasonal changes in the $\delta^{13}\text{C}$ of POM.

First, the $\delta^{13}\text{C}$ of POM often decreases with depth in part because of internal recycling of the DIC pool (Quay *et al.*, 1986), and it is possible that by sampling only the epilimnion and metalimnion of the water column we did not capture the full range of variation in the $\delta^{13}\text{C}$ of POM. In our study lakes, zooplankton that feed on POM below the metalimnion could have lower $\delta^{13}\text{C}$ signatures. This is unlikely for the *Daphnia* populations in these lakes, because their $\delta^{13}\text{C}$ signatures are generally within 0.5‰ of the average $\delta^{13}\text{C}$ of POM (Fig. 3). However, variable feeding depth may explain why the $\delta^{13}\text{C}$ of *H. franciscanus* is 2.2‰ (SD = 1.04, $n = 32$ pair-wise comparisons) lower than *Daphnia*, although they have a similar C : N ratio (Fig. 2).

Second, the $\delta^{13}\text{C}$ of zooplankton may be temporally decoupled from the $\delta^{13}\text{C}$ of POM, if the rate of isotopic change in POM is faster than the tissue turnover of different zooplankton taxa. Grey (2001) found that *Daphnia hyalina* Leydig reached isotopic equilibrium within 2 weeks in a diet switch experiment, but there is little known about the relative isotopic turnover rates of *Daphnia* and POM in natural lakes. Although our time series are short, adding a time lag to the regression models did not improve the ability of POM $\delta^{13}\text{C}$ to predict temporal change in zooplankton $\delta^{13}\text{C}$. In SHL-D, the temporal pattern of $\delta^{13}\text{C}$ was similar among zooplankton species (*D. pulicaria* and *H. franciscanus*), although the $\delta^{13}\text{C}$ of POM tended to decline over the season. In this case, the $\delta^{13}\text{C}$ of POM does not explain the temporal variability of zooplankton $\delta^{13}\text{C}$. Alternatively, it is possible that changes in zooplankton $\delta^{13}\text{C}$ reflect seasonally correlated changes in lipid concentration. Goulden *et al.* (1998) found that the lipid content of *Daphnia catawba* increased with increasing food concentration. Similarly, Arts, Robarts & Evans (1993) found that storage lipids of *Diatomus sicilis* Forbes

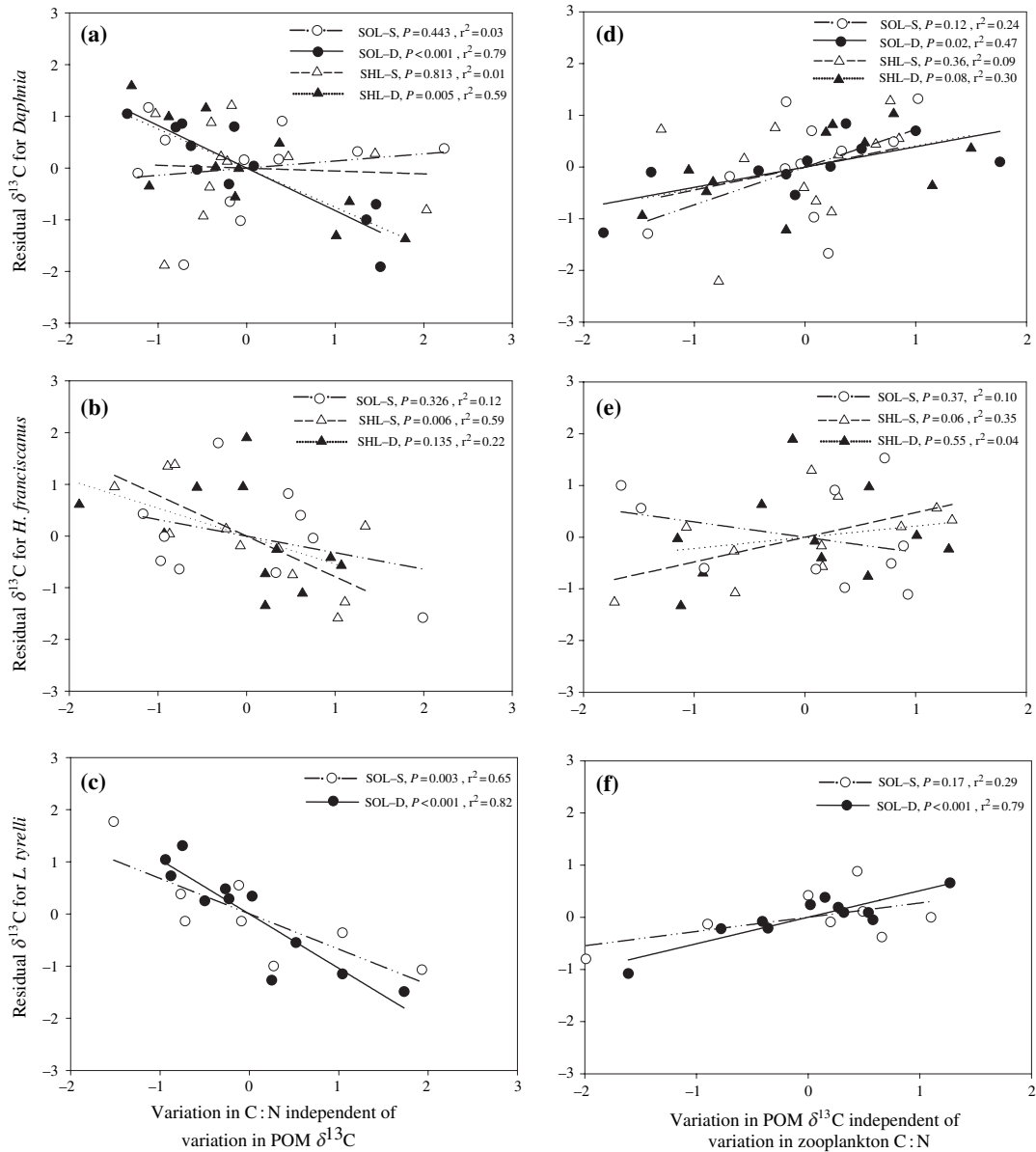


Fig. 4 Partial regression plots of the predictors of a multiple regression model relating the C : N of the zooplankton, and the average $\delta^{13}\text{C}$ of POM, to the $\delta^{13}\text{C}$ of zooplankton. The vertical axes are the residuals from ordinary least squares (OLS) regression of zooplankton $\delta^{13}\text{C}$, in the absence of the predictor variable on the x -axis. The predictor variable is either zooplankton C : N (a, b, c) or POM $\delta^{13}\text{C}$ (d, e, f). The horizontal axes are residuals from OLS regression of either C : N versus POM $\delta^{13}\text{C}$ (a, b, c), or POM $\delta^{13}\text{C}$ versus C : N (d, e, f). The P -values shown in each panel are for the regression coefficients of the entire model, which is shown in Table 1. R^2 values are coefficients of determination, and represent the amount of variation in the $\delta^{13}\text{C}$ of zooplankton that is explained by the predictor variable (e.g. C : N) if the other predictor variable is held constant (e.g. POM $\delta^{13}\text{C}$).

increased during periods of high algal abundance. Large temporal variation of zooplankton lipids is well documented (Arts & Wainman, 1998), but is rarely addressed in the context of stable isotope analysis.

Third, the $\delta^{13}\text{C}$ of POM may not reflect the $\delta^{13}\text{C}$ of zooplankton diet because of selective feeding behaviour. A size fraction of POM ($<41\ \mu\text{m}$) may mask

considerable isotopic heterogeneity (Pel *et al.*, 2003). Calanoids can feed preferentially on detritus, protozoans or different species of algae that do not reflect the average POM $\delta^{13}\text{C}$. Selective feeding also complicates the previous two issues, because the isotopic composition of POM may vary with depth, and components of POM can have different temporal

Fig. 5 Logistic regression of C : N and $\delta^{13}\text{C}$ for all data, excluding *Daphnia* from SOL-S and SHL-S (because of higher temporal variability in their $\delta^{13}\text{C}$). Logistic regression equations are shown for all species ($\delta^{13}\text{C} = -36.1/(1 + 0.85 e^{-0.33\text{CN}})$, $r^2 = 0.52$), all copepods ($\delta^{13}\text{C} = -36.26/(1 + 0.28 e^{-0.20\text{CN}})$, $r^2 = 0.94$), and for *L. tyrelli* and *Daphnia* together ($\delta^{13}\text{C} = -35.42/(1 + 4.3 e^{-0.61\text{CN}})$, $r^2 = 0.67$).

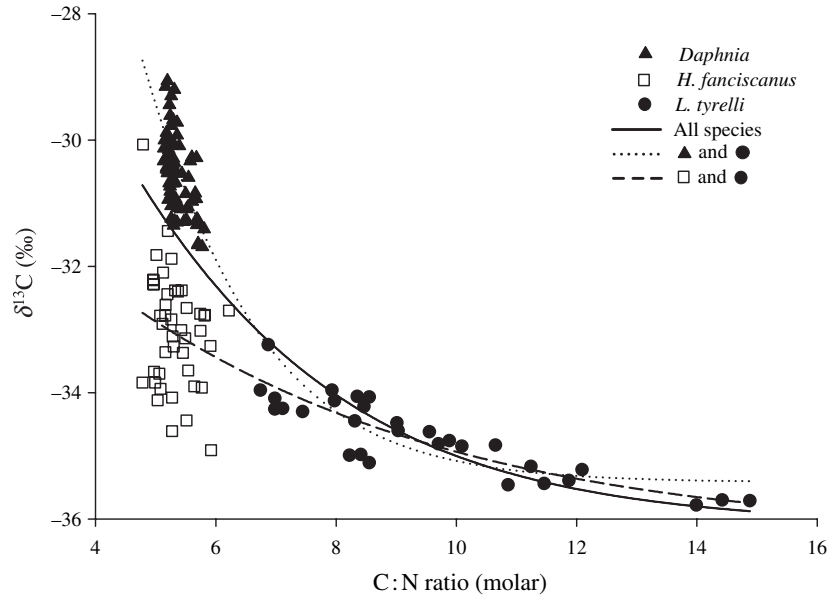
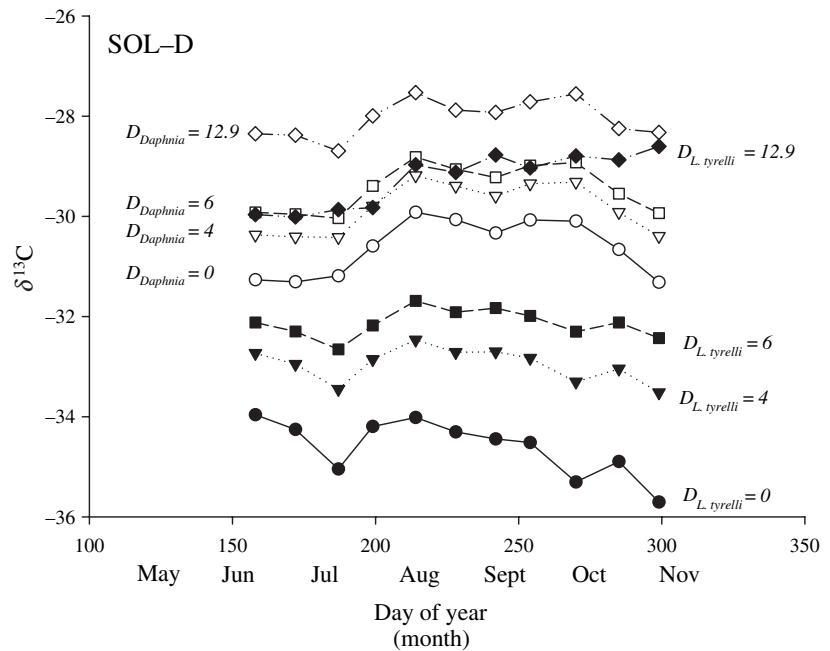


Fig. 6 Sensitivity analysis of McConnaughey & McRoy's (1979) lipid normalisation technique. D is the isotopic difference between lipids and proteins and varies from 0 to 12.9‰ (see text). Closed symbols are for *L. tyrelli*, and open symbols are for *Daphnia rosea* at SOL-D.



patterns (Pel *et al.*, 2003). Large isotopic heterogeneity at the base of the food chain complicates our ability to interpret pathways of carbon flow in the pelagic habitat of lakes.

Distinguishing dietary variation from total isotopic variation is a significant challenge for ecologists that use stable isotopes to infer consumer dietary carbon sources. France (1995) suggested that the feeding diversity of consumers can mask the hypothesised negative relationship between C : N and $\delta^{13}\text{C}$. In our

study, temporal variability in the $\delta^{13}\text{C}$ of food sources may similarly mask a relationship between C : N and $\delta^{13}\text{C}$ of zooplankton at a given lake site. Detecting a significant negative relationship between C : N and $\delta^{13}\text{C}$ in zooplankton depends on the tissue turnover time of the zooplankton taxa and the degree of isotopic variability of their food sources. This may be particularly unlikely for *Daphnia*, because it has a small seasonal range of C : N and a short generation time. The seasonal patterns of *Daphnia* C : N were

similar among all sites, but were uncorrelated with $\delta^{13}\text{C}$ at the shallower sites (SHL-S and SOL-S). The $\delta^{13}\text{C}$ of *Daphnia* was strikingly variable at SOL-S, despite *Daphnia* having a similar temporal C : N pattern as SOL-D (Pearson's $R = 0.84$). SOL is subject to water level drawdown, which results in a reduced water residence time at SOL-S (Nowlin *et al.*, 2004). We suspect that this hydrology results in large temporal variability in the $\delta^{13}\text{C}$ of *Daphnia* food sources.

The strongest evidence that C : N explains temporal variability of zooplankton $\delta^{13}\text{C}$ comes from our data for *L. tyrelli* in SOL. It is generally acknowledged that temporal variation in lipid content, which has a high C : N, can increase intraspecific or interstage variability of zooplankton C : N (Sterner & Hessen, 1994; Villar-Argaiz *et al.*, 2002). The temporal variability of C : N in *L. tyrelli* is large compared with that in *Daphnia* and *H. franciscanus*, and compared with the interspecific variation in C : N among the 24 zooplankton genera reviewed by Elser *et al.* (2000) (Fig. 1). The C : N ratio of *L. tyrelli* increased by approximately 8 over the sampling period at both sites in SOL. This seasonal range is similar to *Mysis relicta* (Leggett, 1998), but is small compared with interstage variation in the C : N ratio of the calanoid *Mixodiaptomus laciniatus* (Villar-Argaiz *et al.*, 2002). For *L. tyrelli*, the seasonal decrease of $\delta^{13}\text{C}$ concurrent with the increase in C : N, at both sites of SOL (Figs 2 & 3), supports the hypothesis that changes in lipid content explain the seasonal $\delta^{13}\text{C}$ patterns for this species. An alternate hypothesis is that the $\delta^{13}\text{C}$ of *L. tyrelli* food sources declined over the season, although neither the $\delta^{13}\text{C}$ of POM nor *Daphnia* declined over the same time period.

The negative relationship between C : N and $\delta^{13}\text{C}$ among zooplankton taxa is also consistent with the hypothesis that zooplankton with a high C : N have higher concentrations of lipids that have low $\delta^{13}\text{C}$ signatures (Fig. 5). There are several important implications of this relationship for interpreting the $\delta^{13}\text{C}$ of zooplankton. For example, an organism with a low C : N (e.g. *Daphnia*) can increase its lipid concentration (say by 10% by weight) without a substantial change in whole body C : N. A similar increase in lipid in an organism with an already high C : N (e.g. *L. tyrelli*) will result in a greater increase in C : N. This is because lipids have a higher concentration of carbon by weight than either proteins or carbohy-

drates, and the relationship between lipid concentration and C : N is an increasing nonlinear function (McConnaughey, 1978). Therefore, a consumer with a low C : N (i.e. *Daphnia*) may experience large changes in $\delta^{13}\text{C}$ despite small seasonal changes in C : N. The exact form of the relationship between zooplankton $\delta^{13}\text{C}$ and C : N will depend on the $\delta^{13}\text{C}$ of zooplankton lipids, and the relationship between lipid and C : N.

Should we normalise $\delta^{13}\text{C}$ based on C : N ratio?

McConnaughey & McRoy (1979) developed a method to normalise the $\delta^{13}\text{C}$ of an organism depending on its C : N ratio, and the average difference between lipids and other body tissues (approximately 6‰). However, normalisation of $\delta^{13}\text{C}$ only estimates the $\delta^{13}\text{C}$ of the non-lipid fraction of a consumer, and therefore excludes any dietary acquisition and storage of lipids (Goulden *et al.*, 1998).

Deciding whether to normalise the $\delta^{13}\text{C}$ of consumers depends on the question of interest. Consider the seasonal increase in the C : N of *L. tyrelli*, concurrently with a seasonal decline in its $\delta^{13}\text{C}$. This could be interpreted as a seasonal accumulation of dietary lipids that have a low $\delta^{13}\text{C}$. In this case, normalising the $\delta^{13}\text{C}$ based on C : N would exclude lipids from the dietary analysis. As food sources (POM) typically have a lower concentration of lipid than consumers, if we extracted lipids then the $\delta^{13}\text{C}$ of *L. tyrelli* might better 'match' the $\delta^{13}\text{C}$ of POM. However, as lipids are typically dietary in zooplankton, both the $\delta^{13}\text{C}$ of the lipids and the lipid-free component of zooplankton are useful for dietary analyses. In organisms where changes in lipid content reflect changes in synthesis (Chamberlain *et al.*, 2004), rather than accumulation from diet, it is more reasonable to extract lipids or normalise $\delta^{13}\text{C}$ signatures based on C : N. In general, lipid normalisation is more suitable if we are interested in a dietary analysis of a consumer's proteins and carbohydrates.

How do we normalise $\delta^{13}\text{C}$ based on C : N ratio?

If we are interested in tracing the non-lipid component of a consumer's diet then normalisation procedures may be helpful, although there are some methodological considerations. The two main assumptions of McConnaughey & McRoy's (1979) lipid normalisation technique are: (i) there is a

consistent and positive nonlinear relationship between lipid content and C : N, and (ii) there is a constant difference between the $\delta^{13}\text{C}$ of lipid and proteins/carbohydrates in the body of an organism ($D = 6\text{‰}$). There is some empirical support for the first assumption (McConnaughey, 1978; Lesage, 1999; Schmidt *et al.*, 2003), but its generality is certainly not well established. It is often assumed that temporal variation in lipid content can increase intraspecific or interstage variability of C : N because of the high C : N of lipids (Sterner & Hessen, 1994; Villar-Argaiz *et al.*, 2002), but the relationship between C : N and lipid content is rarely quantified.

The second assumption also has some empirical support, but certainly deserves further study. Parker (1964) found highly variable isotopic differences between the $\delta^{13}\text{C}$ of lipids and the bulk organism (0.5–15‰). Lipid biosynthesis discriminates against ^{13}C (Monson & Hayes, 2002), and contributes to isotopic variability among different FA (van Dongen *et al.*, 2002; Veefkind, 2003). In a size fraction of marine zooplankton, Veefkind (2003) found that the median difference between the $\delta^{13}\text{C}$ of a particular FA and bulk zooplankton varied from -1 to -9‰, but the weighted average difference (based on relative FA abundance) was approximately 6.5‰. Normalisation of $\delta^{13}\text{C}$ signatures are quite sensitive to the assumption that $D = 6\text{‰}$ (Fig. 6), so, if normalisation is used, researchers should independently measure D , and verify the relationship between lipid and C : N.

Interpreting isotopic differences between zooplankton species

Isotopic differences between species can be related to differences in feeding behaviour and differences in body composition, yet the latter is rarely considered when interpreting the $\delta^{13}\text{C}$ of pelagic zooplankton. Considering the seasonal and interspecific variation in zooplankton lipid content, may help explain the seasonal variation in the $\delta^{13}\text{C}$ of pelagic zooplankton (Grey *et al.*, 2001; Bastviken *et al.*, 2003; Pace *et al.*, 2004), and variation of zooplankton $\delta^{13}\text{C}$ among lakes (Karlsson *et al.*, 2003; Vadeboncoeur *et al.*, 2003).

Previously we suggested using *Daphnia* as a baseline to compare the isotopic signatures between zooplankton among lakes (Matthews & Mazumder, 2003). The lower $\delta^{13}\text{C}$ of *H. franciscanus* compared with *Daphnia* suggests that the $\delta^{13}\text{C}$ of *H. franciscanus*'s diet is

significantly lower than *Daphnia*. In this case, normalisation would probably change only the average $\delta^{13}\text{C}$ of each species, but have little effect on the average difference between species or their relative temporal pattern of $\delta^{13}\text{C}$. However, we cannot compare the $\delta^{13}\text{C}$ of *Daphnia* or *H. franciscanus* to *L. tyrelli* because they have different C : N ratios, and current normalisation approaches are sensitive to parameter assumptions. Resolving this challenge not only requires measuring the $\delta^{13}\text{C}$ of lipids over time, but may also require knowledge about the timing of lipid storage and utilisation in different zooplankton species.

Our data supports the hypothesis that temporal change in body composition (C : N) can significantly affect the $\delta^{13}\text{C}$ of pelagic zooplankton. As lipids are primarily dietary in pelagic zooplankton (Arts & Wainman, 1998), dietary studies of zooplankton using $\delta^{13}\text{C}$ would benefit from a more detailed consideration of lipids. In general, our work demonstrates that stable isotope analysts should carefully consider the consequences of dietary lipids in the interpretation of consumer $\delta^{13}\text{C}$.

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