Food web ecology of zooplankton communities in lakes

by

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Abstract

I used natural abundances of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to examine the food web structure of lake zooplankton communities. I focused on modeling isotopic variation with respect to trophic variation ($\delta^{15}N$) and to variation in dietary carbon sources ($\delta^{13}C$). The isotopic patterns suggest that zooplankton food webs have reticulate connections between food chains, and a large diversity of interactions between consumers and their resources.

Variation in the $\delta^{13}C$ of zooplankton depended on taxonomic identity, body composition, and habitat specialization. In Sooke Lake Reservoir, seasonal variation in the $\delta^{13}C$ of zooplankton was mainly related to variation in lipid content and the $\delta^{13}C$ of lipids. This has significant consequences for interpreting the pathways of terrestrial carbon through plankton food webs. In Council Lake, variation in the $\delta^{13}C$ of zooplankton among taxa was related to habitat specialization, and indicates taxon-specific exploitation of allochthonous resources. Using a cage experiment, I confirmed that $\delta^{13}C$ can indicate habitat specialization of zooplankton. Among lakes, my data suggest that zooplankton communities can readily exploit carbon produced below the epilimnion.

Large inter- and intra-lake variation in the $\delta^{15}N$ of zooplankton suggests significant trophic variation within zooplankton communities. In a year long study, annual averages of taxa specific $\delta^{15}N$ matched our expectations about the feeding ecology of zooplankton. However, short term variation in the $\delta^{15}N$ of herbivorous zooplankton (like *Daphnia*) was decoupled from seasonal variation in the $\delta^{15}N$ of invertebrate predators. This suggests there are multiple food chains within the plankton community (i.e. grazing chain, microbial chain), and that the strength of each food chain may vary among lakes or seasonally within a lake. This seasonal variation in the food web structure of zooplankton has significant consequences for how we model and consider the trophic position of individual fish.

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Chapter 1

Introduction

Zooplankton communities are ecologically diverse, and temporally and spatially dynamic. Recently, Leibold and Norberg (2004) proposed that zooplankton communities have the three necessary features of complex adaptive systems. First they have sustained diversity and individuality of components. In this context, zooplankton species, life stages, clones, hybrids, and individuals can act as system components. Second, interactions among these components can change in response to external processes. For example, the feeding behaviours of components in a zooplankton community respond to seasonal and spatial changes in the resource environment. Third, an autonomous process, such as competitive exclusion, can select among components as a result of species interactions. As a result, the composition of zooplankton communities changes seasonally in response to interactions among system components and the environment.

Zooplankton communities respond in various ways to changes in food resources. Along a lake resource gradient, the species composition of zooplankton may 'turnover' in response to the quantity, quality, and edibility of the available resources (Leibold et al. 1997). Tessier and Woodruff (2002b) argue that the distribution of *Daphnia* grazers among lakes reflects an adaptive match of exploitation ability with the resource environment. This adaptive match can also occur seasonally within a lake, as species composition responds to available resources. However, the rate of species turnover within a lake is likely constrained by both regional (e.g. dispersal) and local processes (e.g. species interactions) (Leibold et al. 1997). An adaptive match may also be possible if the

resident species change their feeding behaviours seasonally in response to compositional, temporal, and spatial variation in food sources (Anderson 1967, Kling et al. 1992). Large behavioural flexibility of zooplankton feeding behaviours means that the same species can exploit different resources depending on the environmental and food web context (Burns and Schallenberg 2001).

Despite the diversity of zooplankton feeding behaviours, certain common trade-offs lead to repeatable behavioural patterns in the field. *Daphnia* face a trade-off between temperature and food in a stratified water column, which can lead to vertical migration (Kessler and Lampert 2004b). *Daphnia* also face a trade-off between high food quality and low minimum resource requirements, which may explain the occurrence of efficient grazers in nutrient rich environments (Tessier and Woodruff 2002b). Copepods also face numerous trade-offs that govern their feeding behaviour and life history. For example, copepods adjust their levels of dietary carotenoids that confer resistance to parasitism, in order to decrease vulnerability to visual predators (Van Der Veen 2005). Copepods also trade-off egg production in the summer in order to survive the winter and produce eggs in time for the spring phytoplankton bloom. This could generate characteristic seasonal patterns of lipid accumulation and egg production (Arts et al. 1993; Arts et al. 1992).

Zooplankton communities, as complex adaptive systems, also face community level trade-offs that can lead to repeatable patterns of food web structure (Norberg 2004). Such tradeoffs are typically harder to study because they involve community wide processes that are difficult to experimentally manipulate (e.g. dispersal, species turnover). The adaptive capacity of a zooplankton community describes the ability of the community to maintain a certain process (e.g. grazing efficiency of primary consumers)

during environmental change. This capacity is proportional to the trait variance of the zooplankton community, which describes the distribution of traits for each species in the community (Norberg 2004). Trait variance is a simplifying way to link ecosystem function with community composition. For example, a community's grazing efficiency may be proportional to the average zooplankton body size. The adaptive capacity of a zooplankton community is proportional to a community's ability to acquire, or maintain, a composition of zooplankton that efficiently exploits the available resources. In general, the exploitation efficiency of a zooplankton community in a given lake depends on the 1) trait variance of the resident zooplankton community, 2) the trait variance in the regional pool of zooplankton, and 3) the rate of zooplankton dispersal among lakes.

The trait variance of a zooplankton community includes the amount of behavioural flexibility in the feeding behaviour of its components. In terms of exploitation efficiency, the trait variance of a single component may be larger than the difference among two other components. For example, variation in the feeding behaviour within a species of calanoid copepod may be greater than the difference in feeding behaviour between two *Daphnia* species. In this thesis, I focus on the variation in the feeding behaviour of various system components of zooplankton communities. I use stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to explore the diversity of feeding behaviours of zooplankton, and, in doing so, attempt to quantify the behavioural component of trait variance in zooplankton communities.

Thesis work

At the outset of this thesis work, I had three main questions. First, is zooplankton food web structure similar among lakes? Second, do the dominant members of

zooplankton communities maintain similar feeding behaviours over the season despite seasonal changes in the resource environment? Third, how do zooplankton communities respond to seasonal changes in the spatial distribution of resources in lakes? Algal biomass typically peaks in the metalimnion of coastal oligotrophic lakes, but it is unclear if zooplankton communities can efficiently exploit this resource (Williamson et al. 1996; Cole et al. 2002a). Therefore, the goal of this work was to examine how feeding behaviours of zooplankton change in response to seasonally and spatially variable resources (Norberg 2004).

Stable isotopes are increasingly utilized for studying zooplankton ecology. At the beginning of my thesis work (2001) only a few studies had used $\delta^{13}C$ to determine the dietary carbon sources of zooplankton (del Giorgio and France 1996; Gu et al. 1999; Leggett et al. 2000; Grey et al. 2001). Several studies had used $\delta^{15}N$ to estimate the trophic position of fish (Cabana and Rasmussen 1994; Vander Zanden et al. 1999), but only two studies had explicitly considered trophic variation of zooplankton (Kling et al. 1992; Graham 1997).

There are several reasons why stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) are useful tools for studying food webs. Differences in $\delta^{13}C$ occur at the base of the food chain based on the conditions in which primary consumers fix carbon (Riebesell et al. 2000). For example, algae are more "choosy" at high compared to low CO_2 concentration, and, therefore, algae assimilate relatively more ^{12}C than ^{13}C during photosynthesis at high CO_2 concentration. These differences in $\delta^{13}C$ at the base of the food chain can be used to indicate the proportion of carbon that contributes to the diet of a particular consumer (see Post 2002). Like carbon, there are several processes that can lead to variation in the $\delta^{15}N$

at the base of food chains (Robinson 2001). However, $\delta^{15}N$ is useful for food web studies because consumers tend to preferentially excrete ^{14}N, so the relative proportion of ^{15}N to ^{14}N increases up the food chain (termed trophic enrichment). The magnitude of trophic enrichment varies among species (Vanderklift and Ponsard 2003), but the average enrichment is typically between 2 and 4‰ (Post 2002; Vanderklift and Ponsard 2003). Together $\delta^{13}C$ and $\delta^{15}N$ are useful for determining the diet of consumers in speciose communities, and for consumers where gut content analysis is impractical (Ponsard and Arditi 2000).

In this thesis I use natural abundances of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to investigate the diversity of feeding behaviours within zooplankton communities, and how these behaviours change over the season. Specifically, I used $\delta^{13}C$ and $\delta^{15}N$ to detect 1) differences in feeding behaviour between zooplankton species (Kling et al. 1992), 2) seasonal change in food web structure of zooplankton communities, and 3) spatial differences in resource exploitation of zooplankton communities in lakes with thermally structured resource distributions (i.e. deep algal maxima).

Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were useful tools to probe the food web structure of zooplankton communities, and investigate the seasonal dynamics of zooplankton feeding interactions. Below, I outline the primary objectives, and rationale for each thesis chapter. In chapters 2 and 3, I describe the general patterns of isotopic variation among zooplankton, and then suggest ways to isolate trophic from isotopic variation (Chapter 4). In chapters 5 and 6, I refine some of the common interpretations of zooplankton $\delta^{13}C$ and develop a model that allowed me to test hypotheses about habitat specialization in

zooplankton communities. I used this model to quantify differential exploitation of allochthonous resources by various zooplankton taxa (Chapter 7).

Chapter 2: Compositional variation of $\delta^{13}C$ and $\delta^{15}N$ in zooplankton communities

The biggest challenge for stable isotope ecologists is to isolate meaningful ecological information from isotopic variation. Early studies recognized that variation in $\delta^{15}N$ among lakes was related to differences in the sources of nitrogen rather than trophic variation (Cabana and Rasmussen 1996). Therefore, to compare the trophic position of a consumer among lakes we have to account for baseline variation in $\delta^{15}N$ (Post 2002). Previous studies used a bulk zooplankton size fraction as a baseline for the pelagic habitat (Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1999). In this chapter, I test whether there is isotopic heterogeneity within zooplankton communities that might be related to trophic variation. I propose that *Daphnia* is a useful baseline consumer in isotope studies, because the $\delta^{13}C$ and $\delta^{15}N$ of *Daphnia* probably reflects the primary sources of carbon that support pelagic food chains.

Chapter 3: Modeling trophic position of fish using zooplankton $\delta^{15}N$

In this chapter, I compiled all the available literature regarding seasonal patterns of zooplankton $\delta^{15}N$. I found large seasonal variation in the $\delta^{15}N$ of zooplankton that is clearly unrelated to seasonal changes in trophic position. Seasonal variation of *Daphnia* $\delta^{15}N$ depends on seasonal variation in the $\delta^{15}N$ of its food sources, which, in turn, depends on the biogeochemical processes involved in nitrogen cycling (Lehmann et al. 2004). What are the consequences of this baseline variation at the bottom of the food chain for estimating the trophic position of fish? If baseline variation is small, then a

seasonal average of *Daphnia* $\delta^{15}N$ is sufficient for estimating the trophic position of fish. However, large variation in the $\delta^{15}N$ of *Daphnia* could contribute to isotopic variation among individual fish that grow at different rates. I use a model of juvenile sockeye growth coupled with time series of zooplankton $\delta^{15}N$ to examine if variation in $\delta^{15}N$ among individual fish is related to trophic variation.

Chapter 4: Trophic variation in zooplankton communities

In chapter 2, I compared the $\delta^{15}N$ of different zooplankton taxa among lakes, and found that copepods had a consistently higher $\delta^{15}N$ than *Daphnia* or *Holopedium*. In chapter 4, I test whether these isotopic differences vary seasonally for zooplankton taxa with well known feeding behaviours. I chose Council Lake for this study because it had two predominant herbivores (*Holopedium* and *Daphnia*), two invertebrate predators (*Chaoborus, Epischura*), and a presumed omnivore (*Leptodiaptomus tyrelli*). In this year long study, I measured the $\delta^{15}N$ of the dominant members of the zooplankton community and their putative food sources. My goal was to detect temporal variation in zooplankton community structure using year long time series of zooplankton $\delta^{15}N$.

Chapter 5: Temporal variation in zooplankton C:N and $\delta^{13}C$

In this chapter, I propose that the body composition of zooplankton significantly affects how we interpret zooplankton $\delta^{13}C$. Lipids have a low $\delta^{13}C$ due to discrimination against ^{13}C during lipid biosynthesis (DeNiro and Epstein 1977). In lakes, lipid is primarily synthesized by algae and then transferred to zooplankton (Arts and Wainman 1998). There is little modification of the $\delta^{13}C$ of lipids during the uptake and assimilation by zooplankton (Grice et al. 1998). Given that lipids are primarily dietary in freshwater

zooplankton (Arts and Wainman 1998), the $\delta^{13}C$ of lipid is useful for dietary analyses. This chapter was an attempt to relate variation in zooplankton lipid content (indicated by C:N ratio) with the $\delta^{13}C$ of zooplankton. I argue that lipid normalization of $\delta^{13}C$ (to a lipid level of zero) excludes important dietary information about the sources of carbon that fuel pelagic production.

Chapter 6: Stoichiometry of carbon stable isotopes in zooplankton

In chapter 5, I empirically addressed the relationship between zooplankton C:N and $\delta^{13}C$. The negative relationship between zooplankton C:N (an indicator of lipid content) and $\delta^{13}C$ provided clear evidence that lipids are important for interpreting the $\delta^{13}C$ of zooplankton. In chapter 6, I further test this hypothesis. I developed a stoichiometric model to explain the form of the relationship between lipid content and zooplankton (Fig. 6.2), and the negative relationship between zooplankton C:N and $\delta^{13}C$ (Fig. 6.4). I parameterized the model using a year long study from Sooke Lake Reservoir, and then used the model to fit time series data of zooplankton $\delta^{13}C$ from two other lakes of contrasting productivity.

Chapter 7: Habitat specialization and allochthony of zooplankton

In this final chapter, I found that zooplankton specialize in their choice of feeding habitat in a water column where allochthonous and autochthonous resources vary with depth. In Council Lake, algal biomass peaks in the hypolimnion (Davies et al. 2004b), but do zooplankton exploit this resource? I used the $\delta^{13}C$ of zooplankton and POM to test if zooplankton differentially exploit hypolimnetic resources. Using a cage experiment, I

tested whether differences in the $\delta^{13}C$ among zooplankton taxa reflect habitat specialization or selective feeding on algae in the epilimnion (Grey et al. 2001).

Chapter 2: Compositional and interlake variability of zooplankton affect baseline stable isotope signatures

Citation:

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Abstract

Zooplankton are commonly used to establish a baseline isotopic signature for pelagic production in lakes. Our objective was to evaluate this approach by quantifying among- and within-lake variability of δ^{13}Cand δ^{15}N for different taxa of pelagic zooplankton. We measured the δ^{13}C and δ^{15}N of *Daphnia*, *Holopedium*, and calanoid copepods from four lakes sampled from June to November 2001, and from eight additional lakes sampled once in midsummer. In the four lakes with temporal sampling, within-lake differences due to taxonomic grouping accounted for 36.7% of the variance in δ^{15}N and 41.7% of the variance in δ^{13}C. Among all lakes, the δ^{15}N of calanoid copepods was on average 2.55‰, and 2.44‰ higher than *Daphnia* or *Holopedium*, respectively, whereas the δ^{13}C of calanoid copepods was 2.19‰ and 2.23‰ lower than *Daphnia* or *Holopedium*, respectively. If ^{15}N fractionation is similar among species, the differences in δ^{15}N suggest that calanoid copepods either feed at a higher trophic position in the food web, or they have a consistently higher baseline δ^{15}N signature than *Daphnia* or *Holopedium* among lakes. Differences in δ^{13}C suggest that zooplankton taxa in the pelagia of lakes have different food sources. We conclude that species composition and feeding behaviours of the zooplankton community should be considered before making among lake comparisons of food web structure. We show that *Daphnia* is a useful isotopic baseline for organisms that rely on primary production in lakes.

Introduction

Aquatic ecologists frequently use stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) to measure the food source and trophic position of aquatic consumers, and to make inferences about food web structure (Kling et al. 1992; Cabana and Rasmussen 1994; Vander Zanden et al. 1999; Post 2002). Comparisons of food web structure among and within ecosystems rely on a baseline isotopic signature for each system. Without an ecosystem specific isotopic baseline, results do not account for the large among system variations in the $\delta^{15}N$ and $\delta^{13}C$ at the base of food webs (Rounick and Winterbourn 1986; Cabana and Rasmussen 1996; Lake et al. 2001). Recent multiple lake food web analyses used primary consumers, including pelagic zooplankton, as an isotopic baseline for other members of the lake community (Kling et al. 1992; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1999; Post 2002). Each of these studies used a slightly different method of baseline correction that was tailored to address a question of specific ecological interest. Each method, except Kling et al.'s (1992), used a size fraction of pelagic zooplankton to develop the baseline (Vander Zanden and Rasmussen 1999; Post 2002), or as part of the baseline itself (Cabana and Rasmussen 1994).

The substantive goal of a baseline is to reflect the isotopic signature of the primary source of production for the food web (Cabana and Rasmussen 1994; Post 2002). However, choosing and finding an appropriate baseline depends on the spatial and temporal context of the ecological question under consideration. For example, Cabana and Rasmussen (1994) modeled food chain length using one size fraction of zooplankton (< 250μm) as the baseline $\delta^{15}N$ signature to estimate the trophic position of invertebrate predators, planktivorous fish, and piscivorous fish. A zooplankton size fraction was

chosen as a baseline because isotopic signatures of primary consumers are less temporally and spatially variable than primary producers (Cabana and Rasmussen 1996). However, this method assumes that the mixture of different zooplankton taxa within a given size fraction is representative of the $\delta^{13}C$ and $\delta^{15}N$ of the primary food source for the pelagic food web.

An alternative to using a zooplankton size fraction would be to use a single taxonomic grouping, with a known feeding behaviour, as an isotopic baseline for production in the habitat where it feeds (*sensu* Kling et al. 1992; Post 2002). For example, Kling et al. (1992) used a copepod that was known to be herbivorous as a baseline for an omnivorous copepod. This scenario was ideal because both species, the baseline and the omnivore, were biologically related and likely had similar temporal and spatial integration of food source isotopic signatures. In other cases, researchers have used mussels as a pelagic indicator species to compare among sampling sites, and to correct for among system variation in baseline isotopic signatures (Fry 1999; McKinney et al. 1999; Lake et al. 2001; Post 2002). A recent study by Post (2002) indicated that mussels and snails reflect the isotopic signatures of the pelagic and littoral environment, respectively. In his study, the $\delta^{13}C$ of mussels was not statistically different from the median of a time series for a size fraction (>150μm) of bulk pelagic zooplankton (Post 2002).

Previous attempts to establish a lake-specific isotopic baseline using pelagic zooplankton (Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1999; Post 2002) relied in part on size fractions of total zooplankton, and did not address possible isotopic differences due to species composition. Within a lake, different taxa of pelagic

zooplankton can have substantially different $\delta^{15}N$ and $\delta^{13}C$ signatures (Kling et al. 1992; Grey and Jones 1999). Among taxa, variability depends not only on foraging behaviour and trophic interactions (Kling et al. 1992; Meili et al. 1996; Grey and Jones 1999; Jones et al. 1999; Grey and Jones 2001), but may also depend on taxon-specific baseline $\delta^{13}C$ and $\delta^{15}N$ signatures. Regardless of the cause of variation, significant heterogeneity of stable isotopic signatures within a zooplankton size fraction may lead to bias in multiple lake food web comparisons.

Since the pioneering study by Kling et al. (1992), no study has measured the $\delta^{13}C$ and $\delta^{15}N$ of different pelagic zooplankton taxa in multiple lakes to determine if there are consistent interspecific isotopic differences within and among lakes. The goal of this study is to provide better information on the isotopic signatures of zooplankton in order to advance our understanding of baseline determination in the pelagia of lakes. To this end, we quantify among and within lake variation in the isotopic signatures of the dominant taxonomic groups of pelagic zooplankton communities for 12 coastal lakes in British Columbia. We also discuss factors that may affect the $\delta^{13}C$ and $\delta^{15}N$ of different zooplankton taxa, and suggest when *Daphnia* is an appropriate isotopic baseline for organisms that rely on pelagic production.

Methods

Zooplankton collection and analysis

We collected zooplankton samples every two or three weeks from June to November 2001 from four lakes in the Greater Victoria region in British Columbia: Council Lake (COL), Elk Lake (ELL), Sooke Lake Reservoir (SOL), and Shawnigan

Lake (SHL). We also sampled another eight lakes once during the sampling period. Zooplankton were collected with a 64 μm mesh, 50 cm diameter Wisconsin net from the entire water column or to a maximum depth of 30 m. Zooplankton were left overnight at 4°C in filtered lake water (GF F) or deionized water to evacuate gut contents. Within 24 hours of collection we sorted live zooplankton into three categories, calanoid copepods (C), *Daphnia* (D), or *Holopedium* (H), and dried each sample at 60°C. For isotopic analysis, our goal was to get ~1mg of zooplankton tissue for each sample. Samples consisted of approximately 40-80 *Daphnia*, 80-150 calanoid copepods, or 20-50 *Holopedium*. Each sample of calanoid copepods was a mixture of adult and late stage calanoid copepodids, and had a mean body size of >1mm. Five lakes had all three taxa in sufficient abundance for isotopic analysis, three lakes had only calanoid copepods and *Daphnia*, two lakes had only calanoid copepods and *Holopedium*, and two lakes had only *Daphnia* and *Holopedium* (Table 2.1). Samples were analyzed at the University of Waterloo Environmental Isotope Laboratory (Waterloo, Ontario, Canada) on an Isochrom Continuous Flow Stable Isotope Ratio Mass Spectrometer coupled to a Carlo Erba Elemental Analyzer. The precision for both $\delta^{13}C$ and $\delta^{15}N$ was <0.1‰. The samples were analyzed for $\delta^{13}C$, $\delta^{15}N$, percent carbon, and percent nitrogen. The carbon to nitrogen ratio (C:N) is reported as a molar ratio.

The handling time of invertebrates, while unpreserved, can have a significant effect on both the $\delta^{13}C$ and the $\delta^{15}N$ within 24 hours of sample collection (Kaehler and Pakhomov 2001). To test for an effect of sample handling, we compared the $\delta^{13}C$ and $\delta^{15}N$ of *Daphnia* left overnight in filtered lake water to *Daphnia* frozen within four hours of collection. We found no effect of sample handling in any of the three lakes we tested,

for either $\delta^{13}C$ (ELL: $F_{1,4} = 0.044$, $p = 0.844$; SHL: $F_{1,12} = 3.383$, $p = 0.091$; SOL: $F_{1,11} = 1.795$, $p = 0.207$) or $\delta^{15}N$ (ELL: $F_{1,4} = 0.035$, $p = 0.862$; SHL: $F_{1,12} = 2.87$, $p = 0.116$; SOL: $F_{1,11} = 0.557$, $p = 0.471$).

Statistical Analysis

We used a random effects nested ANOVA to apportion variance of $\delta^{15}N$, $\delta^{13}C$ and C:N ratio of *Daphnia*, *Holopedium,* and calanoid copepods to among and within lake differences. We used variance components from this analysis to compare the relative variability of our dependent variables ($\delta^{15}N$, $\delta^{13}C$, and C:N ratio) among and within lakes. This approach allowed us to quantify how much of the total variance is explained by among lake differences (a lake effect), compared to the variance explained by within lake differences due to taxonomic grouping (a taxa effect). For this hierarchical analysis, the taxon effect is nested within the main lake effect.

The null expectation of our nested ANOVA was that neither the lake effect, nor the effect of taxon nested within lakes would account for a significant component of the total variance in $\delta^{15}N$, $\delta^{13}C$ or the C:N ratio of zooplankton. Rejecting the null hypothesis for the lake effect would support the need for baseline correction of isotopic signatures among lakes. Rejecting the null hypothesis for the taxon effect would indicate that zooplankton community composition should be considered for baseline determination. We included C:N ratio in the analysis because it does not vary much among lakes, and thus, provided a good reference variable to compare with $\delta^{13}C$ and $\delta^{15}N$.

We performed this analysis for Council Lake, Elk Lake, Sooke Lake Reservoir, and Shawnigan Lake, which were the four lakes we sampled multiple times. Council and Shawnigan Lake both had *Daphnia*, *Holopedium,* and calanoid copepods, whereas Sooke

and Elk Lake had only *Daphnia* and calanoid copepods (Table 2.1). Due to the unbalanced nature of zooplankton community composition in these study lakes, our nested ANOVA was unbalanced, with 4 lakes as part of the main lake effect, and 2 or 3 zooplankton taxa making up the taxa effect nested within each lake. To account for this unbalanced analysis, we used the recommended residual maximum-likelihood method to estimate variance components (Robinson 1987; Rusak et al. 2002).

We tested for isotopic homogeneity of zooplankton $\delta^{15}N$ and $\delta^{13}C$ as per the method of Ponsard and Arditi (2000): $\delta_{taxa} = \delta_{base} + \Delta \pm \sigma_{\Delta}$, where σ_{Δ} is the standard deviation of the isotopic enrichment (Δ) for multiple species. If the ratio of the observed group variance (σ_g^2) to the variance in fractionation (σ_{Δ}^2) was significantly greater than 1 (using a one tailed *F*-test), then we rejected the null hypothesis of isotopic homogeneity for that group. The values of σ_{Δ}^2 ($\sigma_{\Delta N}^2$=0.98, *n*=56; $\sigma_{\Delta C}^2$=1.3, *n*=107) from Post (2002), when used for a single taxon of zooplankton, are conservative estimates of variation in trophic enrichment. By using this test for a single taxon over time, we are not testing homogeneity of diet, as did Ponsard and Arditi (2000), but rather temporal homogeneity of isotopic variance resulting from changes in diet and baselines. For example, if a group with calanoids and *Daphnia* has a significantly higher σ_g^2 than $\sigma_{\Delta N}^2$, either taxa have different trophic positions but share a common baseline, or taxa have different baselines and differences in $\delta^{15}N$ are not solely a result of trophic variation. Although we did not, and perhaps could not, measure δ_{base} for each taxon, this approach allowed us to determine if isotopic variation within a group is larger than we would expect based on variable fractionation from a common baseline signature.

We examined among-lake patterns of within-lake differences in the isotopic signatures of *Daphnia*, *Holopedium,* and calanoid copepods. For each lake our dependent variable was the difference between the $\delta^{15}N$ or $\delta^{13}C$ of two zooplankton taxa present in that lake. From our twelve study lakes, we used eight to compare *Daphnia*-calanoid copepod isotope signatures, seven to compare *Holopedium*-calanoid isotope signatures, and seven to compare *Daphnia*-*Holopedium* isotope signatures. This approach allowed us to quantify within lake variability of zooplankton taxa, independent of among lake differences in isotopic baselines.

Results

Among and within lake variability of $\delta^{13}C$ and $\delta^{15}N$

In our nested ANOVA the lake effect accounted for 56.4% of the total variance in $\delta^{15}N$, but did not account for a significant component of the variation in $\delta^{13}C$ or C:N ratio (Table 2.2). We found a significant taxa effect for $\delta^{13}C$ ($p < 0.001$), $\delta^{15}N$ ($p < 0.001$), and C:N ratio ($p < 0.001$) (Table 2.2). Within lake differences due to taxonomic groupings accounted for 36.7% of the variance in $\delta^{15}N$, 41.7% of the variance in $\delta^{13}C$, and 62.5% of the variance in C:N ratio (Table 2.2).

The nested ANOVA indicated isotopic differences among taxa within zooplankton communities. To identify the source of these differences for Council Lake (COL), Elk Lake (ELL), Sooke Lake Reservoir (SOL), and Shawnigan Lake (SHL) we ran a one-way ANOVA and Tukey's post-hoc tests for each lake (Table 2.3). In all four lakes, calanoid copepods had the highest mean $\delta^{15}N$. However, in COL, the mean $\delta^{15}N$ of *Daphnia* and calanoid copepods were not statistically different. The $\delta^{13}C$ of calanoid

copepods was significantly lower than $\delta^{13}C$ of *Daphnia* or *Holopedium* in SHL and SOL, but was not significantly different from *Daphnia* in COL or ELL. Seasonal variation in $\delta^{13}C$ was typically small (<3‰) for individual taxa within a lake (Table 2.4). Only the calanoids in SOL showed a clear temporal trend. In this case, the $\delta^{13}C$ declined from -34.0‰ to -35.7‰ throughout the sampling season. Seasonal changes in $\delta^{15}N$ was variable among and within species. The $\delta^{15}N$ of calanoids increased from June to November in both SOL (4.7-6.6‰) and SHL (7.8-10.7‰). Over the same time period the $\delta^{15}N$ of *Daphnia* increased in SOL (2.1-3.5‰) and SHL (4.9-7.4‰). The $\delta^{15}N$ of *Daphnia* and calanoids in Council Lake was lowest in June (3.4‰ for both taxa), increased to a maximum in July (~5.5‰ for both taxa), and then declined through to September (Table 2.4). The difference in $\delta^{15}N$ between *Daphnia* and calanoids was seasonally variable in Elk Lake, but the $\delta^{15}N$ of calanoids was higher than *Daphnia* for all sampling dates.

Homogeneity of temporal isotopic variance

The temporal variation in $\delta^{15}N$ of individual taxon was small and always significantly less than interspecific variance in trophic fractionation (Table 2.4). In groups with multiple taxa, we rejected the null hypothesis of isotopic homogeneity for three out of four lakes. In all such groupings, variation in $\delta^{15}N$ was more heterogeneous than would be expected by variability in Δ_N alone. For $\delta^{13}C$, the temporal variance of individual taxon was small in all cases except Elk Lake (Table 2.4). We only rejected the hypothesis of homogeneity of $\delta^{13}C$, for groups of zooplankton in Elk Lake and Sooke Lake Reservoir.

Interlake comparisons of taxa specific differences

To place our observations of within-lake differences into a larger regional context, we compared the results from Council Lake, Elk Lake, Sooke Lake Reservoir, and Shawnigan Lake to eight other lakes in the Victoria region. In all cases where calanoid copepods were present, they had a higher mean $\delta^{15}N$ than either *Daphnia* or *Holopedium*, and in all but two lakes, calanoid copepods had a lower $\delta^{13}C$ than *Daphnia* or *Holopedium* (Fig. 2.2a). For the eight lakes where calanoid copepods and *Daphnia* occurred together, the $\delta^{15}N$ of calanoid copepods was on average 2.55‰ higher (t = 5.817, df = 7, $p < 0.001$), and $\delta^{13}C$ was on average 2.19‰ lower (t = 3.94, df = 7, p = 0.006) than *Daphnia* $\delta^{15}N$ and $\delta^{13}C$ signatures respectively (Fig. 2.3). In the seven lakes where calanoid copepods and *Holopedium* occurred together, the $\delta^{15}N$ of calanoid copepods was 2.44‰ higher (t = 11.51, df = 6, $p < 0.001$), and the $\delta^{13}C$ was 2.23‰ lower (t = 2.92, df = 6, p = 0.027) than $\delta^{15}N$ and $\delta^{13}C$ of *Holopedium,* respectively (Fig. 2.3). The mean among lake difference between the $\delta^{15}N$ or $\delta^{13}C$ of *Daphnia* and *Holopedium* was not significantly different from zero ($\delta^{15}N$: t = -0.49, df = 6, p = 0.644; $\delta^{13}C$: t = -0.467, df = 6, p = 0.657) (Fig. 2.3).

Discussion

Patterns of zooplankton $\delta^{13}C$ and $\delta^{15}N$ among lakes

The results of our nested ANOVA confirm the need for among-system baseline correction of $\delta^{15}N$, as previously stressed by several studies (Cabana and Rasmussen 1996; McKinney et al. 1999; Vander Zanden et al. 1999; Post 2002). 56.4% of the total

variance in $\delta^{15}N$ of zooplankton was accounted for by differences among lakes. In our study lakes, zooplankton $\delta^{15}N$ varied from 0.72‰ for *Daphnia* in Coquitlam Reservoir to 13.00‰ for calanoid copepods in Elk Lake. This is a large range for basal taxa of the food web, but is comparable to previously reported variability in $\delta^{15}N$ signatures among lakes (Cabana and Rasmussen 1996; Vander Zanden et al. 1999; Lake et al. 2001; Post 2002). In our study, the large among lake variation of individual zooplankton species, and of particulate organic matter (<41μm) (unpublished data), is likely related to anthropogenic activity in the watersheds of our study lakes, as previously suggested by other studies (Cabana and Rasmussen 1996; McKinney et al 1999). However, our study is the first to quantify the importance of zooplankton species composition to the determination of $\delta^{15}N$ baselines. In our dataset, within lake differences in zooplankton taxa accounted for 36.7% of the total variance in $\delta^{15}N$, indicating taxon specific isotopic signatures within zooplankton communities.

Unlike for $\delta^{15}N$ we found no significant among-lake differences in zooplankton $\delta^{13}C$, but found large and significant within-lake differences among zooplankton taxa. Only 17% of the total variance in $\delta^{13}C$ was attributed to among lake variability, whereas 41.7% was accounted for by taxonomic grouping. Though our data provide no support for among lake baseline correction of $\delta^{13}C$, our range in $\delta^{13}C$ of zooplankton among four lakes is small compared to studies with more lakes (France et al. 1997; Grey et al. 2000; Post 2002). Even among the ten lakes where *Daphnia* was present (Table 2.1), the $\delta^{13}C$ only varied from –34.4‰ to –30.3‰, whereas *Daphnia middendorfiana* varied from –44.7‰ to –31.5‰ over a single year in an Alaskan lake (Gu et al. 1999). However, our results show large within-lake variability of $\delta^{13}C$ signatures among zooplankton taxa

(Fig. 2.1). The range of $\delta^{13}C$ within a lake varies from 1.75‰ in Elk Lake to 3.98‰ in Sooke Lake Reservoir (Table 2.3). The magnitude of this variation is not surprising, given previous studies of within lake variation in the $\delta^{13}C$ of different zooplankton taxa (Grey and Jones 1999) and zooplankton size fractions (Post 2002). However, it highlights the importance of zooplankton taxonomic composition in determining $\delta^{13}C$ baselines.

Comparing temporal variance to variance in trophic enrichment

$\delta^{15}N$ values - In the four lakes with temporal sampling, the temporal variance of a single taxon was significantly less than the interspecific variance of Δ_N. This suggests that either ${\sigma_{\Delta N}}^2$ is overestimated in the literature (Post 2002), or intra-taxon temporal variation, resulting from changes in diet, baseline, and fractionation, is small relative to ${\sigma_{\Delta N}}^2$. Improved taxon specific estimates of Δ_N would clearly increase the sensitivity of this approach. In three of four lakes, groups with multiple taxa showed isotopic heterogeneity of $\delta^{15}N$, suggesting that the variation in $\delta^{15}N$ is significantly larger than we would expect from the interspecific variance of Δ_N. This heterogeneity could result from either taxon specific baseline $\delta^{15}N$ signatures, or different trophic positions on a common baseline.

$\delta^{13}C$ values - We found few cases where the temporal variation of a single taxon's $\delta^{13}C$ signatures was significantly larger than interspecific variation in Δ_C. In contrast to the results of Ponsard and Arditi (2000), some of our groupings with multiple taxa showed isotopic heterogeneity. The $\delta^{13}C$ of *Daphnia* and calanoid copepods in Elk Lake are highly variable over time (Table 2.4), and ${\sigma_g}^2$ is significantly greater than ${\sigma_{\Delta C}}^2$. The

high temporal variance in Elk Lake zooplankton has interesting implications for baseline determination. Either both taxa have a radically different feeding behaviour in Elk Lake, compared to other lakes with the same $\delta^{13}C$ baseline, or they have a similar feeding behaviour and Elk Lake has a variable $\delta^{13}C$ baseline. Given the low temporal variability of these same taxa in Shawnigan Lake (Table 2.4), it is more likely that Elk Lake has a seasonally variable baseline.

Given our data, we cannot determine whether among taxa variability is a result of taxon specific baselines, or variability in feeding behaviour. For example, the differences in $\delta^{15}N$ between *Daphnia* and calanoids could be due to different trophic positions, different fractionations from a common baseline, or different baselines. To help discriminate between these possibilities we consider factors that can affect the within lake variability of $\delta^{15}N$ and $\delta^{13}C$ of different zooplankton taxa.

Explaining $\delta^{15}N$ in terms of trophic variation

Trophic food chain models often consider zooplankton communities as a single trophic level of herbivores connecting algae to fish. However, previous studies have shown that both calanoid copepods and *Daphnia* are omnivores, feeding on non-photosynthetic prey including bacteria, ciliates, heterotrophic nanoflagellates (HNF), rotifers, and other micro-zooplankton (Paul et al. 1995; Sanders et al. 1996; Burns and Shallenberg 2001). Species of both *Daphnia* and calanoid copepods have higher survival and reproductive capability when their algal diet is supplemented with heterotrophic organisms (Williamson and Butler 1986; Sanders et al. 1996). *Daphnia* and calanoids both consume HNF, but calanoids are more effective grazers of HNF, particularly under eutrophic conditions (Burns and Shallenberg 2001). From these previous studies, we

know that both *Daphnia* and calanoid copepods can be omnivorous, but to what extent likely depends on lake conditions.

In our study, we used $\delta^{15}N$ to quantify the relative difference between feeding behaviours of different zooplankton taxa. Without isotopic measurements of the algae, protozoans, or rotifers, we have no evidence for what proportion of a zooplankton's diet is algal versus heterotrophic in origin. However, we hypothesize that the among taxa variation in $\delta^{15}N$ is partially a result of calanoid copepods feeding higher in the food chain than the predominantly herbivorous *Daphnia* or *Holopedium* taxa. Among all study lakes, the difference in $\delta^{15}N$ between calanoid copepods and *Daphnia* or *Holopedium* was 2.55 ‰ and 2.44‰, respectively (Fig. 2.3). If we assume an average trophic enrichment of 3.4‰ (Minagawa and Wada 1984; Post 2002), and assume that *Daphnia* and calanoids have the same baseline $\delta^{15}N$, then calanoids are an average of 0.74 (se=0.13, *n*=8) trophic levels higher than *Daphnia*. However, this estimate of trophic variation is confounded by possible differences in taxon specific $\delta^{15}N$ baselines, or differences in fractionation from a common baseline.

Explanations for variation in zooplankton $\delta^{13}C$

In ten of the twelve study lakes, the $\delta^{13}C$ of calanoid copepods was lower than *Daphnia* or *Holopedium* (Fig. 2.2a), resulting in a mean difference (2.0‰) that is significantly greater than zero (Fig. 2.3b). There are two likely explanations for why calanoid copepods were depleted in ^{13}C relative to *Daphnia* or *Holopedium*. First, lipids are isotopically lighter than other body constituents (Tieszen et al. 1983; Kling et al. 1992), and since copepods can store more lipids than other zooplankton taxa (Arts et al.

1993), they may generally have lower $\delta^{13}C$ signatures. Second, calanoid copepods may be feeding on a food source with a lower $\delta^{13}C$ than other zooplankton, either by feeding more selectively or by feeding deeper in a lake than either *Daphnia* or *Holopedium*.

Lipids - The effect of lipids on the $\delta^{13}C$ signature of different zooplankton taxa and the corresponding effects on baseline $\delta^{13}C$ determination are unclear from the current literature. Some studies show that lipids can affect the $\delta^{13}C$ of zooplankton (Kling et al. 1992; Leggett 1998), while others report no significant effect (France 1995; Campbell et al. 2000). Considering the maximum effect of lipids on $\delta^{13}C$ may help bound the interpretation of our data. If lipids in calanoid copepods are ~5‰ depleted from other body tissue (Kling et al 1992), and represent upwards of 65% of the body tissue (maximum estimate from Arts et al. 1993) then lipids could cause a depletion in calanoid copepods of up to 3.25‰. In our study, the difference between the $\delta^{13}C$ of calanoid copepods and *Daphnia* or *Holopedium*, is an average of 2.0‰ with differences as high as 4.4‰ (Fig. 2.3). Therefore, if lipids have no effect on *Daphnia* or *Holopedium* $\delta^{13}C$, the maximum effect of lipids on calanoid copepods cannot fully account for the range in among taxa $\delta^{13}C$ variability. Rather, the feeding behaviour of calanoid copepods, either by selective feeding, or by feeding deeper in the water column than *Daphnia* or *Holopedium*, likely contributes to the discrepancy in $\delta^{13}C$ signatures among zooplankton taxa.

Selective feeding – Among-taxa variability in $\delta^{13}C$ (Grey and Jones 1999; our study) suggests that a single size fraction of POM is not a suitable baseline for the $\delta^{13}C$ of multiple zooplankton taxa. Several studies have shown that the $\delta^{13}C$ of zooplankton is isotopically lighter than size fractions of POM (Kling et al. 1992; Zohary et al. 1994; del

Giorgio and France 1996; Meili et al. 1996; Jones et al. 1999; Grey et al. 2000), and some have attributed these differences to the selective feeding behaviour of zooplankton (Del Giorgio and France 1996; Meili et al. 1996; Jones et al. 1999). POM is a mixture of algae, detritus (allochthonous or autochthonous in origin), bacteria, and small planktonic organisms. Different components within POM can have different $\delta^{13}C$ signatures (Leggett 1998). For example, a more enriched terrestrial $\delta^{13}C$ signature may mask a lighter algal $\delta^{13}C$ signature, especially in lakes with large allochthonous carbon input (Meili et al. 1996; Jones et al. 1999; Grey and Jones 2001). In our study, the $\delta^{13}C$ of POM (<41μm) varied among lakes in step with zooplankton $\delta^{13}C$ (unpublished data). However, since the $\delta^{13}C$ of a single size fraction of POM does not reflect the $\delta^{13}C$ of different zooplankton taxa, it may not reflect the $\delta^{13}C$ of the primary food source that fuels upper trophic levels.

Feeding depth - The $\delta^{13}C$ signature of zooplankton, and how we establish a pelagic baseline, may depend on where different zooplankton taxa feed in a thermally stratified water column. Respired carbon from heterotrophic metabolism is isotopically lighter than the consumed carbon source (Rau 1978), and in clearwater lakes, high epilimnetic respiration relative to production, combined with hypolimnetic metabolism can result in a depletion of the $\delta^{13}C$ of DIC (France et al. 1997). During the period of thermal stratification in lakes, a vertical gradient in the $\delta^{13}C$ of DIC could lead to a vertical gradient in the $\delta^{13}C$ of POM. Many of our study lakes are clearwater systems (mean DOC of 2.9 mgC L^{-1}), and have metalimnetic Chl *a* maxima throughout much of the summer. In addition, the $\delta^{13}C$ signature of metalimnetic POM (<41um) in our study lakes is depleted by >1 ‰ relative to epilimnetic POM (unpublished data), as commonly

reported in other studies (Del Giorgio and France 1996, France et al. 1997). If taxa are feeding at different depths in the water column this could affect baseline $\delta^{13}C$ determination of zooplankton. Unfortunately, we have not quantified the vertical feeding behaviour of each taxon in each of our lakes. However, in Council Lake, a fishless lake with abundant invertebrate predation, the *Daphnia* and *Holopedium* populations have peak abundances (both day and night) in the hypo- and epilimnion respectively (unpublished data). In this lake, the $\delta^{13}C$ of *Daphnia* is significantly lower than the $\delta^{13}C$ of *Holopedium* (Table 2.3). Though perhaps an isolated case, feeding depth could differentially affect the $\delta^{13}C$ of zooplankton taxa and complicate baseline determination.

A combination of lipids, selective feeding, and vertical feeding behaviour in a stratified water column likely influences the $\delta^{13}C$ of zooplankton in our study lakes. However, these competing hypotheses cannot be resolved with the current data; therefore, we cannot quantify the relative magnitudes of the effect of lipids, depth, and selective feeding on the $\delta^{13}C$ of different zooplankton taxa, and the combined effects on baseline $\delta^{13}C$ determination.

Isolating trophic variation from baseline variation

The temporal heterogeneity of $\delta^{15}N$ in a group of zooplankton taxa can result from different feeding behaviours (namely trophic position) and different baselines. Isotopic homogeneity of $\delta^{15}N$ for individual taxa suggests that feeding behaviours coupled with temporal baselines can be relatively stable within a lake. Isotopic heterogeneity of $\delta^{15}N$ for groups of zooplankton suggests that differences among taxa are greater than expected based only on variable fractionation. If this heterogeneity was

solely a result of different isotopic baselines, then among lakes we might expect random variation in the difference between the $\delta^{15}N$ of *Daphnia* and calanoids. However, we found that the $\delta^{15}N$ of calanoid copepods is higher than *Daphnia* in all eight lakes where they co-occurred (Fig. 2.3). Either the baselines are consistently higher for calanoids among lakes, or the variation of within lake taxon specific baseline $\delta^{15}N$ is small compared to trophic variation among taxa. It is also possible that *Daphnia* and calanoids share a common baseline, but calanoids have a larger ^{15}N fractionation factor. Though we cannot explicitly quantify among lake trophic variation due to unknown differences in baselines and fractionation, our data is consistent with the hypothesis that calanoids feed at a higher trophic position than *Daphnia* or *Holopedium*.

Elk Lake (ELL) presents an interesting case where alternate $\delta^{15}N$ baselines may exist for *Daphnia* and calanoid copepods. The average difference in $\delta^{15}N$ between *Daphnia* and calanoid copepods is 4.83‰, but this difference is seasonally variable (range=1.63-6.2‰). The $\delta^{15}N$ of calanoids in ELL follows a similar temporal pattern as in SOL and SHL, but the $\delta^{15}N$ of *Daphnia* in Elk Lake changes dramatically over the season in response to variable nitrogen dynamics, and changes in algal species composition (unpublished data). At this point, we cannot discriminate between feeding behaviour and differences in taxon specific isotopic baselines, however several lines of evidence suggest that both are important in determining the $\delta^{13}C$ and $\delta^{15}N$ baselines for pelagic production in lakes, and in some cases a single taxon may be a more appropriate baseline for the pelagia of lakes.

Daphnia as an isotopic baseline among and within lakes

Several studies use bulk zooplankton to establish a baseline for the pelagia of lakes (Cabana and Rasmussen 1994; Post 2002). Using bulk zooplankton is methodologically simple, but is only suitable if the mixture of taxa reflects the $\delta^{13}C$ and $\delta^{15}N$ of the primary food source in the pelagia. If fish feed non-selectively on a similar size class of zooplankton that is also collected as the baseline, then the isotopic signature of a bulk zooplankton size fraction could accurately reflect fish diet. However, if fish feed selectively by zooplankton taxa, and taxa are isotopically distinct, then using a bulk size fraction of zooplankton could introduce error into the estimate of fish trophic position. To estimate the maximum amount of this error, we calculated a range of zooplankton $\delta^{15}N$ for each lake (Fig. 2.1, and 2.2). For lakes with temporal sampling, the among-lake mean of the maximum amount of error is 0.90 (SE=0.17, *n*=4) trophic levels (Fig. 2.1). For all lakes together, the mean of the maximum error is 0.64 (SE=0.10, *n*=12) trophic levels (range = 0.2 to 1.35) (Fig. 2.2). Consider Elk Lake as an example, which has a maximum error of 1.35 trophic levels. We could achieve this maximum error using two different approaches to baseline determination. If we used a bulk size fraction that was made up of primarily *Daphnia*, then our error would be a maximum if fish fed only on calanoids. Likewise, we could incur maximal error if we used only *Daphnia* as our baseline and fish fed only on calanoids. The actual amount of error for Elk Lake likely varies between 0 and 1.35 trophic levels, and ultimately depends on the $\delta^{15}N$ of the size fraction of bulk zooplankton collected, the zooplankton species composition, and the foraging behaviour of the fish species of interest.

In this paper we present *Daphnia* as an alternate, but complementary method for measuring the baseline $\delta^{13}C$ and $\delta^{15}N$ in the pelagia of lakes. *Daphnia*, as a short-lived organism, does not provide the same temporal integration as mussels (Fry 1999; Post 2002), but is better suited for finer scale temporal integration of pelagic $\delta^{13}C$ or $\delta^{15}N$ signatures. Mussels are commonly used to integrate the temporal variance in the $\delta^{15}N$ or the $\delta^{13}C$ of pelagic primary production (McKinney et al. 1999; Post 2002). Mussels are most suitable for the comparison of processes that affect the $\delta^{13}C$ and $\delta^{15}N$ over large temporal scales, because they integrate the isotopic signature of the pelagia over a longer period of time than zooplankton (Cabana and Rasmussen 1996; Post 2002). The temporal variation in the $\delta^{13}C$ and $\delta^{15}N$ of *Daphnia* can be large, as in Elk Lake where it suggests that the pelagic baseline varies throughout the season. This result is both a limitation and strength of using *Daphnia* as a baseline. For example, coarse sampling of a zooplankton taxa with a short tissue turnover time may miss ecologically important sources of production that have substantially different isotopic signatures. This originally motivated the use of long-lived consumers, such as mussels, as pelagic baselines (Cabana and Rasmussen 1996; Post 2002). However, a time series of the $\delta^{15}N$ of *Daphnia,* with a carefully chosen temporal resolution, may be useful to detect, for example, fine scale seasonal patterns of anthropogenic activities in recreational or residential lakes. In this case, it is better to use a single species of zooplankton (or multiple species), because changes in species composition will likely increase temporal variability in the $\delta^{15}N$ and $\delta^{13}C$ of a bulk size fraction of zooplankton.

A potentially significant drawback of using *Daphnia* as a baseline, is that the enrichment of ^{15}N per trophic level depends on the C:N ratio of the ingested algae

(Adams and Sterner 2000). Further research comparing the $\delta^{15}N$ of *Daphnia* among lakes with food sources of varying C:N ratios would be valuable for establishing *Daphnia* as a baseline indicator of $\delta^{15}N$ in the pelagia of lakes. Using zooplankton taxa other than *Daphnia*, such as copepods, may be problematic for two reasons. First, if copepods have high lipid content, $\delta^{13}C$ may change seasonally or vary among lakes in response to variation in lipid storage. Second, variable calanoid omnivory may complicate the interpretation of $\delta^{15}N$ among lakes, or the temporal pattern of $\delta^{15}N$ within a lake.

Zooplankton communities are complex, but this complexity is rarely addressed in the context of isotopic baseline determination. Future research should focus on the among lake variability of individual zooplankton taxa, and the primary factors that affect the relative isotopic differences between taxa (see variation in Fig. 2.3). For example, zooplankton taxa that coexist in the same pelagic environment may partition food resources either by habitat selection in the water column, or by food selectivity on the basis of size or quality. Better consideration of zooplankton trophic structure and food sources could help resolve the causes of variation in the isotopic signatures at upper trophic levels. This complexity of trophic structure within the pelagic zooplankton community may have larger food web scale consequences. Using stable isotopes, we can determine whether differences at the base of the food chain propagate up the food chain to planktivorous fish and beyond. Pelagic fish are often size or taxa selective when foraging (Kerfoot 1982; Johnston and Mathias 1994), or may switch between prey of different quality (Mazumder et al. 1990a). Since different zooplankton taxa have different isotopic signatures, this may help us track the diet of fish. If zooplankton are used appropriately as a baseline, the variation in upper trophic levels can more accurately

reflect variation in the isotopic signature of zooplankton. If used poorly, then variation in zooplankton trophic structure (or baselines) will propagate as error in estimates of trophic position and food source.

Tables

Table 2.1 : Zooplankton species in the lake survey.

Zooplankton species were grouped as *Daphnia* (D), *Holopedium* (H), or calanoid copepods (C). N is the number of sampling dates.

Lake (N)	**Lake ID**	**Zooplankton taxon**	**Categories**
Council Lake (8)	COL	*Leptodiaptomus tyrelli*	C
		Daphnia pulex	D
		Holopedium gibberum	H
Elk Lake (9)	ELL	*Hesperodiaptomus franciscanus*	C
		Daphnia pulicaria	D
Sooke Reservoir (11)	SOL	*Leptodiaptomus tyrelli*	C
		Daphnia rosea	D
Shawnigan Lake (11)	SHL	*Hesperodiaptomus franciscanus*	C
		Daphnia pulicaria	D
		Holopedium gibberum	H
Coquitlam Reservoir (1)	COQ	unidentified calanoid	C
		Daphnia rosea	D
		Holopedium gibberum	H
Lubbe Reservoir (1)	LUL	*Leptodiaptomus tyrelli*	C
		Daphnia rosea	D
		Holopedium gibberum	H
Deception Reservoir (1)	DER	*Hesperodiaptomus franciscanus*	C
		Daphnia middendorffiana	D
Seymour Reservoir (1)	SER	*Daphnia rosea*	D
		Holopedium gibberum	H
Cusheon Lake (1)	CUL	*Hesperodiaptomus franciscanus*	C
		Daphnia pulex	D
Butchart Lake (1)	BUL	*Leptodiaptomus tyrelli*	C
		Holopedium gibberum	H
Goldstream (1)	GOL	*Leptodiaptomus tyrelli*	C
		Daphnia rosea	D
		Holopedium gibberum	H
Japan Gulch Reservoir (1)	JPR	*Epischura nevadensis*	C
		Holopedium gibberum	H

Table 2.2 : Nested ANOVA table for zooplankton lake survey.
The design is unbalanced because Council and Shawnigan Lake have three taxa, and Elk and Sooke Lake have only two. Variance components are estimated using maximum likelihood.

Variable		**DF**	**MSQ**	**F-stat**	**P-value**	**Percent variance**
$\delta^{13}C$						
	Lake	3	45.5	2.08	0.20	17.0
	Taxa(Lake)	6	21.6	10.5	<0.001	41.7
	Residual	77	20.6			41.3
$\delta^{15}N$						
	Lake	3	170.3	5.3	0.04	56.4
	Taxa(Lake)	6	31.9	47.2	<0.001	36.7
	Residual	77	0.68			6.9
C:N						
	Lake	3	22.5	1.4	0.34	0.001
	Taxa(Lake)	6	16.1	18.0	<0.001	62.5
	Residual	77	0.90			37.5

Table 2.3 : Within lake variability of zooplankton $\delta^{15}N$ and $\delta^{13}C$.

ANOVA was performed for zooplankton grouped by taxa; *Daphnia* (D), *Holopedium* (H) and calanoid copepods (C). Groups that are significantly different based on Tukey's post hoc tests ($p<0.05$) are grouped by letters *a*,*b*, and *c*.

Lake	***F*-stat**	***p*-value**	r^2	**Taxa (mean)**
$\delta^{15}N$				
Council Lake	$F_{2,17}$= 10.4	0.001	0.55	H(2.88)^a, D(3.89)^b, C(4.70)^b
Elk Lake	$F_{1,14}$= 68.4	<0.001	0.83	D(8.40)^a, C(13.0)^b
Shawnigan Lake	$F_{2,26}$= 38.6	<0.001	0.75	H(5.90)^a, D(5.98)^a, C(8.95)^b
Sooke Reservoir	$F_{1,20}$= 123.0	<0.001	0.85	D(3.14)^a, C(5.71)^b
$\delta^{13}C$				
Council Lake	$F_{2,17}$=5.9	0.011	0.41	D(-34.0)^a, C(-33.6)^a, H(-32.0)^b
Elk Lake	$F_{1,14}$=1.7	0.218	0.11	C(-30.6)^a, D(-28.8)^a
Shawnigan Lake	$F_{2,26}$=15.7	<0.001	0.55	C(-32.1)^a, H(-30.4)^b, D(-30.3)^b
Sooke Reservoir	$F_{1,20}$=276.9	<0.001	0.93	C(-34.6)^a, D(-30.6)^b

Table 2.4 : Test of homogeneity of temporal variance of $\delta^{13}C$ and $\delta^{15}N$

Zooplankton categories were *Daphnia* (D), *Holopedium* (H), and calanoid copepods (C), and were from the four lakes with seasonal sampling. We compared within taxa temporal variance of isotopic composition (σ_T^2) to the interspecific variance in $\delta^{15}N$ and $\delta^{13}C$ enrichment derived by Post (Fig. 2.6, 2002) ($\sigma_{\Delta N}^2$= 0.960, N= 56; $\sigma_{\Delta C}^2$ = 1.69, N= 107) using a one tailed F-test ($F= \sigma_T^2/\sigma_\Delta^2$) (Ponsard and Arditi 2000). We reject the null hypothesis of isotopic homogeneity when F is significantly greater than one. ** $p<0.01$, * $p<0.05$, NS non-significant.

Lake	Group	F-value $\delta^{15}N$	Range $\delta^{15}N$	F-value $\delta^{13}C$	Range $\delta^{13}C$	N
Council Lake						
	D	<1	2.2	<1	2.6	7
	H	<1	1.8	2.0 NS	3.2	5
	C	<1	2.1	<1	2.1	8
	D, H, C	1.0 NS		<1		20
Elk Lake						
	D	1.7 NS	4.3	2.6 *	6.9	9
	C	<1	2.2	6.5 **	8.7	7
	D, C	7.0 **		4.5 **		16
Shawnigan Lake						
	D	<1	2.5	<1	2.5	11
	H	<1	1.6	<1	2.8	7
	C	1.3 NS	3.1	<1	1.3	11
	D, H, C	3.3 **		<1		29
Sooke Reservoir						
	D	<1	1.3	<1	1.4	11
	C	<1	1.9	<1	1.7	11
	D, C	2.1 *		2.6 **		22

Figures

Figure 2.1 : Pelagic zooplankton food web structure of four lakes.

Council Lake (COL), Elk Lake (ELL), Shawnigan Lake (SHL), and Sooke Lake Reservoir (SOL). We interpret the within lake range in $\delta^{15}N$ as the maximum amount of error introduced to a estimate of fish trophic position that neglects species composition. Assuming a mean trophic level enrichment of 3.4‰ for $\delta^{15}N$, the maximum number of trophic levels worth of error in the estimation of fish trophic position is 0.54 for COL, 1.35 for ELL, 0.90 for SHL, and 0.76 for SOL. The mean maximum amount of this error is 0.90 (SE=0.17, n=4).

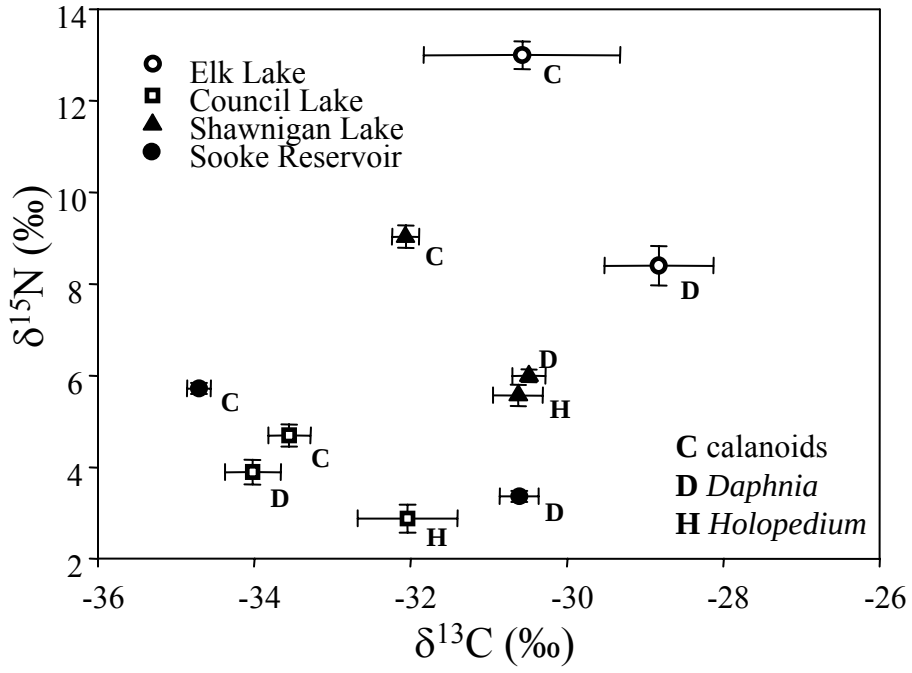

Elk Lake
Council Lake
Shawnigan Lake
Sooke Reservoir
C calanoids
D Daphnia
H Holopedium
δ15N (‰)
δ13C (‰)
14
12
10
8
6
4
2
-36
-34
-32
-30
-28
-26

Figure 2.2 : $\delta^{13}C$ and $\delta^{15}N$ of different zooplankton taxa in 12 lakes.

Lakes are grouped by different compositions of the zooplankton community. Error bars for the four lakes with temporal coverage are ± one SE of the seasonal mean. Dashed lines are for clarity of interpretation. The mean maximum number of trophic levels worth of error in the estimation of fish trophic position is 0.64 (SE= 0.10, N= 12).

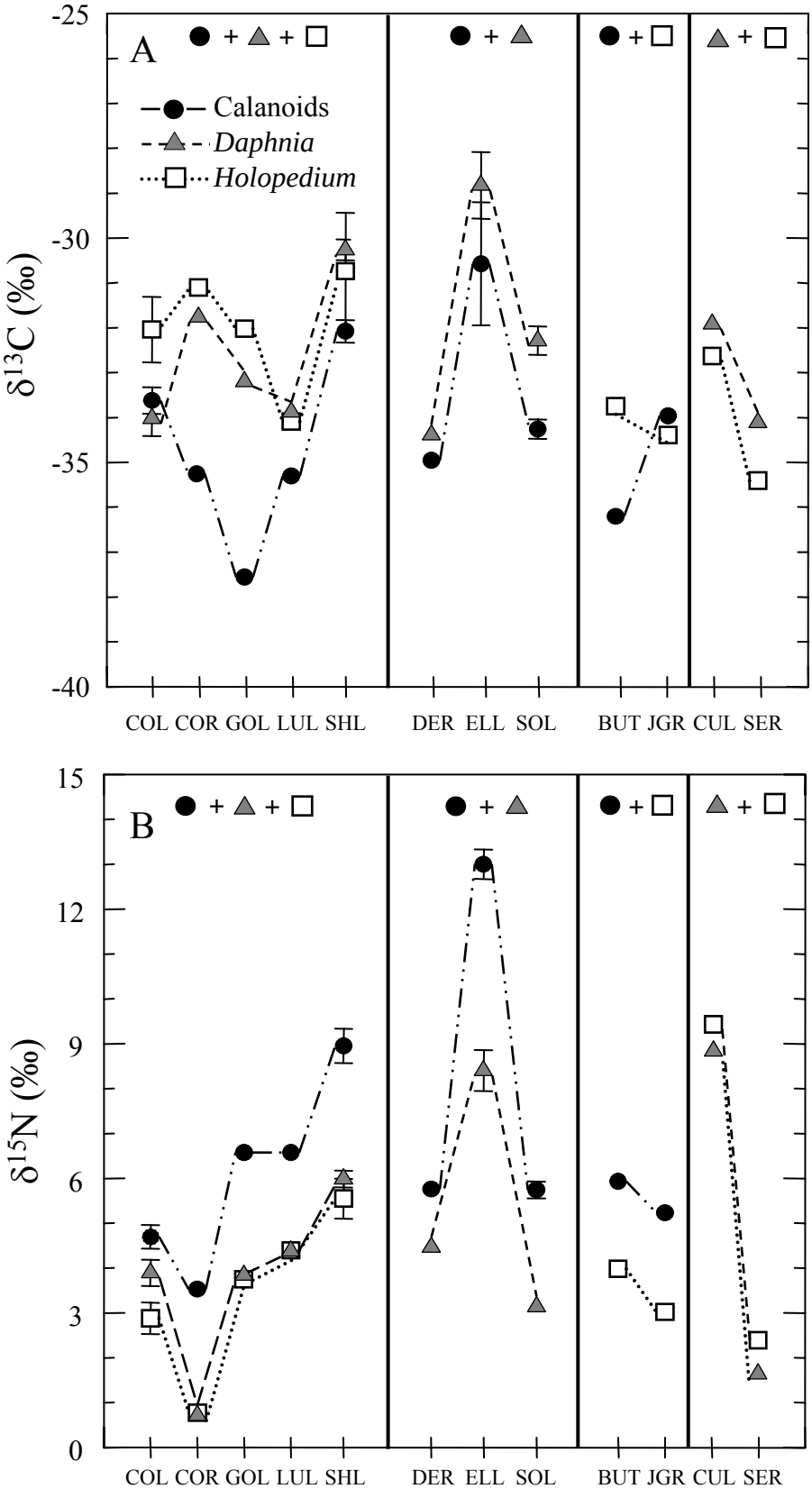
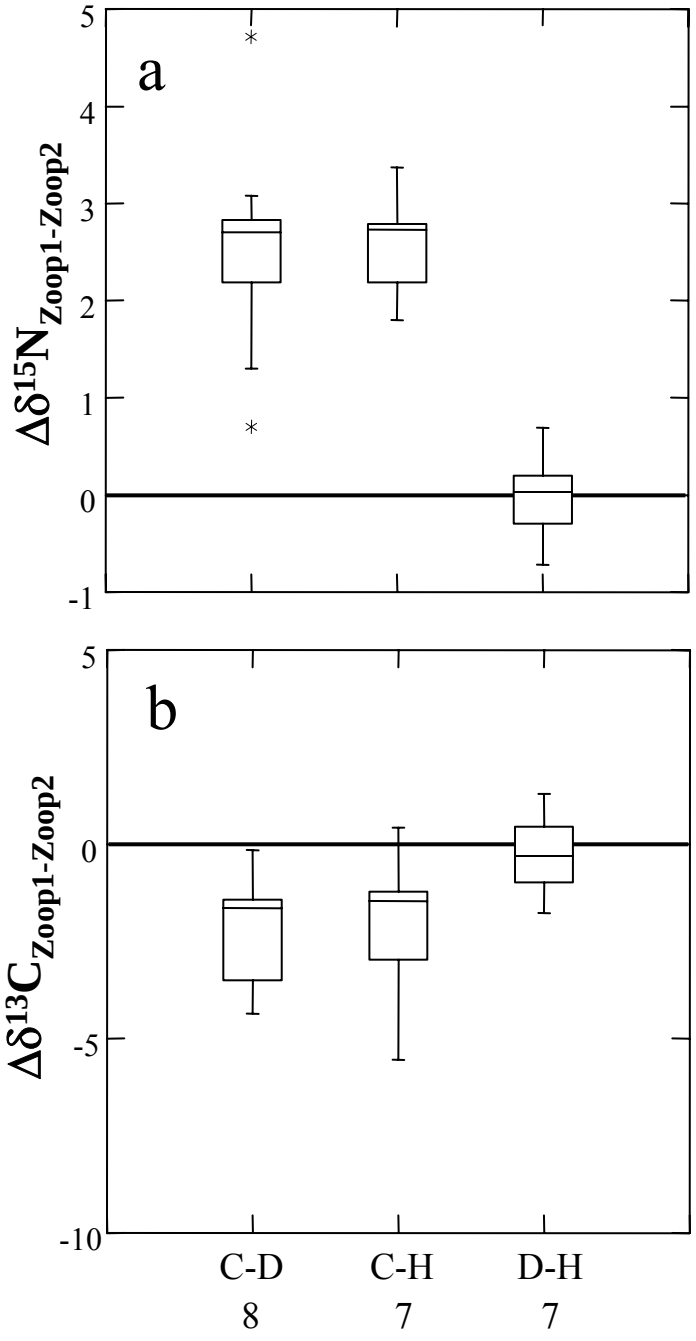

A
Calanoids
Daphnia
Holopedium
δ13C (‰)
-25
-30
-35
-40
B
δ15N (‰)
15
12
9
6
3
0
COL COR GOL LUL SHL
DER ELL SOL
BUT JGR CUL SER

Figure 2.3 : Boxplots of differences between zooplankton taxa.

Zooplankton taxa are calanoid copepods (C), *Daphnia* (D), and *Holopedium* (H). Number of lakes denotes the subset of lakes where the two taxa were sampled. a) Mean calanoid $\delta^{15}N$ is 2.55‰ higher than *Daphnia* (t_7=5.8, p<0.001) and 2.44‰ higher than *Holopedium* (t_6=11.5, p<0.001). b) Mean calanoid $\delta^{13}C$ is 2.19‰ lower than *Daphnia* (t_7= 3.94, p= 0.01) and 2.23‰ lighter than *Holopedium* (t_6=2.92, p=0.03). Mean difference between *Daphnia* and *Holopedium* $\delta^{13}C$ and $\delta^{15}N$ is not significantly different from zero ($\delta^{15}N$: t_6=-0.49, p=0.64; $\delta^{13}C$: t_6=0.47, p=0.66).


a
b
$\Delta\delta^{15}N_{Zoop1-Zoop2}$
$\Delta\delta^{13}C_{Zoop1-Zoop2}$
5
4
3
2
1
0
-1
-5
-10
C-D
C-H
D-H
8
7
7

Chapter 3: Consequences of large temporal variability of zooplankton $\delta^{15}N$ for modeling fish trophic position and variation

Citation:

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Abstract

We use a temporal integration model (TIM) to determine how estimates of trophic variation, using $\delta^{15}N$, depend on consumer growth dynamics and temporal isotopic variation ($\delta^{15}N$) of food sources. Consumers are rarely in isotopic equilibrium with their food sources, so instantaneous comparisons between the $\delta^{15}N$ of a consumer and its diet provide little information about trophic variation, even if the trophic positions of the diet are known. In this paper, we focus on the trophic link between zooplankton and planktivorous fish. We first review the extent of temporal variability of zooplankton $\delta^{15}N$, and then examine the consequences of this variability for understanding the isotopic composition of planktivorous fish communities. We use time series of $\delta^{15}N$ for *Daphnia*, calanoid copepods, and particulate organic matter (>200 μm) to generate theoretical diets for a model juvenile sockeye over a typical growing season. We use a TIM to predict the isotopic trajectory of individual juveniles feeding on these diets, and explore how variance in growth rate and isotopic enrichment ($\Delta_{\delta N}$) can affect estimates of trophic position and intrapopulation isotopic variability. In general, we found that using a seasonal average of *Daphnia* $\delta^{15}N$ to estimate the trophic position of planktivorous fish is nearly equivalent to using a TIM. However, temporal variation in the $\delta^{15}N$ of food sources, coupled with individual differences in the growth rate of consumers, can contribute to intrapopulation isotopic variation of consumers, and lead to correlations between consumer size and $\delta^{15}N$.

Introduction

Ecologists are increasingly using average $\delta^{15}N$ values to estimate the trophic position of consumers, and the variance in $\delta^{15}N$ (intra- or interspecific) to estimate trophic variation. The most reliable method for estimating trophic position is to measure the elevation of $\delta^{15}N$ from a baseline ($\delta^{15}N_{base}$), divide by an average enrichment per trophic level ($\Delta_{\delta N}$), and add the trophic position of the baseline (λ_{base}) (Post 2002). Using a baseline helps account for large inter-system variation in $\delta^{15}N$ that is unrelated to trophic variation (Vander Zanden and Rasmussen 1999, Post 2002, Matthews and Mazumder 2003). However, residual variation of $\delta^{15}N$ (i.e., intrapopulation variation) may not be synonymous with trophic variation, because of large temporal, compositional, and spatial isotopic variability of food sources (Maruyama et al. 2001; Melville and Connolly 2003; Needoba et al. 2003), and large variance in $\Delta_{\delta N}$ (Post 2002). In a recent review, Vanderklift and Ponsard (2003) suggest that taxon-specific estimates of the variance in $\Delta_{\delta N}$ may improve our ability to account for intrapopulation variation of $\delta^{15}N$. In the present study, we investigate how antecedent temporal variability in the $\delta^{15}N$ of a consumer's food sources affects estimates of trophic position, and the interpretation of intrapopulation isotopic variance.

Temporal, spatial, and compositional variability of the consumer's food sources affect how we choose $\delta^{15}N_{base}$ (Cabana and Rasmussen 1996; Post 2002; Chapter 2). For example, measuring the trophic position of fish in lakes may require a baseline for both the pelagic (e.g. mussels, bulk zooplankton, *Daphnia*) and benthic (snails, *Chironomids*) food chains (Post et al. 2000; Matthews and Mazumder 2003; Vadeboncoeur et al. 2003),

because fish utilize both pelagic and benthic resources. This approach relies, in part, on the assumption that consumers are in temporal isotopic equilibrium with each baseline.

Current baseline approaches for estimating the trophic position of freshwater fish address temporal variability of $\delta^{15}N$ in various ways. Intuitively, researchers trade-off their sampling effort with how well they expect their baseline to match the temporal variability in the $\delta^{15}N$ of the consumer's food sources. For example, Cabana and Rasmussen (1994) used a size fraction of zooplankton (<250 μm) as a baseline to estimate the trophic position of fish, in order to help account for the high temporal variance in $\delta^{15}N$ of primary producers. Post (2002) extended this approach to account for spatial variability at the base of lake food webs by using mussels and snails as baseline species for pelagic and littoral lake habitats, respectively. Recently, we suggested using *Daphnia* as a baseline for the pelagic habitat (Matthews and Mazumder 2003), but we did not provide a general model to measure the trophic position of planktivorous fish. Here we compare two approaches to estimate trophic variation of planktivorous fish, using either a seasonal average or a time series of *Daphnia* $\delta^{15}N$. These approaches are general, and are applicable to other systems where it is possible to make repeated measurements of a consumer's food source or baseline, and measure or model the growth dynamics of the consumer.

Individual zooplankton species exhibit large seasonal variation in $\delta^{15}N$ (Graham 1997; Leggett et al. 2000). How do we account for this large variation in our estimates of fish trophic position and trophic variation using $\delta^{15}N$? Diet switch experiments confirm that the isotopic turnover time of consumers frequently lags the isotopic turnover time of their diets (Hesslein et al. 1993; Herzka and Holt 2000; Harvey et al. 2002). As a result,

we expect isotopic dis-equilibrium between planktivores and zooplankton if there is considerable temporal variation in the $\delta^{15}N$ of zooplankton. Using a seasonal average of zooplankton $\delta^{15}N$ as $\delta^{15}N_{base}$ will help mitigate this dis-equilibrium. However, estimates of fish trophic position might be sensitive to the pattern of temporal variation in zooplankton $\delta^{15}N$, and so in some cases a seasonal average may not be appropriate. In other words, the isotopic dis-equilibrium between zooplankton and fish will depend on the antecedent temporal variation of zooplankton $\delta^{15}N$ and the isotopic turnover of fish tissue.

In this paper, we first survey the literature to quantify the extent of temporal variation in the $\delta^{15}N$ of zooplankton. Second, we present data on the temporal variability of $\delta^{15}N$ in zooplankton communities from six sites in four lakes from a previous study (Matthews and Mazumder 2003). Third, we use a temporal integration model (TIM) from Harvey et al. (2002) to calculate $\delta^{15}N_{base}$, and to explore how intrapopulation isotopic variation depends on individual variation in consumer growth dynamics. For this application, we use juvenile sockeye in Lake Washington as a model system. In Lake Washington and other coastal lakes, juvenile sockeye are obligate planktivores and selectively feed on different zooplankton taxa (Mazumder and Edmundson 2002; Ballantyne et al. 2003). In addition, we can realistically model fry growth over a time period (May to November) that matches many studies that have repeatedly sampled the $\delta^{15}N$ of multiple zooplankton species. Finally, we compare two models, a seasonal average model (SAM) and a TIM, which both use time series of zooplankton $\delta^{15}N$, to estimate the trophic position and trophic variation of fish.

Methods

Literature survey: assessing the extent of zooplankton $\delta^{15}N$ variability

We summarized data from 15 lakes to quantify the extent of temporal variation in the $\delta^{15}N$ of size fractions of plankton and individual taxa of zooplankton. To our knowledge this is an exhaustive survey of the available literature. We used Grafula 3 (v2.10) to digitize data from figures of published papers and from a thesis (Table 3.3). We classified all size fractions of plankton as particulate organic matter (POM) and aggregated all zooplankton species into broad taxonomic groups.

The temporal variation of $\delta^{15}N$ for individual zooplankton taxa

We collected zooplankton from six sites in four coastal lakes on Vancouver Island, British Columbia: Sooke Lake (SOL) and Shawnigan Lake (SHL), Council Lake (COL), and Elk Lake (ELL). In SOL and SHL, we sampled every 2 or 3 weeks from June to November 2001 at two stations, 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)). These two lakes are morphologically similar (SOL: 6.1 km^2, SHL: 5.5 km^2) and are in adjacent catchments (within 4 km). They are temperate, warm monomictic, and typically have mean summer epilimetic chlorophyll *a* concentrations <2 $\mu g\ L^{-1}$ (Davies et al. 2004a). We sampled zooplankton from Council Lake and Elk Lake eight times between May and September 2001. Council Lake is a 0.16 km^2 (max depth 17 m) oligotrophic lake, and Elk Lake is a 2.46 km^2 (max depth 20 m) meso-eutrophic lake. Detailed morphometrics (Spafard et al. 2002), nutrient dynamics (Nowlin 2003; Davies 2004), and productivity of these systems (Davies et al. 2004b) are available elsewhere.

We collected zooplankton for isotopic analysis with a large Wisconsin net (50 cm diameter, 64 μm mesh) from the entire water column, or from a maximum depth of 30 m. We used a smaller net (30 cm diameter, 64 μm mesh) to collect zooplankton for identification and enumeration. Zooplankton handling, sorting, and stable isotope analysis is described in detail in Matthews and Mazumder (2003). Briefly, we picked different zooplankton taxa from a bulk zooplankton size fraction, dried them at 60°C, and packaged them in tin capsules. The number of individuals per sample varied depending on the species and size, to approximate 1 mg of dry zooplankton tissue for isotopic analysis. Zooplankton biomass was calculated using an optical counting program (Z-count), and published length weight regressions (Culver et al. 1985; Yan and Mackie 1987).

We collected particulate organic matter (POM) >200 μm from the epilimnion and metalimnion using a 6 m section of Tygon tubing and a vertically oriented Niskin sampler, respectively. We filtered at least 20 L of lake water though a 200 μm Nitex mesh, and then backwashed the POM onto precombusted (550°C for 1 hour) 25 mm GF-C filters (Whatman). Filters were dried overnight at 60°C and packaged in tin cups. $\delta^{15}N$ analyses were conducted on an isochrom continuous flow isotope ratio mass spectrometer coupled to a Carlo Erba elemental analyzer at the University of Waterloo Environmental Isotope Lab (Waterloo, Ontario, Canada), with a precision of <0.1‰.

Temporal integration model (TIM) for predicting the trophic position of fish

We modified a model developed for predicting the temporal change in isotopic composition following a diet switch between two food sources with different isotopic

compositions (Hesslein et al. 1993; Harvey et al. 2002). We used equation 1 to calculate the isotopic signature of a fish at day *t* (δ_t),

$$\delta_t = \frac{\delta_{t-1} \times B_{t-1} + (B_t - B_{t-1}) \times ((\delta_{fs-1} + \delta_{fs}) \div 2 + f)}{B_t} - [m \times (\delta_{t-1} - ((\delta_{fs-1} + \delta_{fs}) \div 2 + f))] \quad (1)$$

where 'B' is the biomass, 'δ_{fs-1}' is the isotopic signature of the food source on day t-1, 'm' is a metabolic turnover constant, and 'f' is the fractionation factor between the food source and the consumer. To simplify the data requirements of bioenergetics analysis (Harvey et al. 2002), we coupled the model with simulated growth trajectories of sockeye juveniles (*Oncorhynchus* nerka) in Lake Washington (Ballantyne et al. 2003). In Lake Washington, sockeye grow from 1 to 12.5 g from the beginning of May to November (Ballantyne et al. 2003). We modeled sockeye juvenile growth using a simple recursive logistic growth model, with a maximum juvenile weight of 12.5 g, and a growth rate (μ; g day^{-1}) that we chose at random from a normal distribution (typically, $\mu = 0.03$, $\sigma_\mu^2 = 0.001$). We used the variance in specific growth rate of juvenile sockeye from Ballantyne et al. (2003) to parameterize our simulations of growth dynamics. For the purposes of our simulations, we assumed that juveniles at the start of the simulation (1.0 g) were in isotopic equilibrium with their zooplankton diet. We used m=0.0005 in all of our simulations (following Harvey et al. 2002), and performed a sensitivity analysis of this parameter (m = 0.0005 to 1.0). All simulations were done using S-plus 2000.

To include natural variability in trophic fractionation among individual sockeye juveniles, we randomly chose a enrichment factor for each juvenile from a normal distribution ($\Delta_{\delta N} = 3.4‰$, $\sigma_\Delta^2 = 0.17$), where $\Delta_{\delta N}$ is the average enrichment per trophic

level (Post 2002), and σ_{Δ}^2 is a field estimate of the variance in trophic enrichment of $\delta^{15}N$ for Lake Trout (*Salvelinus namaycush*) (Vander Zanden and Rasmussen 2001). This field estimate is similar to a more recent estimate for muscle tissue of fish raised on a fixed diet ($\sigma_{\Delta}^2 = 0.12$, n=6) from Vanderklift and Ponsard (2003). We also conducted simulations using an interspecific estimate, not specific to freshwater fish ($\sigma_{\Delta}^2 = 0.98$; Post 2002), in order to address the model's sensitivity with respect to variation in trophic enrichment.

Simulated fish diets for the TIM

For each simulation, we projected the isotopic trajectory of 1000 fish fed on one of four theoretical diets: 1) POM >200 μm, 2) only *Daphnia*, 3) only calanoids, and 4) the predicted $\delta^{15}N$ of the macrozooplankton community. In Council Lake, we included a simulated diet of only *Holopedium*. We linearly interpolated the $\delta^{15}N$ for each simulated diet, so as to get a daily resolution of $\delta^{15}N$ between sampling dates. To calculate the predicted $\delta^{15}N$ of a macrozooplankton diet we used the percent biomass composition (Table 3.1), the percent nitrogen, and the $\delta^{15}N$ of different zooplankton taxa to predict the $\delta^{15}N$ signature of the macrozooplankton community. For each sampling date, we calculated the percentage contribution of nitrogen from each taxon as the proportion of the biomass times its nitrogen content. We normalized the proportions to sum to one and multiplied them by the observed $\delta^{15}N$ for the corresponding zooplankton taxa. We then calculated the predicted $\delta^{15}N$ of the macrozooplankton community as the sum of the contributions of $\delta^{15}N$ by each taxon. Due to the low densities of cyclopoids and *Holopedium* at certain times of year, and the small size of cyclopoids, we were unable to

make isotopic measurements of these taxa for all of the sampling dates. Previous research demonstrates that the difference in $\delta^{15}N$ is typically <0.5‰ between *Daphnia* and *Holopedium* (Matthews and Mazumder 2003), and <1.0‰ between calanoids and cyclopoids (Table 3.3). Therefore, when the abundances of *Holopedium* and cyclopoids were low (typically <15% of biomass), we substituted the $\delta^{15}N$ of *Daphnia* and calanoids for *Holopedium* and cyclopoids, respectively. We acknowledge that the generality of the small differences between these taxonomic groupings has not been thoroughly tested.

To get a broader range of isotopic patterns for our diet simulations, we linearly interpolated time series of *Daphnia* $\delta^{15}N$ for each lake in our literature survey (Table 3.3). We interpolated the $\delta^{15}N$ of *Daphnia* for each day from May to September (Day of Year (DOY): 121 to 243), and separately for June to October (DOY: 152 to 273). We generated these two sets of times series because the sampling patterns over the season differed among lakes. We used these two sets in our TIM simulations to evaluate the effect of intrapopulation variation in juvenile sockeye growth rate (σ_μ^2) on the magnitude and pattern of intrapopulation variation of juvenile sockeye $\delta^{15}N$.

Comparison of baseline approaches for estimating the trophic position of fish

We compared two baseline approaches to predict the $\delta^{15}N$ of sockeye juveniles, both based on time series of zooplankton $\delta^{15}N$. First, we used a seasonal average of the $\delta^{15}N$ for each diet and added an average fractionation factor of 3.4‰ (Post 2002) to predict the mean $\delta^{15}N$ of juveniles (SAM). Second, we used the temporal integration model (TIM), with f = 3.4‰, to predict the $\delta^{15}N$ of juveniles based on the observed

temporal isotopic variability of different diets. We interpreted differences between these predictions ($\Delta_{SAM-TIM}$) as the potential amount of error in our estimates of fish trophic position depending on our model selection. We calculated $\Delta_{SAM-TIM}$ (in ‰ units) for all the simulations we performed in this paper, including those done as part of the sensitivity analysis.

Results

Quantification of temporal variation from previous studies

Results from the literature survey support conclusions reached by Matthews and Mazumder (2003) regarding isotopic differences among zooplankton taxa within a lake. Despite large isotopic variation for individual taxa among lakes, within a lake the $\delta^{15}N$ of zooplankton follow established feeding behaviours (Table 3.3). Typically, the more herbivorous zooplankton (like *Daphnia*) have the lowest $\delta^{15}N$ signatures, followed by variably omnivorous copepods (calanoids and cyclopoids) and invertebrate predators (*Bythotrephes*, *Chaoborus*, and *Leptodora*). However, there is considerable unexplained variation in the relative differences between taxa among and within lakes (Table 3.3). The seasonal range of $\delta^{15}N$ for *Daphnia* varied from 1.3‰ at SOL-D to 10.9‰ in Crooked Lake (Fig. 3.1).

Results and interpretation of the temporal integration model (TIM)

We used the temporal patterns in the $\delta^{15}N$ of zooplankton taxa from our six study sites (Fig. 3.2) as the theoretical diets for juvenile sockeye, and used the temporal integration model (TIM) to predict the $\delta^{15}N$ of juveniles (Fig. 3.3). Each simulation generated a distribution of $\delta^{15}N$ values for juvenile sockeye feeding exclusively on a

defined diet. The degree of separation between the regions of predicted $\delta^{15}N$ varied among lakes (Fig. 3.3), and was greatest in Elk Lake. The diets made up of *Daphnia* and POM typically overlapped, except in Council Lake (2001) where POM and *Holopedium* diets overlapped. In general, the mean difference between peaks depended on differences in the $\delta^{15}N$ between diets and the pattern of temporal variation in the diet (Fig. 3.2). The variance of each peak depended primarily on the variance in trophic enrichment (σ_{Δ}^2) (Fig. 3.4), however the variance in Fig. 3.4A results from individual sockeye juveniles growing at different rates on a fixed diet whose $\delta^{15}N$ is increasing over the season (from SOL-D).

Variation in growth rates among individuals (σ_{μ}^2) led to an increase in intrapopulation variance by the end of the simulation. This was particularly noticeable when the intrapopulation variation in isotopic enrichment was negligible (as in Fig. 3.5; $\sigma_{\Delta}^2 = 0.008$). This result was insignificant when we used higher estimates of σ_{Δ}^2 (e.g. 0.17, as in Fig. 3.3). Given the pattern of temporal variation in *Daphnia* $\delta^{15}N$ for Council Lake (2002), fast growing fish had a higher $\delta^{15}N$ than slow growing fish at the end of the simulation (Fig. 3.5). Therefore, variation in growth rate, coupled with an increasing $\delta^{15}N$ of *Daphnia* early in the time series, generated a positive relationship between juvenile size and $\delta^{15}N$ (see Fig. 3.6A). This was a general result for *Daphnia* time series that had abrupt increases in $\delta^{15}N$ early in the season (Fig. 3.1A). Abrupt decreases in *Daphnia* $\delta^{15}N$ early in the season (Fig. 3.1B) led to negative relationships between juvenile size and $\delta^{15}N$ (Fig. 3.6A). The total amount of intrapopulation variation of juvenile sockeye $\delta^{15}N$ at the end of the simulations was positively related to the intrapopulation variation in growth rate (σ_{μ}^2), but the relationship depended on the specific pattern of *Daphnia*

$\delta^{15}N$ (Fig. 3.6B). Overall, the timing and interaction between juvenile sockeye growth and change in *Daphnia* $\delta^{15}N$, along with individual differences in isotopic enrichment, can contribute to isotopic variation among individual juvenile sockeye.

Comparison of baseline approaches for estimating the trophic position of fish

To compare different approaches of predicting the $\delta^{15}N$ of a planktivore, we computed the difference in predictions between the TIM and a seasonal average model ($\Delta_{SAM-TIM}$). In general, the average differences were small (Fig. 3.7). For a juvenile sockeye feeding exclusively on *Daphnia*, the average $\Delta_{SAM-TIM}$ among lakes was insignificant (mean = 0.06‰, range = -0.66 to 0.68, N = 30). Among all simulated diets the average difference was similarly small, but had a wider range (mean = 0.10‰, range = -0.9 to 1.6, Fig. 3.7).

Discussion

Specific taxa or bulk size fractions for $\delta^{15}N_{base}$?

Previous approaches for estimating the trophic position of fish relied to some degree on size fractions of zooplankton (Cabana and Rasmussen 1994; Vander Zanden et al. 1999; Post 2002). Different zooplankton taxa have unique isotopic signatures (Meili et al. 1996; Matthews and Mazumder 2003) and can exhibit distinct seasonal patterns of $\delta^{15}N$ (Graham 1997; Leggett et al. 2000; Grey et al. 2001; Fig. 3.2). But, does the added precision of separating different zooplankton taxa warrant the extra effort for estimating fish trophic position? In general this depends on the specific research question, and must be tailored to a particular study system (Post 2002).

There are advantages and disadvantages of using size fractions of plankton to estimate the trophic position of fish, as opposed to using a single zooplankton taxon. From our literature survey we found that size fractions of plankton typically had the highest coefficient of variation for a given lake site (14 out of 19 time series in Table 3.3). This higher variation likely results from baseline variation in the primary consumers, in addition to changes in the zooplankton composition of the size fraction. In our 6 study sites, the predicted $\delta^{15}N$ of the macrozooplankton community accounted for 82% of the temporal variation in the $\delta^{15}N$ of POM (>200μm) ($F_{1,52} = 230.9$, $p < 0.001$, $r^2 = 0.82$), but was consistently higher than POM by ~1.5‰. This low $\delta^{15}N$ of POM is likely a result of large algae, which we routinely observed in the >200 μm size fraction. Similarly in Lake Ontario, Kiriluk et al. (1995) found that the $\delta^{15}N$ of net plankton (>153 μm) was 1.2‰ when *Bosmina* and diatoms were abundant, but was 12.1‰ when cyclopoid copepods dominated the same size fraction (Kiriluk et al. 1995). When there is isotopic heterogeneity within a plankton size fraction, the amount of error in estimates of fish trophic position will partly depend on how well the composition of the size fraction actually represents the diet of the fish. Fish are often size selective foragers and readily switch between zooplankton taxa depending on availability (Mazumder et al. 1990a; Ballantyne et al. 2003). Size fractions of plankton can approximate natural changes in fish diet, if they account for the natural changes in the relative abundance of different zooplankton taxa. Currently we know that different zooplankton taxa have distinct $\delta^{15}N$ signatures (Matthews and Mazumder 2003), and that this is a robust pattern among lakes (Table 3.3). However, if isotopic differences between zooplankton taxa result from different baseline sources of nitrogen (as opposed to trophic variation), then a size

fraction might effectively average out the isotopic variability within the zooplankton community that is unrelated to trophic variation. If this is the case, then a time series of a size fraction may successfully capture baseline variation. This is why it is so critical to distinguish between baseline and trophic variation (Post 2002), and to determine to what extent isotopic heterogeneity within the zooplankton community actually reflects trophic rather than baseline variation.

Estimating the trophic position of fish using time series of plankton $\delta^{15}N$

Temporal variation of zooplankton $\delta^{15}N$ is substantial and might be largely unrelated to trophic variation (Fig. 3.1). Robinson (2001) argues that a consumer's $\delta^{15}N$ signature represents the integration of the nitrogen cycle up to the consumer's position in the food web, and Needoba et al. (2003) highlight the uncertainty in the causes of $\delta^{15}N$ variation among phytoplankton taxa. Read together, these studies suggest that temporal variation in $\delta^{15}N$ of a zooplankton taxon does not necessarily indicate trophic variation. Indeed, $\delta^{15}N$ signatures of zooplankton are frequently influenced by changes in nitrogen cycling (Leggett et al. 2000; O'Reilly et al. 2002). A striking example is from Lake Tanganyika, where O' Reilly et al. (2002) demonstrated how a change in the nitrogen source can rapidly change the stable isotope representation of a pelagic food web. Following an upwelling event of NH_4 with a high $\delta^{15}N$, zooplankton had a $\delta^{15}N$ signature similar to their planktivorous consumers (O'Reilly et al. 2002). It is still unknown whether this type of isotopic distortion is a common feature of lakes. Nevertheless, large temporal variation of zooplankton $\delta^{15}N$ (Fig. 3.1) means that using a seasonal average or

a time series of zooplankton $\delta^{15}N$ could give different estimates for the trophic position of planktivorous fish.

Using seasonal averages (SAM) - The trophic position of an individual fish can be estimated as the elevation of $\delta^{15}N$ from the baseline ($\delta^{15}N_{base}$) divided by an average enrichment per trophic level, plus the trophic position of the baseline (Eq. 2).

$$\lambda_{base} + (\delta^{15}N_{fish} - \delta^{15}N_{base})/\Delta_{\delta N} \qquad (2)$$

Where $\Delta_{\delta N}$ is the average enrichment per trophic level, and λ_{base} is the trophic level of the consumer used as the baseline (Post 2002). This estimate assumes that the consumer's tissue is in isotopic equilibrium with its baseline (Post 2002). Given the large temporal variability in zooplankton $\delta^{15}N$ (Fig. 3.1; Table 3.3), neither point estimates of POM nor point estimates of individual zooplankton species provide robust measures of $\delta^{15}N_{base}$. As such, a seasonal average is always preferable to point estimates.

In some cases, the seasonal average of single zooplankton taxon can be a useful baseline ($\delta^{15}N_{base}$) for measuring the trophic position of fish. *Daphnia* is a convenient baseline zooplankton taxon (Matthews and Mazumder 2003), because we can use a seasonal average of *Daphnia* $\delta^{15}N$ for $\delta^{15}N_{base}$, and define $\lambda_{base}=\lambda_{Herbivore}=\lambda_{Daphnia}= 2$. This assumes that *Daphnia* is predominantly an herbivore, and thus belongs to the "2^{nd}" trophic level of the classical pelagic food chain. This model can then be used to estimate the relative trophic position of other zooplankton taxa, or other POM size fractions relative to the seasonal average of *Daphnia* using Eq. 2.

Using a time series and a temporal integration model (TIM) - The purpose of the temporal integration model (TIM) is to use the temporal variability of consumer food sources explicitly in estimates of trophic position and variation. The TIM scales the

temporal variability of the consumer's food source to match the turnover time of the consumer's tissue. For example, to model the zooplankton-planktivore trophic link we could define $\lambda_{base}=\lambda_{Daphnia}$; but, instead of using a seasonal average of *Daphnia* $\delta^{15}N$ as $\delta^{15}N_{base}$, we could determine $\delta^{15}N_{base}$ using Eq. 1 and a time series of *Daphnia* $\delta^{15}N$. For example, the TIM can predict isotopic trajectories of planktivores that feed exclusively on *Daphnia*. If we consider the average of these simulated planktivores as the "3rd" trophic level, we can use the average $\delta^{15}N$ of simulated planktivores as $\delta^{15}N_{base}$ for the planktivore trophic level (with $\lambda_{base}=3$). Used in this way $\delta^{15}N_{base}$ differs slightly from Post's (2002) definition. Here $\delta^{15}N_{base}$ is the average isotopic signature of the modeled consumer that feeds on the trophic level below it. Therefore, fish collected from the field can have isotopic signatures below $\lambda_{base}=3$. Comparing the variance in $\delta^{15}N$ of field estimates around $\lambda_{base}=3$ could then give us some indication of trophic variation at the third trophic level.

The utility of the TIM depends primarily on the isotopic separation between food sources and the variance in trophic enrichment (σ_{Δ}^2). Variance in growth rate can contribute to intrapopulation variance (variance of peaks in Fig. 3.4A), but our approach is most sensitive to σ_{Δ}^2 (compare Fig. 3.4B and 3.4C). Ideally, researchers should use an estimate of σ_{Δ}^2 that is specific to the consumer of interest. A recent review suggests that this approach may soon be possible given the considerable interest in this area of research (Vanderklift and Ponsard 2003).

The TIM model was not very sensitive to the isotopic turnover constant *m* (Table 3.2), probably because of the large temporal variability of zooplankton $\delta^{15}N$, and because we only modeled the growth phase of juvenile sockeye. Several previous diet switch

studies suggest that *m* has a small impact on isotopic change compared to growth dilution (Hesslein et al. 1993; Vander Zanden et al. 1998). This is particularly the case for poikilotherms such as fish, which have a low basal metabolic rate (Herzka and Holt 2000).

Using the TIM model

The TIM model can easily be extended to model the trophic position of consumers in other food chains as long as we scale the temporal isotopic variability of the food source to the turnover time of the consumer. To use this model one would need information about the growth dynamics of the consumer, and a temporal series of a baseline taxon or a food source with a known trophic position (such as *Daphnia* for planktivorous fish). Although this approach is data intensive, it forces the user to consider the effects of uncertainty in growth rate and trophic enrichment on the accuracy of trophic position estimates using $\delta^{15}N$. Modeling multiple steps in the food chain with this type of approach will likely fail in complex food webs, because uncertainties in growth dynamics and trophic fractionation will propagate up the food chain. Post's (2002) approach is clearly better suited to make inferences about food chain length, and the trophic position of top predators. The model we present is intended for interpreting isotopic variation at adjacent trophic levels and for identifying the significance of isotopic differences among individual consumers.

Post (2002) models trophic position of fish using a long lived primary consumer, such as mussels, as a baseline with $\lambda_{Mussels}=2$. An advantage of this approach is that a long lived consumer naturally integrates the changing isotopic signatures of the environment (Post 2002). Long lived consumers, unlike a coarse time series of

zooplankton, continuously sample the environment, and might be more likely to pick up short term baseline variation (O'Reilly et al. 2002). This is particularly useful when you can spatially and temporally match a consumer with a baseline species that has a known feeding behaviour (*sensu* Kling et al. 1992). But there are situations where this might not be possible. For example, to estimate the trophic position of a rapidly growing fish larva (Herzka and Holt 2000), it might be more reasonable to measure the temporal variability of the larvae's food source, and use an observed variance in growth rates to interpret intrapopulation trophic variation (Fig. 3.5). Likewise, TIM could also be useful for feeding experiments to estimate $\Delta_{\delta N}$ (Vanderklift and Ponsard 2003), particularly if the $\delta^{15}N$ of food sources change over the experiment (as might be the case for live feed).

In using TIM to calculate $\delta^{15}N_{base}$, it is important to consider if the added complexity is worthwhile, compared to the simpler SAM approach. Among all our simulated diets, the difference between models ($\Delta_{SAM-TIM}$) is almost always less than 1‰ (Fig. 3.7), which amounts to less than half a trophic level (since $\Delta_{\delta N} \sim 3.4$‰). In many cases, $\Delta_{SAM-TIM}$ is not much larger than the analytical precision of replicate field samples. Therefore, in most of the circumstances modeled here, the choice of models will not significantly alter the interpretation of stable isotope data from the field. However, in our model simulations, all the fish started at the same biomass and grew over time in a single cohort. Ultimately, the difference between models will depend on the timing of growth among individual consumers, the interaction with of the isotopic change in food sources, and the sampling resolution of food sources and consumers. In general, the TIM model is most useful for short time series where large changes in the $\delta^{15}N$ of food sources occur early in the growth of the consumer.

It is still an open question how precisely we can expect to, or need to, determine trophic position. For example, how significant is half a trophic level of variation? For studies of contaminant accumulation, half a trophic level can be quite significant, depending on the rate of contaminant accumulation up the food chain. For example, the slope of the relationship between $\delta^{15}N$ and mercury (Cabana and Rasmussen 1994) is steeper than several other contaminants (Kidd et al. 1995; Kiriluk et al. 1995), and even a 0.5‰ (< 0.2 trophic levels) error in the predicted $\delta^{15}N$ of a planktivorous fish can lead to >30% error in the prediction of its mercury concentration. For investigating trophic energetics, a 0.5‰ difference in $\delta^{15}N$ is probably inconsequential. However, half a trophic level of variation (~1.7‰) within a planktivorous fish community could be significant, particularly since fish predators at the top of lake food chains only vary by two trophic levels among lakes (Post et al. 2000). Within a population, if an individual fish is half a trophic level higher than the average population (because it has made an earlier transition to piscivory), then this could have significant consequences for its overall fitness (Post 2003). Using $\delta^{15}N$ to measure trophic position is a continuing challenge for ecologists, because there are many sources of intrapopulation variation in $\delta^{15}N$ that are unrelated to trophic variation. In general, ecological arguments based on stable isotope variation can be strengthened by using independent sources of dietary data (Matthews and Mazumder 2004), or data on growth dynamics (Post 2003).

Interpreting intrapopulation and temporal variation of $\delta^{15}N$

Determining whether size-based variation in $\delta^{15}N$ is actually related to trophic variation is a common problem in many ecological studies. Body size of fish is often

correlated with $\delta^{15}N$ (Beaudoin et al. 1999; Fry et al. 1999), and this relationship is commonly interpreted as a ontogenetic shift to higher trophic positions (Guiguer et al. 2002). However, relationships between body size and $\delta^{15}N$ can also result from other processes, including shifts in habitat use (Genner et al. 2003), and temporal variability of food sources (Fig. 3.6A). One of the important features of the TIM is that it can provide a prediction about the expected relationship between body size (or growth rate) and $\delta^{15}N$.

A practical extension of the TIM we used here would be a model that linked consumer growth dynamics with the isotopic composition and abundance of food sources. This would help address covariation between consumer growth rate and $\delta^{15}N$ of food sources, which could complicate interpretations of temporal isotopic patterns and intrapopulation variation of consumers. In lakes, for example, if the food quality of algae declines following a switch from uptake of NO_3^- to nitrogen fixation, this may lead to a correlated decline in both the $\delta^{15}N$ and abundance of the zooplankton community. In a similar vein, growth rate of juvenile sockeye may be correlated with the $\delta^{15}N$ of their food source. If juveniles grow faster by feeding on zooplankton with a higher $\delta^{15}N$, this could lead to a positive relationship between body size and $\delta^{15}N$. To determine if the resulting intrapopulation variation is related to trophic variation it is essential to determine to what extent $\delta^{15}N$ variation in the plankton community, both among and within species, is attributable to trophic variation.

For calculating the trophic position of planktivorous fish, the SAM (Seasonal Average Model) is much simpler to use and yields equivalent information as the TIM (Fig. 3.7). The TIM approach is more widely applicable for determining the integrative feeding history of consumers in general, and for interpreting intrapopulation isotopic

variation in particular. For example, TIM can provide an estimate of isotopic variation that is unrelated to trophic variation, and a prediction about the relationship between consumer body size and $\delta^{15}N$. Different types of temporal variation in food sources, coupled with variation in the growth dynamics of consumers, will lead to different amounts of intrapopulation isotopic variation (Fig. 3.6B). Large directional change in the $\delta^{15}N$ of a consumer's food source, particularly early in a consumer's ontogeny, will lead to more intrapopulation isotopic variation, because small differences in growth rate can lead to large isotopic differences in the biomass fixed during this time (Fig. 3.5). In comparison, smaller random isotopic variation will lead to less variation among individuals, even if individuals are growing at different rates. In either case, the intrapopulation variation resulting from differences in growth rate is unrelated to trophic variation, unless the temporal variation in the food source itself is related to trophic variation. Therefore, the TIM can provide an expected variance of a consumer population that feeds on a defined diet over time, and so can be used as a null model to test for the presence of trophic variation within a population (*sensu* Matthews and Mazumder 2004). There is an emerging body of literature that is interpreting intrapopulation variability in stable isotopes as evidence of individual diet variation (Gu et al. 1997; Beaudoin et al. 1999). However, these studies do not explicitly state the magnitude of variance required for evidence of individual specialization. Future studies should consider how the TIM model affects the use of isotopic mixing models and our interpretations of intra-individual variation of inert tissues (such as hair, feathers, or scales). In general, the temporal integration modeling approach is critical for linking isotopic variation among individuals to individual differences in diet.

Tables

Table 3.1 : Zooplankton biomass composition in 4 lakes

Taxonomic composition of zooplankton biomass for Sooke Lake Reservoir (SOL-S, SOL-D), Shawnigan Lake (SHL-S, SHL-D), Council Lake (COL), and Elk Lake (ELL). Percentages are a seasonal average of the percentage of the total zooplankton biomass on each sampling date.

Lake site	Percentage of zooplankton biomass: average (SE)				
	Daphnia spp.	calanoids	*Holopedium*	cyclopoids	Other
SOL-S	54.8 (3.8)	21.5 (2.7)	0	18.2 (1.9)	4.4 (1.3)
SOL-D	26.1 (2.8)	55.9 (2.7)	0	13.0 (2.8)	3.8 (0.5)
SHL-S	50.0 (2.7)	15.0 (1.9)	11.1 (3.6)	16.8 (1.2)	7.1 (1.0)
SHL-D	50.7 (3.7)	23.2 (1.9)	1.7 (1.2)	14.0 (1.6)	10.3 (1.3)
COL	36.5 (5.5)	33.8 (3.3)	24.1 (6.3)	0	5.6 (1.9)
ELL	68.4 (8.1)	19.6 (5.0)	0	5.0 (2.6)	7.1 (2.6)

Table 3.2 : Sensitivity analysis of *m* parameter in TIM.

The effect on the final estimate of fish $\delta^{15}N$ resulting from variability in the rate of metabolic tissue turnover. Parameter estimates in the literature for freshwater fish range from 0 to 0.025 day^{-1} (Hesslein et al. 1993; Harvey et al. 2002). The mean and variance is calculated for a simulation of 1000 fish that fed only on calanoids at SHL-S.

Parameter 'm'	Mean (variance)
0.0005	12.41 (0.054)
0.0070	12.46 (0.052)
0.01	12.49 (0.054)
0.05	12.78 (0.044)
0.10	13.07 (0.036)
0.25	13.56 (0.029)
0.35	13.71 (0.028)
0.50	13.81 (0.028)
0.80	13.81 (0.027)
1.0	13.73 (0.026)

Table 3.3 : Literature survey of zooplankton $\delta^{15}N$ time series.

Summary of the seasonal average, coefficient of temporal variance (variance/mean), and range of $\delta^{15}N$ for different zooplankton taxa and size fractions of POM in 15 lakes.

Lake (source)	Type of Plankton	Mean (‰)	CV	$\delta^{15}N$ Range	Sampling days
Buffalo Pound	POM	5.8	0.37	6.7	8
(Graham 1997)	*Daphnia*	7.9	0.18	4.1	7
	cyclopoid	7.6	0.29	6.5	8
Council Lake (2001)	POM	2.6	0.23	1.8	8
(Chapter 2)	*Daphnia*	3.9	0.18	2.2	7
	Holopedium	2.9	0.24	1.8	5
	calanoid	4.7	0.15	2.1	8
Council Lake (2002)	POM	1.2	1.62	9.2	27
(Chapter 4)	*Daphnia*	2.4	0.69	6.7	28
	Diaptomus	5.2	0.19	4.3	15
	Epischura	4.9	0.10	4.5	10
	Holopedium	2.9	0.61	7.9	17
	Chaoborus	5.1	0.15	5.8	28
Crooked Lake	POM	5.4	0.66	8.4	8
(Graham 1997)	*Daphnia*	9.4	0.45	10.9	8
	cyclopoid	9.7	0.29	8.0	8
	Leptodora	11.1	0.15	3.8	7
Elk Lake (ELL)	POM	7.5	0.22	3.9	8
(Chapter 2)	*Daphnia*	8.6	0.14	3.9	8
	calanoid	13.0	0.06	2.2	7
Katepwa Lake	POM	5.5	0.44	7.0	6
(Graham 1997)	*Daphnia*	8.8	0.37	9.4	7
	cyclopoid	12.7	0.27	9.5	8
	Leptodora	10.9	0.24	7.6	7
Lake 110	POM	6.3	0.22	3.9	7
(Kidd et al. 1999)	*Chaoborus*	7.1	0.08	1.5	7
Lake 227	POM	8.3	0.21	5.0	6
(Kidd et al. 1999)	*Chaoborus*	9.4	0.10	2.6	6
Lake Diefenbbaker	POM	8.7	0.14	3.2	8
(Graham 1997)	*Daphnia*	12.2	0.11	4.4	8
	cyclopoid	12.6	0.29	10.3	8
	Leptodora	12.4	0.34	9.4	6
Last Mountain Lake	POM	7.8	0.16	2.8	7
(Graham 1997)	*Daphnia*	6.5	0.22	4.7	8
	cyclopoid	9.3	0.25	6.6	8
	Leptodora	10.4	0.25	6.8	6

Loch Ness	POM	7.6	0.29	8.8	12
(Grey et al. 2001)	POM	10.6	0.25	9.6	12
	Daphnia	6.1	0.19	3.0	6
	calanoid	8.4	0.11	2.3	6
	cyclopoid	8.8	0.05	1.2	6
	Bythotrephes	10.5	0.04	0.8	6
Lake Ontario (41)	POM (>0.7 μm)	7.7	0.38	8.4	8
(Leggett et al. 2000)	POM (20-44 μm)	6.0	0.59	9.3	10
	Bosmina	6.3	0.38	8.8	6
	Daphnia	8.1	0.36	7.3	12
	calanoid	12.4	0.37	13.7	14
	cyclopoid	12.3	0.31	11.3	16
Lake Ontario (81)	POM (>0.7 μm)	6.1	0.41	8.0	9
(Leggett et al. 2000)	POM (64-110 μm)	6.6	0.26	6.5	11
	POM (110-210 μm)	10.1	0.35	9.6	5
	POM (210-295 μm)	11.1	0.08	2.5	6
	Bosmina	7.5	0.25	6.4	10
	Daphnia	7.5	0.31	4.6	3
	calanoid	11.4	0.23	7.4	10
	cyclopoid	10.7	0.35	13.1	11
Shawnigan Lake	POM				11
(SHL-S)		5.6	0.15	2.9	
(This study)	*Daphnia*	6.4	0.11	2.3	11
	calanoid	9.1	0.12	3.3	11
Shawnigan Lake	POM				11
(SHL-D)		5.7	0.12	2.5	
(Chapter 2)	*Holopedium*	5.8	0.12	2.2	8
	Daphnia	6.0	0.11	2.5	11
	calanoid	9.1	0.13	3.1	11
Smith Lake	POM	3.3	0.57	7.2	26
(Gu et al. 1994)	*Daphnia*	4.4	0.25	6.3	49
	Diaptomus	6.9	0.10	2.4	10
	Heterocope	6.9	0.10	2.0	10
Sooke Reservoir	POM				11
(SOL-S)		3.3	0.38	3.9	
(This study)	*Daphnia*	3.6	0.17	2.1	11
	calanoid	6.0	0.06	1.1	11
Sooke Reservoir	POM				11
(SOL-D)		3.7	0.24	3.3	
(Chapter 2)	*Daphnia*	3.1	0.14	1.3	11
	calanoid	5.7	0.11	1.9	11
Lake Suwa	*Bosmina*	10.9	0.12	3.1	5
(Toshioka et al. 1994)	calanoid	12.6	0.13	4.8	13
	Leptodora	13.1	0.14	5.3	13

Figures

Figure 3.1 : Time series of *Daphnia* $\delta^{15}N$ from a literature survey.

The figure is split based on the simulation analyses in Fig. 3.6. (A) shows time series of *Daphnia* $\delta^{15}N$ that resulted in either a significant positive relationship ($p < 0.05$) between juvenile sockeye size and $\delta^{15}N$ (denoted by *), or no relationship. (B) shows time series with a significant negative relationship between juvenile sockeye size and $\delta^{15}N$ (Fig. 3.6 A). The line through Smith Lake is a LOWESS plot (Tension = 0.5) for multiple years of data.

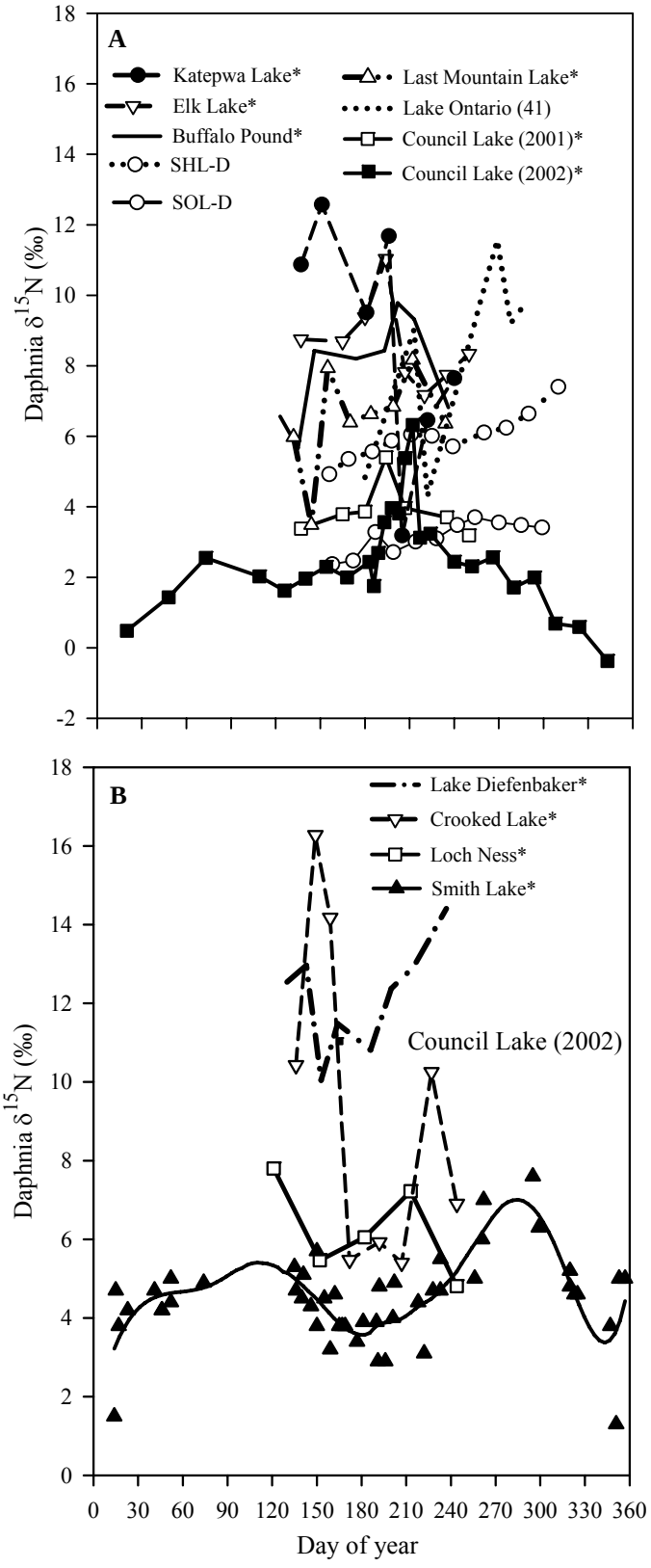

A
Katepwa Lake*
Elk Lake*
Buffalo Pound*
SHL-D
SOL-D
Last Mountain Lake*
Lake Ontario (41)
Council Lake (2001)*
Council Lake (2002)*
Daphnia δ15N (‰)
B
Lake Diefenbaker*
Crooked Lake*
Loch Ness*
Smith Lake*
Council Lake (2002)
Daphnia δ15N (‰)
Day of year

Figure 3.2 : Seasonal $\delta^{15}N$ of *Daphnia*, calanoids, *Holopedium*, and POM >200μm. Error bars are on ± 1SE of the mean. When error bars are not present, only a single sample was analyzed for that sampling date. Filled symbols for SHL and SOL are the deep sites (from Matthews and Mazumder 2003), and open symbols are the shallow sites (see text).

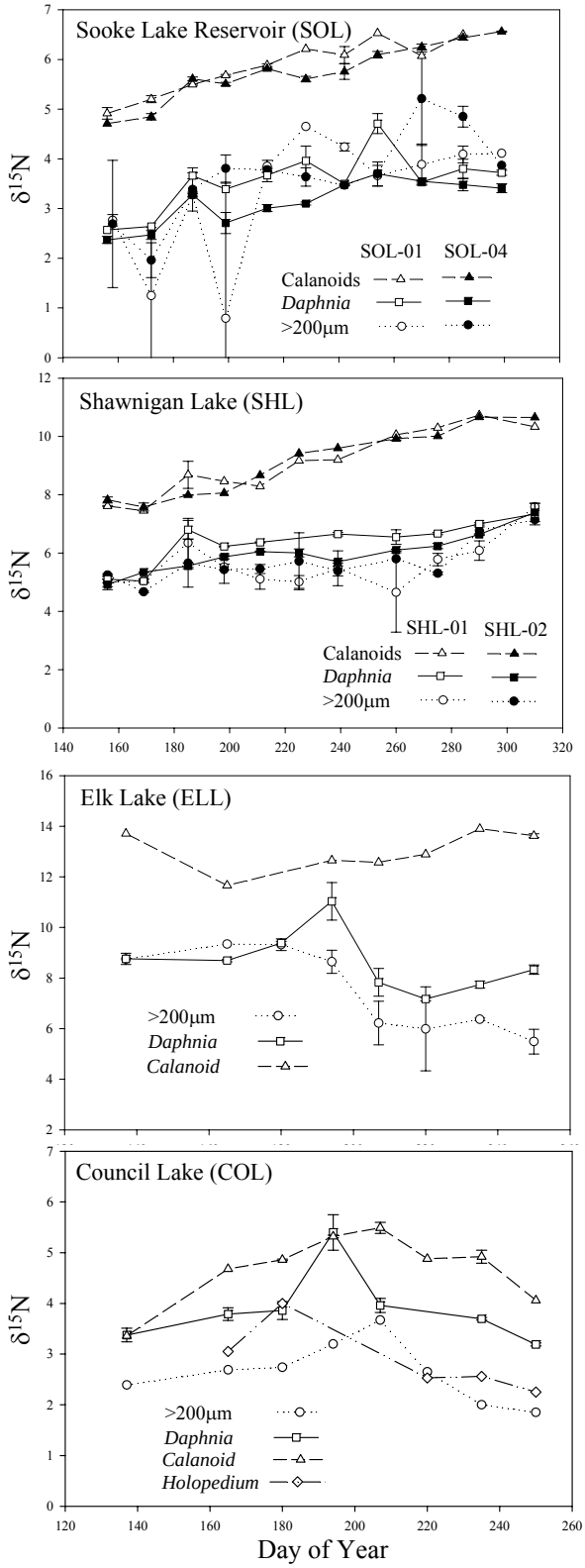

Sooke Lake Reservoir (SOL)
δ15N
SOL-01
SOL-04
Calanoids
Daphnia
>200µm
Shawnigan Lake (SHL)
δ15N
SHL-01
SHL-02
Calanoids
Daphnia
>200µm
Elk Lake (ELL)
δ15N
>200µm
Daphnia
Calanoid
Council Lake (COL)
δ15N
>200µm
Daphnia
Calanoid
Holopedium
Day of Year

Figure 3.3 : Intrapopulation isotopic variance of juvenile sockeye.

Each distribution represents a predicted region for a juvenile sockeye feeding exclusively on a defined diet for our four study lakes (POM>200 μm; *Daphnia*, *Holopedium*, macrozooplankton, or calanoids). The $\delta^{15}N$ of macrozooplankton is calculated as described in the text. The TIM simulations were done for each diet category from day 152 to 273 (June to Oct), with parameters $\mu = 0.03$, $\sigma_{\mu}^2 = 0.01$, $\sigma_{\Delta}^2 = 0.17$.

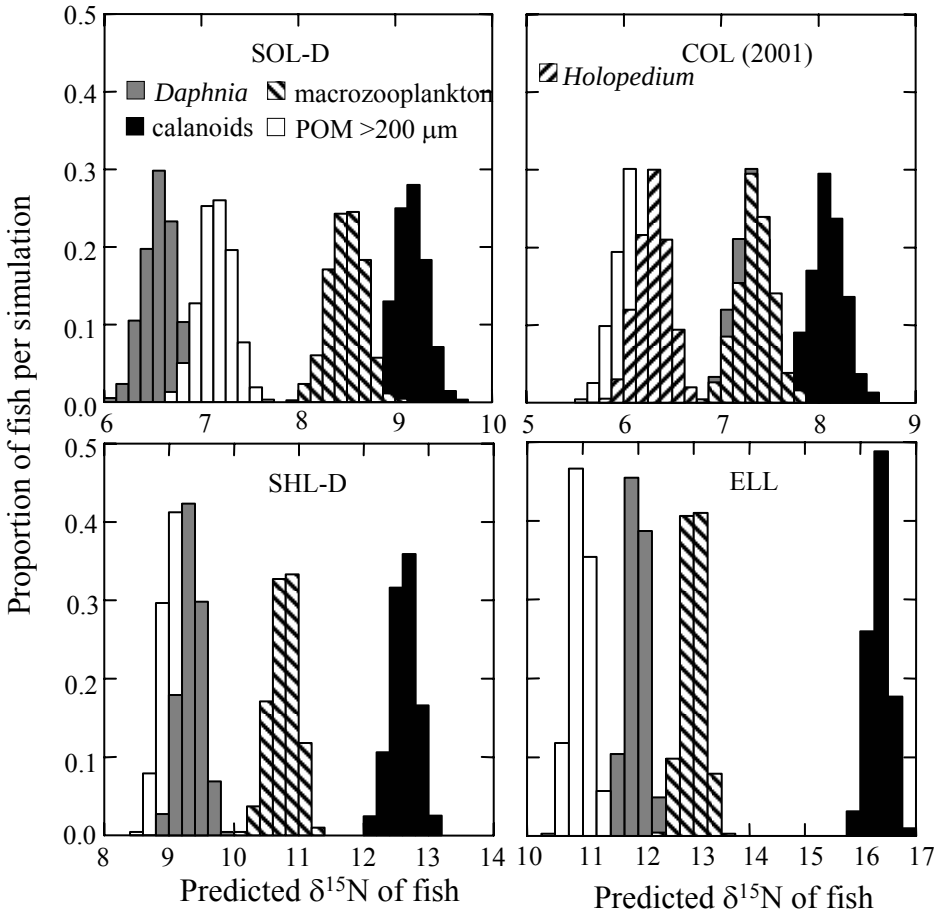

SOL-D
Daphnia
macrozooplankton
calanoids
POM >200 μm
COL (2001)
Holopedium
SHL-D
ELL
Proportion of fish per simulation
Predicted δ15N of fish
Predicted δ15N of fish

Figure 3.4 : Sensitivity analysis of the temporal integration model

Done with respect to the variance in trophic fractionation σ_{Δ}^2 for diets at SOL-D. Hatching is the same as in Fig. 3.3. (A) Shows the amount of variance attributed primarily to differences in growth rate among individual juvenile sockeye. (B) Shows a typical increase in variance if we increase the σ_{Δ}^2 to a value for freshwater fish (σ_{Δ}^2= 0.17) (Vander Zanden and Rasmussen 2001). (C) Shows how the variance changes using an interspecific estimate of variance from Post (2002) (σ_{Δ}^2= 0.98).

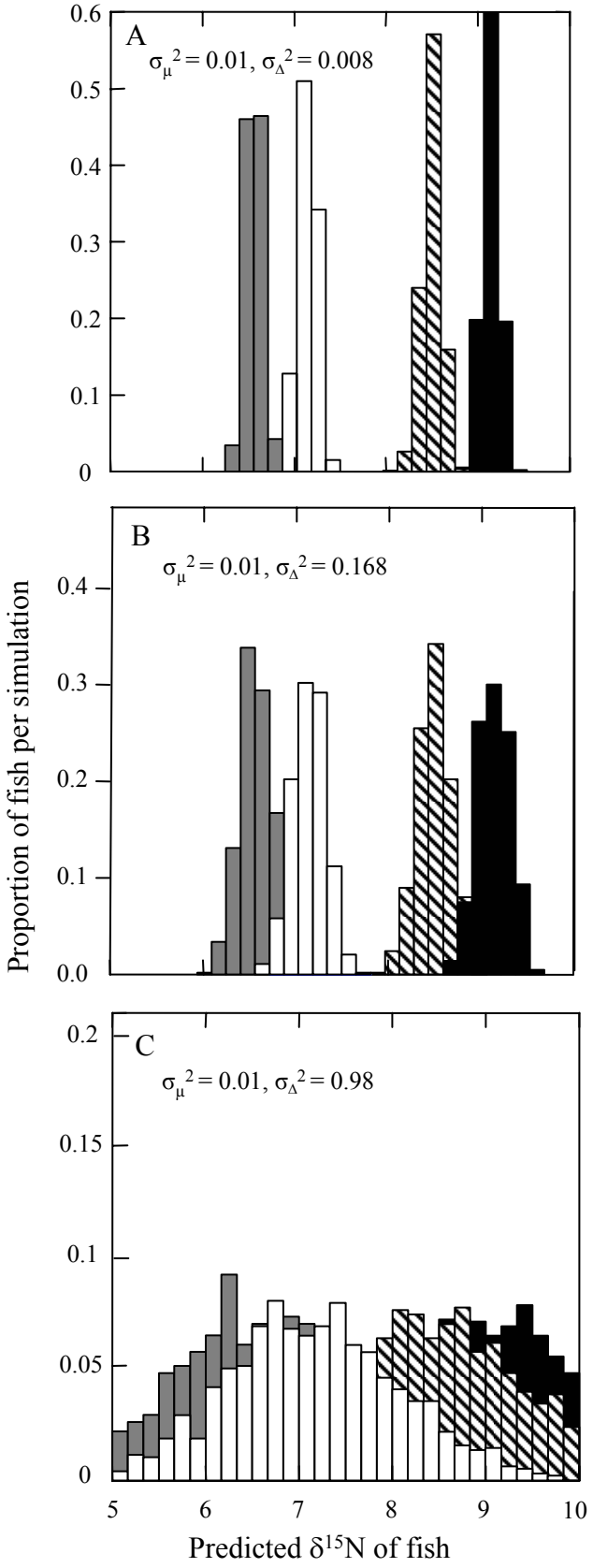

A
$\sigma_\mu^2 = 0.01$, $\sigma_\Delta^2 = 0.008$
B
$\sigma_\mu^2 = 0.01$, $\sigma_\Delta^2 = 0.168$
C
$\sigma_\mu^2 = 0.01$, $\sigma_\Delta^2 = 0.98$
Proportion of fish per simulation
Predicted δ^{15}N of fish

Figure 3.5 : Isotopic trajectory of juvenile sockeye in a TIM simulation.

Trajectory of juvenile sockeye (symbols - left axis) in a TIM simulation based on *Daphnia* $\delta^{15}N$ from Council Lake 2002 (solid line - right axis) (Table 3.3). The simulation was from day 152 to 273 (June to Oct), with parameters $\mu = 0.03$, $\sigma_\mu^2 = 0.01$, $\sigma_\Delta^2 = 0.008$. The dotted line (left axis) is the predicted $\delta^{15}N_{base}$ for the planktivore trophic level, calculated as the seasonal average of *Daphnia* $\delta^{15}N + 3.4‰$. The open circles are a trajectory of a fish with an average growth rate which is similar to the TIM's prediction for $\delta^{15}N_{base}$. The difference between predictions is $\Delta_{SAM-TIM}$ (Fig. 3.7).

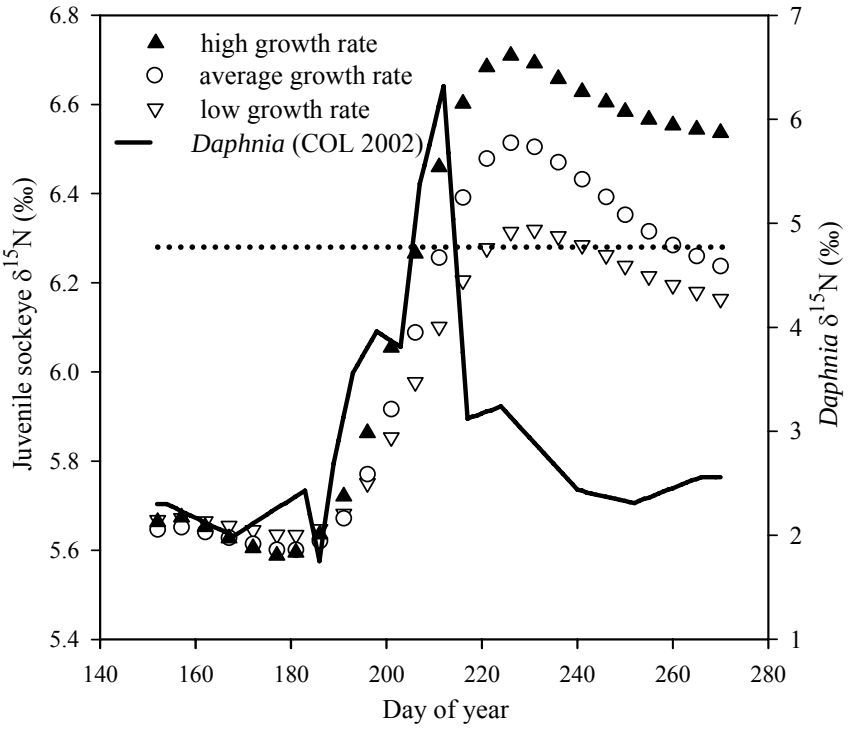

high growth rate
average growth rate
low growth rate
Daphnia (COL 2002)
Juvenile sockeye δ15N (‰)
Daphnia δ15N (‰)
Day of year
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
7
6
5
4
3
2
1
140
160
180
200
220
240
260
280

Figure 3.6 : Applications of the TIM model.

(A) Shows lines from linear regressions (N= 1000; data points not shown) for 4 simulations where there was a significant ($p< 0.05$) relationship between juvenile sockeye weight and $\delta^{15}N$ at the end of a TIM simulation. Here, all simulations were done from day 121 to 243 (May to September) with parameters $\mu= 0.03$, $\sigma_{\mu}^2 = 0.01$, $\sigma_{\Delta}^2 = 0.17$. For clarity, intrapopulation variation in $\delta^{15}N$ is shown as Z-scores for individual juvenile sockeye at the end of the simulation. (B) Shows the effect of increasing variation in growth rate among individuals (σ_{μ}^2) on the total amount of intrapopulation isotopic variation at the end of several TIM simulations. Lines are the same as in (A), as are the parameters μ and σ_{Δ}^2 for the simulations.

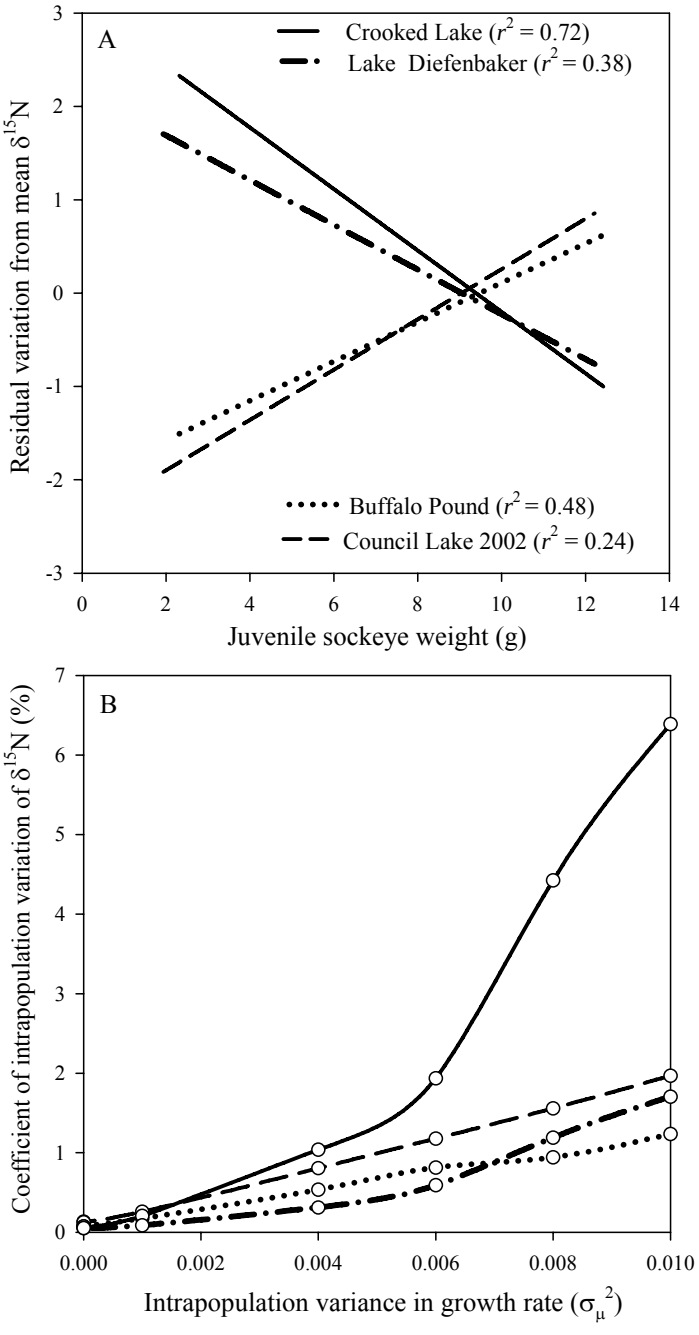
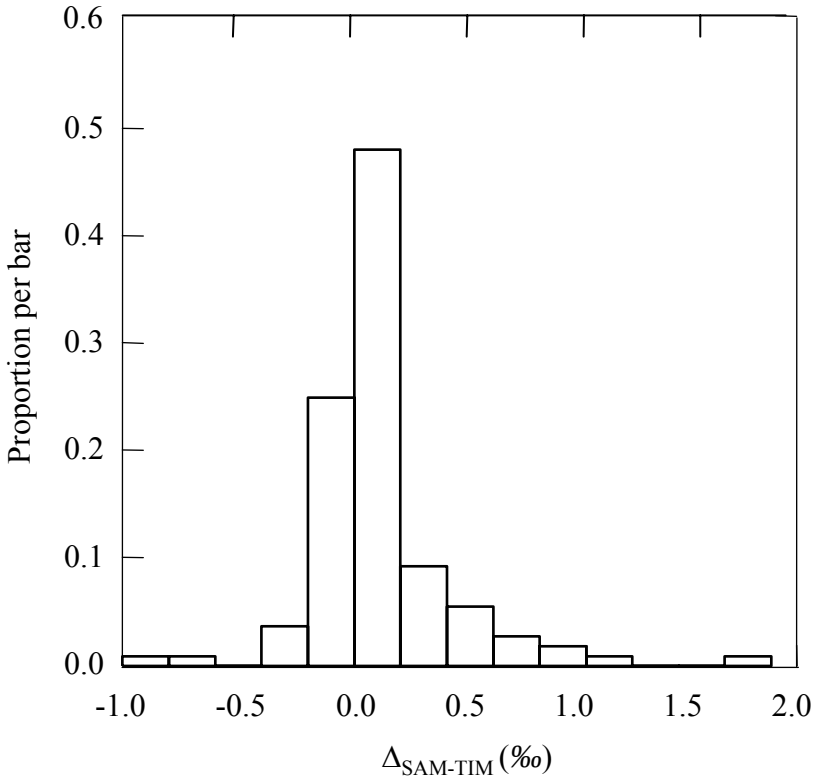

A
Crooked Lake (r^2 = 0.72)
Lake Diefenbaker (r^2 = 0.38)
Buffalo Pound (r^2 = 0.48)
Council Lake 2002 (r^2 = 0.24)
Residual variation from mean $\delta^{15}N$
Juvenile sockeye weight (g)
B
Coefficient of intrapopulation variation of $\delta^{15}N$ (%)
Intrapopulation variance in growth rate (σ_μ^2)

Figure 3.7 : Comparison of baseline models for the trophic position of fish.

Histogram of the difference between baseline approaches ($\Delta_{SAM-TIM}$ in ‰ units) for calculating the trophic position of juvenile sockeye. Here we plot the results from 108 simulations based on different interpolated time series and model modifications, including all the simulations used to generate Fig. 3.3 (different diets), Fig. 3.4 (sensitivity analysis), and Fig. 3.6 (*Daphnia* only).


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion per bar
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
$\Delta_{SAM-TIM}$ (‰)

Chapter 4: Distinguishing trophic from isotopic variation ($\delta^{15}N$) in zooplankton communities

Citation:

Matthews, B., and A. Mazumder. Distinguishing trophic from isotopic variation ($\delta^{15}N$) in zooplankton communities. *Submitted*.

Abstract

We measured the $\delta^{15}N$ of particulate organic matter (POM), *Daphnia* (D), *Holopedium gibberum* (H), *Leptodiaptomus tyrelli* (LT), *Epischura nevadensis* (E), *and Chaoborus trivittatus* (C) over an annual cycle in Council Lake, a pristine oligotrophic fishless lake. We also examined size-based variation in the $\delta^{15}N$ of zooplankton over the same time period. Annual averages of the $\delta^{15}N$ of plankton (C, LT, E > D, H > POM) reflected established differences in feeding behaviours. Body size was correlated with $\delta^{15}N$ in *Daphnia*, *Chaoborus*, and *Epischura* which could indicate size-based trophic variation, selective feeding, or physiological differences in ^{15}N enrichment. During Jul and August, the $\delta^{15}N$ of D, H, and LT were all briefly higher than the $\delta^{15}N$ of E and C, despite little temporal variation in the $\delta^{15}N$ of POM (< 41 μm). However, the $\delta^{15}N$ of larger size fractions of POM increased rapidly prior to D, H, and LT, and could explain some of the temporal variability of zooplankton $\delta^{15}N$. Detailed time series of isotopic differences among zooplankton species may help detect seasonal changes in zooplankton feeding behaviours, and trace multiple pathways of nitrogen flow (i.e. food chains) in planktonic food webs.

Introduction

Measuring the temporal variability of zooplankton $\delta^{15}N$ is a novel approach for quantifying seasonal changes in the trophic structure of zooplankton communities. Several studies have documented large seasonal changes in the $\delta^{15}N$ of individual zooplankton taxa (Graham 1997; Leggett et al. 2000). Most of this temporal variation is unlikely to be related to trophic variation, because of large seasonal isotopic variability of primary producers (Leggett et al. 2000; Lehmann et al. 2004). The $\delta^{15}N$ of *Daphnia* can change by > 7‰ over a summer in response to changes in biogeochemical processes that alter the $\delta^{15}N$ of its food sources (Leggett et al. 2000). Isotopic differences between zooplankton taxa more likely represent trophic variation (Kling et al. 1992; Matthews and Mazumder 2003; Karlsson et al. 2004), but no study has examined if time series of $\delta^{15}N$ for multiple zooplankton taxa can indicate seasonal changes in their relative feeding behaviours.

The trophic position of freshwater zooplankton varies among species and within a species over time. Life-history omnivory is a common feature of many invertebrate predators (Moore et al. 1994; Branstrator et al. 2000), and even late instars can exhibit varying degrees of omnivory (Moore et al. 1994). Nauplii of calanoid copepods are herbivorous, and can mature into herbivores, detritivores, and omnivores (Anderson 1967; Chow-Fraser and Wong 1986). Adult calanoid copepods can switch foraging strategies depending on the available food sources (Burns and Schallenberg 2001), and may alternate between an algal and microbial-based food chain depending on the time of year (Porter 1996).

In this paper, we examine whether seasonal changes in the $\delta^{15}N$ of different zooplankton taxa support expectations about trophic variation in different zooplankton instars, sizes, and taxa. To do so, we sampled a pristine fishless coastal oligotrophic lake (Council Lake), and measured the $\delta^{15}N$ of several common zooplankton taxa that have well known feeding behaviours. We measured the $\delta^{15}N$ of particulate organic matter (POM), *Daphnia* and *Holopedium* (predominantly herbivores), *Epischura* and *C. trivittatus* (invertebrate predators), and a calanoid copepod species (*L. tyrelli*) that is omnivorous (Anderson 1967). We sampled at least monthly for an entire year, because changes in biogeochemical processes significantly affect the isotopic dynamics of particulate organic matter (POM) (Lehmann et al. 2004). We found that the temporal variation in the $\delta^{15}N$ of zooplankton was uncoupled from the $\delta^{15}N$ of a small size fraction of POM (POM <41μm), and that the $\delta^{15}N$ of the more herbivorous taxa briefly exceeded the $\delta^{15}N$ of invertebrate predators.

Methods

We sampled zooplankton and particulate organic matter <41 μm (POM) in Council Lake (48º31' 123º41') 28 times between 14 Feb 2002 and 17 Mar 2003. During lake stratification (May to Nov) we collected samples every two weeks, except in Jul when we sampled every 3-4 days. Council Lake is a warm monomictic oligotrophic lake that rarely gets permanent ice cover. We collected zooplankton during the day with a 64 μm mesh, 50 cm diameter Wisconsin net by vertical tows through the entire water column during the day, and horizontal tows through the epilimnion. We froze the zooplankton samples within 4 h of collections, and later sorted out composite samples

(~0.5 mg) from five taxonomic groups (Table 4.1): *Daphnia*, *Holopedium gibberum* (*Holopedium*), *Leptodiaptomus tyrelli* (*L. tyrelli*), *Epischura nevadensis* (*Epischura*), *and Chaoborus trivittatus* (*C. trivitattus*). Prior to isotopic analysis, we measured the body size of all zooplankton 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 composite sample of cladocerans we selected individuals of varying sizes, but for copepods we tended to exclude the smaller copepodid stages because large copepods (>1 mm) were present year round (Annual range of average size: *L. tyrelli* 0.88 to 1.64 mm, *n*= 15; *Epischura* 1.14-2.19 mm, *n*= 10). *Chaoborus* instars II, and III only appeared in our samples in the middle of June to the end of July, whereas instar IV larvae were present year round.

In the spring of 2002, we used a Niskin bottle to collect lake water from 3 depths (2, 8, 16 m). Starting in May 2002, and for the rest of the study, we sampled 6 depths (~2, 6, 8, 10, 12, 14 m), except in July when we sampled 9 depths (1, 3, 5, 7, 8, 9, 12, 14, 16 m). We chose these depths so that during the stratified period we could sample 2 to 3 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 pre-combusted (550°C for 1 hour) 25 mm GF-C 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). For 7 dates in July, we also filtered >4 L for POM 41-200 μm ($POM_{41\text{-}200}$) from 3 depths (3, 9, and 14m), and picked out clumps of POM from our zooplankton net samples that had large aggregations of *S.*

lapponica (POM_{SL}), but that also included microplankton. 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 POM_{41} filters by extracting them in 95% ethanol at 4°C overnight, and analyzing the extracts on a spectrophotometer (Ultraspec® 2000, Amerhsam) using a 10-cm quartz cell. All stable isotope samples were analyzed on a Finnigan 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* standard in every sample run, and its precision was <0.2‰ for $\delta^{15}N$ and $\delta^{13}C$ within and among all sample runs.

We compiled limnological data for Council Lake for the summer of 2002 (May to Sept) from an ongoing research program investigating the water quality of lakes and reservoirs in Victoria, British Columbia (Table 4.2). Details about the limnological sampling procedures and sample analysis for this data are available elsewhere (Davies 2004). Monthly measurements during the summer were made for the limnological parameters shown in Table 4.2.

Results

Temporal and size-based variation in the $\delta^{15}N$ of zooplankton

Over the entire sampling season the average $\delta^{15}N$ of the invertebrate predators (*Epischura* and *C. trivitattus*) was about 2.4‰ higher than the herbivores (*Daphnia* and *Holopedium*) (Table 4.1). The seasonal average $\delta^{15}N$ of *L. tyrelli* was within 0.5‰ of *Epischura* and *C. trivitattus*, but the difference depended on the time of year (Fig. 4.1). During July and August, the $\delta^{15}N$ of *Daphnia* and *Holopedium* increased and surpassed

the $\delta^{15}N$ of *Epischura* and *C. trivitattus*. The $\delta^{15}N$ of *L. tyrelli* was similar to *Epischura* and *C. trivittatus* during the unstratified period, but then reached a maximum at the end of July concurrently with the maximum $\delta^{15}N$ of *Daphnia* (Fig. 4.1, 4.2).

Isotopic differences among zooplankton taxa depended primarily on the time of year (Fig. 4.1), and, for some taxa, secondarily on zooplankton body size (Figs. 4.3, 4.4, 4.5). The range of *Daphnia* $\delta^{15}N$ was 10.9‰ over the entire sampling period, and *Daphnia* body size accounted for 25% of the seasonal variability ($F_{1,93} = 31.4$, $r^2 = 0.25$, $p < 0.001$; Fig. 4.3A). Using non-parametric rank correlation (samples ranked for each sampling day), we found that larger *Daphnia* had a higher $\delta^{15}N$ than smaller *Daphnia* (Fig. 4.3B; Spearman's rank correlation: $r_s = 0.786$, $p < 0.001$). Isotopic differences between large (> 1.8 mm) and medium sized (< 1.8 mm) *Daphnia* (categorized *post hoc* based on Fig. 4.4A) depended on the time of year (Fig. 4.4B), but the $\delta^{15}N$ of large *Daphnia* was an average of 1.1‰ (SD = 0.9, $N = 19$) higher than medium sized *Daphnia*. Only large *Daphnia* ever exceeded 5‰ (Fig. 4.4B). In contrast, the size of *Holopedium* was uncorrelated with $\delta^{15}N$ ($r = 0.01$, $p = 0.42$), and its body size stayed within a narrow range (0.8 to 1.2 mm) from July to September, even though the amplitude of variation in $\delta^{15}N$ was similar to *Daphnia* (Fig. 4.1B).

In July and August, when instars II, III, and IV of *C. trivittatus* were all present in the water column, we found that $\delta^{15}N$ of *C. trivittatus* increased by about 0.5‰ per mm of head length (Fig. 4.5). The slope of the positive relationship between size and $\delta^{15}N$ was not significantly different between months (ANCOVA: $F_{1,59} = 0.004$, $p = 0.81$), but the intercept was slightly higher in August. The sharp drop in the $\delta^{15}N$ of *Chaoborus* 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 $\delta^{15}N$. However, the $\delta^{15}N$ of instar IV larvae also declined by ~1 ‰ in June, and then increased over the summer.

Seasonal variation in body size and $\delta^{15}N$ 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 $\delta^{15}N$ (7.3‰), but two weeks later a similar sized sample (1.56 mm) had a $\delta^{15}N$ 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. However, more detailed studies might be able to use $\delta^{15}N$ to detect ontogenetic trophic variation in copepod communities.

Temporal change in the composition of POM

The range in the $\delta^{15}N$ of POM <41 μm (POM_{41}) was 12.5‰ over the entire study period (Fig. 4.2; Table 4.1). Excluding 20 Jan, 2003, the total range of $\delta^{15}N$ for POM_{41} was 6.0‰ (Min= -1.5, Max= 4.5‰). The average range in the $\delta^{15}N$ of POM_{41} over the entire water column was 2.3‰ (SE= 0.27, n= 27). During the stratified period (May to October), neither the average nor the temporal patterns of POM_{41} $\delta^{15}N$ were significantly different among strata (Table 4.1; RM-ANOVA, $F_{2,3}$= 1.17, p= 0.42; $F_{30,45}$= 1.14, p= 0.34). During July and August, when the $\delta^{15}N$ of *Daphnia*, *Holopedium*, and *L. tyrelli* reached their maxima, the $\delta^{15}N$ of POM_{41} did not increase substantially. As a result, the average isotopic difference between *Daphnia* and POM_{41} ($\Delta_{D\text{-}POM41}$) was 3.1‰ during July and August (Range: 2.2 to 5.6‰), and only 1.4‰ (Range 0.6 to 2.1‰) for the rest of stratification. The $\delta^{15}N$ of larger size fractions of POM ($POM_{41\text{-}200}$, and POM_{SL})

increased rapidly prior to the peak $\delta^{15}N$ of *Daphnia*, *Holopedium,* and *L. tyrelli* (Fig. 4.2B). The $\delta^{15}N$ of $POM_{41\text{-}200}$ peaked on July 17th just prior to the peak $\delta^{15}N$ for *Holopedium* (26 July) and *Daphnia* (31 July), whereas the $\delta^{15}N$ of POM_{SL} fraction increased to a maximum on July 31st that was higher than either *Daphnia* or *Holopedium*. We cannot explain the large isotopic excursion of POM_{41} on 20 Jan, 2003. Lehmann et al. (2004) also found a high $\delta^{15}N$ 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 a maximum on the 12th and 17th of July. On these two days, the average carbon concentration (POC) of POM in the water column (0.17 mgC L^{-1}) was ~30% lower than during the rest of stratification (Fig. 4.2A). This week of low POC with a high C:N (low food quality) occurred a week before the maximum $\delta^{15}N$ of *Daphnia*. However, Chl *a* was not unusually low during this time period. The Chl *a* of POM_{41} was typically less than 1 μgL^{-1} and was lowest in the winter and at the beginning of July and August (Fig. 4.2A).

Vertical variation in the composition of POM

Previous zooplankton sampling in Council Lake revealed that *Daphnia* feed in the hypolimnion during the day and night during July (unpubl. data). Therefore, in July, we increased our sampling frequency and depth resolution of POM to test for potential effects of feeding depth on the $\delta^{15}N$ of zooplankton. In July, we found that the $\delta^{15}N$ of POM did not increase with depth, and that the C:N was highest in the metalimnion and

lowest in the hypolimnion (Fig. 4.6). Size fractionated Chl *a* revealed that algal abundance was highest in the hypolimnion, particularly in larger size fractions.

The $\delta^{13}C$ of POM_{41} declined with depth during the period of stratification, and increased slightly with depth during the unstratified period (Fig. 4.7, 4.8). In July, the $\delta^{13}C$ of $POM_{41\text{-}200}$ was lowest in the hypolimnion, where it reached $\delta^{13}C$ values as low as POM_{SL} (Fig. 4.8). In contrast, the average $\delta^{15}N$ of $POM_{41\text{-}200}$ in July was 2.6‰, and there was no significant effect of depth ($F_{2,19} = 2.59$, $p = 0.10$).

Mismatch between zooplankton and POM $\delta^{13}C$

The $\delta^{13}C$ of zooplankton depended on the taxonomic grouping (and the size class for *Daphnia*), during both the stratified (ANOVA: $F_{5,74}= 87.5$, $p< 0.001$) and unstratified period ($F_{3,30}= 45.6$, $p< 0.001$; Fig. 4.8). The $\delta^{13}C$ of *L. tyrelli* and both size classes of *Daphnia* were lower than the $\delta^{13}C$ of POM_{41} at all depths during stratification, whereas the $\delta^{13}C$ of *Chaoborus*, *Epischura* and *Holopedium* were all within the $\delta^{13}C$ range of POM_{41} over the water column (Fig. 4.8). The $\delta^{13}C$ of POM_{SL} and $POM_{41\text{-}200}$ in the hypolimnion were the only measured POM fractions that could account for the low $\delta^{13}C$ of *Daphnia* and *L. tyrelli* during the stratified period. Despite similar patterns of $\delta^{15}N$ in *Daphnia* and *Holopedium*, only the $\delta^{13}C$ of large *Daphnia* and *L. tyrelli* declined concurrently with the decline in the $\delta^{13}C$ of hypolimnetic POM_{41} following lake stratification (Fig. 4.8).

Discussion

Seasonal averages of zooplankton $\delta^{15}N$ qualitatively reflect established feeding strategies of different taxa (Chapter 2, Chapter 3). However, in this paper we show that the $\delta^{15}N$ of zooplankton can change substantially over the season, even in a pristine lake with no public access or development in the watershed. We also found that the isotopic difference in $\delta^{15}N$ between two zooplankton taxa strongly depends on the time of year (Fig. 4.1), and that the $\delta^{15}N$ of more herbivorous zooplankton can temporarily have a higher $\delta^{15}N$ than invertebrate predators.

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

Our results provide some of the first evidence that larger *Daphnia* can have a higher $\delta^{15}N$ than smaller *Daphnia* (Fig. 4.3, 4.4), which could indicate a size-based transition in diet to food sources with a higher $\delta^{15}N$ (e.g. $POM_{41\text{-}200}$ or POM_{SL}). However, we are uncertain if zooplankton in Council Lake can directly graze larger size fractions of POM, or whether size-based variation in $\delta^{15}N$ is positively related to trophic position. The maximum particle size ingested by cladocerans increases with body size (Burns 1968), and experimental studies have found that *Daphnia* have a higher fecundity when their algal diet is supplemented with ciliates, heterotrophic nanoflagellates, and bacteria (Sanders et al. 1996). We were able to visually confirm, by looking at fecal pellets, that copepods are able to ingest *S. lapponica* (which makes up POM_{SL}), but we found no evidence of *S. lapponica* in the gut of large *Daphnia.*

Large variation in the $\delta^{15}N$ of zooplankton food sources could explain why the $\delta^{15}N$ of large *Daphnia*, *Holopedium*, and *L. tyrelli* increased above the $\delta^{15}N$ of more

omnivorous taxa (*Epischura* and *C. trivitattus*). The $\delta^{15}N$ of POM_{41} does not substantially increase prior to, or during, the increase in the $\delta^{15}N$ of *Daphnia*, *Holopedium*, and *L. tyrelli*. However, the $\delta^{15}N$ of larger size fractions of POM ($POM_{41\text{-}200}$, and POM_{SL}) both increase rapidly in early July, and the $\delta^{15}N$ of $POM_{41\text{-}200}$ peaks just prior to the maximum $\delta^{15}N$ of large *Daphnia* (Fig. 4.2, 4.3). It might be possible that large *Daphnia* (>1.8 mm) can feed on larger POM size fractions, but this is unlikely for *Holopedium*. *Holopedium* grazes smaller sized particles than large *Daphnia*, and in July is less abundant in the hypolimnion of Council Lake (which is the primary origin of POM_{SL}). An alternate possibility is that smaller edible algae, within POM_{41}, follow a similar $\delta^{15}N$ pattern as POM_{SL}. This is only plausible if both large and small algae use a similar source of dissolved inorganic nitrogen, and have a similar discrimination against ^{15}N during uptake (but see Needoba et al. 2003). This would explain why the $\delta^{15}N$ of *Daphnia* and *Holopedium* increases dramatically (Fig. 4.2) even if they do not graze directly on POM_{SL}. If this is the case, it is still surprising that the $\delta^{15}N$ of smaller *Daphnia* did not increase concurrently with *Holopedium*.

In future studies, it could be informative to compare the change in zooplankton $\delta^{15}N$ with changes in the $\delta^{15}N$ of DIN (*sensu* Leggett et al. 2000). This is somewhat analogous to comparing the $\delta^{13}C$ of zooplankton to the $\delta^{13}C$ of dissolved inorganic carbon (DIC) (Pace et al. 2004), and could help determine to what extent zooplankton are tracking the $\delta^{15}N$ of difference DIN sources. The situation for DIN may be more complicated than for DIC, because ammonia and nitrate can have different $\delta^{15}N$ signatures, (Leggett et al. 2000) and there may be large uncertainty in the ^{15}N fractionation among algal species and for bacteria during DIN uptake (Hoch et al. 1992;

Needoba et al. 2003). Although measuring the $\delta^{15}N$ of DIN is analytically challenging (particularly in lakes as oligotrophic as Council Lake), it could certainly help future studies determine how well the $\delta^{15}N$ of POM reflects the nitrogen sources of zooplankton.

POM is an isotopically heterogeneous mixture and may contain zooplankton food sources that are in low abundance and potentially undetectable by measuring bulk properties of POM (Pel et al. 2003). Many studies have shown that the $\delta^{13}C$ of zooplankton is substantially lower than the $\delta^{13}C$ of POM (Fig. 4.8), particularly in oligotrophic lakes with high inputs of allochthonous carbon (Grey et al. 2001). However the relationship between the $\delta^{15}N$ of POM and zooplankton is far less clear. In Council Lake, larger size fractions of POM have the highest $\delta^{15}N$, but we have no data or expectation about the isotopic heterogeneity of $\delta^{15}N$ within the POM_{41} size fraction. As a result it is difficult to determine if the $\delta^{15}N$ of POM provides a reasonable estimate of zooplankton food sources. The $\delta^{13}C$ of large *Daphnia* is more similar to the $\delta^{13}C$ of $POM_{41\text{-}200}$ and POM_{SL} then it is to POM_{41}, whereas depth-based variation in the $\delta^{13}C$ of POM_{41} can account for the $\delta^{13}C$ of *Holopedium*, *Epischura*, and *Chaoborus* (Fig. 4.7, 4.8). During stratification, only the $\delta^{13}C$ of large *Daphnia* and *L. tyrelli* declined concurrently with the $\delta^{13}C$ of POM_{41} in the hypolimnion (Fig. 4.8). This suggests that both these taxa can exploit carbon in the hypolimnion, but there is still a large mismatch between the $\delta^{13}C$ of POM_{41} and *Daphnia*. This difference can only be partially explained by differences in lipid content (Matthews and Mazumder 2005a; Matthews et al. unpubl. data). The low $\delta^{13}C$ of large POM size fractions suggests that some zooplankton can

directly graze these size fractions. However, an alternate explanation, which matches our argument for $\delta^{15}N$, is that the $\delta^{13}C$ of these large POM fractions may reflect the $\delta^{13}C$ of smaller more edible algae whose $\delta^{13}C$ is masked by other carbon sources in POM_{41} (Pel et al. 2003).

Vertical variation of zooplankton feeding

In Council Lake, spatial segregation of zooplankton in the water column further confounds our interpretation of $\delta^{15}N$ patterns. Large *Daphnia* have an extremely low $\delta^{13}C$ suggesting they are feeding on POM in the hypolimnion (Fig. 4.8), which matches our observations that large *Daphnia* do not feed in the epilimnion during the day or night (unpubli. data). In comparison, *Holopedium* and smaller *Daphnia* are more abundant in the epilimnion and both have a higher $\delta^{13}C$ (Fig. 4.8). It is somewhat surprising that zooplankton with divergent $\delta^{13}C$ signatures during stratification (e.g. large *Daphnia* and *Holopedium* in Fig. 4.8) have such similar seasonal $\delta^{15}N$ patterns (Fig. 4.1B). However, very different processes can lead to the spatial, compositional, and temporal isotopic heterogeneity of the $\delta^{13}C$ and $\delta^{15}N$ of POM and zooplankton. For example, the low $\delta^{13}C$ of POM in the hypolimnion (Fig. 4.7) might result from algae incorporating recycled CO_2 that has a lower $\delta^{13}C$. This is plausible for Council Lake because Chl *a* is highest in the hypolimnion in both small and large size fractions of POM (Fig. 4.6). If zooplankton can differentially exploit carbon in the hypolimnion, this could contribute to the large differences in $\delta^{13}C$ among taxa (Fig. 4.8). However, carbon cycling in the hypolimnion will not necessarily influence the vertical distribution of POM $\delta^{15}N$ (Fig. 4.6). Indeed, seasonal patterns of POM $\delta^{13}C$ and $\delta^{15}N$ over the water column are not always correlated

or controlled by the same processes (Lehmann et al. 2004), so zooplankton feeding at different depths could have very different $\delta^{13}C$ signatures but similar $\delta^{15}N$ patterns.

Interpreting size-based variation of plankton isotopic signatures

It is a common difficulty to determine if size-based variation of a consumer's $\delta^{15}N$ reflects trophic variation (Genner et al. 2003), or just size-based variation in the $\delta^{15}N$ of food sources (e.g. algae of different sizes). For example, larger *Daphnia* may feed on components of a microbial or algal food web that have a higher trophic position than algae, leading to the relationship in Fig. 4.3. But, we have not confirmed that size-based variation in the $\delta^{15}N$ of POM is positively correlated with trophic variation. For example, *S. lapponica* is a large colonial algae (100-250 μm) that is closely related to crysophytes, and therefore, may have the morphological capacity for phagotrophy (Pipes and Leedale 1992). The high $\delta^{15}N$ of POM_{SL} is consistent with *S. lapponica* being mixotrophic, but we can only speculate about a potential influence of mixotrophy on the $\delta^{15}N$ of pelagic zooplankton in Council Lake.

In Council Lake, it is likewise difficult to determine if the positive relationship between $\delta^{15}N$ and the head length of *C. trivittatus* provides evidence of life-history omnivory (*sensu* Branstrator et al. 2000). *Chaoborus* is a size-selective predator whose gape width increases throughout its ontogeny. Therefore, we might predict a positive relationship between *Chaoborus* body size and $\delta^{15}N$ (Fig. 4.5). In order to attribute this relationship to trophic variation, we have to determine if the isotopic variation among prey items reflects trophic variation. For example, if *C. trivittatus* in Council Lake only consumes *Daphnia,* then the relationship in Fig. 4.5 may result from size selective

foraging on a *Daphnia* population that has a positive relationship between $\delta^{15}N$ and daphnid body size (Fig. 4.3A). Interpreting Fig. 4.5 is even more difficult if *C. trivittatus* also feeds on copepods, because the copepods have a similar $\delta^{15}N$ as large *Daphnia* during July and August. In either case, without independent evidence that size-based isotopic variation in *Daphnia* is related to trophic variation, our data provide little isotopic evidence for omnivory in *C. trivittatus*. However, *Chaoborus* is 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 degree of omnivory of different larval instars at different times of year.

Physiological differences associated with body size could also lead to positive relationships between zooplankton size and $\delta^{15}N$, but the current research is ambiguous. Some studies suggest that $\delta^{15}N$ may increase with organism age (Adams and Sterner 2000; Overman and Parrish 2001), whereas others have found no correlation between age and $\delta^{15}N$ (Minagawa and Wada 1984). Adams and Sterner (2000) found that the $\delta^{15}N$ of *Daphnia magna* increased by ~0.4‰ after 5 days of starvation, which could lead to a positive relationship between *Daphnia* size and $\delta^{15}N$ if larger *Daphnia* are more susceptible to starvation (Threlkeld 1976). In Council Lake, the summertime peak of large *Daphnia* $\delta^{15}N$ coincided with their maximum C:N (>8.0) during the stratified period (Average C:N: 7.0, SD= 0.87). Since the C:N of *Daphnia* is positively correlated with lipid content (Matthews et al. unpubl. data), large *Daphnia* with a high C:N are unlikely starving in Council Lake at the end of July. Alternatively, higher ingestion rates of large *Daphnia* could decrease digestion efficiency and lead to higher $\delta^{15}N$ (Power et al. 2003). There are several physiological mechanisms that could lead to size-based

differences in $\delta^{15}N$, but no studies have attempted to resolve these issues for *Daphnia* or any other zooplankton taxa.

Uncertainties in trophic enrichment

Uncertainties in trophic enrichment currently confound all our attempts at extracting information about trophic variation from isotopic variation. Temporal variation in trophic enrichment ($\Delta_{\delta N}$) between zooplankton and their diet may partially explain the decoupling of $\delta^{15}N$ between zooplankton and POM_{41}. Adams and Sterner (2000) found that the $\delta^{15}N$ of *Daphnia magna* increased by ~2.5‰ concurrently with an increase in the C:N of *Daphnia's* food source (*Scenedesmus aculeatus*) 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 $\delta^{15}N$ between *Daphnia* and POM <41 μm for all sampling dates ($\Delta_{D\text{-}POM41}$= -15.4 + 1.7 (C:N), $p< 0.01$), and for all dates excluding 20 Jan 2003 ($\Delta_{D\text{-}POM41}$= -8.6 + 1.03 (C:N), $p< 0.01$). Therefore, our data for $\Delta_{D\text{-}POM41}$ is consistent with the direction but not the magnitude of Adams and Sterner's (2000) experimental result that trophic enrichment of *Daphnia* increases with increasing C:N of the food source. In contrast, for *Holopedium* there was no significant linear relationship between the C:N of POM and $\Delta_{H\text{-}POM41}$ ($\Delta_{H\text{-}POM41}$= -5.1 + 0.7 (C:N), $p= 0.17$). Given the rapid increase in the $\delta^{15}N$ of large size fractions of POM (Fig. 4.2), and the uncertainty about the component of POM_{41} that zooplankton assimilate, we believe it is unlikely that a 4‰ increase in the $\delta^{15}N$ of *Daphnia* and *Holopedium* is solely a result of an increase in the C:N of POM_{41}.

Detecting trophic variation in plankton using $\delta^{15}N$

Plankton communities can exhibit remarkable temporal variation in the $\delta^{15}N$ of their constituent parts, but long and detailed time series of taxa-specific zooplankton $\delta^{15}N$ might be a promising approach to detect sub-seasonal variation in zooplankton community trophic structure. This approach depends on whether we can isolate a component of the temporal variation in $\delta^{15}N$ that is related to trophic variation. Recently, Perga and Gerdeaux (*In press*) found that the $\delta^{15}N$ of zooplankton in Lake Geneva had a seasonal range of >10‰, and monthly variations were seasonally correlated among the resident taxa (*Daphnia*, copepods, and *Leptodora*). In contrast, the $\delta^{15}N$ patterns of zooplankton taxa in Council Lake, that have comparable tissue turnover times, were seasonally uncorrelated. For example, the $\delta^{15}N$ of *L. tyrelli* generally tracked the $\delta^{15}N$ of *C. trivittatus*, except for a few weeks in July and August when it followed the $\delta^{15}N$ of *Daphnia* and *Holopedium*. This suggests that *L. tyrelli* switches its feeding behaviour during this time period to more closely match the food sources of *Daphnia* and *Holopedium*. This is consistent with a previous study that found *L. tyrelli* was variably omnivorous depending on the availability of food sources (Anderson 1967). In comparison, the $\delta^{15}N$ of *Epischura* was always within 0.5‰ of *C. trivittatus,* which is consistent with *Epischura*'s more predatory style of feeding (Wong and Sprules 1986).

The $\delta^{15}N$ of zooplankton gives us an indication of the temporal integration of the nitrogen cycle leading up to a certain point in the food chain (Robinson 2001). It is possible that divergent seasonal patterns in $\delta^{15}N$ of different zooplankton taxa can indicate how taxa can differentially exploit alternate pathways of nitrogen transfer (i.e. different food chains). Time series of isotopic differences between zooplankton taxa may

help detect multiple pathways of nitrogen transfer to different species of zooplankton in food webs where algal and microbial food chains are intertwined (Porter 1996). However, isotopic differences between two zooplankton species depend on the antecedent temporal variation in the $\delta^{15}N$ of their food sources, differences in their relative rates of nitrogen turnover, and differences in their trophic enrichment. Currently, the biggest limitation to this approach is the uncertainty in trophic enrichment among zooplankton species.

Tables

Table 4.1 : Summary of $\delta^{15}N$ for zooplankton in Council Lake.
Averages of the zooplankton are for all measurements made during the entire sampling period 14 Feb, 2002 to 17 Mar, 2003. POM measurements in the Epilimnion (E), Metalimnion (M), and Hypolimnion (H) are from sampling dates during the stratified period (5 May, to 20 Oct).

Plankton category	Mean (SE)	25th and 75th percentiles	N
Chaoborus trivittatus	5.1 (0.1)	4.5, 5.6	28
Epischura nevadensis	4.9 (0.2)	4.4, 5.2	10
Leptodiaptomus tyrelli	5.2 (0.3)	4.7, 6.0	15
Holopedium gibberum	2.9 (0.4)	1.9, 3.2	18
Daphnia	2.4 (0.3)	1.7, 2.9	28
Daphnia spp. (> 1.8 mm)	2.9 (0.3)	2.0, 3.8	22
Daphnia spp. (< 1.8 mm)	1.8 (0.3)	1.4, 2.4	25
POM (< 41μm)	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

Table 4.2 : Limnological characteristics of Council Lake.

Summaries are for the period May to Aug 2002. 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 POM < 41 μm (see text).

Parameter	Mean (SE) (μg L^{-1})
Surface area	16 ha
Max depth	17 m
Secchi depth	7.8 m
TP	5.7
TN	77.0
DOC	2200
Chl *a* (< 41 μm)	
E	0.50 (0.27)
M	0.69 (0.30)
H	0.89 (0.41)
Chl *a* (> 0.7 μm)	
E	0.54 (0.16)
M	0.85 (0.37)
H	1.78 (0.48)

Figures

Figure 4.1 : Seasonal variation of $\delta^{15}N$ in Council Lake zooplankton

(A) and (B) Temporal variability of plankton $\delta^{15}N$ in Council Lake in 2002 to 2003. Error bars are +/- one standard error. The period of thermal stratification is shown with dotted vertical lines.

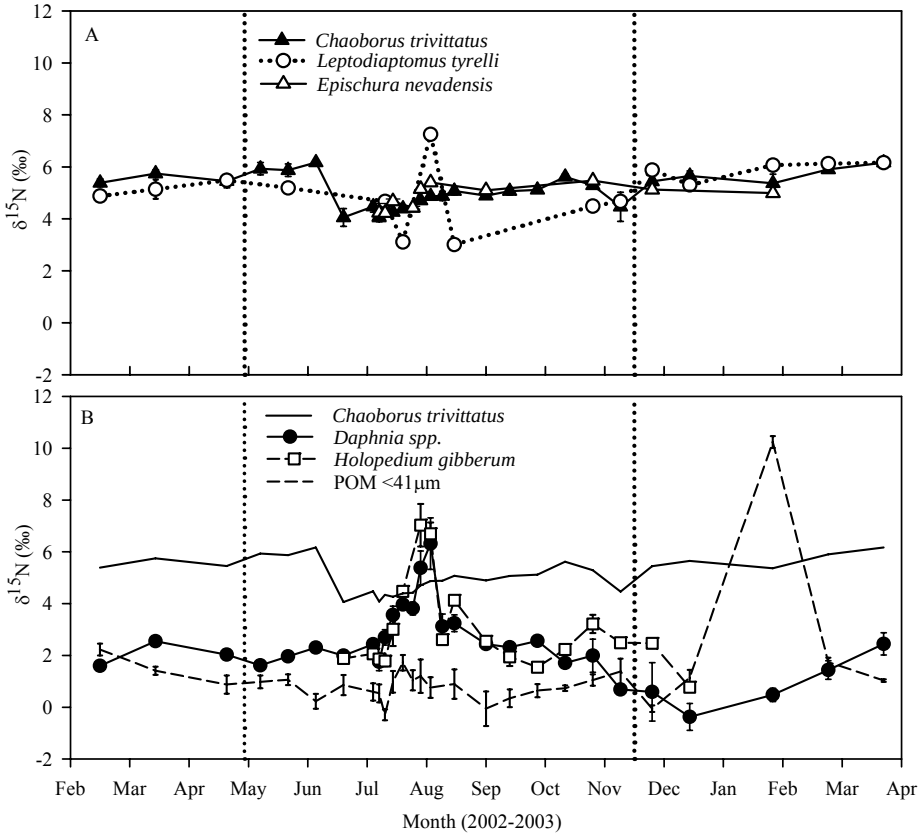

A
Chaoborus trivittatus
Leptodiaptomus tyrelli
Epischura nevadensis
δ15N (‰)
B
Chaoborus trivittatus
Daphnia spp.
Holopedium gibberum
POM <41μm
δ15N (‰)
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr
Month (2002-2003)

Figure 4.2 : Comparison of zooplankton and POM $\delta^{15}N$ in Council Lake.

(A) Temporal variability of average water column Chlorophyll *a*, particulate organic carbon (PC), and C:N ratio for POM < 41 μm from Fig. 4.1. Chl *a* values are shown in μg L^{-1} (seasonal average of 0.65 μg L^{-1}, ~0.11 on shown scale). (B) Comparison of *Daphnia* $\delta^{15}N$ with the $\delta^{15}N$ of other plankton size fractions, and a fraction dominated by *S. lapponica* (POM_{SL}).

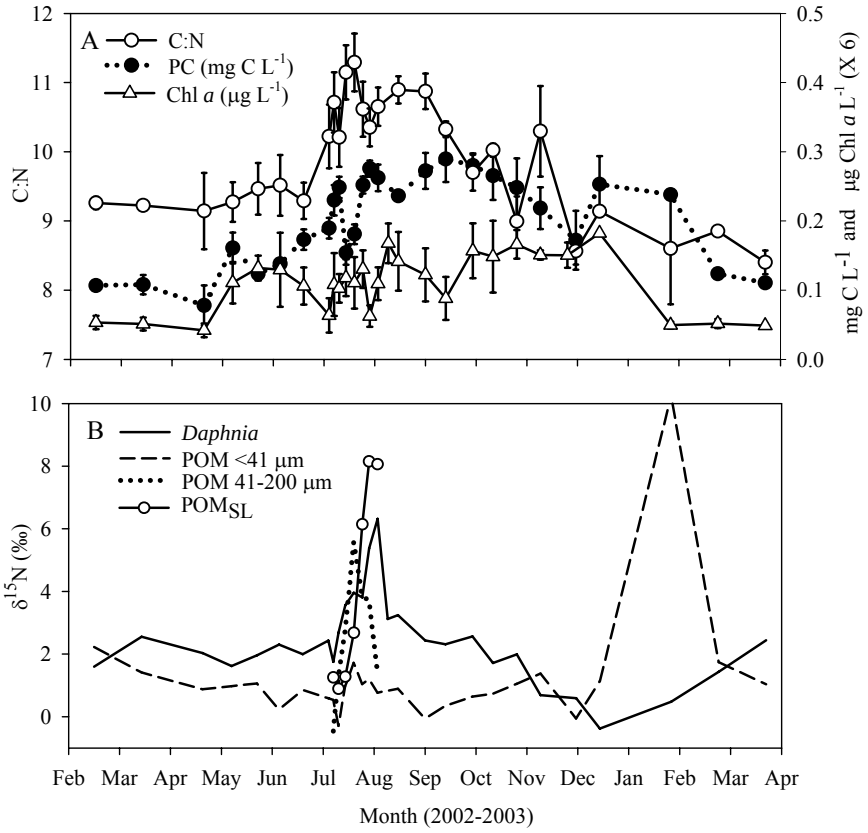
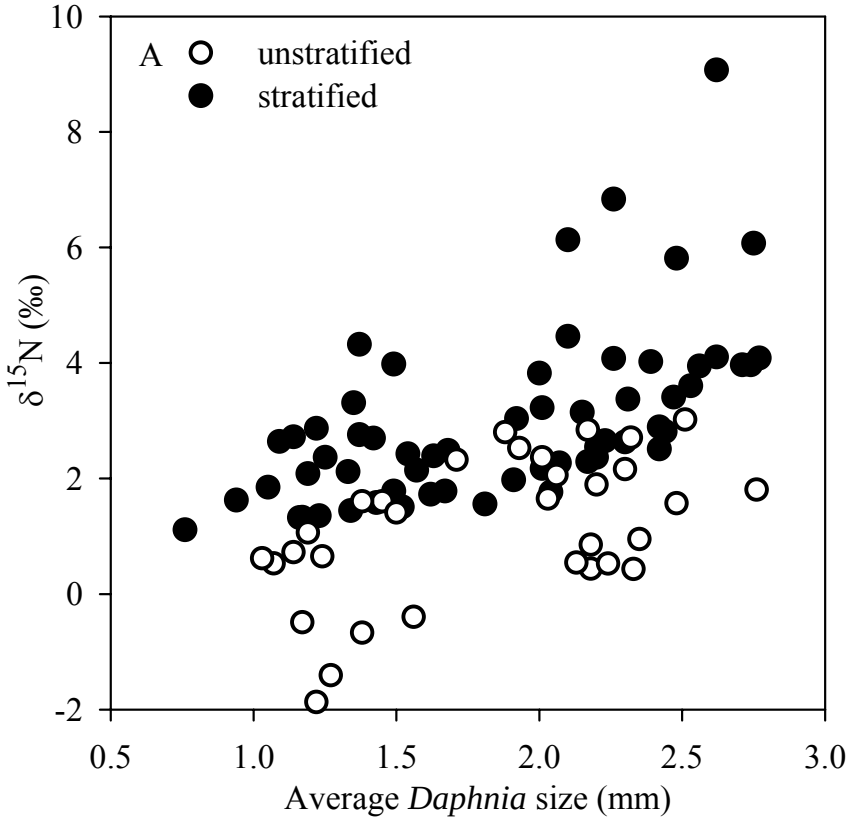

A
C:N
PC (mg C L-1)
Chl a (µg L-1)
C:N
mg C L-1 and µg Chl a L-1 (X 6)
B
Daphnia
POM <41 µm
POM 41-200 µm
POMSL
δ15N (‰)
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr
Month (2002-2003)

Figure 4.3 : Body size $\delta^{15}N$ relationships in *Daphnia*.

(A) Relationship between average *Daphnia* body size and $\delta^{15}N$, for all samples picked in Council Lake over the entire study period. Filled and open circles are from the thermally stratified and unstratified periods respectively.(B) Relationship between *Daphnia* size rank and the $\delta^{15}N$ rank for each sampling day with 2 or more samples (maximum 7). We have applied a random jitter to reduce overlapping points. Spearman's rank correlation is positive significant ($r_s = 0.786$, $p < 0.001$).


A
unstratified
stratified
10
8
6
4
2
0
-2
δ15N (‰)
0.5
1.0
1.5
2.0
2.5
3.0
Average Daphnia size (mm)

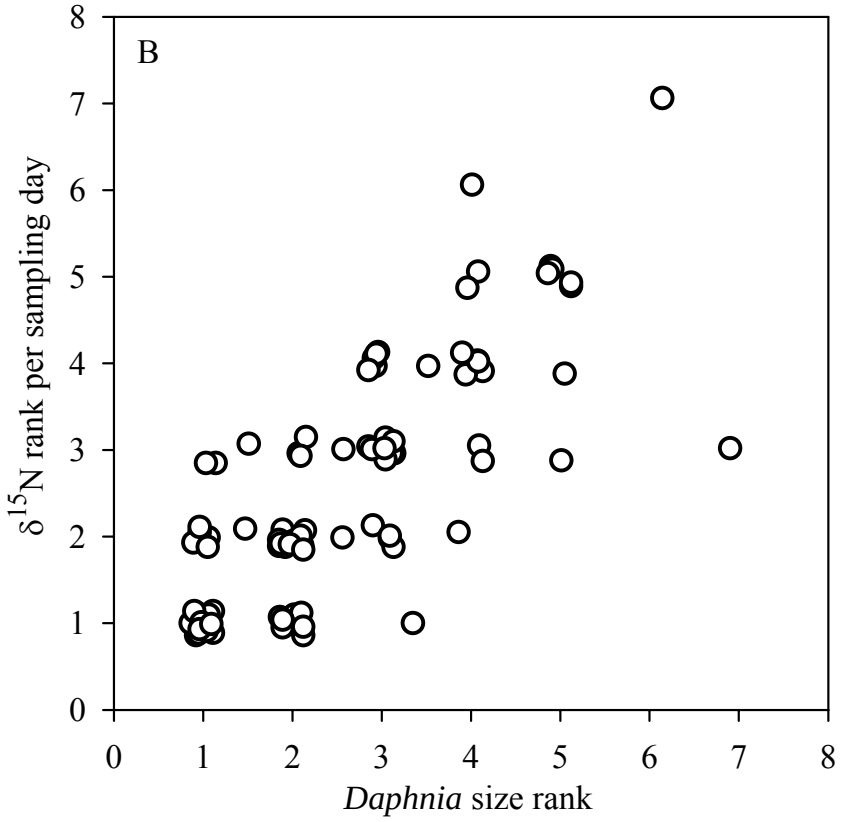

B
8
7
6
5
4
3
2
1
0
δ15N rank per sampling day
0
1
2
3
4
5
6
7
8
Daphnia size rank

Figure 4.4 : Size and $\delta^{15}N$ of *Daphnia* in Council Lake.

(A) Average size of picked *Daphnia* samples over the season. We classified large (closed circles >1.8 mm) and medium sized (open circles <1.8 mm) *Daphnia post hoc* based on a natural division in the data. (B) $\delta^{15}N$ of each size class over the sampling season.

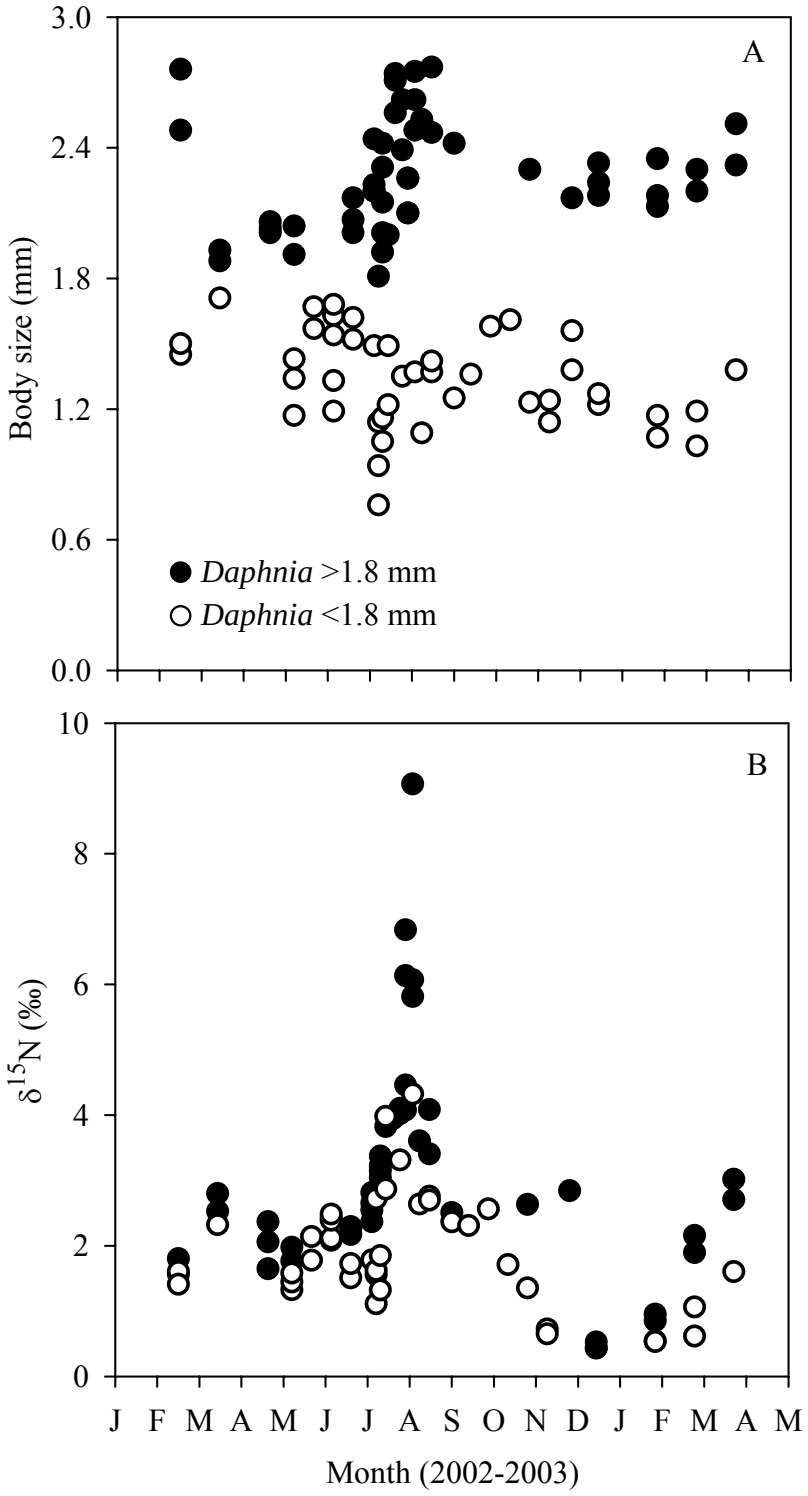

A
B
Body size (mm)
3.0
2.4
1.8
1.2
0.6
0.0
● Daphnia >1.8 mm
○ Daphnia <1.8 mm
δ15N (‰)
10
8
6
4
2
0
J F M A M J J A S O N D J F M A M
Month (2002-2003)

Figure 4.5 : Relationship between head length and $\delta^{15}N$ for *Chaoborus trivittatus.* Samples were from July (open triangles) and Aug (solid triangles) in Council Lake. Vertical dotted lines denote instar separation following Fedorenko and Swift (1972). Each data point is a composite of multiple individuals. The regression equation for July is $\delta^{15}N = 3.8$ (SE= 0.12) + 0.54(SE= 0.10)•Length ($F_{1,41} = 28.48$, $p < 0.001$, $r^2 = 0.41$), and for Aug is $\delta^{15}N = 4.2$ (SE= 0.16) + 0.59(SE= 0.13)•Length ($F_{1,18} = 26.6$, $p < 0.001$, $r^2 = 0.53$).

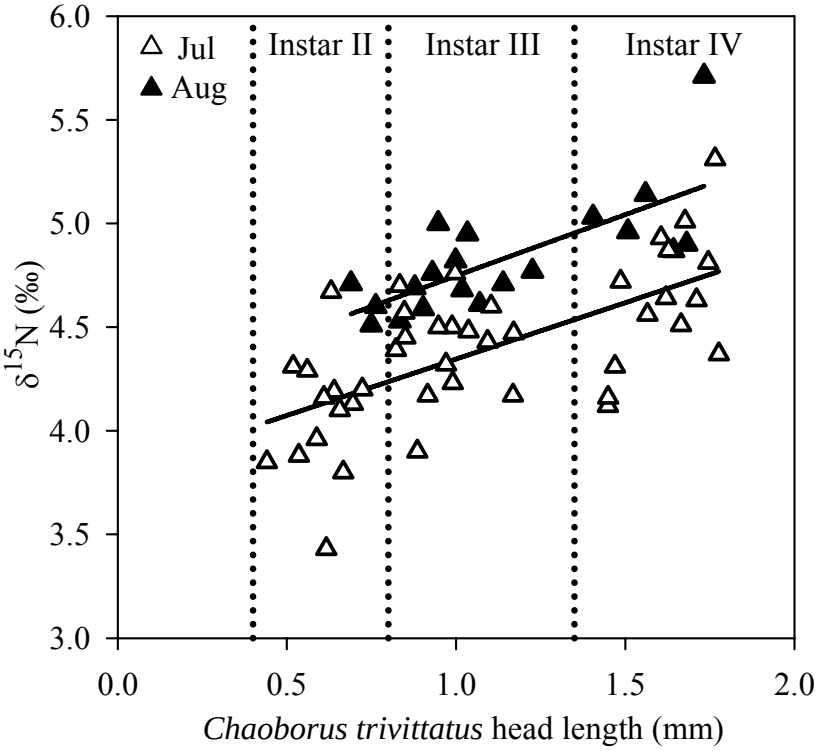

6.0
5.5
5.0
4.5
4.0
3.5
3.0
δ15N (‰)
Jul
Aug
Instar II
Instar III
Instar IV
0.0
0.5
1.0
1.5
2.0
Chaoborus trivittatus head length (mm)

Figure 4.6 : Vertical distribution of zooplankton food resources in Council Lake

Depth profiles of Chlorophyll *a*, particulate carbon (PC), molar C:N ratio, and $\delta^{15}N$ for POM < 41μm in July. Error bars are all +/- one standard error based on 7 sampling dates in July. The temperature profile was taken on 12 July, 2002 at the deepest site in Council Lake.

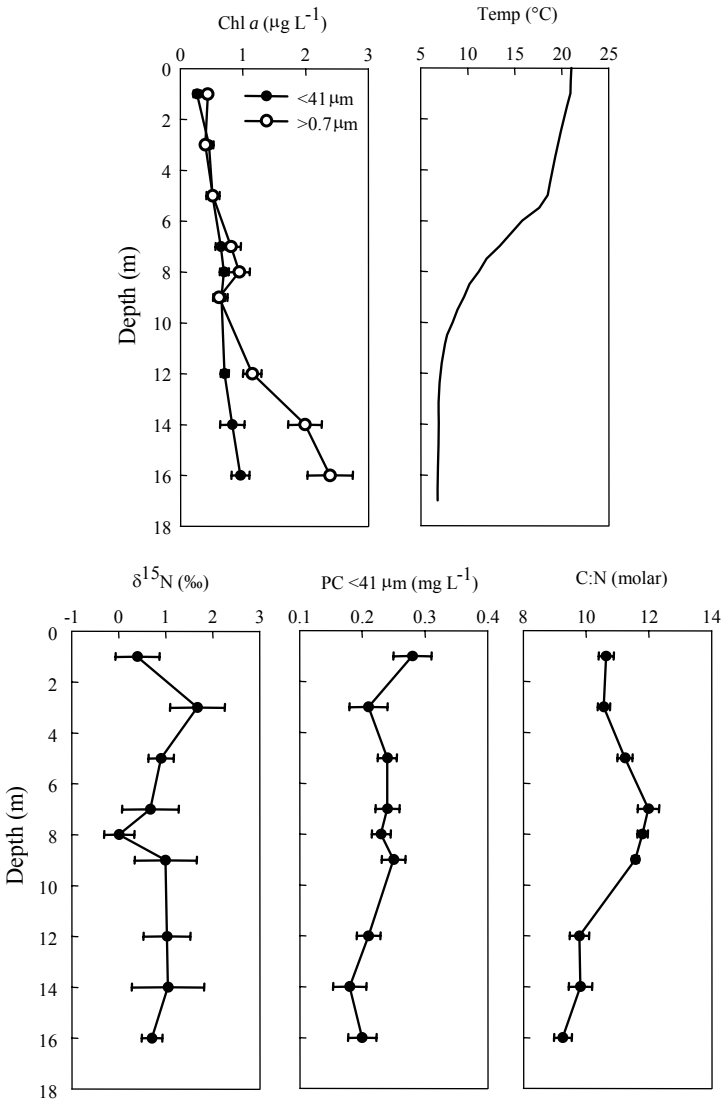

Chl a (µg L-1)
Temp (°C)
Depth (m)
<41 µm
>0.7µm
δ15N (‰)
PC <41 µm (mg L-1)
C:N (molar)

Figure 4.7 : Vertical distribution of POM $\delta^{13}C$ in Council Lake

Profiles of $\delta^{13}C$ of POM <41μm are for the stratified (*n*= 21) and unstratified (*n*= 8) period in Council Lake. Error bars are +/- one standard error based on the number of times we sampled each depth.

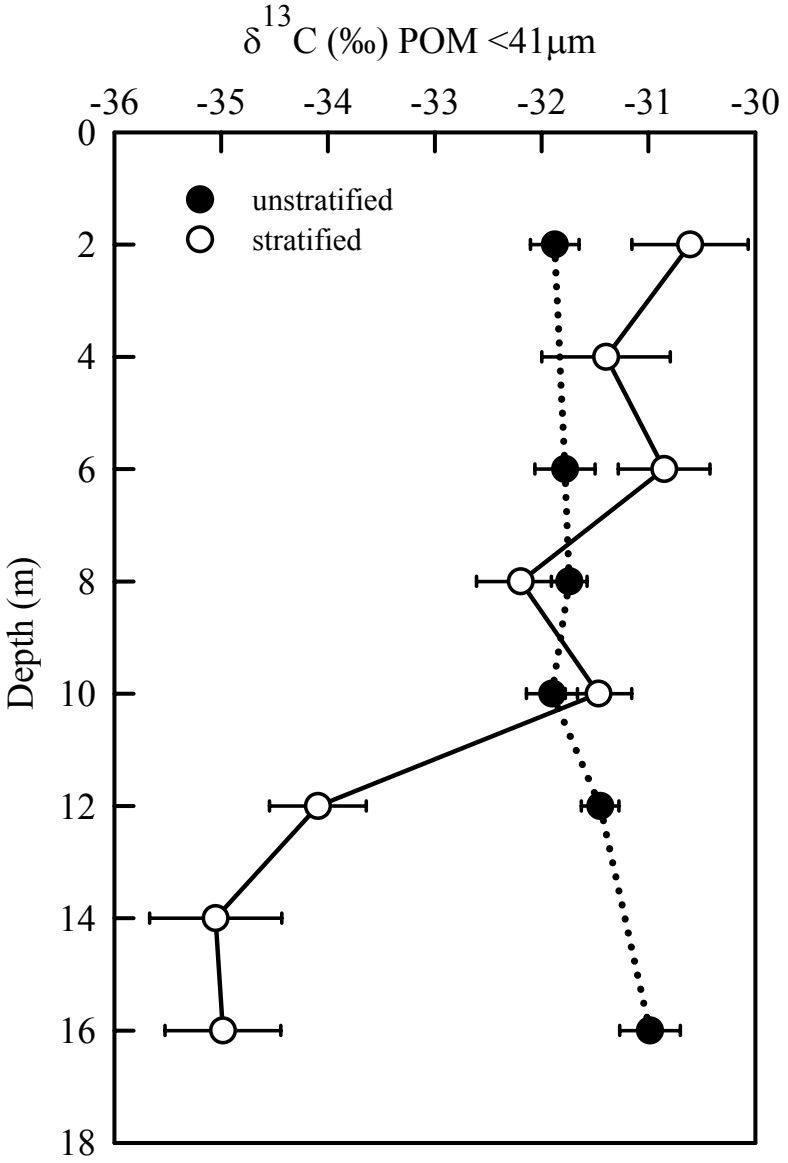
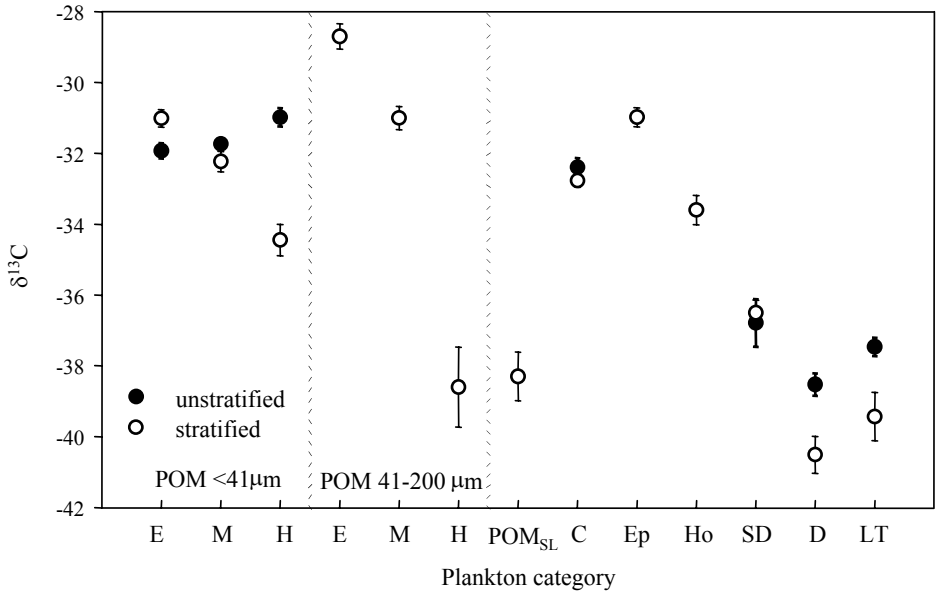

δ13C (‰) POM <41μm
-36
-35
-34
-33
-32
-31
-30
0
2
4
6
8
10
12
14
16
18
Depth (m)
unstratified
stratified

Figure 4.8 : Comparison of $\delta^{13}C$ for plankton in Council Lake

Average $\delta^{13}C$ of plankton during the unstratified (closed circles) and stratified period (open circles) in Council Lake. POM 41-200 μm and POM <41 μm were sampled from the epilimnion (E: 2-6 m), metalimnion (M: 6-10 m), and hypolimnion (H: 10-16 m). POM_{SL} included aggregations of a large colonial algae *S. lapponica* (see text). Most zooplankton taxa were present year round (C: *Chaoborus trivitattus*, SD: *Daphnia* <1.8 mm, D: *Daphnia* >1.8mm, and LT: *Leptodiaptomus tyrelli*), but others (Ep: *Epischura*, Ho: *Holopedium gibberum*) were only sampled twice during the unstratified period (data not shown).


-28
-30
-32
-34
-36
-38
-40
-42
δ13C
unstratified
stratified
POM <41µm
POM 41-200 µm
E
M
H
E
M
H
POMSL
C
Ep
Ho
SD
D
LT
Plankton category

Chapter 5: Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}C$

Citation:

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Abstract

$\delta^{13}C$ 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 $\delta^{13}C$ of different zooplankton taxa are rarely considered. Here, we investigate to what extent temporal variation in the $\delta^{13}C$ of POM (<41μm) and the C:N of zooplankton can explain the temporal variability in $\delta^{13}C$ of freshwater zooplankton. We compare temporal patterns of $\delta^{13}C$ and C:N for *Daphnia*, *Hesperodiaptomus franciscanus* and *Leptodiaptomus tyrelli* over a six month period at four sites in two oligotrophic lakes. In all three taxa, seasonal variation in zooplankton C:N explained more of the variation in zooplankton $\delta^{13}C$ than did the $\delta^{13}C$ of POM. This suggests that variation in the lipid content of zooplankton can strongly influence temporal variation of $\delta^{13}C$ in zooplankton. Using these data, we evaluate procedures that estimate the $\delta^{13}C$ of only the non-lipid component of zooplankton. If zooplankton lipids are primarily dietary in origin, than extracting lipids or "normalizing" $\delta^{13}C$ based on C:N will exclude a major dietary source, and therefore may be inappropriate. We conclude that temporal variation in body composition (C:N) of zooplankton can significantly influence the temporal variation of zooplankton $\delta^{13}C$ signatures.

Introduction

The stable carbon isotope ^{13}C is commonly used to quantify energy flow in lake food webs (Vander Zanden and Rasmussen 1999; Post *et al.* 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 $\delta^{13}C$ of pelagic resources, and are less temporally variable than primary producers (Kling *et al.* 1992; Cabana and Rasmussen 1996; Post 2002; Matthews and Mazumder 2003). Understanding the sources of temporal variability in $\delta^{13}C$ 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 $\delta^{13}C$ of zooplankton food sources including lake productivity and respiration (France *et al.* 1997), lake trophic status (Grey *et al.* 2000), and sources of allochthonous dissolved inorganic carbon (DIC) (Aravena *et al.* 1992). Within a lake, seasonal variation in the $\delta^{13}C$ of zooplankton food sources is influenced by seasonal variations in the $\delta^{13}C$ signature of DIC (Quay *et al.* 1986; Leggett *et al.* 1999; Jonsson *et al.*2001), the composition of particulate organic matter (POM) (Grey *et al.* 2001), algal productivity (Hollander and McKenzie 1991), algal species composition (Zohary *et al.* 1994) and algal lipid synthesis (van Dongen *et al.* 2002).

The amplitude of temporal variation in the $\delta^{13}C$ of bulk plankton varies from <3‰ to 20‰ among lakes (Zohary *et al.* 1994). Some of this variability is due to changes in species composition, since different zooplankton taxa can have different $\delta^{13}C$ signatures (Matthews and Mazumder 2003; Pel et al. 2003). However, individual zooplankton species can also exhibit large seasonal changes in their $\delta^{13}C$ signature

(Leggett *et al.* 1999; Pel et al. 2003), and few studies have explored the potential sources of this temporal variability (but see Gu *et al.* 1999). It is challenging to determine the sources of variability in zooplankton $\delta^{13}C$, because it is difficult to isolate the $\delta^{13}C$ of distinct food sources (Grey *et al.* 2001). Temporal change in POM $\delta^{13}C$ is the most obvious source of temporal variability in zooplankton $\delta^{13}C$ signatures, but to our knowledge no study has tested this directly. The $\delta^{13}C$ of bulk zooplankton is typically lower than various size fractions of POM (typically <30μm) (del Giorgio and 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 $\delta^{13}C$ signatures (del Giorgio and France 1996). In addition, isotopic heterogeneity within plankton communities may confound the relationship between the $\delta^{13}C$ of zooplankton and POM (Matthews and Mazumder 2003; Pel *et al.* 2003).

Seasonal changes in zooplankton body composition (C:N) may account for some temporal variability in the $\delta^{13}C$ of individual species. C:N is positively related to lipid content (Schmidt *et al.* 2003; Lesage 1999; McConnaughey 1978), and lipids are depleted in ^{13}C relative to proteins and carbohydrates (Parker 1964; Tieszen *et al.* 1983). The $\delta^{13}C$ of synthesized lipids in freshwater and marine algae are on average 12.9‰ (range 5.5 to 15.8‰) 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 and Hedges 1996).

There are two general approaches to determine if zooplankton lipids affect zooplankton $\delta^{13}C$ signatures. First, we could either measure the $\delta^{13}C$ of zooplankton fatty acids directly (Pel et al. 2003), or measure the $\delta^{13}C$ of zooplankton before and after lipid extraction (Kling et al. 1992). It is labour intensive to measure temporal patterns in the $\delta^{13}C$ of individual fatty acids 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 $\delta^{13}C$ analysis (Kelly 2000), under the rationale that synthesized lipids do not reflect the $\delta^{13}C$ of the organism's food source (Tieszen *et al.* 1983). However, lipids in freshwater zooplankton are typically dietary in origin (Goulden and Place, 1990; Arts 1998), so both the $\delta^{13}C$ of lipids and lipid-extracted tissue are valuable for analyses of the diet of zooplankton. Removing lipids prior to isotope analysis, or "normalizing" the $\delta^{13}C$ of zooplankton based on their C:N ratio (*sensu* McConnaughey and McRoy 1979; Leggett 1998), might be contrary to the goals of a dietary analysis using $\delta^{13}C$.

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 $\delta^{13}C$ among or within lakes. Since C:N is positively correlated with lipid content (McConnaughey 1978; Lesage 1999; Schmidt *et al.* 2003), and the $\delta^{13}C$ of lipid is lower than other body constituents (Parker 1964; Tieszen *et al.* 1983), we might expect a negative relationship between C:N and $\delta^{13}C$ in freshwater zooplankton. To test this hypothesis we could measure the C:N and $\delta^{13}C$ 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 $\delta^{13}C$ signatures (Post 2002; Matthews and Mazumder 2003). An alternative is to look for a relationship between C:N and $\delta^{13}C$ in an individual species found at a given lake site.

Detecting a negative relationship between C:N and $\delta^{13}C$ for a single zooplankton species may be challenging because the range of C:N is often small at any one time. However, if we found large variation in $\delta^{13}C$ concurrently with low variability in C:N, this would indicate a limited impact of lipids on $\delta^{13}C$ signatures. In an attempt to increase the range of observed C:N (see Villar-Argaiz et al. 2002), we collected zooplankton over a six month period. The drawback of this approach is that temporal variability in the $\delta^{13}C$ of food sources may mask a relationship between C:N and $\delta^{13}C$. To test for a relationship between C:N and $\delta^{13}C$ 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 $\delta^{13}C$ signatures (Quay *et al.* 1986).

The goals of this study are 1) to partition the temporal variability of zooplankton $\delta^{13}C$ between variation in the $\delta^{13}C$ of POM and variation in the C:N ratio of zooplankton, and 2) to evaluate the applicability of the widely used lipid normalization technique (McConnaughey and McRoy 1979) for interpreting variability of zooplankton $\delta^{13}C$ signatures (Kline 1999; Schmidt et al. 2003). To accomplish these goals, we compare seasonal changes in $\delta^{13}C$ of *Leptodiaptomus tyrelli* Poppe, a calanoid copepod with a seasonally variable C:N, with seasonal changes in $\delta^{13}C$ of *Hesperodiaptomus fransciscanus* Lilljeborg and *Daphnia spp.* which both have a smaller seasonal range of

C:N. We compare these patterns to temporal variability in the $\delta^{13}C$ of POM (<41μm) collected in the epilimnion and metalimnion over the same time period.

Methods

We collected zooplankton samples every two or three 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 <2μg L^{-1} (Davies et al. 2004). Sooke Lake Reservoir 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 (>5m) over the summer and autumn, which results in a higher flushing rate at SOL-S compared to the other three sites (Nowlin *et al.* 2004).

We collected particulate organic matter (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 hour) 25 mm GF-C filters (Whatman). 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 hours of collection we sorted live zooplankton into different categories. From both sites

in Shawnigan Lake we picked out *Daphnia pulicaria* Forbes and the dominant calanoid species *Hesperodiaptomus franciscanus*. *Leptodiaptomus tyrelli* were also present in Shawnigan Lake but were not abundant enough in our samples for isotopic analysis. Both sites of Sooke Lake Reservoir 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 Sooke Lake Reservoir.

Our goal for isotopic analysis was to get ~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‰). Samples were analysed for $\delta^{13}C$, percent carbon and percent nitrogen. Herein, C:N is expressed as a molar ratio.

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

We used multiple regression to determine how much temporal variation in zooplankton $\delta^{13}C$ signatures could be accounted for by variation in the C:N ratio of zooplankton and the $\delta^{13}C$ of POM (<41 μm). For each sampling date, we took the average $\delta^{13}C$ and C:N sample for each taxon at each site, and the average $\delta^{13}C$ of POM (<41μm) in the epilimnion and metalimnion. This simplification was justified because the average difference between the $\delta^{13}C$ of epilimnetic and metalimnetic POM was <0.5‰. For the analysis in Table 5.1 and Fig. 5.4, we standardized 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 and Keough 2002). Adding a two week lag

to the $\delta^{13}C$ of POM did not improve the ability of POM to predict temporal change in the $\delta^{13}C$ of zooplankton (Matthews unpublished data), therefore we used a zero lag for all regression models.

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

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

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

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

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

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

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

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

To determine the sensitivity to the parameter *D* of McConnaughey and McRoy's (1979) lipid normalization, we normalized our isotopic data from SOL-D using equation 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, 12.9‰; SD, 3.8; N, 6: van Dongen et al. 2002).

Results

Among all sites, the range of variation in C:N for *Daphnia* (5.1 to 5.9) and *H. franciscanus* (4.8 to 6.0) was small compared to *L. tyrelli* (6.7 to 14.7), and was small compared to interspecific variation in the C:N of 24 zooplankton genera reviewed by Elser *et al.* (2000) (Fig. 5.1a). The seasonal pattern in the C:N of zooplankton depended on species, but was similar among sites for the same species (Fig. 5.2). *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 Sooke Lake Reservoir. 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 $\mu gC\ ind^{-1}mm^{-1}$ (October).

The $\delta^{13}C$ of particulate organic matter (POM; <41μm) was similar in the epilimnion (0-6m) and metalimnion (8-14m), based on paired t-tests (SOL-S: $\delta^{13}C_{epi\text{-}meta}$=0.71‰, t_9=2.39, p=0.041; SOL-D: $\delta^{13}C_{epi\text{-}meta}$=0.37‰, t_8=1.46, p=0.182; SHL-S: $\delta^{13}C_{epi\text{-}meta}$=0.04‰, t_7=0.172, p=0.87: SHL-D: $\delta^{13}C_{epi\text{-}meta}$=0.35‰, t_6=2.26, p=0.07), and overall, the average difference was <0.5‰ ($\delta^{13}C_{epi\text{-}meta}$=0.39‰, t_{33}=2.99, p=0.005). Considering all sites, zooplankton had a lower $\delta^{13}C$ than the average $\delta^{13}C$ 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 $\delta^{13}C$ of *Daphnia* was only significantly different from the average $\delta^{13}C$ of POM at SOL-S ($\delta^{13}C_{POM\text{-}Daphnia}$=1.67‰, t_{10}=4.711, p<0.001). At all sites, both calanoid species always had a lower $\delta^{13}C$ signature than either *Daphnia* or average POM (Fig. 5.3).

Seasonal variability of zooplankton $\delta^{13}C$ was generally small in our lakes (1.75 to 4.7‰), compared to the range of *Daphnia* $\delta^{13}C$ in 18 lakes gathered from the literature (20.6‰) (Fig. 5.1b). The pattern of temporal variation in $\delta^{13}C$ depended on the site and species (Fig. 5.3). In all four basins the $\delta^{13}C$ 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: 4.70‰, CV, 3.3%; SHL-D: Range, 2.58‰, CV, 2.6%). The $\delta^{13}C$ of *L. tyrelli* declined throughout the sampling period of 2001 at both sites in Sooke Lake Reservoir concurrently with seasonal increases in C:N ratio (Fig. 5.2, 5.3).

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

Multiple regression analysis revealed that the temporal change in the $\delta^{13}C$ of zooplankton was better predicted by the changes in C:N ($ß_{C:N}$) than the $\delta^{13}C$ of POM ($ß_{\delta^{13}C}$). Five of the nine regression models found $ß_{C:N}$ significant, compared to two that found $ß_{\delta^{13}C}$ significant (Table 5.1). In the five significant regression models, $ß_{C:N}$ was larger than $ß_{\delta^{13}C}$ and had the opposite sign (Table 5.1). As expected, changes in the $\delta^{13}C$ of zooplankton were generally positively related to the $\delta^{13}C$ of POM ($ß_{\delta^{13}C}>0$) when the C:N of zooplankton was held constant (Fig. 5.4d, e, f), and negatively related to the C:N of zooplankton ($ß_{C:N}<0$) when the $\delta^{13}C$ of POM was held constant (Fig. 5.4a, b, c). The larger $ß_{C:N}$ compared to $ß_{\delta^{13}C}$, suggests that observed changes in zooplankton C:N had a larger impact on the $\delta^{13}C$ of zooplankton than did changes in the $\delta^{13}C$ of POM. The empirical relationship between C:N and $\delta^{13}C$ depends on the taxa considered, but in general the slope of the relationship is steeper for *Daphnia* than copepods (Fig. 5.5).

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

Changing the parameters of the lipid normalization technique developed by McConnaughey & McRoy (1979) changed the seasonal average $\delta^{13}C$ of each zooplankton species, the average difference between zooplankton species and, in some cases, the temporal pattern of $\delta^{13}C$ (Fig. 5.6). For example, in SOL-D the seasonal average difference in $\delta^{13}C$ between *Daphnia* and *L. tyrelli* was 4.0‰ ($\delta^{13}C_{D\text{-}L}$). At intermediate levels of *D* (6‰; McConnaughey and McRoy 1979), $\delta^{13}C_{D\text{-}L}$ decreased to 2.7‰ following normalization. At high levels of *D (*12.9‰; van Dongen, Schouten & Damste, 2002) $\delta^{13}C_{D\text{-}L}$ declined to 1.2‰, and the temporal trend in $\delta^{13}C$ of *L. tyrelli*

actually reversed, and converged with the $\delta^{13}C$ of *Daphnia* by the end of the 2001 sampling season (Fig. 5.6).

Discussion

Seasonal variation of zooplankton $\delta^{13}C$

Temporal variability in the C:N of zooplankton and changes in the $\delta^{13}C$ of their food sources both affect the interpretation of seasonal patterns of zooplankton $\delta^{13}C$ (Zohary *et al.* 1994; Thompson *et al.* 2000). Among lakes, the $\delta^{13}C$ of zooplankton is predictable from the $\delta^{13}C$ of POM, if we account for an average depletion of zooplankton $\delta^{13}C$ from POM (del Giorgio and France 1996). Within a lake, large seasonal changes in the $\delta^{13}C$ of POM will certainly influence the seasonal pattern of zooplankton $\delta^{13}C$, provided that zooplankton assimilate that POM. In our study lakes, which have small seasonal changes in the $\delta^{13}C$ of POM, temporal variation of zooplankton body composition (C:N) accounted for most of the temporal variation in zooplankton $\delta^{13}C$ (Fig. 5.4). There are several reasons why changes in the $\delta^{13}C$ of zooplankton might not respond to seasonal changes in the $\delta^{13}C$ of POM.

First, the $\delta^{13}C$ of POM often decreases with depth in part due to 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 $\delta^{13}C$ of POM. In our study lakes, zooplankton that feed on POM below the metalimnion could have lower $\delta^{13}C$ signatures. This is unlikely for the *Daphnia* populations in these lakes, because their $\delta^{13}C$ signatures are generally within 0.5‰ of the

average $\delta^{13}C$ of POM (Fig. 5.3). However, variable feeding depth may explain why the $\delta^{13}C$ of *H. franciscanus* is 2.2‰ (SD= 1.04, N= 32 pair-wise comparisons) lower than *Daphnia*, even though they have similar C:N ratios (Fig. 5.2).

Second, the $\delta^{13}C$ of zooplankton may be temporally decoupled from the $\delta^{13}C$ 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 two 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 $\delta^{13}C$ to predict temporal change in zooplankton $\delta^{13}C$. In SHL-D, the temporal pattern of $\delta^{13}C$ was similar among zooplankton species (*Daphnia pulicaria* and *H. franciscanus*), even though the $\delta^{13}C$ of POM tended to decline over the season. In this case, the $\delta^{13}C$ of POM does not explain the temporal variability of zooplankton $\delta^{13}C$. Alternatively, it is possible that changes in zooplankton $\delta^{13}C$ 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 et al. (1993) found that storage lipids of *Diaptomus sicilis* Forbes increased during periods of high algal abundance. Large temporal variation of zooplankton lipids is well documented (Arts and Wainman, 1998), but is rarely addressed in the context of stable isotope analysis.

Third, the $\delta^{13}C$ of POM may not reflect the $\delta^{13}C$ of zooplankton diet because of selective feeding behaviours. A size fraction of POM (<41μm) 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 $\delta^{13}C$. 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 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 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 hypothesized negative relationship between C:N and $\delta^{13}C$. In our study, temporal variability in the $\delta^{13}C$ of food sources may similarly mask a relationship between C:N and $\delta^{13}C$ of zooplankton at a given lake site. Detecting a significant negative relationship between C:N and $\delta^{13}C$ 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 of 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 $\delta^{13}C$ at the shallower sites (SHL-S and SOL-S). The $\delta^{13}C$ 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). Sooke Lake Reservoir 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 $\delta^{13}C$ of *Daphnia* food sources.

The strongest evidence that C:N explains temporal variability of zooplankton $\delta^{13}C$ 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 C:N (Sterner and Hessen 1994; Villar-Argaiz et al. 2002). The temporal variability of C:N in *L. tyrelli* is large compared to that in *Daphnia* and *H. franciscanus,* and compared to the interspecific variation in C:N among the 24 zooplankton genera reviewed by Elser *et al.* (2000) (Fig. 5.1). The C:N ratio of *L. tyrelli* increased by ~8 over the sampling period at both sites in Sooke Lake Reservoir. This seasonal range is similar to *Mysis relicta* (Leggett 1998), but is small compared to interstage variation in the C:N ratio of the calanoid *Mixodiaptomus laciniatus* (Villar-Argaiz et al. 2002). For *L. tyrelli*, the seasonal decrease of $\delta^{13}C$ concurrent with the increase in C:N, at both sites of SOL (Fig. 5.2, 5.3), supports the hypothesis that changes in lipid content explain the seasonal $\delta^{13}C$ patterns for this species. An alternate hypothesis is that the $\delta^{13}C$ of *L. tyrelli* food sources declined over the season, although neither the $\delta^{13}C$ of POM nor *Daphnia* declined over the same time period.

The negative relationship between C:N and $\delta^{13}C$ among zooplankton taxa is also consistent with the hypothesis that zooplankton with a high C:N have higher concentrations of lipids that have low $\delta^{13}C$ signatures (Fig. 5.5). There are several important implications of this relationship for interpreting the $\delta^{13}C$ of zooplankton. For example, an organism with a low C:N (e.g. *Daphnia*) can increase their 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 carbohydrates, and the relationship between lipid concentration and C:N is an increasing non-linear function (McConnaughey 1978).

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

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

McConnaughey and McRoy (1979) developed a method to normalize the $\delta^{13}C$ of an organism depending on its C:N ratio, and the average difference between lipids and other body tissues (~6‰). However, normalization of $\delta^{13}C$ only estimates the $\delta^{13}C$ 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 normalize the $\delta^{13}C$ 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 $\delta^{13}C$. This could be interpreted as a seasonal accumulation of dietary lipids that have a low $\delta^{13}C$. In this case, normalizing the $\delta^{13}C$ based on C:N would exclude lipids from the dietary analysis. Since food sources (POM) typically have a lower concentration of lipid than consumers, if we extracted lipids then the $\delta^{13}C$ of *L. tyrelli* might better "match" the $\delta^{13}C$ of POM. However, since lipids are typically dietary in zooplankton, both the $\delta^{13}C$ 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 normalize $\delta^{13}C$ signatures based on C:N. In general, lipid

normalization is more suitable if we are only interested in a dietary analysis of a consumer's proteins and carbohydrates.

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

If we are interested in tracing the non-lipid component of a consumer's diet then normalization procedures may be helpful, although there are some methodological considerations. The two main assumptions of McConnaughey and McRoy's (1979) lipid normalization technique are: 1) there is a consistent and positive non-linear relationship between lipid content and C:N, and 2) there is a constant difference between the $\delta^{13}C$ of lipid and proteins/carbohydrates in the body of an organism (D=6‰). There is some empirical support for the first assumption (McConnaughey 1978; Lesage 1999; Schmidt *et al.* 2003), but its generality is 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 and 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 $\delta^{13}C$ of lipids and the bulk organism (0.5 to 15‰). Lipid biosynthesis discriminates against ^{13}C (Monson and Hayes 2002), and contributes to isotopic variability among different fatty acids (FA) (van Dongen et al. 2002; Veefkind 2003). In a size fraction of marine zooplankton, Veefkind (2003) found that the median difference between the $\delta^{13}C$ of a particular FA and bulk zooplankton varied from –1 to -9‰, but the weighted average difference (based on relative FA abundance) was ~6.5‰. Normalization of $\delta^{13}C$ signatures is quite sensitive to the assumption that D=6‰ (Fig. 5.6), so, if normalization

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 $\delta^{13}C$ of pelagic zooplankton. Considering the seasonal and interspecific variation in zooplankton lipid content, may help explain the seasonal variation in the $\delta^{13}C$ of pelagic zooplankton (Grey et al. 2001; Bastviken *et al.* 2003; Pace *et al.* 2004), and variation of zooplankton $\delta^{13}C$ 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 and Mazumder 2003). The lower $\delta^{13}C$ of *H. franciscanus* compared to *Daphnia* suggests that the $\delta^{13}C$ of *H. franciscanus*'s diet is significantly lower than *Daphnia*. In this case, normalization would probably only change the average $\delta^{13}C$ of each species, but have little effect on the average difference between species or their relative temporal pattern of $\delta^{13}C$. However, we cannot compare the $\delta^{13}C$ of *Daphnia* or *H. franciscanus* to *L. tyrelli* because they have different C:N ratios, and current normalization approaches are sensitive to parameter assumptions. Resolving this challenge not only requires measuring the $\delta^{13}C$ of lipids over time, but may also require knowledge about the timing of lipid storage and utilization in different zooplankton species.

Our data support the hypothesis that temporal change in body composition (C:N) can significantly affect the $\delta^{13}C$ of pelagic zooplankton. Since lipids are primarily dietary in pelagic zooplankton (Arts and Wainman 1998), dietary studies of zooplankton using $\delta^{13}C$ 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 $\delta^{13}C$.

Tables

Table 5.1 : Multiple regression analysis of zooplankton $\delta^{13}C$.

We use C:N and the $\delta^{13}C$ of POM to predict the $\delta^{13}C$ of zooplankton for each taxa at each site. $ß_{C:N}$ is the multiple regression coefficient that represents the C:N of zooplankton, and $ß_{POM\delta^{13}C}$ represents the $\delta^{13}C$ 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 ($p< 0.05$).

Taxa **Lake Site**	$ß_{C:N}$	$ß_{POM\delta^{13}C}$	$ß_{C:N}$ x $ß_{POM\delta^{13}C}$	***F*-ratio**	***P*-value**	R^2
Daphnia						
SOL-S	0.34	0.73	NS	$F_{(2,9)} = 1.7$	0.236	0.27
SOL-D	-0.77*	0.60*	-0.35 (0.09)*	$F_{(3,8)} = 40.1$	<0.001	0.94
SHL-S	-0.11	0.44	NS	$F_{(2,9)} = 0.7$	0.559	0.14
SHL-D	-0.79*	0.41	NS	$F_{(2,9)} = 7.5$	0.01	0.62
H. franciscanus						
SOL-S	-0.33	-0.30	NS	$F_{(2,8)} = 1.2$	0.354	0.23
SHL-S	-0.79*	0.48	NS	$F_{(2,9)} = 7.0$	0.015	0.61
SHL-D	-0.57	0.22	NS	$F_{(2,9)} = 1.4$	0.296	0.24
L. tyrelli						
SOL-S	-0.83*	0.27	NS	$F_{(2,8)} = 13.7$	0.006	0.82
SOL-D	-1.22*	0.51*	NS	$F_{(2,9)} = 103.2$	<0.001	0.96

Figures

Figure 5.1 : Literature survey of zooplankton C:N and $\delta^{13}C$.

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

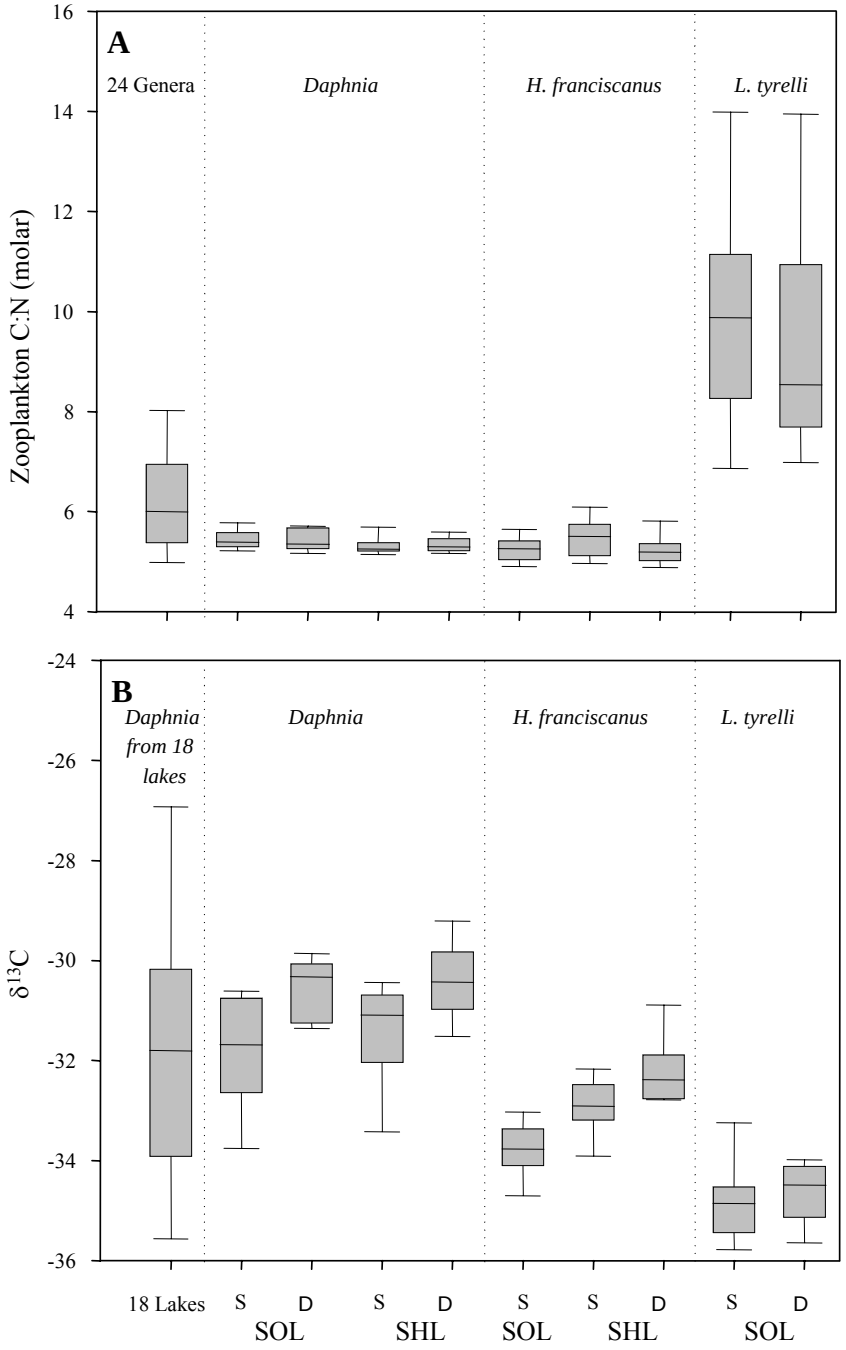

A
24 Genera
Daphnia
H. franciscanus
L. tyrelli
Zooplankton C:N (molar)
16
14
12
10
8
6
4
B
Daphnia from 18 lakes
Daphnia
H. franciscanus
L. tyrelli
δ13C
-24
-26
-28
-30
-32
-34
-36
18 Lakes
S
D
S
D
S
S
D
S
D
SOL
SHL
SOL
SHL
SOL

Figure 5.2 : Seasonal variation of zooplankton C:N

Seasonal pattern of C:N ratio for three zooplankton species 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).

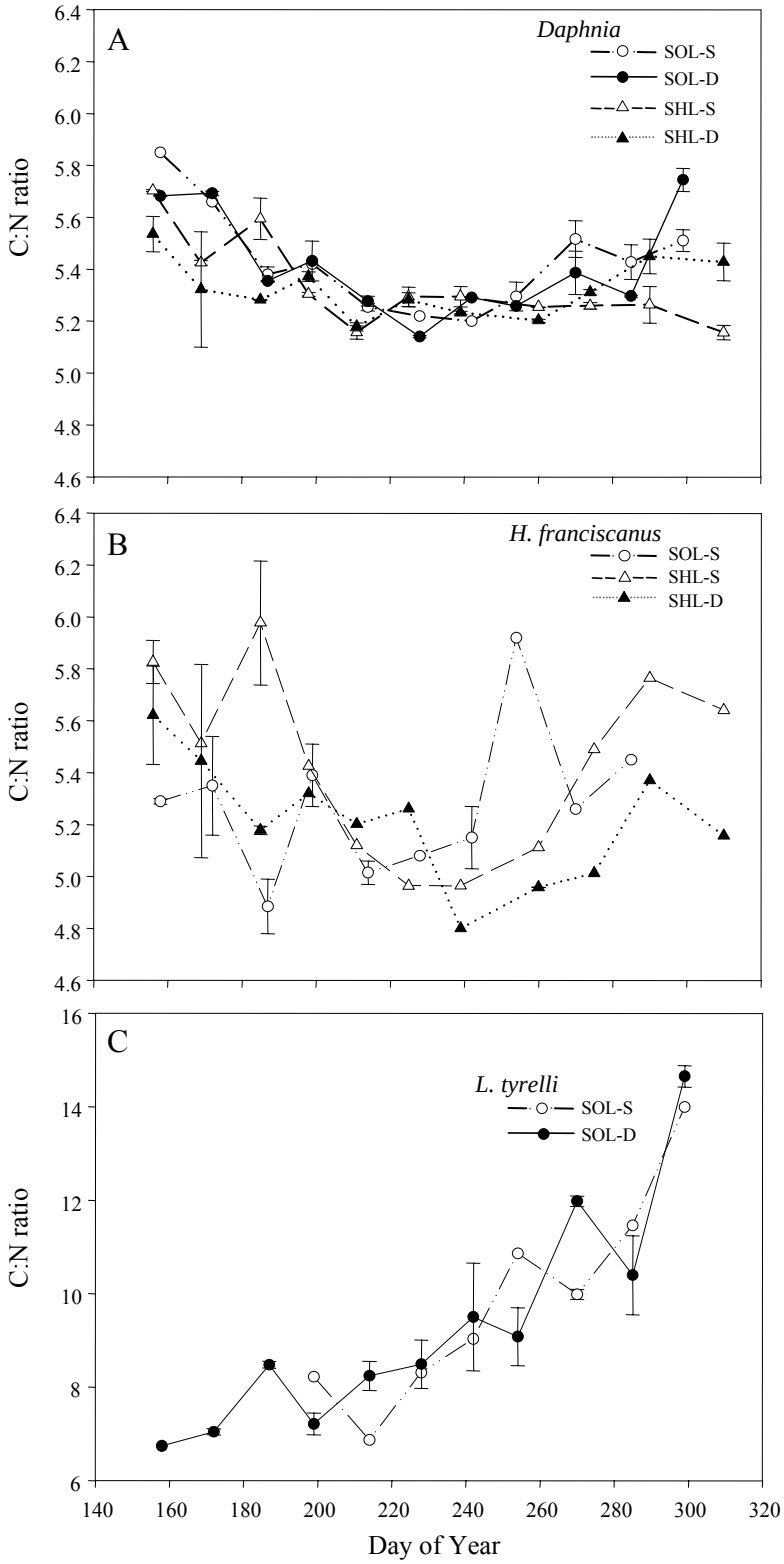

A
Daphnia
SOL-S
SOL-D
SHL-S
SHL-D
B
H. franciscanus
SOL-S
SHL-S
SHL-D
C
L. tyrelli
SOL-S
SOL-D
C:N ratio
Day of Year

Figure 5.3 : Seasonal $\delta^{13}C$ of zooplankton from SOL-D, SOL-S, SHL-D, SHL-S. Error bars are ± 1SE and where they are not present, only a single sample was taken for that sampling date.

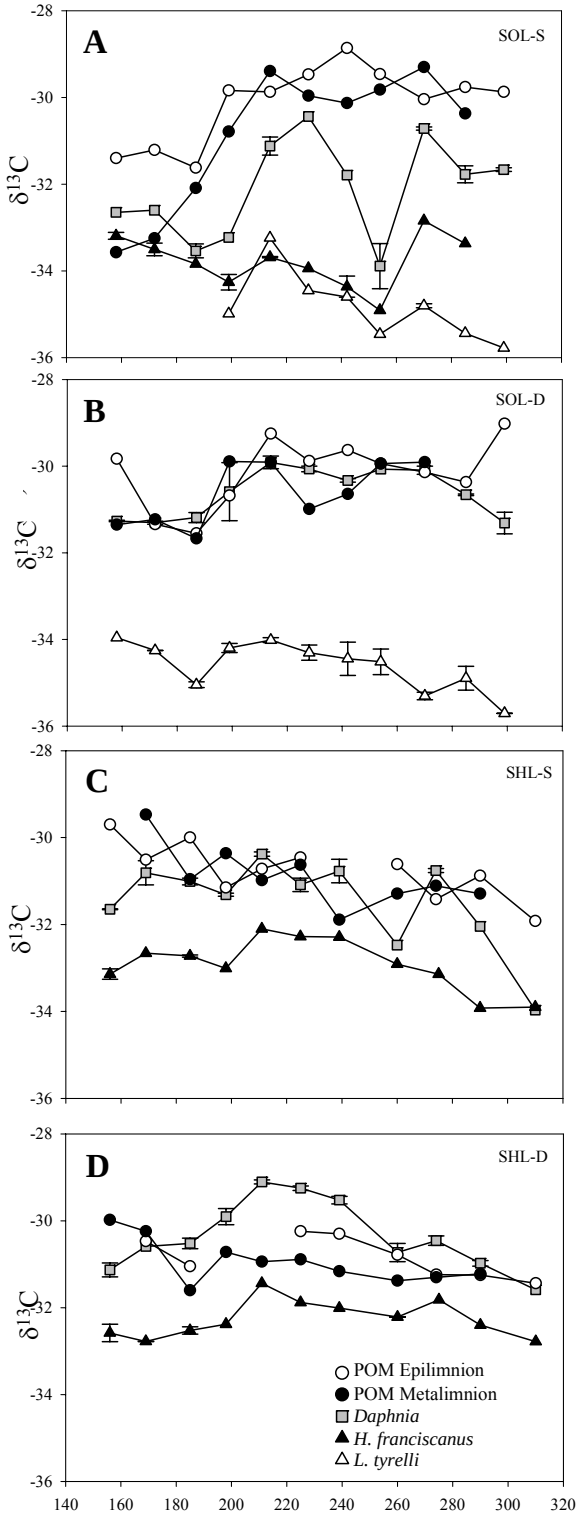

A
SOL-S
B
SOL-D
C
SHL-S
D
SHL-D
δ13C
-28
-30
-32
-34
-36
140
160
180
200
220
240
260
280
300
320
Day of Year
POM Epilimnion
POM Metalimnion
Daphnia
H. franciscanus
L. tyrelli

Figure 5.4 : Partial regression plot for POM $\delta^{13}C$ and zooplankton C:N and $\delta^{13}C$.

These multiple regression model relate the C:N of the zooplankton, and the average $\delta^{13}C$ of POM, to the $\delta^{13}C$ of zooplankton. The vertical axes are the residuals from ordinary least squares (OLS) regression of zooplankton $\delta^{13}C$, in the absence of the predictor variable on the x-axis. The predictor variable is either zooplankton C:N (a, b, c) or POM $\delta^{13}C$ (d, e, f). The horizontal axes are residuals from OLS regression of either C:N versus POM $\delta^{13}C$ (a, b, c), or POM $\delta^{13}C$ 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 5.1. R^2 values are coefficients of determination, and represent the amount of variation in the $\delta^{13}C$ of zooplankton that is explained by the predictor variable (e.g. C:N) if the other predictor variable is held constant (e.g. POM $\delta^{13}C$).

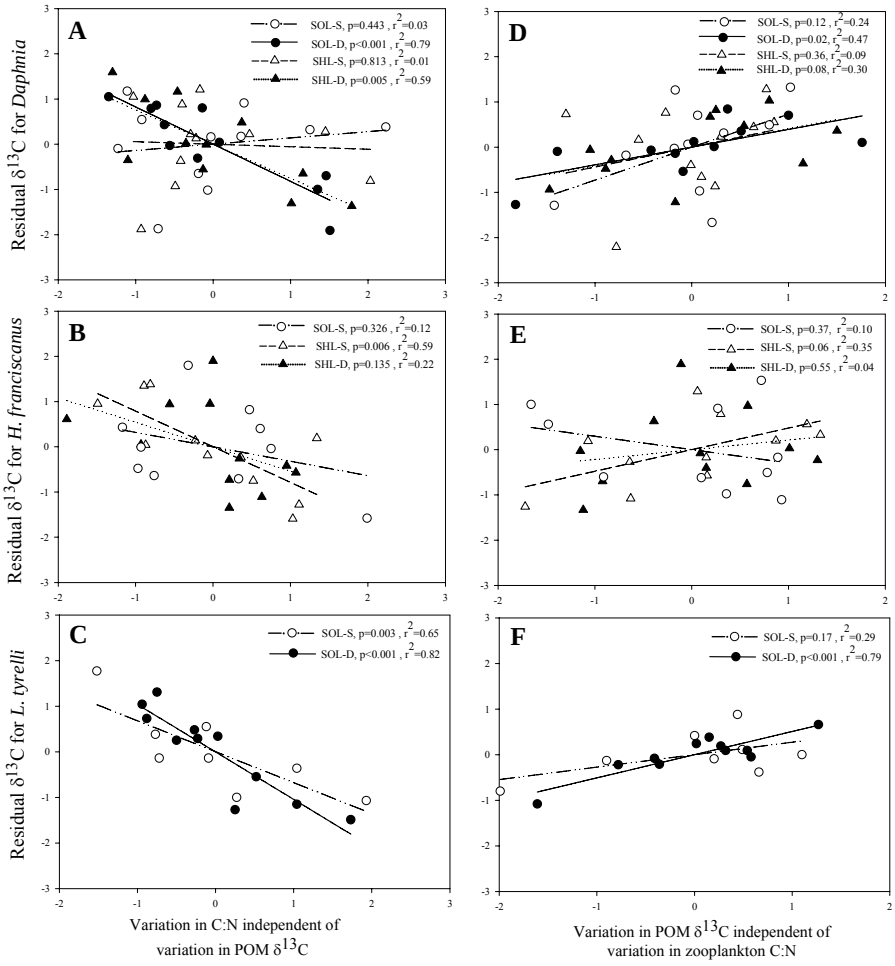

A
SOL-S, p=0.443 , r2=0.03
SOL-D, p<0.001 , r2=0.79
SHL-S, p=0.813 , r2=0.01
SHL-D, p=0.005 , r2=0.59
B
SOL-S, p=0.326 , r2=0.12
SHL-S, p=0.006 , r2=0.59
SHL-D, p=0.135 , r2=0.22
C
SOL-S, p=0.003 , r2=0.65
SOL-D, p<0.001 , r2=0.82
D
SOL-S, p=0.12 , r2=0.24
SOL-D, p=0.02, r2=0.47
SHL-S, p=0.36, r2=0.09
SHL-D, p=0.08, r2=0.30
E
SOL-S, p=0.37, r2=0.10
SHL-S, p=0.06 , r2=0.35
SHL-D, p=0.55 , r2=0.04
F
SOL-S, p=0.17 , r2=0.29
SOL-D, p<0.001 , r2=0.79
Residual δ13C for Daphnia
Residual δ13C for H. franciscanus
Residual δ13C for L. tyrelli
Variation in C:N independent of variation in POM δ13C
Variation in POM δ13C independent of variation in zooplankton C:N

Figure 5.5 : Empirical relationships between C:N and $\delta^{13}C$ of zooplankton.

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

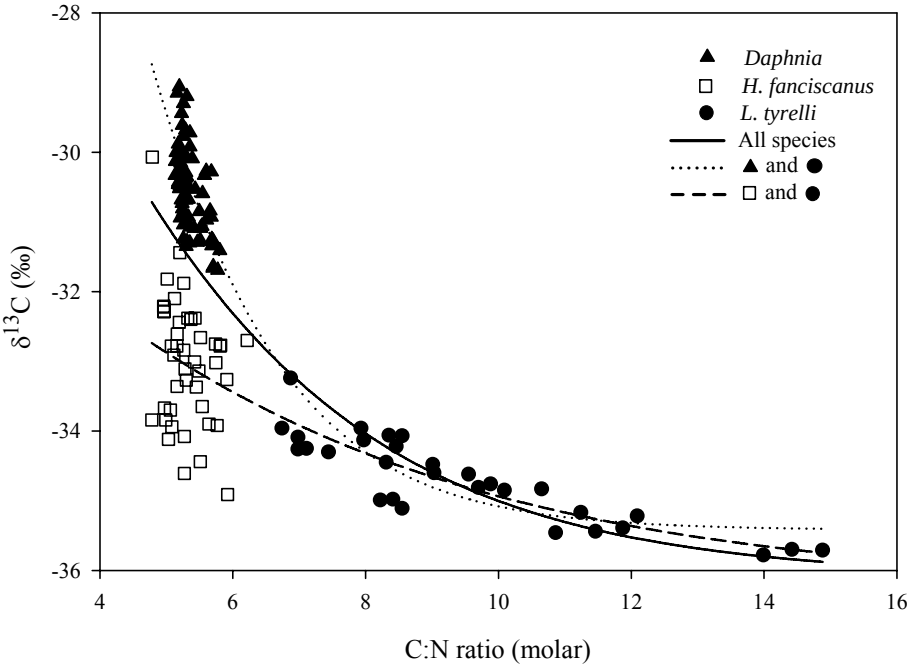
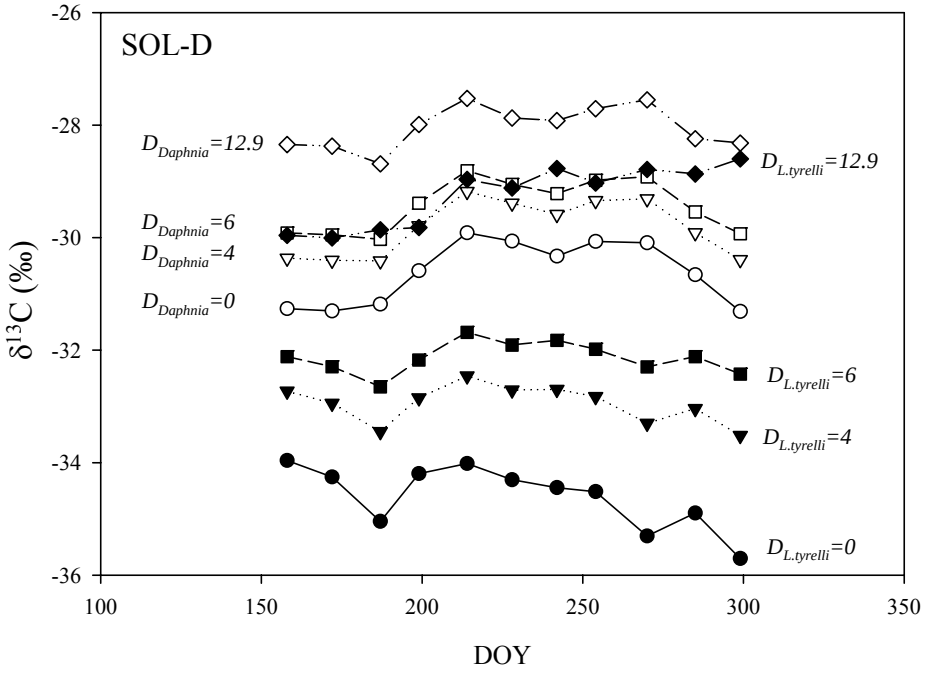

-28
-30
-32
-34
-36
4
6
8
10
12
14
16
δ13C (‰)
C:N ratio (molar)
Daphnia
H. fanciscanus
L. tyrelli
All species
▲ and ●
□ and ●

Figure 5.6 : Sensitivity analysis of a lipid normalization model

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. Normalization model is from McConnaughey and McRoy (1979).


SOL-D
$D_{Daphnia}$=12.9
$D_{Daphnia}$=6
$D_{Daphnia}$=4
$D_{Daphnia}$=0
$D_{L.tyrelli}$=12.9
$D_{L.tyrelli}$=6
$D_{L.tyrelli}$=4
$D_{L.tyrelli}$=0
δ13C (‰)
DOY
-26
-28
-30
-32
-34
-36
100
150
200
250
300
350

Chapter 6: The stoichiometry of carbon stable isotopes in zooplankton

Citation:

Matthews, B., K. Young, J. Grey, R.W. Campbell, J.M. Davies, and A. Mazumder. The stoichiometry of carbon stable isotopes in zooplankton. *Submitted*.

Abstract

Multiple sources of carbon fuel pelagic production in lakes. The $\delta^{13}C$ of zooplankton can indicate the major sources of carbon that reach upper trophic levels. However, it remains unclear why the $\delta^{13}C$ of zooplankton is typically lower than their putative food sources, particularly in oligotrophic lakes. Here, we propose that the low $\delta^{13}C$ of zooplankton lipids partially accounts for the low $\delta^{13}C$ of zooplankton relative to particulate organic matter (POM). We developed a stoichiometric model of zooplankton body composition to explain seasonal variation in zooplankton C:N (as an indicator of lipid content) and $\delta^{13}C$. In Sooke Lake Reservoir (SOL) the low $\delta^{13}C$ of zooplankton lipids almost entirely accounted for the low $\delta^{13}C$ of *Leptodiaptomus tyrelli*. Our model explains the negative relationship between C:N and $\delta^{13}C$ in an oligotrophic (SOL) and mesotrophic lake (Lough Erne). In a eutrophic lake (Pluβsee), the negative relationship was not present and lipid normalization of zooplankton $\delta^{13}C$ had little impact on seasonal $\delta^{13}C$ patterns. We propose that both the $\delta^{13}C$ of lipids and lipid extracted tissue can complement dietary analyses of zooplankton that seek to determine the relative importance of different carbon sources for zooplankton production.

Introduction

Over the past 30 years, researchers have used the $\delta^{13}C$ of zooplankton to test many fundamental ecological questions about carbon cycling in lakes. The $\delta^{13}C$ of *Daphnia* is highly variable among lakes and can reach very low $\delta^{13}C$ signatures (range -25.4 to -46‰; reviewed in Matthews and Mazumder 2005a). It is still unclear how to account for the large variation of zooplankton $\delta^{13}C$ among and within lakes. Rau (1978) first hypothesized that algal re-incorporation of biogenic CO_2 with a low $\delta^{13}C$ may lead to the unexpectedly low $\delta^{13}C$ of freshwater zooplankton. In support of this hypothesis, France et al. (1997) found that zooplankton with low $\delta^{13}C$ were more common in oligotrophic lakes with high heterotrophic activity, and low ratios of production to respiration. Schindler et al. (1997) found that the $\delta^{13}C$ of zooplankton was lowest in experimental lakes that were a net source of CO_2, and concluded that food web structure could alter whole lake metabolism. More recent studies have used the $\delta^{13}C$ of zooplankton to estimate the balance of carbon flow between littoral and pelagic habitats (Vadeboncoeur et al. 2003; Karlsson and Bystrom 2005). Vadeboncoeur et al. (2003) argued that higher rates of periphyton growth in oligotrophic lakes could lead to larger differences in $\delta^{13}C$ between pelagic (low $\delta^{13}C$) and littoral consumers (high $\delta^{13}C$). Building on this study, Karlsson and Bystrom (2005) concluded that top consumers in oligotrophic lakes relied heavily on littoral resources.

The low $\delta^{13}C$ of zooplankton provides suggestive evidence that multiple carbon (e.g. terrestrial, methane) sources fuel pelagic production (Grey et al. 2000; Karlsson et al. 2003; Bastviken et al. 2003). In a survey of lakes with a large range of total

phosphorus (TP: 7 to 780 μgL^{-1}) Grey et al. (2000) argued that zooplankton rely more on allochthonous carbon sources in oligotrophic lakes, because the $\delta^{13}C$ of zooplankton was lower than particulate organic matter (POM) in these lakes. In a survey of oligotrophic lakes (TP: 2.9 to 11.3 $\mu g\ L^{-1}$) with a large range of dissolved organic carbon (DOC: 2 to 9 mg C L^{-1}), Karlsson et al. (2003) found that the $\delta^{13}C$ of zooplankton had a small range among 15 lakes (range~6‰), but was always lower than all the measured sources of organic matter (POM, sediment, terrestrial). As a result, Karlsson et al. (2003) assumed that zooplankton fed selectively on phytoplankton with a low $\delta^{13}C$, and concluded that zooplankton depend more on allochthonous carbon in oligotrophic lakes because of a higher energy mobilization of terrestrial organic matter by heterotrophic bacterioplankton (Karlsson et al. 2003). In 3 eutrophic lakes, Bastviken et al. (2003) suggested that the low $\delta^{13}C$ of zooplankton might result from the incorporation of biogenic methane into zooplankton food webs. Bastviken et al. (2003) could account for the $\delta^{13}C$ of zooplankton in their study lakes (-36 to -42‰) if zooplankton incorporated a small fraction (<5 to 15%) of carbon from methanotrophic bacteria.

Large isotopic heterogeneity among zooplankton food sources within POM poses a major challenge for the interpreting the $\delta^{13}C$ of zooplankton (Pel et al. 2003). For example, zooplankton could have a lower $\delta^{13}C$ than POM if they selectively feed on a food source that has a low $\delta^{13}C$ that only makes up a small carbon fraction of POM (e.g. phytoplankton, methanotrophs). In Loch Ness, a large humic lake with a carbon pool dominated by allochthonous organic carbon, the $\delta^{13}C$ of zooplankton declined during the summer below the $\delta^{13}C$ of POM but stayed above the $\delta^{13}C$ of diatoms (Grey et al. 2001). This pattern suggests that zooplankton relied heavily on autochthonous carbon in the

summer, even though 40-50% of zooplankton carbon came from allochthonous sources annually (Grey et al. 2001). Similarly, in a whole lake experiment, Pace et al. (2004) used the $\delta^{13}C$ of *Daphnia* to argue that 50% of zooplankton carbon originates from allochthonous sources. In un-manipulated lakes it is difficult to confirm if allochthonous and autochthonous carbon have different $\delta^{13}C$ signatures (Karlsson et al. 2003). By experimentally manipulating the $\delta^{13}C$ of DIC, Pace et al. (2004) were able to ensure that algal carbon in the epilimnion had a higher $\delta^{13}C$ than carbon from terrestrial sources.

Despite significant progress in using zooplankton $\delta^{13}C$ to study carbon cycling in lakes, few studies have considered how zooplankton lipid affects our interpretation of zooplankton $\delta^{13}C$. Lipids have a low $\delta^{13}C$ relative to proteins and carbohydrates due to isotopic discrimination against ^{12}C during lipid synthesis (DeNiro and Epstein 1977). Zooplankton lipid can either be produced *de novo* or accumulated from their food sources. If zooplankton primarily synthesize lipid via their own metabolic pathways, then the $\delta^{13}C$ of zooplankton (with lipid) may not match with the $\delta^{13}C$ of their diet. Alternatively, if zooplankton preferentially accumulate and store dietary lipids from algae (Arts and Wainman 1998), then the $\delta^{13}C$ of POM, which has a low lipid content compared to zooplankton, might mask the $\delta^{13}C$ of algal lipids. Regardless, the $\delta^{13}C$ of zooplankton lipids could account for the low $\delta^{13}C$ of zooplankton compared to POM (del Giorgio and France 1996), variation in $\delta^{13}C$ among zooplankton taxa (Matthews and Mazumder 2003), and the large temporal variation of zooplankton $\delta^{13}C$ compared to POM (Zohary et al. 1994). A few researchers have directly measured the $\delta^{13}C$ of zooplankton with and without lipids (Kling et al. 1992, Leggett 1998; Sato et al. 2002), and have generally found small differences in zooplankton $\delta^{13}C$ before (WB) and after

(LE) lipid extraction ($\Delta_{LE\text{-}WB}$). The average $\Delta_{LE\text{-}WB}$ from these studies is 1.9‰ (SD= 1.0, *n*= 14) (copepods: 1.2‰ ; *Mysis relicta*: 2.5‰ ; *Calanus finmarchicus*: 1.4‰), and as a result, several studies have discounted lipid as a significant explanation for the low $\delta^{13}C$ of zooplankton (France 1995, del Giorgio and France 1996, Matthews and Mazumder 2003). However, none of these studies directly tested whether variation in zooplankton lipid content affects the seasonal patterns of zooplankton $\delta^{13}C$, isotopic differences among taxa, or discrepancies between POM and zooplankton.

In the current study, we 1) develop a generalized model to explain variation in zooplankton $\delta^{13}C$ based on variation in zooplankton lipid content, 2) calibrate the model using data from Sooke Lake Reservoir (SOL), and 3) apply the model in two other lakes (Lough Erne, and Pluβsee) that have contrasting trophic status. The model uses a stoichiometric approach to relate zooplankton C:N with lipid content. We test the model using data from SOL, which include the $\delta^{13}C$ of zooplankton (*Daphnia* and *Leptodiaptomus tyrelli*) with and without lipids, and the $\delta^{13}C$ of their respective bulk lipids. We chose SOL because previous work has shown large seasonal variation in the C:N of zooplankton, but small seasonal and depth variation in the $\delta^{13}C$ of POM. Finally, we use our model to develop a lipid normalization technique and compare it with another existing model (McConnaughey and McRoy 1979). Overall, we found that lipids could almost entirely explain the low $\delta^{13}C$ of zooplankton relative to POM in SOL, and our model could explain the negative relationship between C:N and $\delta^{13}C$ observed in two lakes.

Methods

Sooke Lake Reservoir (48°33'N, 123°41'W) is a 605 ha oligotrophic reservoir with a summertime total phosphorus (TP) of ~3 μg L^{-1}, and chlorophyll *a* (chl *a*) typically <1 μg L^{-1} (Davies et al. 2004). Lough Erne (54°12'N, 07°30'W) is a 10 950 ha mesotrophic lake in Northern Ireland (TP ~20-60 μg L^{-1}, chl *a* ~ 6-10 μg L^{-1}), and Pluβsee (54°10'N, 10°26'E) is a small (14 ha) eutrophic lake in northern Germany (TP ~ 110-130 μg L^{-1}, chl *a* ~ 100-150 μg L^{-1}).

Zooplankton were collected at the deepest site (70 m) of Sooke Lake Reservoir every two weeks during stratification (May to Nov), and monthly during the mixed period (Nov to Apr). The entire sampling period was from 16 April 2002 to 25 March 2003. We did multiple vertical tows from a maximum depth of 30 m, using a 50 cm Wisconsin net (64 μm). We froze the zooplankton samples at -80°C within 4 h of collection, and then sorted them into either *Daphnia spp*. or *Leptodiaptomus tyrelli*. After sorting, we freeze dried the samples, and used approximately 1 mg of *Daphnia* and *L. tyrelli* for isotopic analysis. Zooplankton were collected and processed in a similar way from Lough Erne (LER) and Pluβsee (PLU), except the zooplankton were sorted fresh, and dried at 60 °C overnight before stable isotope analysis. Cladocerans in PLU were a mix of *Diaphanosoma brachyurum*, and *Daphnia spp.* (*D. cucullata*, *D. galeata*, *D. hyalina* and their hybrids).

Analysis of lipid $\delta^{13}C$

We extracted the lipids from 1-5 mg of freeze-dried *L. tyrelli* and *Daphnia* from SOL using a 4:2:1 (v/v/v) mixture of chloroform:methanol:water (Kainz et al. 2002). We

pooled the organic layer from the extraction and three subsequent washes with chloroform, and evaporated the pooled extract to 4 ml. We then transferred replicate 2 ml samples into pre-weighed smooth-walled 10X8 mm flat base tin capsules (Elemental Microsystems Limited). After evaporating the chloroform, we weighed the tin capsules to determine percent lipid as a proportion of dry weight. The average analytical precision of percent lipid measurements was <1% based on 26 paired replicates. We then used the same tin capsule with the dried lipid extract for stable isotopes analysis. To measure the $\delta^{13}C$ of the lipid extracted tissue, we transferred organic matter in the methanol layer into the same types of tin capsules and dried them overnight at 60°C.

We collected particulate organic matter (POM) in SOL from 6 different depths on each sampling day using a 6 L Niskin sampler. Sampling depths varied slightly each day, so as to get 2 samples from the epilimnion (~0-6 m), metalimnion (8-14 m), and hypolimnion (14-30 m) during lake stratification. 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). We dried filters overnight at 60°C and packed them in tin cups for isotopic analysis. We also analyzed chlorophyll *a* by extracting the filters in 95% ethanol at 4°C overnight, and analyzing the extracts on a spectrophotometer (Ultraspec® 2000, Amerhsam) using a 10-cm quartz cell.

Stable isotope samples from SOL were analyzed on an elemental analyzer coupled to a Finnigan 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* standard in every sample run, and its precision was <0.1 ‰ for $\delta^{13}C$ within and between all sample runs. For the analysis of lipid samples we also included a

methanol standard to extend the range of $\delta^{13}C$ among standards and ensure linearity at low $\delta^{13}C$ signatures. A small aliquot of methanol was put in a tin cup and immediately dropped into the combustion chamber, in order to minimize evaporative effects on its $\delta^{13}C$ signature. The $\delta^{13}C$ of the methanol standard was -42.9 ‰ (SE= 0.09 ‰). Replicate analysis of the same lipid extract yielded an average analytical precision of 0.2‰ (*n*= 24). Samples from PLU and LER were analyzed following Grey et al. (2001).

Lipid class analysis

For 11 dates during stratification, we also extracted lipids from *L. tyrelli* for lipid class analysis. We picked between 100-150 individuals of *L. tyrelli* and placed them in an extraction medium of 8:4:3 (v/v/v) chloroform:methanol:water, to which we had added a known concentration of 3-hexadecanone as an internal standard. Extraction and analysis of lipid classes was done following Campbell et al. (2004). Samples were analyzed on a MK-5 TLC/FID analyzer with the following analytical standards: tripalmitin (triacylglycerides: TAG), palmitic acid (free fatty acids: FFA), cholesterol (sterols: ST), and DL-α-Phosphatidylcholine, dipalmitoyl (polar lipids: PL).

Stoichiometric model of zooplankton

The purpose of the stoichiometric model is to explain 1) the observed non-linear positive relationship between zooplankton C:N and lipid content, and 2) the observed non-linear negative relationship between zooplankton C:N and $\delta^{13}C$ (non-lipid extracted). The model assumes that zooplankton are made up of three main biochemical building blocks that have fixed stoichiometric ratios, including proteins ($C_{59}H_{94}N_{16}O_{19}S_{0.5}$), lipids

($C_{18}H_{36}O_2$), and carbohydrates (a mix of both chitin ($C_9H_{15}O_6N_1$), and primary carbohydrate ($C_6H_{12}O_6$)). The C:N of zooplankton is then,

$$C:N = M\frac{C_{carbo}\Phi_{carbo} + C_{protein}\Phi_{protein} + C_{lipid}\Phi_{lipid}}{N_{carbo}\Phi_{carbo} + N_{protein}\Phi_{protein}} \qquad (1)$$

where M is the molar mass ratio for carbon and nitrogen (i.e. 14.01 g mol^{-1} / 12.01 g mol^{-1}), C_x and N_x are respectively the carbon or nitrogen content for each biomolecule, and Φ_x is the dry mass proportion.

To obtain a relationship between Φ_{lipid} and C:N, we assumed that carbohydrate is a fixed fraction (k) of the remaining dry weight without lipids, in which case the carbohydrate and protein fraction are expressed as:

$$\Phi_{carbo} = k(1-\Phi_{lipid}) \qquad (2)$$

$$\Phi_{protein} = (1-k)(1-\Phi_{lipid}) \qquad (3)$$

which when substituted into equation (1) yields:

$$C:N = M\frac{k(C_{carbo} - C_{protein}) + C_{prot} + \Phi_{lipid}\left[k(C_{protein} - C_{carbo}) + C_{lipid} - C_{protein}\right]}{k(N_{carbo} - N_{protein}) + N_{protein} + \Phi_{lipid}\left[N_{protein}(k-1) - N_{carbo}\right]} \qquad (4)$$

To include chitin in the model, we assumed that a fraction (*c*) of the total carbohydrate carbon pool (C_{carbo}) was made up of chitin (C_{chitin}), and the rest was primary carbohydrate (C_{pc}) such that:

$$C_{carbo} = C_{chitin}c + (1-c)C_{pc} \qquad (5)$$

$$N_{carbo} = cN_{chitin} \qquad (6)$$

With these simplifications, C:N can be expressed in terms of the constants in Table 6.1, *k*, Φ_{lipid}, and *c*:

$$C:N = M\frac{0.525 + k(0.064c - 0.125) + \Phi_{lipid}[k(0.125 - 0.064c) + 0.236)]}{0.166 + k(0.06c - 0.166) + \Phi_{lipid}(0.166k - 0.06c - 0.166)} \qquad (7)$$

To estimate the parameters *k* and *c*, we fit Eq. 7 to our observed data for zooplankton lipid content and C:N in SOL. To do this we minimized the sum of the squares (by Ordinary Least Squares (OLS) parameter estimation) of the observed differences between the predicted and observed C:N. From the resulting parameters (Table 6.1), equation 7 can be simplified to:

$$C:N = M\frac{(0.50 + 0.26\Phi_{lipid})}{(0.13 - 0.13\Phi_{lipid})} \quad (8)$$

and re-arranged for Φ_{lipid} as:

$$\Phi_{lipid} = \frac{(0.111C:N - 0.5)}{(0.119C:N + 0.26)} \quad (9)$$

The $\delta^{13}C$ of zooplankton can then be calculated based on a carbon weighted average of each biochemical pool of carbon as follows using the parameters in Table 6.1:

$$\delta^{13}C = \frac{0.05573*\delta^{13}C_{carbo}*(1-\Phi_{lipid}) + 0.23845*\delta^{13}C_{prot}*(1-\Phi_{lipid}) + 0.44791*\delta^{13}C_{lipid}*\Phi_{lipid}}{0.29417 + 0.15374*\Phi_{lipid}} \quad (10)$$

Assuming that the $\delta^{13}C$ of carbohydrates and proteins are the same (following McConnaughey and McRoy 1979), we can substitute equation 9 into equation 10, and predict the $\delta^{13}C$ of zooplankton based on 1) zooplankton C:N, 2) the average difference between $\delta^{13}C$ of lipids and carbohydrates and proteins ($\Delta_{C\text{-}L}$), and 3) the $\delta^{13}C$ of either lipids, carbohydrates, or proteins. We estimated $\Delta_{C\text{-}L}$ by calculating the average daily difference in $\delta^{13}C$ between zooplankton lipid and lipid extracted zooplankton samples from SOL ($\Delta_{LE\text{-}L}$ in Table 6.2). We then used this estimate of $\Delta_{C\text{-}L}$, and OLS parameter estimation (to estimate the $\delta^{13}C_{LE}$) to model the expected non-linear relationship between zooplankton C:N and $\delta^{13}C$ for SOL, and LER.

To normalize the $\delta^{13}C$ of zooplankton to $\Phi_{lipid} = 0$ (following McConnaughey and McRoy 1979), we predicted the $\delta^{13}C$ of lipid extracted tissue ($\delta^{13}C_{LE}$) using the $\delta^{13}C$ of zooplankton (non-lipid extracted), $\Delta_{C\text{-}L}$ (estimated using daily $\Delta_{LE\text{-}L}$ measurements), and zooplankton C:N. For clarity we have shown our lipid normalization model (Eq. 11) in terms of percent lipid (Φ_{lipid}), which can be predicted from C:N using equation 9.

$$\delta^{13}C_{LE} = \frac{\delta^{13}C\,(0.29417 + 0.15374\Phi_{lipid}) + 0.44791\Delta_{C\text{-}L}\Phi_{lipid}}{0.29417 + 0.15374 * \Phi_{lipid}} \quad (11)$$

We then compared our normalization approach (Eq. 11) with McConnaughey and McRoy's (1979) normalization procedure, and compared the prediction of $\delta^{13}C_{LE}$ from both models with our observed values for the $\delta^{13}C$ of lipid extracted tissue.

Results

Temporal variation in plankton C:N and lipid content

L. tyrelli and *Daphnia* had contrasting seasonal patterns of C:N in SOL. *Daphnia* had its lowest C:N during periods of stratification, and its highest C:N in the winter and spring (Range = 2.0, Fig. 6.1A). The C:N of *L. tyrelli* reached a maximum near the end of stratification, and then declined over the winter and spring (range = 11.0, Fig. 6.1A). In contrast to SOL, the seasonal pattern of zooplankton C:N in PLU was similar among taxa, and in both LER and PLU the range of zooplankton C:N was between 2.5 and 6.2 (Table 6.3, Fig. 6.5, 6.6). The C:N of zooplankton in SOL had a strong positive relationship with percent lipid content (Fig. 6.2). *Daphnia* had a narrow seasonal range of lipid content (12.9 to 26.8%, *n*= 11) compared to *L. tyrelli* (24.9 to 60.2%, *n*= 19). The

high lipid content of *L. tyrelli* was due to a high concentration of triacylglycerides (TAG), which was the main lipid class of *L. tyrelli* during the stratified period (Fig. 6.1B).

Temporal variation of plankton $\delta^{13}C$

The $\delta^{13}C$ of POM in SOL varied from -26.9 to -31.6‰ over the entire sampling period (n= 117; Fig. 6.3). The sampling depth (covariate) and day of year (categorical) explained 65.7% of the variance in POM $\delta^{13}C$ (ANOVA: depth $F_{1,96}$= 31.4, $p < 0.001$; date $F_{19,96}$= 8.2, $p < 0.001$). The sampling day of year (DOY) explained nearly three times more variation in POM $\delta^{13}C$ than did sampling depth (coefficient of partial determination: $r_{DOY}^2 = 0.73$, $r_{depth}^2 = 0.25$). The variance of POM $\delta^{13}C$ was higher during stratification ($F_{83,32} = 2.44$, $p < 0.01$), as was the daily average range of $\delta^{13}C$ over the water column (stratified = 2.34‰, n = 13, unstratified = 1.11‰, n = 7) (shaded area in Fig. 6.3).

In general the $\delta^{13}C$ of zooplankon was than the $\delta^{13}C$ of POM in SOL, but this depended on the tissue type. The $\delta^{13}C_{WB}$ (whole body) of *Daphnia* in SOL was 4.1‰ lower (SD = 0.93, n= 20) than the average $\delta^{13}C$ of POM, and 2.4‰ higher (SD = 1.3, n= 20) than the $\delta^{13}C_{WB}$ of *L. tyrelli* (Fig. 6.3). In comparison, lipid extracted *Daphnia* ($\delta^{13}C_{LE}$) was only 0.62‰ greater than the $\delta^{13}C_{LE}$ of *L. tyrelli* (Paired-t-test: t_8= 4.7, p = 0.002), and was not significantly different from the $\delta^{13}C$ of POM (Paired-t-test: *Daphnia*, t_9= 1.69, p= 0.125). The $\delta^{13}C_{LE}$ of *L. tyrelli* was 0.81‰ lower than the $\delta^{13}C$ of POM (Paired t-test: t_{16}= 5.50, p< 0.001), and the $\delta^{13}C_L$ of *L. tyrelli* was always less than the $\delta^{13}C_L$ of *Daphnia* (Average daily Δ = 5.96‰, SD= 1.03, n= 9). In PLU the range of zooplankton $\delta^{13}C$ was 2 to 5 times larger than for zooplankton in either SOL or LER,

despite small seasonal variation in zooplankton C:N (Table 6.3, Fig. 6.6). Unlike in SOL (Fig. 6.4) and LER (Fig. 6.5A), in PLU there was no significant negative relationship between zooplankton C:N and $\delta^{13}C$, and lipid normalization had little influence on seasonal patterns (Fig. 6.6).

Stoichiometric modeling

In SOL, the stoichiometric model of zooplankton accounted for over 90% of the variation in the relationship between zooplankton lipid content and C:N (model $r^2 = 0.91$; Fig. 6.2). Using Eq. 11, we were able to predict the form of the relationship between C:N and $\delta^{13}C$ in both SOL (Fig. 6.4) and LER (Fig. 6.5B), but not in PLU (Fig. 6.6C). Since the temporal variation in the $\delta^{13}C$ of POM was small in SOL, our model could explain over 70% of the variation in the $\delta^{13}C$ of zooplankton (model $r^2 = 0.73$; Fig. 6.4). In LER, the model explained 66% of the variation in zooplankton $\delta^{13}C$ (1999: $r^2 = 0.66$, 2003: $r^2 = 0.66$). In PLU there is a positive relationship between C:N and $\delta^{13}C$ ($r^2 = 0.19$, $p < 0.001$).

Compared to McConnaughey and McRoy (1979), our lipid normalization model (Eq. 11) improved predictions of $\delta^{13}C_{LE}$ for *L. tyrelli* (Fig. 6.7; both models using $\Delta_{LE\text{-}L} = 9.2$), but neither model (both parameterized using $\Delta_{LE\text{-}L} = 5.1$) could accurately predict the unexpectedly high $\delta^{13}C_{LE}$ of *Daphnia* (Fig. 6.7).

Discussion

We found that the low $\delta^{13}C$ of zooplankton lipids can explain why the $\delta^{13}C$ of zooplankton is lower than POM in SOL. Our stoichiometric model can predict the form of relationship between percent lipid and C:N (Fig. 6.2), and between C:N and $\delta^{13}C$ of zooplankton (Fig. 6.4, 6.5). The lipid normalization model (Eq. 11) could be calibrated

for other consumers by modifying the relationship in Fig. 6.2, and independently measuring $\Delta_{C\text{-}L}$ (Eq. 11, Table 6.1). This general modeling approach is widely applicable to ecosystem studies that wish to quantify the carbon sources of a consumer's lipids, proteins, and carbohydrates.

Seasonal patterns of zooplankton lipid content

The lipid content of zooplankton typically increases with food concentration (Lee et al. 1971; Goulden and Place 1990; Arts et al. 1993) and food quality (Cowgill et al. 1984). For example, the lipid content of *Daphnia pulex* in a eutrophic lake varied from 10 to 40%, and was positively correlated with edible algal biomass (Arts et al. 1993). Cowgill et al. (1984) found that the lipid content of *Daphnia magna* was 9.4% and 23.3% when fed on low and high quality foods, respectively. The lipid content of *Daphnia* in SOL was highest in May (26.9%), and lowest in midsummer (12.9%), but its C:N was not correlated with either the C:N or Chl *a* of POM <41μm (Fig. 6.1). This indicates that seasonal change in the C:N of *Daphnia* in SOL does not respond in a predictable way to changes in the bulk properties of POM. Coastal British Columbia lakes commonly have high algal biomass and productivity in the winter and early spring when epilimnetic water temperature is ~10°C (Davies et al. 2004). As a result, the high C:N of *Daphnia* in the spring may result from relatively high food concentrations (with a high carbon content) at a time of year when water temperatures, and possibly food quality, may not favour rapid growth or reproduction of *Daphnia*.

The high lipid content and large seasonal variability of lipid content in *L. tyrelli* is common among freshwater and marine calanoid copepods (Cavaletto et al. 1989; Walve and Larsson 1999). For example, Cavaletto et al. (1989) found that the lipid

content varied from 42 to 67% in *Limnocalanus macrurus*, and reached as high as 30% in *Senecella calanoides.* In SOL the range of C:N for *L. tyrelli* was nearly 5 times larger than *Daphnia*, but the range of lipid content was only ~3 times larger. This is because the relationship between C:N and lipid content is an increasing non-linear function (Eq. 9; Fig. 6.2). Therefore, organisms with low lipid content (such as *Daphnia*) theoretically have a more seasonally stable C:N even though their lipid content can change by 30% (Arts et al. 1993).

Variation in zooplankton lipid content among lakes correlates with the amount of carbon fixation (as a seasonal average) allocated to phytoplankton lipids (Wainman et al. 1993). Reasons for the variation in seasonal patterns of zooplankton lipid content for different zooplankton taxa remain unclear. Abrupt variation in the C:N of *L. tyrelli* (Fig. 6.1) may reflect changes in recent feeding success over the two week sampling interval. Starving calanoids can loose 50% of their storage lipids within a week (Lee et al. 1971), whereas *Daphnia* can lose most of their storage lipid within a few days (Tessier et al. 1983). It is intriguing that the patterns of seasonal variation in zooplankton lipid content among taxa are opposite in SOL (Fig. 6.1A), but correlated in PLU (Fig. 6.6A). Arts et al. (1992; 1993) found a similar pattern for zooplankton lipid dynamics in two lakes with contrasting food abundance. In an oligotrophic saline lake, Arts et al. (1993) found that *Leptodiaptomus sicilis* reached its highest lipid content (30-45%) in the autumn and spring, whereas *Epischura nevadensis* reached maximum lipid content (40 to 60%) during the summer. In a hypereutrophic lake, Arts et al. (1992) found that the timing of lipid accumulation was similar for *L. sicilis*, *D. pulex*, and *Diacyclops biscuspidatus thomasi*. In all of these species, increases in lipid content were associated with increases

in the triacylglyceride (TAG) storage lipid (Arts et al. 1992, 1993), as we observed for *L. tyrelli* in SOL. The results from PLU and SOL and those of Arts et al. (1992, 1993) suggest that contrasting lipid accumulation could be more common in lakes with low food concentrations. Variable life history strategies and the timing of lipid ingestion, storage, and utilization, may help explain how multiple zooplankton species can co-exist in lakes with low productivity.

Correlation between zooplankton C:N and $\delta^{13}C$ in lakes of varying productivity

Our stoichiometric model predicted the general form of the relationship between zooplankton C:N and $\delta^{13}C$ in SOL (Fig. 6.4) and LER (Fig. 6.5B). The position of this relationship (intercept) depends on the $\delta^{13}C$ of food sources, whereas the shape depends primarily on the isotopic composition of lipids, carbohydrates, and proteins (bigger $\Delta_{C\text{-}L}$ leads to a steeper slope), and secondarily on the relationship between lipid content and C:N (Fig. 6.2). If the $\delta^{13}C$ of food sources change rapidly over the season, then the correlation between C:N and $\delta^{13}C$ will be weaker or not apparent. We believe this is the case in PLU, where the range in zooplankton $\delta^{13}C$ is >20‰ but the variation in zooplankton C:N is only ~3. The reversal of the relationship between C:N and $\delta^{13}C$ in PLU indicates that lipids will explain less of the seasonal variation of plankton $\delta^{13}C$ in lakes with either large seasonal or spatial (for example depth based variation in $\delta^{13}C$) variation in the $\delta^{13}C$ of zooplankton food sources.

The $\delta^{13}C$ of POM in SOL exhibited relatively small variation with respect to season and depth; however, seasonal changes in the $\delta^{13}C$ of zooplankton food sources can still account for some of the unexplained variation in Fig. 6.4. The points circled in Fig.

6.4 are all from 8 May to 19 Jun, 2002, which suggests that seasonal changes in relationship between zooplankton C:N and $\delta^{13}C$ could help identify isotopic shifts in zooplankton diet.

Tracing the diet of zooplankton using $\delta^{13}C_{LE}$ and $\delta^{13}C_L$

Both the $\delta^{13}C$ of lipid extracted tissue ($\delta^{13}C_{LE}$) and of lipid ($\delta^{13}C_L$) can be useful tracers of a consumer's diet. In SOL, the $\delta^{13}C_{LE}$ of zooplankton could be used in combination with the $\delta^{13}C$ of allochthonous and autochthonous carbon to determine the relative contribution of terrestrial carbon sources to pelagic zooplankton (Grey et al. 2001, Pace et al. 2004). In SOL, the $\delta^{13}C$ of terrestrial carbon (evergreen needles) in the watershed is -27.5‰ (SD= 0.8, N= 44) (Perga et al. submitted). Unfortunately we did not measure the $\delta^{13}C$ of algae (or their lipids) in SOL, so we cannot quantify the relative proportion of allochthonous and autochthonous carbon to zooplankton. In a survey of 12 northern Sweden lakes (<1000 m a.s.l.), Karlsson et al. (2003) found that the $\delta^{13}C$ of terrestrial carbon was -27.1 (SD=0.45, N=12), whereas the $\delta^{13}C$ of algae varied from -37.7 to -46.2‰. If the $\delta^{13}C$ of algae in SOL is within this range, then our data suggest that *L. tyrelli* relies more on autochthonous carbon sources than *Daphnia* (Fig. 6.3).

In SOL, the lower $\delta^{13}C_L$ of *L. tyrelli* compared to *Daphnia* (Fig. 6.3) implies that *L. tyrelli* is feeding on a carbon source (which we suspect to be algae) with a substantially lower $\delta^{13}C$ than *Daphnia*. This is consistent with previous studies that suggest that the $\delta^{13}C$ of algae (and their lipids) is masked by terrestrial sources of carbon in POM (del Giorgio and France 1996; Grey et al. 2001). Pel et al. (2003) found that the $\delta^{13}C$ of algal fatty acids (FAs) can vary by 10‰ depending on the algal species, and reach values less

than -40‰. Within algae, the $\delta^{13}C$ of lipid biomolecules can also differ depending on their biosynthetic origin and the $\delta^{13}C$ of their precursors (Chikaraishi et al. 2004). For example, Riebesell et al. (2000) found that various algal lipid classes were 1.9 to 8.5‰ lower than particulate organic carbon. Schouten et al. (1998) found that the average $\delta^{13}C$ of FAs was 0.8 to 8.7‰ lower than other algal cell material. Therefore, the low $\delta^{13}C$ of algal lipids might account for the low $\delta^{13}C$ of zooplankton lipids in SOL (Fig. 6.3). The main alternative to this hypothesis is that zooplankton are feeding on a similar carbon source, and the difference in $\delta^{13}C_L$ between *Daphnia* and *L. tyrelli* is due to taxa specific discrimination against ^{12}C during lipid synthesis. However, other studies have found little fractionation during the ingestion and assimilation of lipids by zooplankton (Grice et al. 1998; Klein Breteler et al. 2002). Therefore, we believe that the differences in $\delta^{13}C_L$ between *L. tyrelli* and *Daphnia* are primarily due to differences in the $\delta^{13}C$ of dietary carbon sources, rather than physiological differences in metabolism.

Several researchers have recently recognized the benefits of incorporating the $\delta^{13}C$ of lipids into dietary analyses (Focken and Becker 1998; Gaye-Siessegger et al. 2004). In feeding experiments with carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*), Focken and Becker (1998) found that the $\delta^{13}C$ of lipids provide reliable tracer of diet. In all individuals (n= 45 tilapia; n= 24 carp), the $\delta^{13}C$ of lipids was within 1‰ of the $\delta^{13}C$ of dietary lipids. The $\delta^{13}C$ of bulk lipids ($\delta^{13}C_L$) can certainly provide valuable information about the source of a consumer's diet, but the interpretation of $\delta^{13}C_L$ depends on the relative balance of dietary acquisition, catabolism, and anabolism of lipids. The $\delta^{13}C_L$ will be lower than dietary carbon sources if the consumer synthesizes a large proportion of lipids. This is because pyruvate decarboxylase discriminates against ^{13}C

during lipid synthesis (DeNiro and Epstein 1977). In contrast, the $\delta^{13}C_L$ will increase following substantial catabolism (Gaye-Siessegger et al. 2004). Gaye-Siessegger et al. (2004) found that the $\delta^{13}C_L$ in carp was closest to the $\delta^{13}C$ of dietary lipids at intermediate feeding levels. Nevertheless, the $\delta^{13}C_L$ in carp was typically within 1‰ of the $\delta^{13}C_L$ of its diet over a large range of feeding levels and lipid content in the diet. In general, the balance of anabolism and catabolism will determine how close the $\delta^{13}C$ of consumer lipids matches with lipids in their food sources.

Lipid extraction and normalization in stable isotope analysis

Removing lipids from consumers prior to isotope analysis allows ecologists to trace the pathways of protein and carbohydrate through the food chain, without the confounding effects of lipid metabolism (Kelly 2000). Lipids typically have a lower $\delta^{13}C$ than proteins or carbohydrates (DeNiro and Epstein 1977), such that extracting lipids prior to stable isotope analysis will reduce the variation in $\delta^{13}C$ among samples that result from differences in lipid content. This approach is particularly useful to compare $\delta^{13}C$ among different organisms, tissues, or samples that have varying lipid content. For example, POM has lower lipid content than zooplankton so the $\delta^{13}C$ of lipid extracted zooplankton should better match with the $\delta^{13}C$ of POM (Fig. 6.3).

Though lipid normalization is also a common practice in stable isotope ecology, there are few reliable techniques to ensure realistic estimates of $\delta^{13}C_{LE}$ (Matthews and Mazumder 2005a). Several researchers have used the lipid normalization technique of McConnaughey and McRoy (1979) to normalize the $\delta^{13}C$ of zooplankton based on the observed C:N ratio (Kline 1999; Schmidt et al. 2003). The model we provide here is

specifically calibrated to zooplankton, and accurately predicts the $\delta^{13}C_{LE}$ of *L. tyrelli* (Fig. 6.7). Future studies should test the assumption that the $\delta^{13}C$ of carbohydrates and proteins are similar, which may help account for the 2‰ discrepancies between the predicted and observed $\delta^{13}C$ of *Daphnia*.

The model we provide for lipid normalization is adaptable to other organisms, and can be modified by changing the relationship between C:N and lipid, and the $\delta^{13}C$ of lipid, proteins, and carbohydrates. If samples do not have a large range of lipid content then a simpler model may suffice to normalize $\delta^{13}C$ values (see Dufour et al. 2001). Dufour et al. (2001) used the positive linear relationship between C:N and Δ_{WB-LE} in a population of arctic char (*Salvelinus alpinus*) to normalize the $\delta^{13}C$ of non-lipid extracted samples. This procedure works provided that the relationship is linear over the range of C:N in the samples, which is not the case for our samples (Fig. 6.2).

Consequences of incorporating zooplankton lipids into food web studies

The $\delta^{13}C$ of lipids are particularly useful and important for determining the origins of zooplankton carbon, because zooplankton can be highly selective in their acquisition and digestion of food sources (Harvey et al. 1987; Cowie and Hedges 1996), and can accumulate and store large amounts of lipid (Arts et al. 1993). For example, the high lipid content of *L. tyrelli* in SOL probably results from the accumulation of TAG storage lipids from algae, in which case the $\delta^{13}C_L$ of *L. tyrelli* might match the $\delta^{13}C$ of algal lipids. Isotopic routing of dietary lipids is a general challenge for the analysis of diet using stable isotopes (Gannes et al. 1997), and is particularly important for organisms like zooplankton that can accumulate up to 60% lipid content (Fig. 6.2).

Measuring the amount and $\delta^{13}C$ of zooplankton lipids will likely help resolve how carbon is cycled from allochthonous sources through pelagic food chains (Grey et al. 2001; Karlsson et al. 2003; Pace et al. 2004). In all these studies, $\delta^{13}C_{WB}$ was used in a mixing model to determine the amount of zooplankton allochthony (i.e. the proportion of allochthonous carbon assimilated by zooplankton). In SOL, the $\delta^{13}C$ of zooplankton could over- or underestimate zooplankton allochthony depending on whether lipids are dietary sources of carbon. If zooplankton accumulate lipids from their diet (Arts and Wainman 1998) then using $\delta^{13}C_{LE}$ (and possibly $\delta^{13}C_{WB}$) will overestimate zooplankton allochthony. Conversely, if zooplankton synthesize lipids *de novo* (*sensu* Chamberlain et al. 2004) then $\delta^{13}C_{WB}$ will underestimate allochthony and $\delta^{13}C_{LE}$ would provide better estimates of allochthony. Seasonal variation in *Daphnia* lipid content likely had little impact on Pace et al.'s (2004) general conclusions, because the experimental manipulation led to a large magnitude of seasonal variation in the $\delta^{13}C$ of algae (as in PLU). Nevertheless, future studies should consider the consequences of the low $\delta^{13}C$ of zooplankton lipids, and the seasonal and among lake variation in zooplankton lipid content.

The $\delta^{13}C$ of lipids may also help detect and quantify the contribution of methane to pelagic food webs (Bastviken et al. 2003). Methane oxidizing bacteria have unique fatty acids that can be traced higher up the food chain (Boschker et al. 1998). Given that lipids are carbon rich molecules, measuring $\delta^{13}C_L$ may be an efficient way to detect a contribution of methane to zooplankton diets. A more detailed approach would measure the $\delta^{13}C$ of the fatty acids specifically associated with methane oxidizing bacteria (Kiyashko et al. 2004).

In conclusion, variation in zooplankton lipid content and the $\delta^{13}C$ of lipids contributes to the large seasonal and interlake variation in zooplankton $\delta^{13}C$. Future studies should test the generality of our stoichiometric model of lipid content and C:N, and determine the amount of among lake variation in $\Delta_{LE\text{-}WB}$, and $\Delta_{LE\text{-}L}$. Multiple tracers ($\delta^{13}C$, FAs, etc…) are likely needed to identify the sources of carbon that are contributing to the differences in $\delta^{13}C_L$ between calanoids and *Daphnia*. In general, we believe that both the $\delta^{13}C$ of lipids and lipid extracted tissue provide useful dietary information about a consumer's carbon sources.

Tables

Table 6.1 : Description of parameters for the stoichiometric model.
All percentages (SE) are expressed in the model as the proportion of dry weight (DW). The carbohydrate pool is a weighted average of chitin and primary carbohydrate. The * indicates that the parameter value was determined by best fit (minimum sum of squares) of the model (Eq. 4) to the data (Fig. 6.2). The † indicates parameters that were calculated as a result of fitted parameters.

Symbol	Parameters and constants	Value (SE)
C_{carbo}	Carbon content of the mixed carbohydrate pool	41.2% †
C_{prot}	Carbon content of protein biomolecule ($C_{59}H_{94}N_{16}O_{19}S_{0.5}$)	52.5%
C_{lipid}	Carbon content of lipid biomolecule ($C_{18}H_{36}O_2$)	76.0%
C_{chitin}	Carbon content of chitin ($C_9H_5O_6N_1$)	46.4%
C_{pc}	Carbon content of primary carbohydrate (CH_2O)	40.0%
N_{carbo}	Nitrogen content of carbohydrate pool	1.1% †
N_{prot}	Nitrogen content of protein	16.6%
N_{chitin}	Nitrogen content of chitin	6.0%
Φ_{carbo}	DW fraction of carbohydrate pool in zooplankton	varies (%)
Φ_{prot}	DW fraction of proteins in zooplankton	varies (%)
Φ_{lipid}	DW fraction of lipids in zooplankton	varies (%)
c	DW fraction of chitin in the carbohydrate pool	20.0% (4.5) *
k	DW fraction of carbohydrate pool when $\Phi_{lipid}= 0$	23.0% (13.5) *
Δ_{C-L}	Difference in $\delta^{13}C$ between lipids and carbohydrates	estimated as Δ_{LE-L}

Table 6.2 : Summary data for zooplankton and POM $\delta^{13}C$ in SOL

Seasonal averages of $\delta^{13}C$ for zooplankton and particulate organic matter (POM) from Sooke Lake Reservoir in 2002-2003. $\Delta_{LE\text{-}WB}$ is the average daily difference between whole body (WB) and lipid extracted (LE) tissue, and $\Delta_{LE\text{-}L}$ is the average daily difference in $\delta^{13}C$ between lipid extracted tissue and lipids (L).

Sample		**$\delta^{13}C$ (SE, ‰)**	**$\delta^{13}C$ Range**	**C:N (SE)**	**C:N Range**	***n* days**
Daphnia (WB)		-33.2 (0.24)	3.8	6.0 (0.11)	2.0	20
Daphnia (LE)		-29.2 (0.20)	2.0	4.7 (0.03)	0.3	10
Daphnia (L)		-34.0 (0.52)	5.3			11
L. tyrelli (WB)		-35.6 (0.22)	4.1	12.8 (0.68)	11.0	20
L. tyrelli (LE)		-29.8 (0.12)	1.9	4.1 (0.04)	0.5	18
L. tyrelli (L)		-39.0 (0.22)	2.4			16
POM	All depths	-29.1 (0.18)	2.9	8.9 (0.12)	2.3	20
	2-6 m (Epi)	-29.2 (0.23)	3.8	9.0 (0.13)	2.5	20
	8-14 m (Meta)	-29.4 (0.22)	3.1	9.0 (0.15)	2.6	19
	14-30 m (Hypo)	-28.7 (0.15)	2.3	8.7 (0.13)	1.9	20
$\Delta_{LE\text{-}WB}$	All zooplankton	5.1 (0.24)	3.9			28
	Daphnia	3.8 (0.12)	1.1			10
	L. tyrelli	5.8 (0.23)	3.8			18
$\Delta_{LE\text{-}L}$	All zooplankton	7.6 (0.36)	7.4			36
	Daphnia	5.1 (0.33)	3.4			14
	L. tyrelli	9.2 (0.11)	2.4			22

Table 6.3 : Summary $\delta^{13}C$ and C:N data for PLU and LER.

Seasonal averages of $\delta^{13}C_{WB}$ (whole body) and C:N for zooplankton in Lough Erne, and Pluβsee.

Sample	**$\delta^{13}C$ (SE,‰)**	**$\delta^{13}C$ Range**	**C:N (SE)**	**C:N Range**	***n* days**
Lough Erne (LER)					
1999 - *E. gracilis*	-33.2 (0.5)	5.3	4.7 (0.2)	2.8	12
2003 - *E. gracilis*	-31.6 (0.6)	6.8	5.7 (0.6)	6.2	12
Pluβsee (PLU) (2004)					
Cladocerans	-33.0 (1.6)	21.6	4.8 (0.1)	2.5	27
Cladocerans LE	-31.9 (1.7)	25.4			
Calanoids	-34.0 (1.5)	21.0	4.9 (0.1)	3.0	27
Calanoids LE	-32.6 (1.7)	25.3			
Cyclopoids	-32.5 (1.1)	17.0	4.8 (0.2)	2.9	27
Cyclopoids LE	-31.0 (1.3)	21.0			

Figures

Figure 6.1 : Seasonal variation of plankton C:N and lipid in SOL

(A) Variation in C:N of *Leptodiaptomus tyrelli* and *Daphnia* in SOL. (B) Variation in the lipid proportion of zooplankton (right scale) of *L. tyrelli* (closed circles) and *Daphnia* (open triangles), and the lipid class composition of *L. tyrelli* (left scale): triacylglyceroles (TAG), phospholipids (PL), and cholesterol (CL). (C) Seasonal variation in the C:N and Chlorophyll *a* of POM <41μm. All error bars are ± 1 SE.

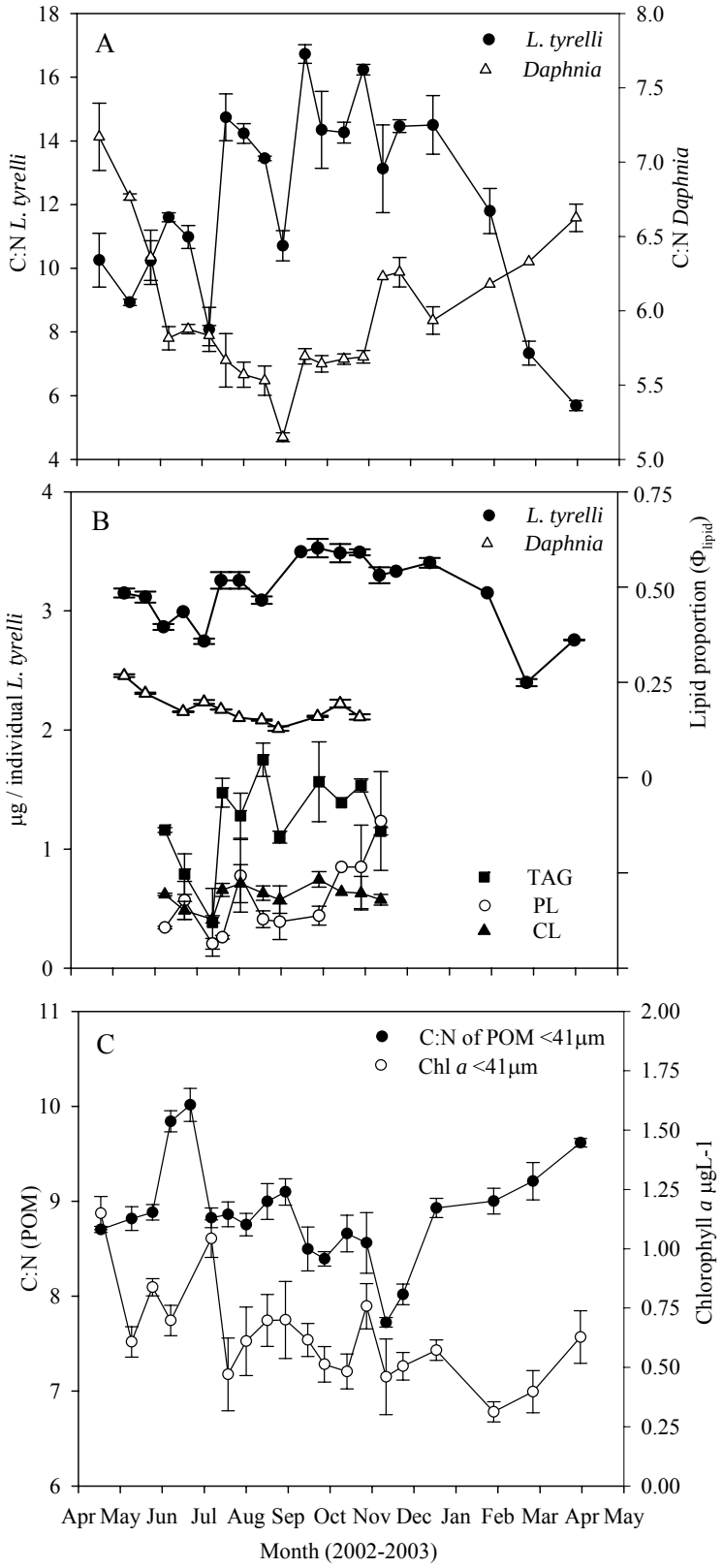

A
L. tyrelli
Daphnia
C:N L. tyrelli
C:N Daphnia
B
L. tyrelli
Daphnia
µg / individual L. tyrelli
Lipid proportion (Φlipid)
TAG
PL
CL
C
C:N of POM <41µm
Chl a <41µm
C:N (POM)
Chlorophyll a µgL-1
Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May
Month (2002-2003)

Figure 6.2 : Modeled relationship between C:N and lipid content.

Relationship between lipid proportion of zooplankton and the C:N of *Daphnia* and *L. tyrelli* from SOL. The plotted line (which is a plot of Eq. 8) is the OLS best fit of Eq. 7 that we used to calculate parameters *k*, and *c* (Table 6.1). The two samples with no lipid are averages of the C:N for all the lipid extracted samples of *Daphnia* and *L. tyrelli* from Fig. 6.3.

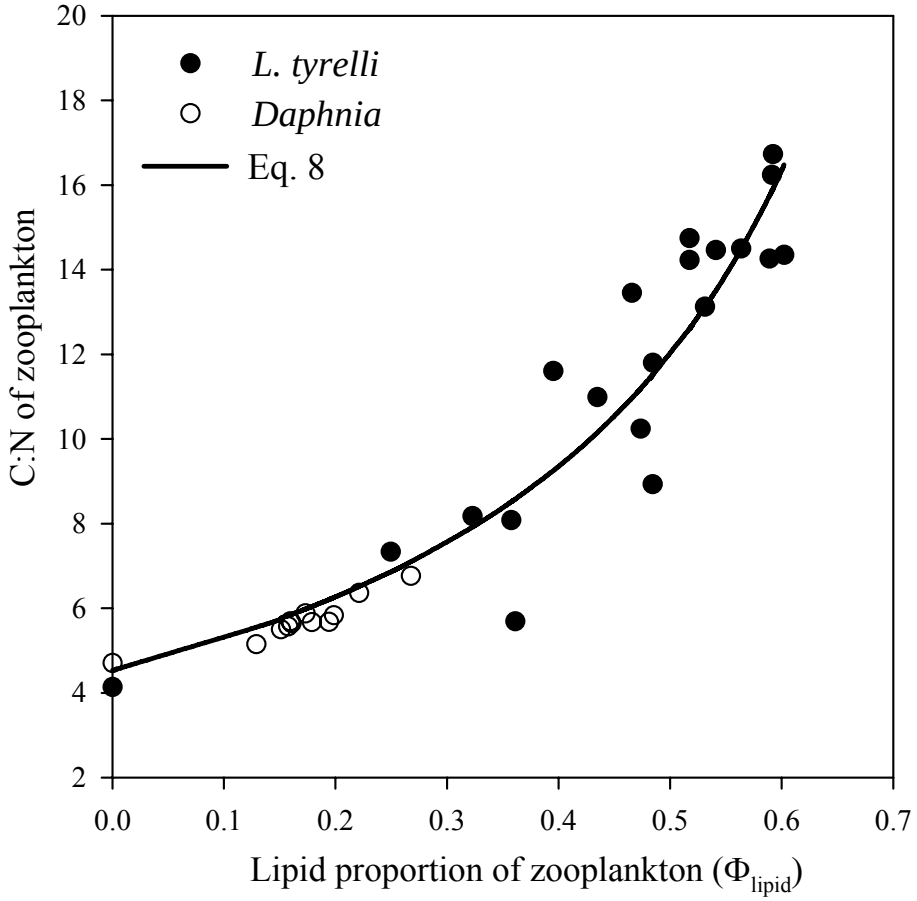

20
18
16
14
12
10
8
6
4
2
C:N of zooplankton
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Lipid proportion of zooplankton (Φ_{lipid})
L. tyrelli
Daphnia
Eq. 8

Figure 6.3 : Seasonal isotopic variation of plankton $\delta^{13}C$ in SOL

Variation in the $\delta^{13}C$ of zooplankton, lipid-extracted zooplankton, and zooplankton lipids in Sooke Lake Reservoir (SOL). The shaded region is the maximum and minimum of POM $\delta^{13}C$ collected from a 6 depth profile (0-30m) on each sampling day. The dotted lines indicate the period of stratification, and all error bars are 1 SD.

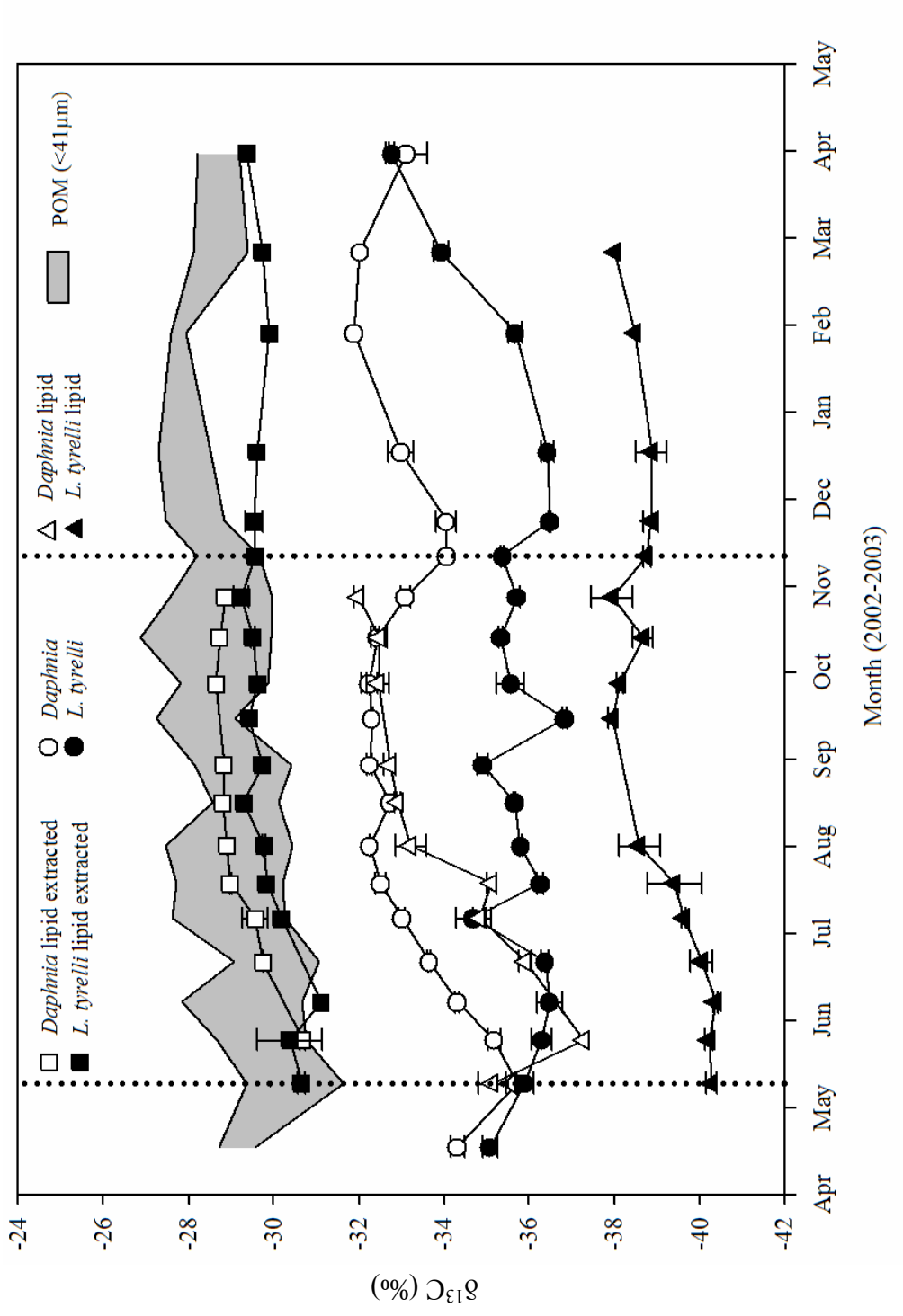

δ13C (‰)
-24
-26
-28
-30
-32
-34
-36
-38
-40
-42
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Month (2002-2003)
Daphnia lipid extracted
L. tyrelli lipid extracted
Daphnia
L. tyrelli
Daphnia lipid
L. tyrelli lipid
POM (<41µm)

Figure 6.4 : Relationship between C:N and $\delta^{13}C$ for zooplankton in SOL.

The line is fitted by OLS parameter estimation using Eq. 11, parameters in Table 6.1, and $\Delta_{C\text{-}L}$=7.6 (Table 6.2 $\Delta_{LE\text{-}L}$ for all zooplankton). The circled points are all from April 16th to Jun 19th, and were included in the curve fitting.

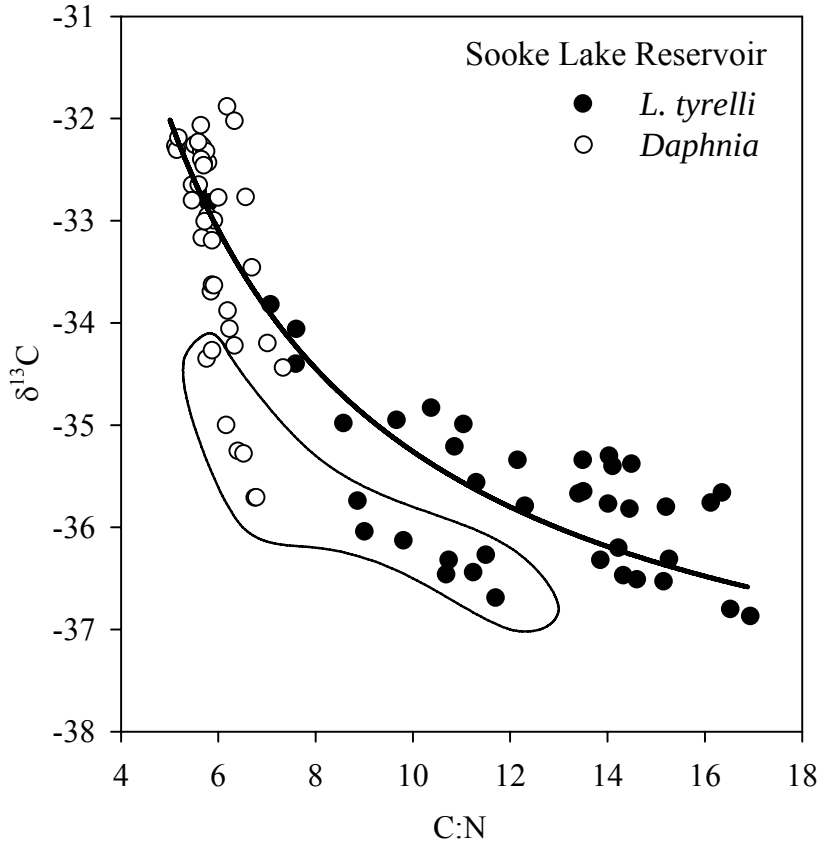

Sooke Lake Reservoir
L. tyrelli
Daphnia
-31
-32
-33
-34
-35
-36
-37
-38
δ13C
4
6
8
10
12
14
16
18
C:N

Figure 6.5 : Seasonal variation in the $\delta^{13}C$ of zooplankton in LER.

(A) Seasonal variation of C:N, $\delta^{13}C$ and $\delta^{13}C_{LE}$ for *Eudiaptomus gracilis* in Lake Erne (LER). (B) The lines are best fits of Eq. 11 as in Fig. 6.4, except $\Delta_{C\text{-}L}$ was also fit simultaneously by OLS regression (1999: $\Delta_{C\text{-}L}$= 9.4 , 2003: $\Delta_{C\text{-}L}$= 6.4). The first data point in 2003 was removed before fitting the model.

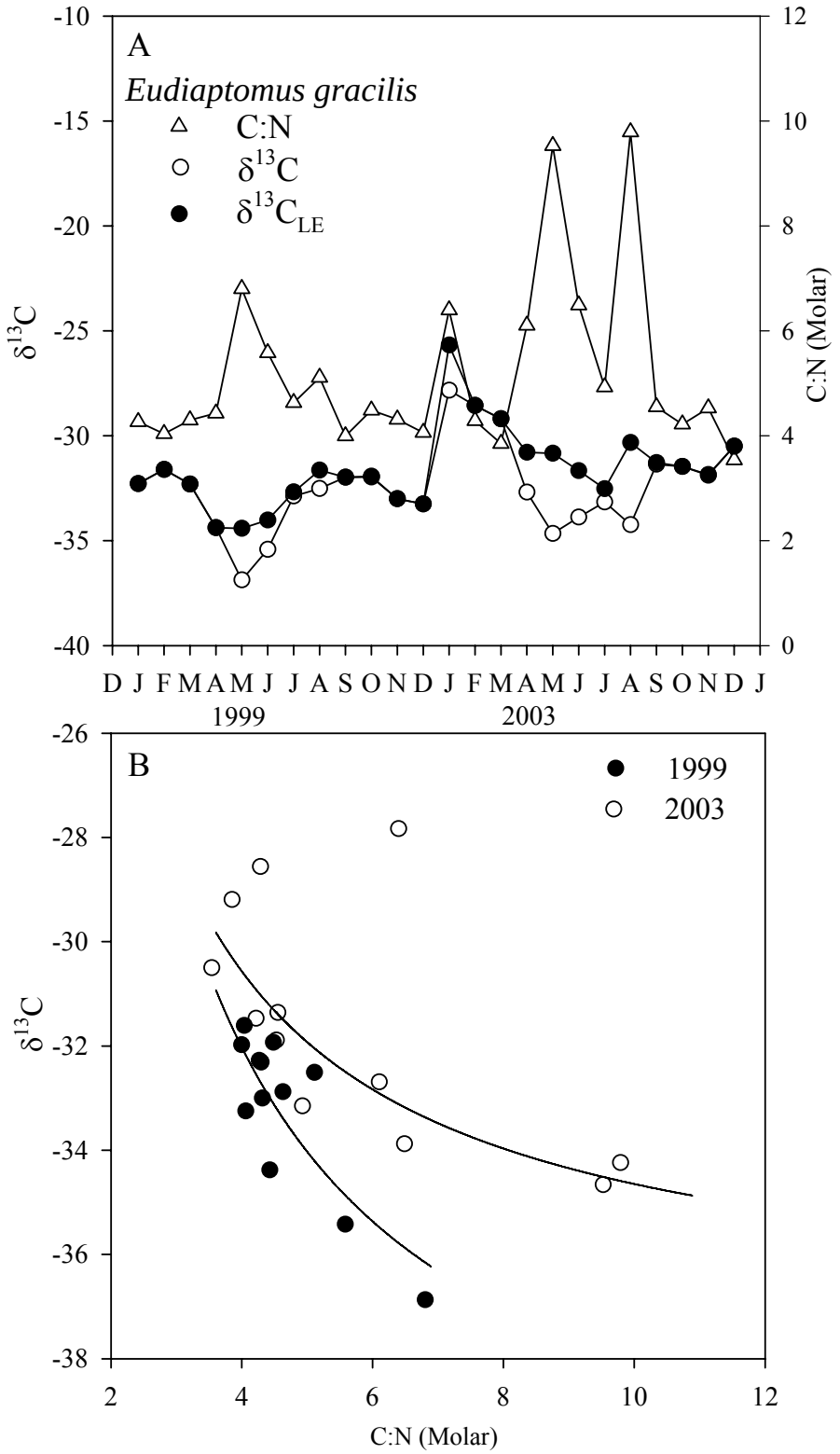

A
Eudiaptomus gracilis
C:N
δ13C
δ13CLE
δ13C
C:N (Molar)
-10
-15
-20
-25
-30
-35
-40
12
10
8
6
4
2
0
D J F M A M J J A S O N D J F M A M J J A S O N D J
1999
2003
B
1999
2003
δ13C
C:N (Molar)
-26
-28
-30
-32
-34
-36
-38
2
4
6
8
10
12

Figure 6.6 : Seasonal variation of zooplankton C:N and $\delta^{13}C$ in PLU.

Seasonal variation of zooplankton C:N (A) and $\delta^{13}C$ (B) in Lake Pluβsee in 2004. (C) Relationship between C:N and $\delta^{13}C$ for Lake Pluβsee zooplankton.

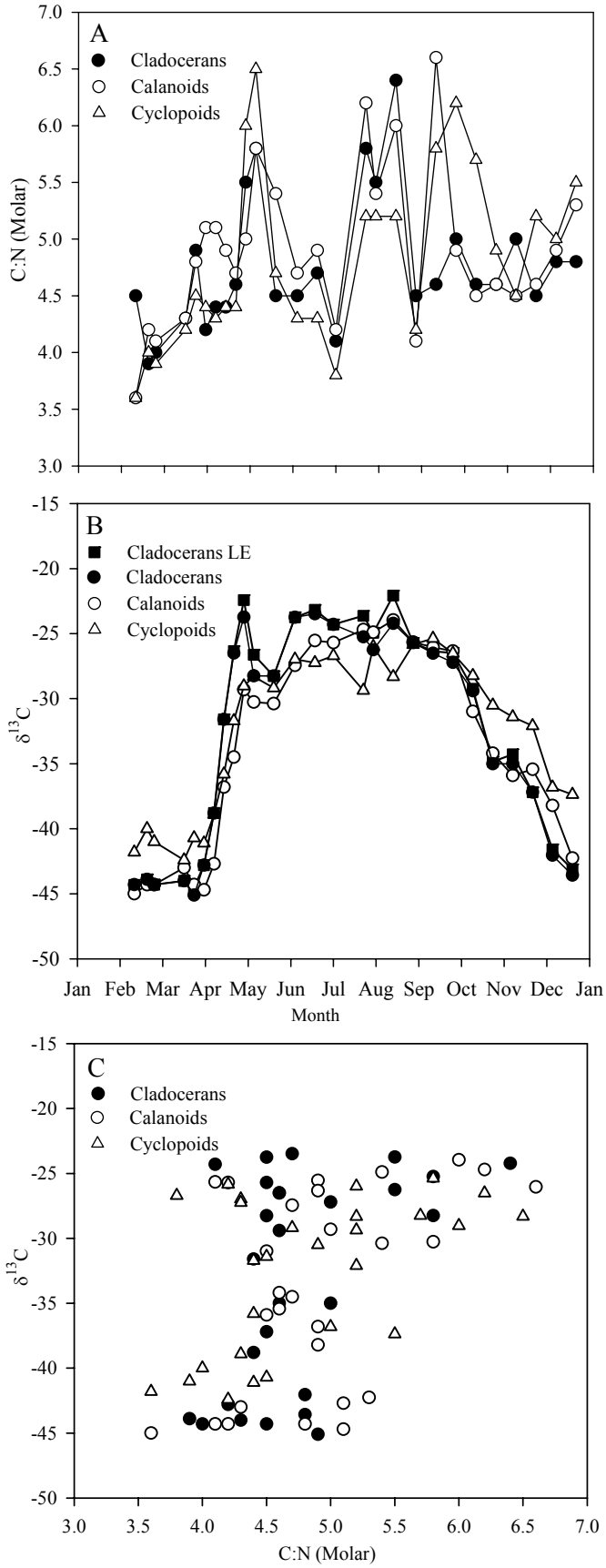
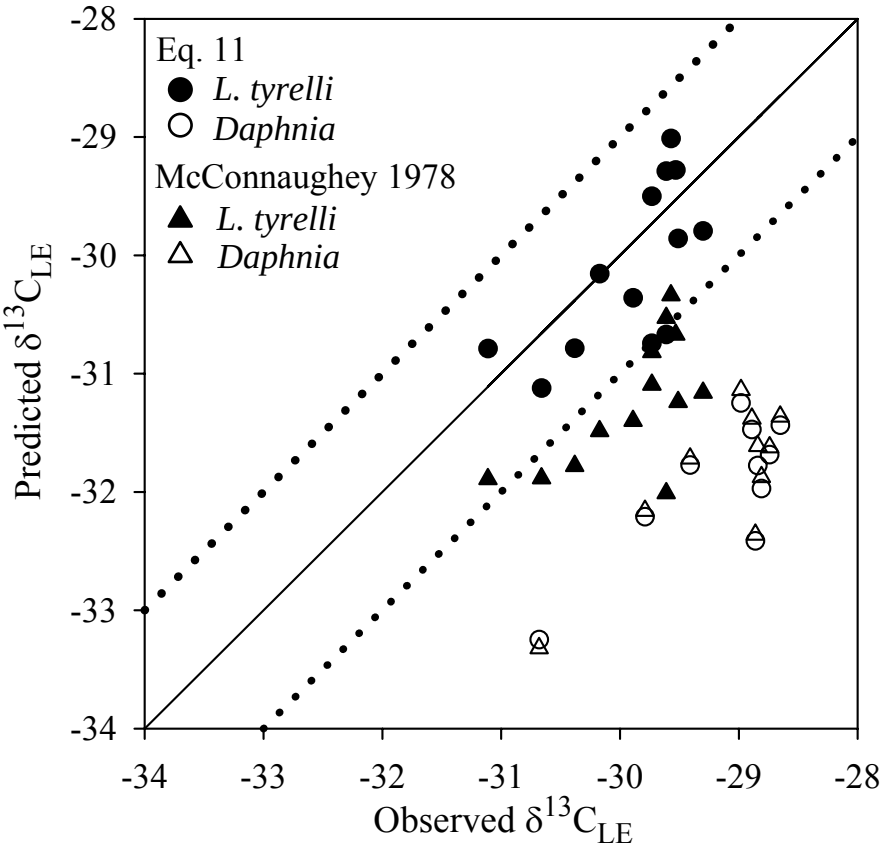

A
Cladocerans
Calanoids
Cyclopoids
C:N (Molar)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
B
Cladocerans LE
Cladocerans
Calanoids
Cyclopoids
δ13C
-15
-20
-25
-30
-35
-40
-45
-50
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan
Month
C
Cladocerans
Calanoids
Cyclopoids
δ13C
-15
-20
-25
-30
-35
-40
-45
-50
3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0
C:N (Molar)

Figure 6.7 : Comparison of lipid normalization models

Circles are predictions from Eq. 11, and triangles are predictions from McConnaughey (1978), but in both models we used the same parameters of $\Delta_{C\text{-}L}$ for each taxa as estimated by $\Delta_{LE\text{-}L}$ in Table 6.2. Neither model could account for the unexpectedly high $\delta^{13}C_{LE}$ of *Daphnia*.


Eq. 11
L. tyrelli
Daphnia
McConnaughey 1978
L. tyrelli
Daphnia
Predicted δ13CLE
Observed δ13CLE
-28
-29
-30
-31
-32
-33
-34

Chapter 7: Habitat specialization and differential exploitation of allochthonous carbon by zooplankton

Citation:

Matthews, B., and A. Mazumder. Habitat specialization and differential exploitation of allochthonous carbon by zooplankton. *Submitted.*

Abstract

Zooplankton vertically partition resources within stratified lakes in response to life history tradeoffs governed by predators, the quantity and quality of food, and abiotic conditions (e.g. UV, temperature, and viscosity). We measured habitat specialization of zooplankton in a fishless oligotrophic lake that has a deep chlorophyll maximum in the summer. During stratification, the quantity and quality of zooplankton food increased with depth and reached a maximum in the hypolimnion. We used a year-long time series of the $\delta^{13}C$ of zooplankton and particulate organic matter (POM) to detect which zooplankton taxa exploited hypolimnetic rather than epilimnetic resources. Since the $\delta^{13}C$ of POM decreased with depth, we could use the $\delta^{13}C$ of zooplankton to detect differences among zooplankton taxa in both their habitat selection and their reliance on allochthonous resources. We used 75L wire mesh enclosures to incubate *Daphnia* at discrete depths in the water column. By doing so, we confirmed that the $\delta^{13}C$ of *Daphnia* can indicate habitat specialization, and depends on the vertical distribution of POM $\delta^{13}C$. We discuss our experimental and comparative results in the context of taxon-specific exploitation of allochthonous versus autochthonous resources. Specifically, we found that zooplankton that specialize in the epilimnion rely more on allochthonous carbon sources than those that specialize in the hypolimnion. Therefore, the composition and feeding behaviours of zooplankton communities determine the fate of allochthonous carbon in lake food webs.

Introduction

Zooplankton communities are complex adaptive systems because they respond to changes in their resources and change the processes that structure their resources (Norberg 2004). Abiotic gradients in the water column of lakes create substantial resource heterogeneity for zooplankton. Gradients of temperature, light, UV, and nutrients interact to determine the quantity, quality, and composition of zooplankton food sources. These abiotic gradients directly affect the production, composition, size structure, and spatial location of autochthonous carbon (Fee 1976; Sterner and Hessen 1994; Park et al. 2004), as well as the transformation and accessibility of allochthonous carbon (Obernosterer and Benner 2004). Abiotic gradients can also indirectly affect resource heterogeneity (through food web interactions) by modifying the interaction between zooplankton grazers and their predators. For example, light and UV can increase the risk for zooplankton feeding in surface waters, and thus, influence habitat selection tradeoffs of zooplankton (Leech and Williamson 2001). Zooplankton communities themselves can also indirectly modify the interaction between abiotic gradients and food web structure. Zooplankton grazing can alter patterns of thermal stratification (Mazumder et al. 1990b), promote the persistence of deep water cholorophyll maxima (Pilati and Wurtsbaugh 2003), and initiate 'cryptic' trophic cascades that affects the size structure but not the abundance of algae (Tessier and Woodruff 2002a). In short, the functional diversity of zooplankton communities interacts with the temporal, spatial, and compositional heterogeneity of their food sources.

As complex adaptive systems, the dynamics of zooplankton communities depend on the traits of the constituent species (e.g. body size, habitat selection) and the tradeoffs

that govern these traits. For example, Tessier and Woodruff (2002b) describe a trade-off in the ability of *Daphnia* grazers to exploit rich and poor quality food. Interestingly, they found that the efficiency and sensitivity of resource use by *Daphnia* is independent of size, (which is contrary to the size efficiency paradigm; see Tessier et al. 2000), and concluded that the trait distributions of their grazer communities have led to an adaptive match between exploitation ability and the resource environment. Extending this to a seasonal perspective, the composition or behaviour of zooplankton species within a community might change over the season so as to match the spatial and temporal variation of allochthonous and autochthonous resources.

There are several mechanisms whereby a change in zooplankton community structure can result in a higher exploitation of resources that vary spatially and seasonally. First, the community could turnover species at a rate that optimizes the acquisition of seasonal resources. This process is likely constrained by rates of zooplankton dispersal and by local interactions (Havel and Shurin 2004). Second, the community could maintain a high diversity of grazers that can sequentially exploit resources as they become available. However, any process that limits the maintenance of zooplankton diversity will inevitably constrain a community's exploitation ability (Leibold et al. 1997). Third, the community could retain a suite of species that are flexible in their feeding behaviour in order to exploit resources as efficiently as they can. There is ample evidence for flexibility of feeding behaviours within zooplankton species that is governed by tradeoffs in resource acquisition. *Daphnia* commonly face a trade-off between food abundance and temperature because high concentration of food is often found in cooler subsurface waters (Fee 1976). In the short term, the population adopts a

distribution that optimizes growth and reproduction (Lampert et al. 2004; Kessler and Lampert 2004b). Within the population there is size-based (Kessler and Lampert 2004a) and individual-based (Kessler 2004) differences in the propensity to exploit sub-surfaces resources. Over a longer term, this trade-off can lead to clone-based differences in sub-surface resource exploitation (Tessier and Leibold 1997). At either time scale, *Daphnia* populations can effectively increase their trait variance and match their acquisition efficiency with their resource environment.

Two important traits structure the feeding relationships of zooplankton in fishless oligotrophic lakes that have deep chlorophyll maxima and large allochthonous carbon subsidies. The first trait is the ability to exploit sub-surface autochthonous food sources (Williamson et al. 1996), and the second trait is the ability to selectively feed on autochthonous versus allochthonous resources (Grey et al. 2001). If the amount of autochthonous carbon increases with depth relative to allochthonous carbon, then the temperature-food trade-off is modified by the presence of allochthonous resources. Therefore, zooplankton taxa can specialize with respect to habitat, or can specialize within a habitat with respect to the size or quality of the food sources. POM is heterogeneous mixture of algae, bacteria, and allochthonous and autochthonous detritus, and these food sources vary with depth. Stable isotope analysis has repeatedly found that the $\delta^{13}C$ of zooplankton is significantly lower than the $\delta^{13}C$ of POM. This suggests there is significant isotopic heterogeneity among zooplankton food sources within POM (Pel et al. 2003), and that zooplankton selectively feed on food sources (e.g. algae) that have a lower $\delta^{13}C$ than the bulk POM (Chapter 6). This mismatch is particularly evident in oligotrophic lakes that have large contributions of allochthonous carbon (Grey et al.

2000; Grey et al. 2001). Previous studies have shown that zooplankton species have different $\delta^{13}C$ signatures (Grey et al. 2001; Matthews and Mazumder 2003), however, no study has investigated if these differences reflect different feeding strategies in general, or whether zooplankton differ in their propensity to exploit sub-surface autochthonous resources in particular.

In this study, we examine how zooplankton can differentially exploit subsurface resources in an oligotrophic lake that has a deep chlorophyll maximum. We chose Council Lake for our study because the $\delta^{13}C$ of POM decreases with depth in the water column. This provides a natural gradient in the $\delta^{13}C$ of food sources that we can use to detect habitat specialization of zooplankton. Our hypotheses were that taxa or size classes of zooplankton that feed deeper in the lake would have a lower $\delta^{13}C$, and rely less on allochthonous carbon sources than other zooplankton. There is considerable debate how well zooplankton exploit subsurface food resources (Williamson et al. 1996; Cole et al. 2002a; Winder et al. 2003). It is well known that deep chlorophyll maxima are common in many types of lakes (Fee 1976), but it is unclear how efficiently these resources are used by different zooplankton communities. Cole et al. (2002a) found that *Daphnia* grew better on epilimnetic resources, but concluded that metalimnetic carbon (although of inferior quality) still contributed to *Daphnia* nutrition. In contrast, several other studies have found that food quality for *Daphnia* increases with depth (Winder et al. 2003; Park et al. 2004; DeMott et al. 2004). There is also considerable uncertainty about the ability of zooplankton communities to exploit allochthonous carbon sources, and few studies have taken a community wide perspective (but see Grey et al. 2001). The mismatch in $\delta^{13}C$ of zooplankton relative to POM is consistent with large allochthonous contributions

of carbon to zooplankton diets (Grey et al. 2001; Pace et al. 2004). However, some models suggest that only a small fraction of allochthonous DOC (<10%) actually contributes to zooplankton production (Cole et al. 2002b).

In this paper, we first test if the spatial gradient in the $\delta^{13}C$ of food sources, which develops seasonally in Council Lake, allows us to detect differences in how zooplankton utilize sub-surface autochthonous resources. If so, this provides insight about how zooplankton communities exploit the available resource gradients. Second, we experimentally manipulate the feeding depth of *Daphnia* (using enclosures) to ensure that the $\delta^{13}C$ is a good indicator of habitat specialization. Third, we use our seasonal $\delta^{13}C$ patterns to determine how different zooplankton can exploit the deep water chlorophyll maxima and allochthonous versus autochthonous sources of carbon.

Methods

Annual collection of plankton in Council Lake (2002-2003)

We sampled zooplankton and particulate organic matter < 41μm (POM) in Council Lake (48º31' 123º41') 28 times between Feb 14, 2002 and Mar 17, 2003. We collected samples at least monthly over the whole sampling period, every two weeks during lake stratification (May to Nov), and every 3-4 days in July. Council Lake is a warm monomictic oligotrophic lake that rarely gets permanent ice cover. We collected zooplankton during the day with a Wisconsin net (64 μm mesh, 50 cm diameter) by vertical and horizontal net tows. We froze bulk zooplankton samples within 4 hrs of collections, and later sorted out composite samples from five taxonomic groups (Table 7.1): *Daphnia*, *Holopedium gibberum* (*Holopedium*), *Leptodiaptomus tyrelli* (*L. tyrelli*),

Epischura nevadensis (*E. nevadensis*), *and Chaoborus trivittatus* (*C. trivitattus*). Prior to isotopic analysis, we measured the average body size of each zooplankton sample using a dissecting microscope, a digital camera, and zooplankton counting software (Z-Count).

We used a Niskin bottle to collect POM from 3 depths (2, 8, 16 m) in the spring of 2002, 9 depths (1, 3, 5, 7, 8, 9, 12, 14, 16 m) in July, and 6 depths (~2, 6, 8, 10, 12, 14 m) for the remainder of the study. We chose these depths so that during the stratified period we would sample 1 to 3 discrete depths in the epilimnion (~0-6m), metalimnion (~6-12m), and hypolimnion (~12-17m). For each sample, we filtered at least 1 L of lake water through a 41 μm Nitex mesh onto pre-combusted (550°C for 1 hour) 25 mm GF-C filters (Whatman). We dried the filters overnight at 60°C and packaged them in tin cups for isotopic analysis. All stable isotope samples were analyzed on a Finnigan Delta Plus Advantage stable isotope ratio mass spectrometer at the Water and Watershed Research Laboratory, at the University of Victoria. *Daphnia* samples from the cage experiment were run with a lower level of helium dilution in order to obtain a sufficient carbon signal. Unfortunately, there was insufficient *Daphnia* biomass by the end of the experiment for $\delta^{15}N$ analysis. We included a powdered *Daphnia* standard in every sample run, and its precision was <0.2‰ within and between all sample runs. In some cases we report $\delta^{13}C$ for zooplankton with their lipids extracted ($\delta^{13}C_{LE}$), as estimated from a previous study (Chapter 6). In most cases we show the uncorrected $\delta^{13}C$ because the seasonal patterns are qualitatively the same.

Design of zooplankton cage experiments

We constructed 36 cylindrical cages (diameter= 0.4m, height= 0.6m, volume~ 75 L) using stainless steel woven wire mesh (Ferrier Wire Good Company Ltd., Toronto,

Canada). The diagonal diameter of the holes was ~320μm, and there were over 500 holes per cm^2. We made a cylindrical frame out of 16 gauge aluminium, wrapped the wire mesh around the outside, and welded the mesh to the frame. We made identical lids for both ends by welding wire mesh onto 14 gauge aluminium rings that we tightly fitted to the cylindrical frame. We surrounded each cage with nylon straps that were stitched together and adjustable to fit snugly around each cage and allow us to vertically suspend the cages at discrete depths.

Temporal Cage Experiments (Jul 21 - Aug 8, 2003)

The two goals of the temporal cage experiment were 1) to test if the differences in $\delta^{13}C$ among zooplankton that we observed in July of 2002 were associated with feeding depth, and 2) to determine the rate of change of *Daphnia* $\delta^{13}C$ in response to a change in feeding depth. We cultured *Daphnia* to have an isotopic signature significantly different from *Daphnia* in Council Lake, and then incubated them *in situ* at different depths in Council Lake. In June we stocked a single 1000 L mesocosm with *Daphnia* from Council Lake, and fed them with *Scenedesmus aculeatus* from a batch culture in the laboratory. The $\delta^{13}C$ of *Daphnia* from the mesocosm at the start of the experiment (Jul 20, 2003) was -19.1‰ (SD= 0.38, N=9). On July 20th, we pumped *Daphnia* from the enclosure into a large bucket and used a plankton splitter to gently divide the *Daphnia* into 45 separate containers. The following day we used 36 of these containers to inoculate 36 cages with *Daphnia* at three discrete depths (4, 8, 14m), and kept 9 containers to measure the initial isotopic composition of *Daphnia*. The initial density of the samples in the 75L cage was 1.3 *Daphnia* L^{-1} (SD= 0.3, N=9), and the initial biomass was 7.0 μg L^{-1} (SD= 3.0, N=9).

We ran the cage enclosure experiment from July 21st to Aug 8th, 2003. Every three days after July 21st (Day 0), we removed 2 traps from each of the three depths. This gave us two replicate time series of 6-7 measurements for each depth. Upon removing each cage from the lake, we rinsed the contents down to the bottom lid and transferred the *Daphnia* into a separate container. We then used filtered lake water (<0.7 μm) to wash off some periphyton growth from the cylindrical part of the trap (not the lids). Starting on day 6, we filtered the periphyton from the wash water onto pre-combusted GF-C filters and analyzed them for $\delta^{13}C$, as above.

On each day of the experiment we collected zooplankton using vertical tows as described above, and POM from 6 depths (2, 4, 7, 9, 12, 16m). We measured the Chlorophyll *a* of POM <41μm, and POM >0.7μm by filtering samples through GF/Fs (Whatman), immersing the filters in 95% ethanol overnight at 4°C, and analyzing the extract on a spectrophotometer.

Profile Cage Experiment (Jul 29 - Aug 8)

The goal of the profile cage experiment was to provide an additional test for an effect of feeding depth on the $\delta^{13}C$ of *Daphnia*. On day 9 of the temporal cage experiment (July 29), we used the 12 removed traps and began the profile cage experiment. In this experiment, we set two replicate cages at 2, 4, 8, 10, 12, and 14m, and incubated them with *Daphnia* from the same stock mesocosm. The $\delta^{13}C$ of *Daphnia* at the start of this experiment was -18.2‰ (SD= 0.23, N= 9). The initial density and biomass of *Daphnia* was 1.3 L^{-1} (SD= 0.20) and 13.8 μg L^{-1}, (SD= 2.5, N= 9) respectively.

Sampling of zooplankton depth distribution

In 2002 and 2003, we sampled the daytime and night time depth distribution of zooplankton for 1 date in July 2002, and 4 dates in July 2003 using a 25L Schindler-Patalas trap (1, 3, 5, 7, 8, 9, 12, 14, 16m). We chose July for this part of the study because data from 2002 revealed that this was the time when the $\delta^{13}C$ gradient of POM was strongest. Since vertical migrations were minimal for all taxa, we pooled depth distribution data for each taxa and calculated the depth at maximum density. We used LOWESS to visually check that this depth of maximum density was the only major peak of zooplankton density in the profile. In 2002, we separated counts of *Daphnia* into two size classes (<1.8 mm and >1.8 mm) to match our size classes of our isotopic data. In 2003, we did not have enough small *Daphnia* in our samples to make isotopic measurements so we only considered large *Daphnia* in our analysis.

Results

Annual variation in the $\delta^{13}C$ of plankton

The range of $\delta^{13}C$ of POM_{41} was large during thermal stratification and small in the winter and spring when the water column was well mixed (Fig. 7.1A). The average range of $\delta^{13}C$ for POM_{41} over the entire water column in Council Lake was 3.8‰ (Min: 0.93‰, Max: 7.8‰). Throughout the stratified period the $\delta^{13}C$ of POM_{41} typically declined with depth, but the steepest gradients were present in July (Fig. 7.2).

Despite the decline in the $\delta^{13}C$ of POM_{41} with depth, the $\delta^{13}C$ of POM_{41} could not account for the entire range of zooplankton $\delta^{13}C$ in Council Lake. Most notably, the $\delta^{13}C$ of *L. tyrelli* and large *Daphnia* was always lower than the lowest $\delta^{13}C$ of POM_{41}

measured on the same day. Normalizing the $\delta^{13}C$ of zooplankton to zero lipid content (Chapter 6) could only partially explain the mismatch between the $\delta^{13}C$ of zooplankton and POM. Although the $\delta^{13}C$ of *Daphnia* and *L. tyrelli* is lower than POM_{41}, the $\delta^{13}C$ of *Daphnia* and *L. tyrelli* declined concurrently with the $\delta^{13}C$ of POM below the epilimnion (Fig 7.1; 7.2), and increased in the fall as the gradient of $\delta^{13}C$ for POM_{41} weakened (Fig. 7.2B). This indicates that zooplankton fed on a fraction of POM (probably algae) that had a lower $\delta^{13}C$ than bulk POM.

Zooplankton differentially responded to seasonal changes in the availability and distribution of allochthonous and autochthonous resources. Seasonal patterns of zooplankton $\delta^{13}C$ depended strongly on the taxonomic grouping, and additionally on the size class for *Daphnia* (Table 7.1, Fig. 7.1). The annual average $\delta^{13}C$ of *Epischura* was ~6‰ higher than *L. tyrelli*, but the differences for a given sampling day were greatest during stratification (Fig. 7.1A). Likewise, the $\delta^{13}C$ of small *Daphnia* (SD) was ~3‰ higher than big *Daphnia* (D), and the biggest differences occurred during stratification (Δ_{SD-D}=-0.5 to 7.8‰; Fig. 7.1C). In July, the average body size of *Daphnia* accounted for ~80% of the variation in both the $\delta^{13}C$ ($F_{1,31}$= 149.1, r^2= 0.82, P< 0.001) and $\delta^{13}C_{LE}$ ($F_{1,31}$= 118.8, r^2= 0.79, P< 0.001) of *Daphnia* (linear regression in Fig. 7.3).

Cage Experiments

The average lake density of *Daphnia* for July was 0.19 *Daphnia* L^{-1} (SD= 0.13, N= 7), and the biomass was 2.2 μgL^{-1} (SD= 0.84, N= 7). This density and biomass is similar to the density (0.15 - 0.26 *Daphnia* L^{-1}) and biomass (2.4 - 2.8 μgL^{-1}) of *Daphnia* remaining in the cages at the end of the temporal and profile experiments.

Temporal Cage Experiment

In July 2003, the $\delta^{13}C$ and C:N of POM decreased with depth, whereas the carbon concentration increased with depth (Fig. 7.4). Chlorophyll *a* was highest in the hypolimnion (Fig. 7.4) particularly in the larger size fractions. Over the entire 18 day experiment, the $\delta^{13}C$ of POM_{41} was lowest in the hypolimnion, and highest in the epilimnion (horizontal lines in Fig. 7.5).

The density of *Daphnia* in the cages declined significantly over the 18 day experiment ($F_{1,55}$= 96.3, P<0.001: ANOVA model with Log density as response variable, lake strata as a fixed factor, and experiment day as the covariate), but the rate of decline was not significantly different among strata (ANOVA interaction term: $F_{2,55}$= 0.105, P= 0.90). Similarly, the rate of decline of *Daphnia* biomass did not depend on the strata (ANOVA interaction term: $F_{2,55}$= 1.36, P= 0.36). At the end of the experiment, the density and biomass of *Daphnia* was 0.15 L^{-1} (SD= 0.06, N= 4), and 2.4 μgL^{-1} (SD= 0.72, N= 4) respectively. Unfortunately, neither epilimnetic trap had enough *Daphnia* remaining on day 18 for $\delta^{13}C$ measurements (Fig. 7.5).

We used a nonlinear least squares analysis to determine if the rate of decline in the $\delta^{13}C$ of *Daphnia* was significantly different among strata. We found that the $\delta^{13}C$ of *Daphnia* in all strata decreased over time, but more rapidly in the hypolimnion, followed by the epilimnion, and metalimnion (Table 7.2). The rate of change in the $\delta^{13}C$ of *Daphnia* in Council Lake (0.045 to 0.094 day^{-1}) was slightly lower than for *Daphnia hyalina* (0.090 to 0.13 day^{-1}) in a lab based diet switch experiment (Grey 2000). We cannot determine if the $\delta^{13}C$ of *Daphnia* reached equilibrium with its POM food source,

because we do not know the $\delta^{13}C$ of the fraction of POM that *Daphnia* actually consumed. However, since the rate of decline in the $\delta^{13}C$ of *Daphnia* is significantly different among depths, this indicates a strong effect of feeding depth on the $\delta^{13}C$ of *Daphnia*.

Profile Cage Experiment

After 9 days the average density and biomass of *Daphnia* declined to 0.26 *Daphnia* L^{-1} (SD= 0.21, N= 12) and 2.8 μg L^{-1} (SD= 2.0, N= 12), respectively. By the end of the experiment, there was no effect of lake strata on the density (ANOVA $F_{2,9}$=0.69, P= 0.53) or biomass (ANOVA $F_{2,9}$= 0.71, P= 0.52) of *Daphnia*. However, we did find a significant effect of feeding depth on *Daphnia* $\delta^{13}C$ (ANOVA: $F_{2,9}$= 48.4, P< 0.001; Fig. 7.6). The $\delta^{13}C$ of *Daphnia* in the metalimnetic (M) traps (7, 9 m) was higher than *Daphnia* in both epilimnetic (E) and hypolimnetic (H) traps (E= -30.2‰, N= 4; M= -27.9‰, N= 4; H= -33.6‰, N= 4).

Depth distribution of zooplankton

In July, the depth at maximum density (DMD) for all zooplankton decreased at night by an average of 2.3 m (SE= 0.86 based on taxa). In an ANOVA with sampling time (day or night) and zooplankton taxa as fixed factors (see Fig. 7.7), the DMD was significantly different among species ($F_{5,29}$= 12.7, P< 0.001) and between times ($F_{1,29}$= 7.08, P=0.013), but the difference between day and night did not depend on the species ($F_{5,29}$= 1.04, P=0.42). Since there was no significant difference in migration patterns among species we averaged all the night and daytime DMDs for all the sampling dates and compared it to their $\delta^{13}C$. We found that the $\delta^{13}C$ of zooplankton was significantly

negatively correlated with the average DMD (Randomized correlation: r= -0.88, P= 0.002, N= 1000, Fig. 7.7). Zooplankton taxa that had maximum densities at deeper depths had a lower $\delta^{13}C$.

Discussion

We found that zooplankton taxa differentially exploit subsurface autochthonous production. By experimentally manipulating the feeding depth of *Daphnia* we confirmed that the $\delta^{13}C$ of zooplankton can provide evidence of habitat specialization, provided the $\delta^{13}C$ of food sources changes with depth (Fig. 7.2). We conclude that different taxa and sizes of zooplankton exploit autochthonous and allochthonous carbon to varying degrees. Therefore the fate of allochthonous carbon depends on the composition of the zooplankton community. If zooplankton grazing permits the deep chlorophyll layer to persist in Council Lake (*sensu* Pilati and Wurtsbaugh 2003), then they may structure the relative availability of allochthonous and autochthonous carbon at different depths in the lake.

Inter- and intraspecific $\delta^{13}C$ patterns

In Council Lake, we found large interspecific variation in the $\delta^{13}C$ of zooplankton (Fig. 7.1). During the stratified period, the $\delta^{13}C$ of *L. tyrelli* and *E. nevadensis* differed by as much as 10‰. The $\delta^{13}C$ of *L. tyrelli* declined concurrently with the $\delta^{13}C$ of POM in the hypolimnion, which indicates that *L. tyrelli* exploits subsurface resources in Council Lake to a greater degree than *E. nevadensis*. This matches observations about their depth distribution and life history (Fig. 7.7). *L. tyrelli* is omnivorous and can switch food sources depending on the lake (Anderson 1967) and the time of year (Chapter 4). It is

also commonly found in the surface waters of other coastal clearwater lakes (BM pers. obs.). In Council Lake, we believe *L. tyrelli* is feeding at deeper depths in order to exploit higher food concentrations (Fig. 7.4). *L. tyrelli* may also be using the hypolimnion as a refuge from invertebrate predation (there are no vertebrate predators in Council Lake), because the depth at maximum density of both invertebrate predators (*Chaoborus* and *Epischura*) is above the hypolimnion (Fig. 7.7).

The $\delta^{13}C$ of *Daphnia* and *Holopedium* diverged following thermal stratification, indicating habitat specialization in the water column. *Holopedium* and small *Daphnia* are specializing on food resources above the hypolimnion, whereas large *Daphnia* are only feeding in the hypolimnion (Fig. 7.1). The $\delta^{13}C$ of *Holopedium* is closest to the $\delta^{13}C$ of terrestrial carbon, suggesting that *Holopedium* rely the most on allochthonous carbon sources (Fig. 7.7). Pace et al. (2004) concluded that 40-55% of particulate organic carbon in the epilimnion of Peter and Paul lake is derived from terrestrial sources. In Council Lake, the C:N of POM decreased with depth, the algal proportion of POM increased with depth, and the $\delta^{13}C$ of POM decreased with depth. Together, these data suggest that the allochthonous and autochthonous carbon sources for zooplankton vary with depth in Council Lake, and that these resources are differentially exploited by different zooplankton taxa.

The coexistence of *Holopedium* and *Daphnia* in Council Lake may involve a trade-off between the ability to exploit high quality food (sub-surface algae) and the minimum resource requirement needed to persist (Tessier and Woodruff 2002b). In the epilimnion, *Holopedium* is faced with a low abundance (~0.2 $mgCL^{-1}$) of poor quality food (C:N >10 $mgCL^{-1}$) that likely has a large contribution of terrestrial carbon (Fig. 7.7).

Holopedium can readily ingest bacteria sized particles (0.5 to 5 μm: Hessen 1985), and *Holopedium* dominated communities can graze a smaller size range of algae than *Daphnia* dominated communities (Cyr and Curtis 1999). So, *Holopedium* may persist and out-compete *Daphnia* in the epilimnion if they trade off their limited ability to exploit high quality food (sub-surface algae) by having a low minimum resource requirement (Tessier and Woodruff 2002b). *Holopedium* is common in oligotrophic lakes (Hessen 1985), and likely has a lower minimum resource requirement than hypolimnetic populations of *D. pulicaria* (Tessier and Woodruff 2002b). Habitat segregation between *Daphnia* and *Holopedium* in Council Lake could result from a trade-off between the ability to exploit rich and poor quality food (Tessier and Woodruff 2002b), provided that allochthonous carbon sources in the epilimnion are of poorer quality than subsurface autochthonous resources. We acknowledge that we have not directly tested this hypothesis, but it is a reasonable inference given the distribution and isotopic composition of food sources in Council Lake (Fig. 7.4).

Different sizes of *Daphnia* in Council Lake also specialize on different habitats. Smaller *Daphnia* were most abundant in the epi- and metalimnion, whereas large *Daphnia* were never present in the epilimnion. This led to a negative linear relationship between average *Daphnia* body size and $\delta^{13}C$ (Fig. 7.3). Several other studies have detected habitat specialization of *Daphnia*, but the reasons for the patterns are generally attributed to tradeoffs between food abundance and predators (Leibold and Tessier 1991; Tessier and Leibold 1997; Winder et al. 2004). For example, Leibold and Tessier (1991) found that two different sized *Daphnia* species, *D. pulicaria* and *D. galeata mendotae*, segregate the water column in response to differing predation regimes. In lakes with high

vertebrate predation the larger species (*D. pulicaria*) was restricted to the hypolimnion, whereas *D. galeata mendotae* always utilizes the epilimnion. In Council Lake, there is no vertebrate predation but there is a high density of invertebrate predators (e.g. *Chaoborus*). *Chaoborus* can induce size-dependent migration patterns in copepods (Neill 1992), and therefore might structure habitat specialization of zooplankton in Council Lake.

Different sized *Daphnia* may specialize at different depths due to their differential ability to exploit food in the hypolimnion (Abrusan 2004) or as a behavioural response to a temperature food trade-off (Lampert et al. 2003). Hypolimnetic populations of *D. pulicaria* have much higher minimum resource requirements but can more efficiently exploit subsurface resources (Tessier and Woodruff 2002b). The high efficiency of hypolimnetic *D. pulicaria* populations might result from the larger mesh size of *Daphnia pulicaria*'s filtering combs compared to other species of comparable size (Abrusan 2004). The mesh size of smaller *Daphnia pulicaria* might not be big enough to efficiently exploit resources in a high viscosity medium. Mesh size is phenotypically plastic (Lampert 1994), so adaptive modification of mesh size during *Daphnia* ontogeny (i.e. increasing mesh size at low food concentration), may allow large *Daphnia* to more efficiently exploit high food quality in the hypolimnion. This is because larger mesh sizes are more efficient for grazing particles in the high viscosity environment of the hypolimnion (Abrusan 2004). In this scenario, the slight upward migration of *Daphnia* at night (~2 m) could be explained as a behaviour to increase the rate of egg development (Winder et al. 2003).

Experimental evidence of habitat specialization

Using $\delta^{13}C$ is a novel and promising approach for studying habitat specialization in zooplankton communities, or in any consumer community where there is a gradient in the $\delta^{13}C$ of food sources. In Council Lake, the strong gradient in the $\delta^{13}C$ of POM with depth makes it an ideal lake to test for habitat specialization (Fig. 7.2). Many other stratified lakes show a decreasing pattern of POM $\delta^{13}C$ with depth (Quay et al. 1986; del Giorgio and France 1996; Jonsson et al. 2001), so this food $\delta^{13}C$ gradient is a useful natural tracer that can detect differences in feeding behaviour of zooplankton among lakes. Matthews and Mazumder (2003) found that the $\delta^{13}C$ of calanoids was lower than the $\delta^{13}C$ of *Daphnia* in 12 stratified lakes. In lakes without a vertical gradient in the $\delta^{13}C$ of POM there was significant difference between the $\delta^{13}C$ of copepods and cladocera (Karlsson et al. 2003). Together these two studies suggest that differences in feeding depth could account for differences in the $\delta^{13}C$ among zooplankton taxa, and could help account for the low $\delta^{13}C$ of zooplankton.

Implications for zooplankton allochthony

The implications of our experimental and comparative results is that the feeding depth of zooplankton and the vertical distribution of resources are fundamental for determining the contribution of allochthonous carbon to zooplankton food webs. Sorting out the pathways of allochthonous carbon through the zooplankton community depends not only on the taxonomic composition of the community, but also on the feeding behaviour of constituent members of the community. This is particularly true in lakes where there is a potential for habitat specialization on deep water resources.

Multiple sources of carbon fuel pelagic production in lakes. The low $\delta^{13}C$ of zooplankton compared to POM, has motivated many researchers to find the 'missing carbon sources' that contribute to zooplankton production (e.g. methane, allochthonous, autochthonous). There are three main explanations for why the $\delta^{13}C$ of zooplankton is typically lower than the $\delta^{13}C$ of POM (del Giorgio and France 1996), none of which are mutually exclusive. First, zooplankton may selectively feed on algal carbon that has a lower $\delta^{13}C$ (del Giorgio and France 1996), and whose signature is masked by other carbon sources that have a higher $\delta^{13}C$ (e.g. terrestrial carbon). Differences in $\delta^{13}C$ among zooplankton taxa might then result from different abilities to exploit autochthonous algal carbon in the epilimnion. Second, zooplankton might accumulate lipids (which have a low $\delta^{13}C$) from their food, and thereby lower their $\delta^{13}C$ relative to their food source (Matthews and Mazumder 2005a). Using a lipid normalization procedure developed in a previous study (Chapter 6), we determined that $\delta^{13}C$ differences among taxa in Council Lake cannot solely be explained by differences in lipid content. Third, zooplankton might feed on carbon from deeper in the water column. Our cage experiments were designed to distinguish between the first and third hypothesis, i.e. whether zooplankton selectively feed on autochthonous production in a single habitat or whether they specialize on different habitats. Our cage experiments confirm that zooplankton feeding at deeper depths have a lower $\delta^{13}C$ (Fig. 7.5, 7.6), and that differences in $\delta^{13}C$ among zooplankton taxa are directly associated with habitat specialization. Therefore, there are multiple pathways that carbon can take through the zooplankton community from allochthonous and autochthonous sources to upper trophic levels in lake food webs. The relative strength of these pathways depends on the spatial

distribution of resources and the composition and behaviour of the zooplankton community.

In Council Lake, different taxa and sizes of zooplankton differentially exploit allochthonous carbon pathways. The proportion of algal carbon in POM increases with depth in Council Lake (Fig. 7.4), and the $\delta^{13}C$ of POM decreases with depth. Therefore, zooplankton with a higher $\delta^{13}C$ signatures rely more on allochthonous carbon. Using data on the $\delta^{13}C$ of algae from Karlsson et al. (2003), we can estimate the allochthonous fraction of carbon assimilated by zooplankton using a simple mixing model (Table 7.3; Karlsson et al. 2003). In 12 northern Sweden Lakes (below a 1000 m altitude), the $\delta^{13}C$ for algae varies from -37.7 to -46.2‰. Using this range of $\delta^{13}C$ for algae, we conclude that *L. tyrelli* and large *Daphnia* rely substantially less on allochthonous carbon than *Epischura*, *Chaoborus*, or *Holopedium* (Table 7.3). Our estimates are not specifically calibrated for Council Lake, because we have not made any measurements of the $\delta^{13}C$ of algae. Although these estimates generally agree with previous studies of zooplankton allochthony (Grey et al. 2001; Karlsson et al. 2003; Pace et al. 2004), no previous study has considered the consequence of a vertical gradient in the $\delta^{13}C$ of POM. Assuming the $\delta^{13}C$ of algae decreases with depth, estimates of zooplankton allochthony depend on the $\delta^{13}C$ gradient of algae, and where different zooplankton feed in the water column. In table 7.3, we calculated estimates of zooplankton allochthony for the case where the average $\delta^{13}C$ of algae is -41.8 (Table 7.3; Karlsson et al. 2003), but the $\delta^{13}C$ of algae decreases with depth at the same rate as the $\delta^{13}C$ of POM (Fig. 7.7). In general, a model that does not include changes in the $\delta^{13}C$ of POM with depth will overestimate

allochthony in epilimnetic zooplankton and underestimate allochthony in hypolimnetic zooplankton.

It is difficult to predict the consequences a vertical $\delta^{13}C$ gradient of POM for the conclusions reached by Pace et al. (2004). Pace et al. (2004) experimentally increased the $\delta^{13}C$ of algae in a whole lake experiment by adding $NaH^{13}CO_2$ with a high $\delta^{13}C$, and concluded that 22-50% of zooplankton carbon are derived from terrestrial sources. However they acknowledged that the data is also consistent with *Daphnia* selectively feeding on endogenous carbon from deeper layers (Pace et al. 2004, Supplementary Information). As in Council Lake, Pace et al. (2004) found that the $\delta^{13}C$ of POM in the metalimnion was higher early in the summer. This is similar to Council Lake, but the pattern is reversed by midsummer. In Peter and Paul Lake, the $\delta^{13}C$ of particulate organic carbon (POC) in the metalimnion became enriched by midsummer following additions of $NaH^{13}CO_2$ but not to the same extent as in the epilimnion. In Council Lake, the $\delta^{13}C$ of POM diverged strongly following stratification, and reached a maximum gradient of ~7‰ over 17 m on Jul 31, 2002. The model that Pace et al. (2004) used would have overestimated the proportion of allochthonous carbon in zooplankton if there was an accessible algal carbon source below the epilimnion that had a lower $\delta^{13}C$. Pace et al. (2004) did not have enough data to include subsurface resources in their models (Pace pers. comm.). On the other hand, *Daphnia* in Paul and Peter lake are migratory, so zooplankton unlikely specialize on deep water autochthonous resources to the same extent as zooplankton in Council Lake.

Zooplankton communities as complex adaptive systems

We have shown that the behaviour of different zooplankton taxa and different sizes determines the fate of allochthonous and autochthonous carbon in Council Lake. The ability to exploit these two carbon sources depends on tradeoffs between temperature, food abundance, food quality, predators and the physical environment (e.g. viscosity). Seasonal changes in feeding behaviour allow different taxa to exploit both allochthonous and autochthonous resources as they become seasonally available. However, zooplankton communities can also structure the supply and habitat structure of their resources, which makes them behave as complex adaptive systems. For example, the persistence of a deep chlorophyll maximum in Council Lake may depend on zooplankton grazing in the epilimnion, because they provide a net downward transport of nutrients to the hypolimnion (Pilati and Wurstbaugh 2003). Interestingly, the zooplankton that feed in the epilimnion, rely more on allochthonous carbon, so there is an interaction between the amount of allochthony in the epilimnion and the persistence of autochthony in the hypolimnion. In other words, *Holopedium* may facilitate food resources for large *Daphnia* in the hypolimnion. In this case, the composition and behaviour of the zooplankton community is structuring the spatial distribution of resources.

The zooplankton community in Council Lake may also structure the availability of allochthonous resources. Normally, zooplankton have little control over the amount and composition of DOC that enters the lake, because it is determined by external watershed processes. However, if zooplankton grazing leads to more light that infringes on the hypolimnion (Mazumder et al. 1990b), then hypolimnetic dissolved organic carbon (DOC) may become more accessible to the heterotrophic pathways. If this is so,

then zooplankton communities can not only change their feeding behaviour in response to seasonal variation in allochthonous inputs (Grey et al. 2001), but also change the accessibility of allochthonous carbon to higher trophic levels.

Tables

Table 7.1 : $\delta^{13}C$ of plankton in Council Lake

Seasonal averages (standard error) for plankton from the entire sampling period Feb 14th, 2002 to Mar 17th, 2003. Range is reported for daily averages.

	$\delta^{13}C_{LE}$	$\delta^{13}C$	Range $\delta^{13}C_{LE}$, $\delta^{13}C$	C:N	Range C:N	N
Chaoborus trivittatus	-31.8 (0.4)	-32.7 (0.5)	3.0, 2.9	5.1	2.4	28
Epischura nevadensis	-31.5 (0.2)	-32.2 (0.2)	6.6, 7.4	5.0	1.7	10
Leptodiaptomus tyrelli	-36.4 (0.2)	-38.4 (0.3)	4.5, 5.7	6.3	3.0	15
Holopedium gibberum	-32.2 (0.3)	-34.1 (0.4)	7.2, 8.0	6.2	2.9	18
Daphnia	-36.6 (0.4)	-38.2 (0.4)	8.2, 8.4	7.0	3.1	28
Daphnia spp. (> 1.8mm)	-37.1 (0.2)	-39.8 (0.2)	6.3, 6.9	7.3	4.3	22
Daphnia spp. (< 1.8mm)	-34.3 (0.3)	-36.6 (0.4)	4.9, 5.7	6.6	3.6	25
POM (< 41μm)						
2 - 6m	-	-31.3 (0.2)	4.3	9.8	4.1	27
6 - 12m	-	-32.1 (0.5)	4.0	10.3	4.2	27
12 - 17m	-	-33.4 (0.5)	7.6	9.4	3.0	27

Table 7.2 : Statistical modeling of temporal cage experiment

Summary of parameter estimates for generalized nonlinear least squares analysis for *Daphnia* $\delta^{13}C$ in the temporal cage experiment in the epilimnion (Epi), metalimnion (Meta), and hypolimnion (Hypo). The model was of the form $\delta^{13}C = \beta_0 * e^{\wedge}(\beta_1 * day)$, and parameter estimates are shown with confidence intervals. All p-values for the parameters of each regression were <0.0001. None of the 95% confidence intervals (CI) for β_1 overlap.

Lake Strata	**β_0 (‰) (CI)**	**β_1 (day^{-1}) (CI)**
Epi (4m)	-17.2	-0.0570
	(-17.8 to -16.4)	(-0.064 to -0.050)
Meta (8m)	-16.9	-0.0453
	(-17.4 to -16.3)	(-0.049 to -0.014)
Hypo (14m)	-16.6	-0.0936
	(-17.5 to -15.7)	(-0.010 to -0.083)

Table 7.3 : Allochthony estimates for zooplankton in Council Lake

Allochthony was calculated using the following mixing model: [1]Zooplankton allochthony = $(\delta^{13}C_{zooplankton} - 0.43 - \delta^{13}C_{algae})/(\delta^{13}C_{allochthnous} - \delta^{13}C_{algae})$. The $\delta^{13}C$ of algae are estimates from Karlsson et al. 2003. We used the average (-41.8‰), minimum (-46.1), and maximum (-37.7) $\delta^{13}C$ of algae to estimate the average, minimum, and maximum amount of zooplankton allochthony. $\delta^{13}C_{allochthnous}$ was -27.5‰ in all cases, and 0.43 is the trophic enrichment from algae to zooplankton (Karlsson et al. 2003). [2]Here we estimate allochthony using the solid line in Fig. 7.7 for algal $\delta^{13}C$. Δ_{1-2} is the difference between the two allochthony model predictions.

Zooplankton taxa	Year	$\delta^{13}C$	Allochthony[1] (Min, Max)	Allochthony[2]	Δ_{1-2}
Holopedium	2002	-31.8	0.67 (0.54, 0.75)	0.55	0.12
	2003	-33.8	0.53 (0.34, 0.64)	0.44	0.09
Epischura	2002	-31.1	0.72 (0.60, 0.78)	0.57	0.14
	2003	-32.0	0.65 (0.51, 0.73)	0.59	0.07
Chaoborus	2002	-32.4	0.63 (0.48, 0.71)	0.61	0.02
	2003	-31.5	0.69 (0.57, 0.76)	0.65	0.04
Leptodiaptomus	2002	-37.7	0.26 (0.00, 0.43)	0.40	-0.14
	2003	-40.5	0.06 (0.00, 0.28)	0.17	-0.11
Daphnia >1.8mm	2002	-39.8	0.11 (0.00, 0.32)	0.28	-0.17
	2003	-41.9	0.00 (0.00, 0.21)	0.05	-0.05
Daphnia <1.8mm	2002	-34.8	0.46 (0.24, 0.59)	0.40	0.05

Figures

Figure 7.1 : Seasonal variation in the $\delta^{13}C$ of plankton in COL

Seasonal patterns in the $\delta^{13}C$ of zooplankton and POM in Council Lake 2002-2003. Vertical dotted lines show the period of thermal stratification. Error bars are +/- one standard error. The shaded region is the maximum and minimum of the $\delta^{13}C$ of POM <41μm collected over the entire water column.

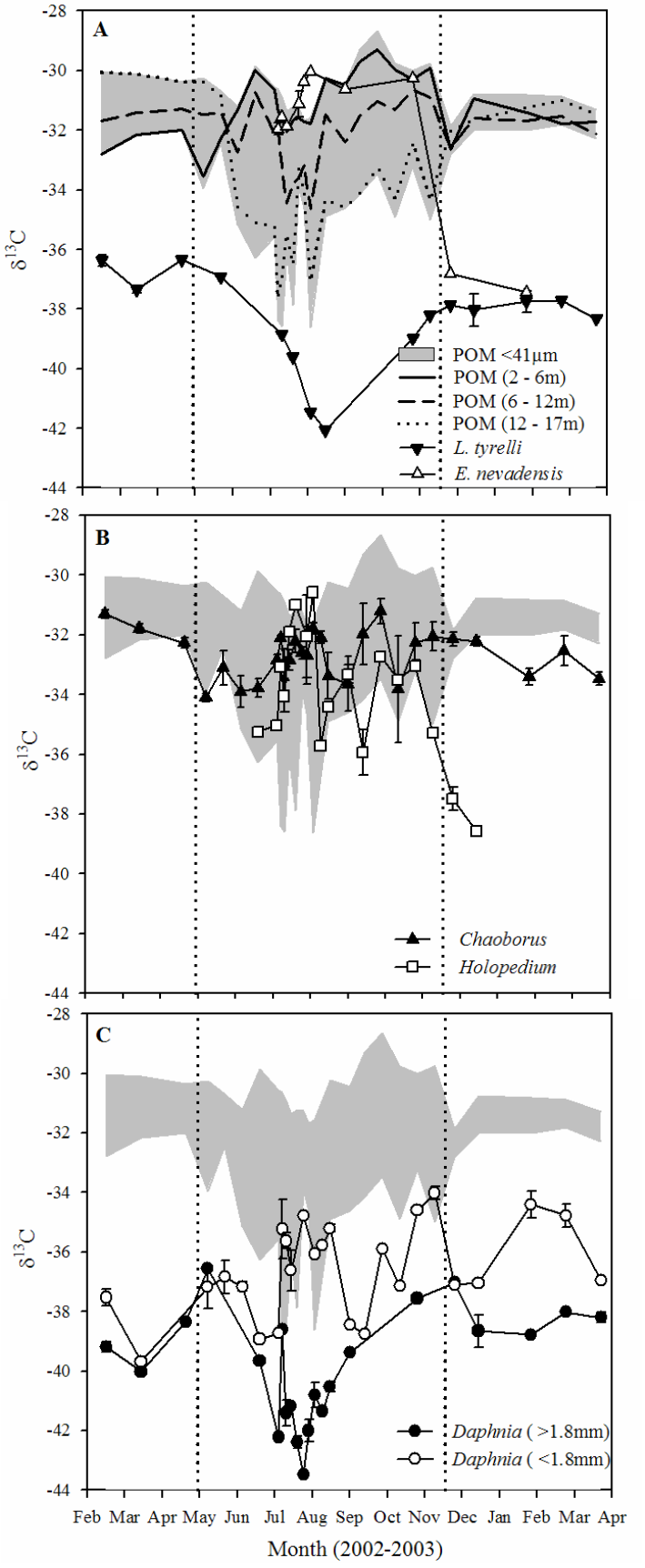

A
B
C
δ13C
-28
-30
-32
-34
-36
-38
-40
-42
-44
POM <41µm
POM (2 - 6m)
POM (6 - 12m)
POM (12 - 17m)
L. tyrelli
E. nevadensis
Chaoborus
Holopedium
Daphnia (>1.8mm)
Daphnia (<1.8mm)
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr
Month (2002-2003)

Figure 7.2 : Vertical gradients in the $\delta^{13}C$ of POM in Council Lake.

Samples were collected from February to Aug 2002 (A) and Sept to March 2003 (B).

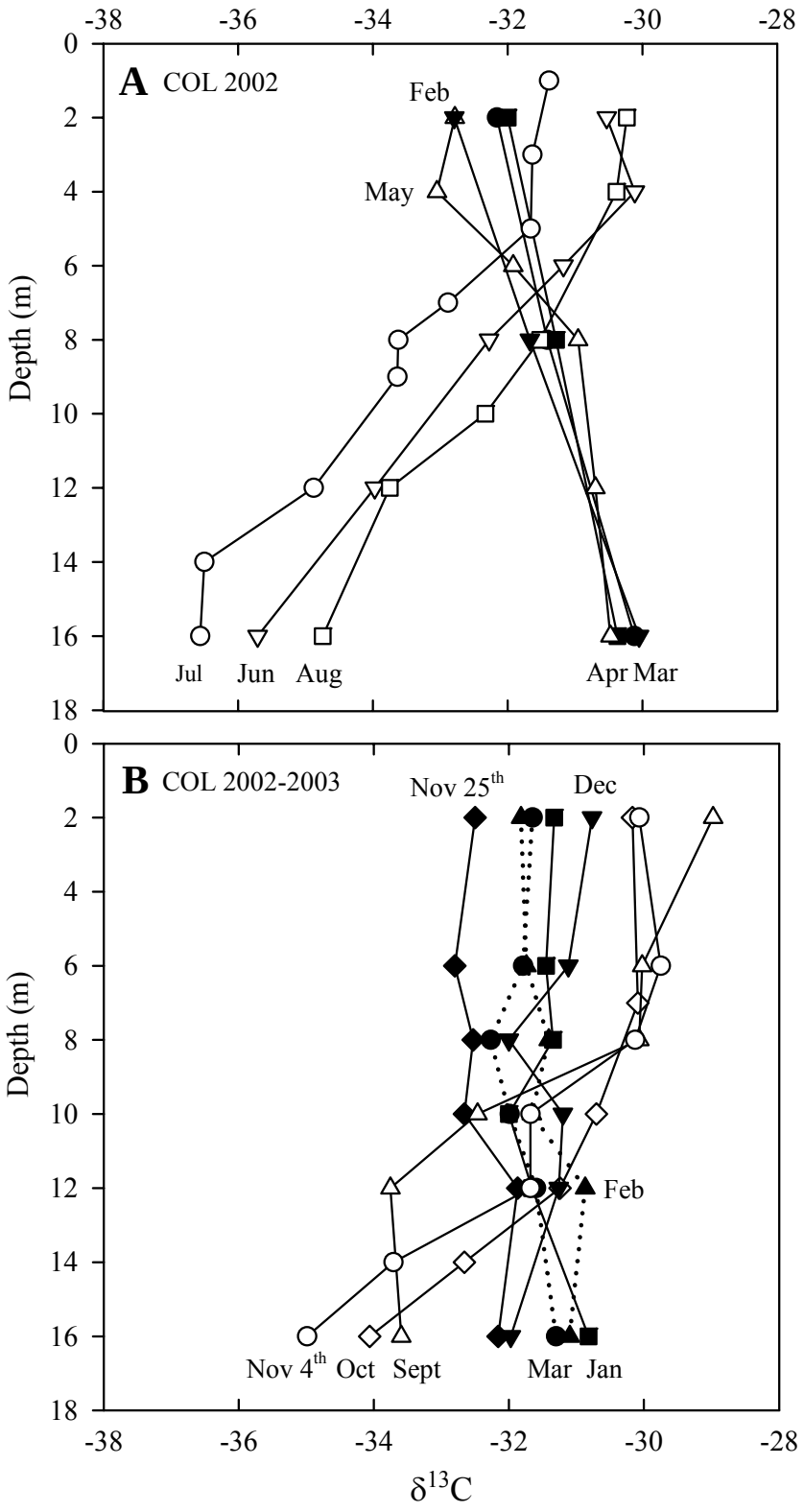

A COL 2002
Feb
May
Jul
Jun
Aug
Apr
Mar
B COL 2002-2003
Nov 25th
Dec
Feb
Nov 4th
Oct
Sept
Mar
Jan
Depth (m)
δ13C
-38
-36
-34
-32
-30
-28
0
2
4
6
8
10
12
14
16
18

Figure 7.3 : Body size $\delta^{13}C$ relationship in Council Lake *Daphnia*

Relationship between the average *Daphnia* body size and $\delta^{13}C$ during July 2002. $\delta^{13}C_{LE}$ is the $\delta^{13}C$ of *Daphnia* following lipid normalization (Chapter 6).

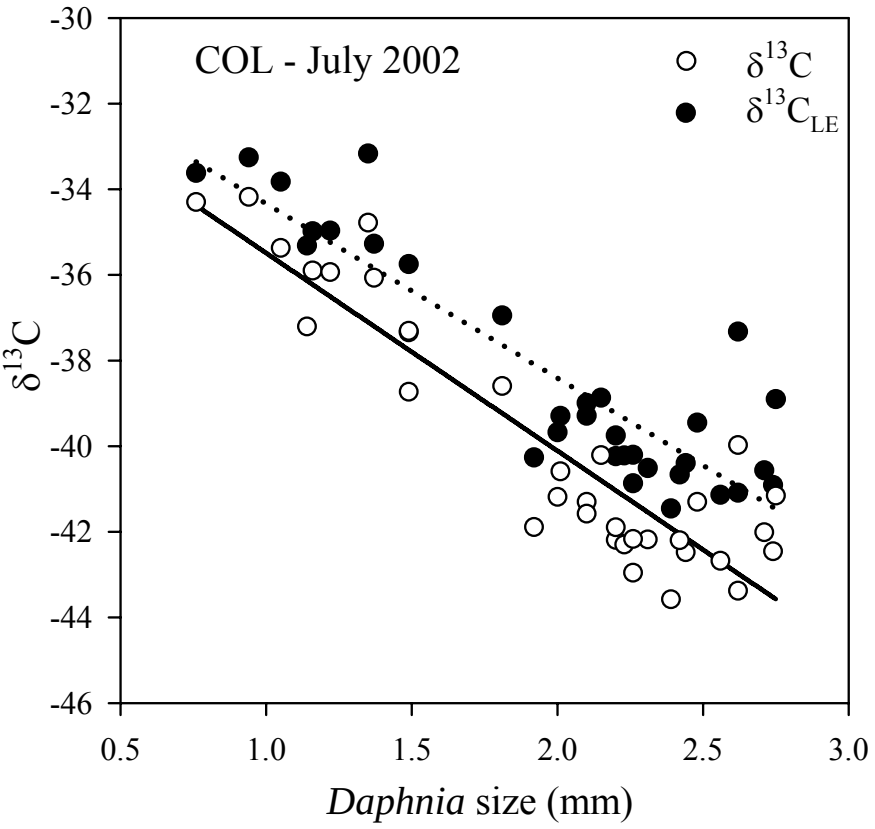

COL - July 2002
δ13C
δ13CLE
-30
-32
-34
-36
-38
-40
-42
-44
-46
δ13C
0.5
1.0
1.5
2.0
2.5
3.0
Daphnia size (mm)

Figure 7.4 : Vertical distribution of POM during cage experiments

Profiles of $\delta^{13}C$, C:N, particulate carbon (PC), chlorophyll *a* (Chl *a*) and temperature for the time period of the cage experiments in 2003. All the closed symbols are for POM <41μm.

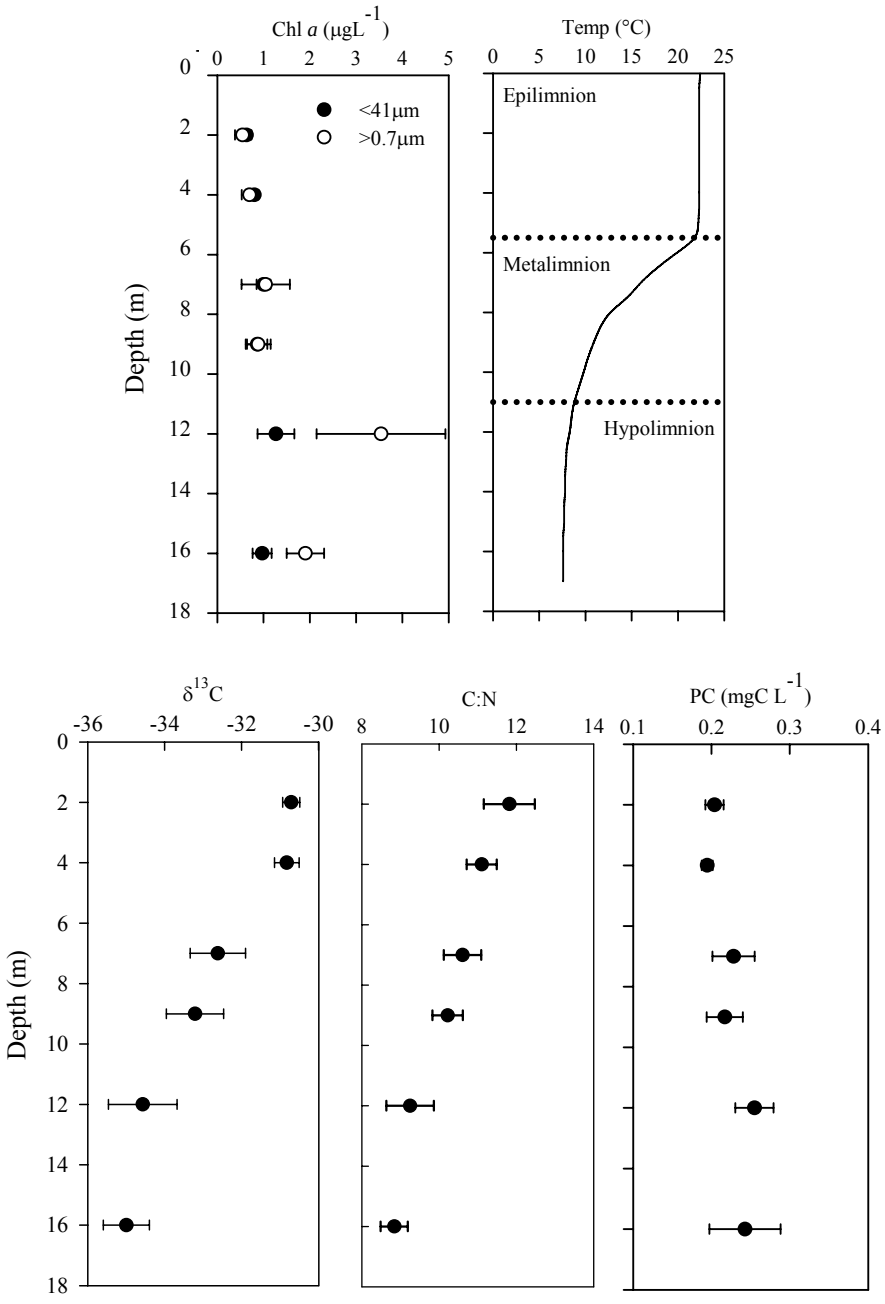

Chl a (μgL-1)
Temp (°C)
Depth (m)
<41μm
>0.7μm
Epilimnion
Metalimnion
Hypolimnion
δ13C
C:N
PC (mgC L-1)

Figure 7.5 : $\delta^{13}C$ of *Daphnia* in the temporal cage experiment.

Symbols are for *Daphnia*, and the dashed, dotted, and solid lines are for POM <41μm at different strata in the lake.

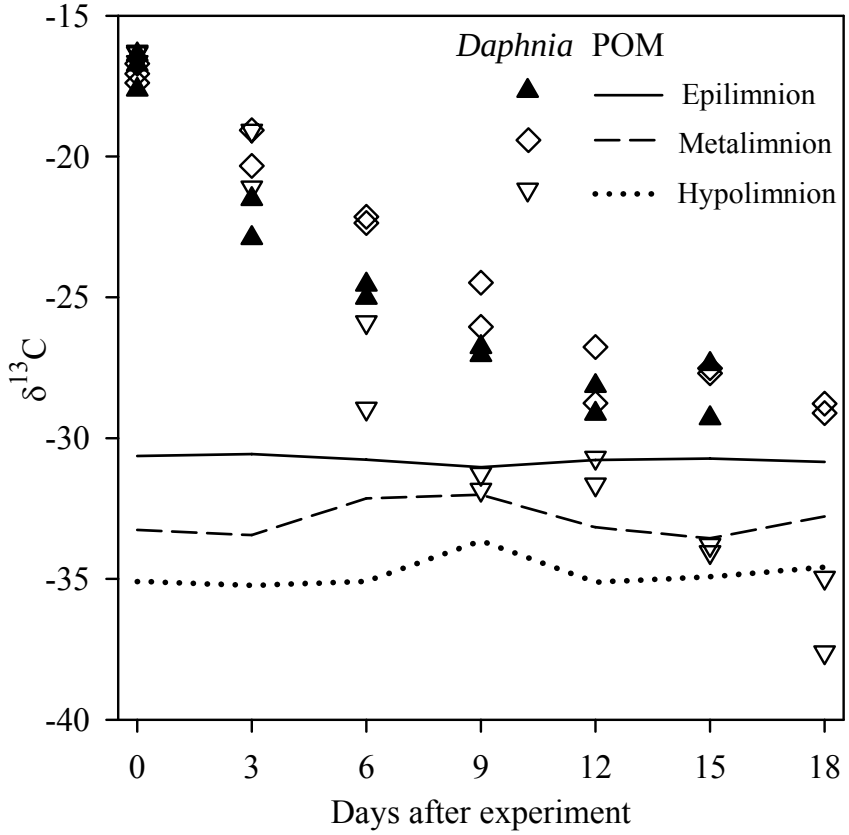

-15
-20
-25
-30
-35
-40
δ13C
0
3
6
9
12
15
18
Days after experiment
Daphnia POM
Epilimnion
Metalimnion
Hypolimnion

Figure 7.6 : $\delta^{13}C$ of *Daphnia* in the profile cage experiment.

The shaded region is the maximum and minimum of the $\delta^{13}C$ of POM <41μm based on repeated sampling over the course of the experiment at the specific cage depth.

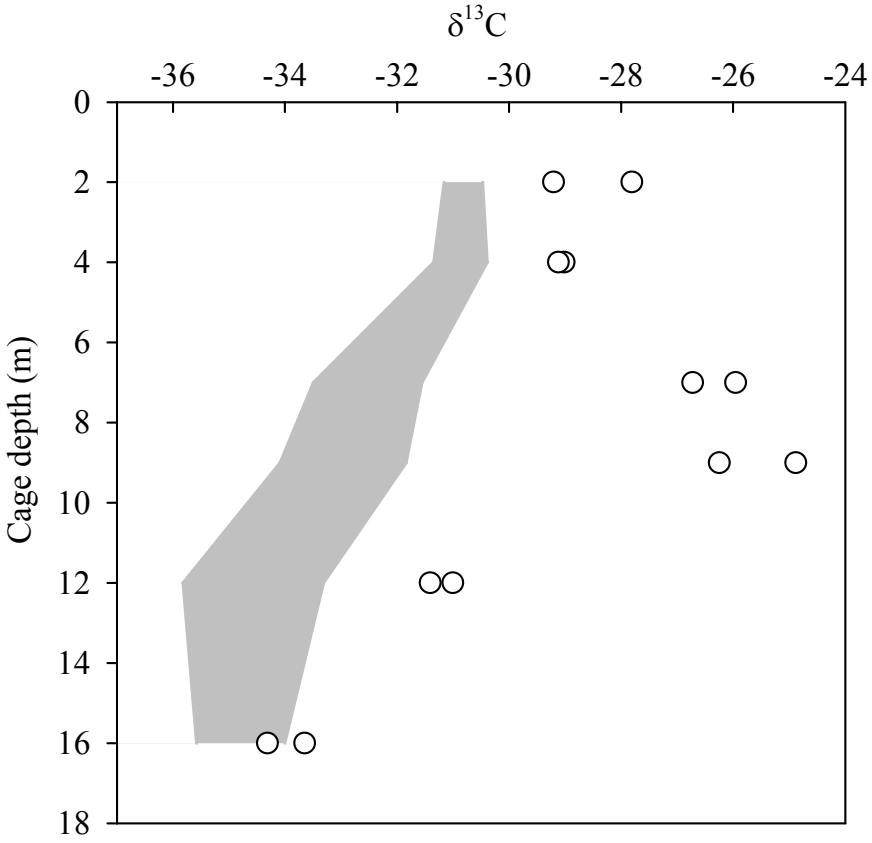
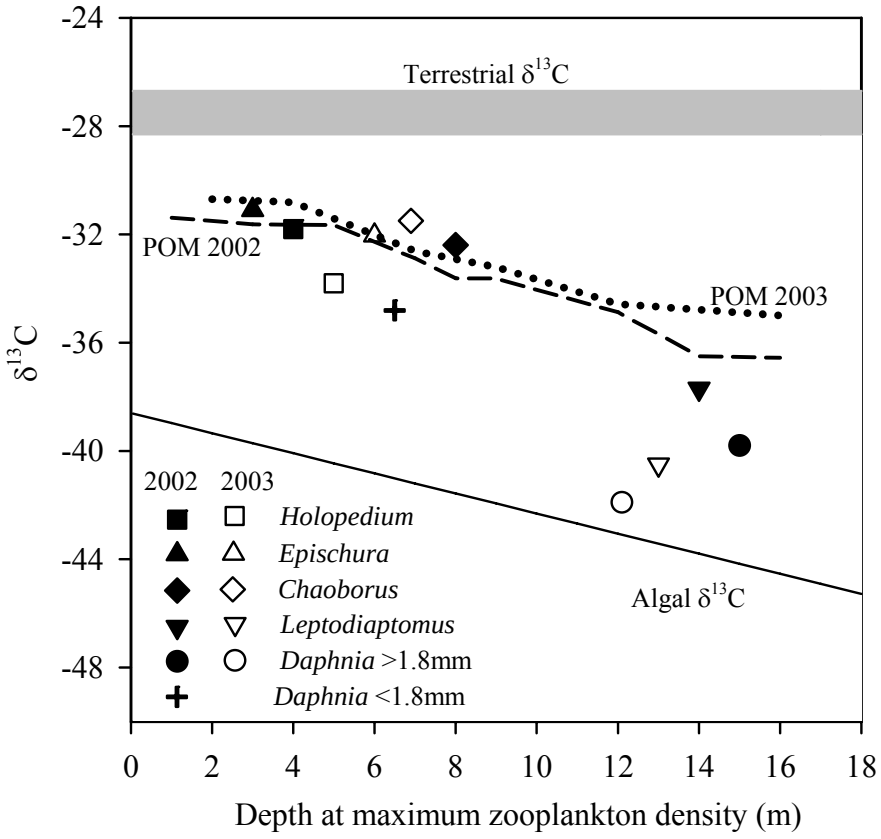

δ13C
-36
-34
-32
-30
-28
-26
-24
0
2
4
6
8
10
12
14
16
18
Cage depth (m)

Figure 7.7 : Habitat specialization and allochthony of zooplankton in Council Lake Relationship between the $\delta^{13}C$ of different zooplankton taxa and sizes and their depth at maximum density. The grey shaded region show the average ± 1 SD of terrestrial carbon. The dashed lines are the $\delta^{13}C$ of POM in midsummer of each year. The solid line is a hypothetical change in the $\delta^{13}C$ of algae with depth (Table 7.3).


-24
-28
-32
-36
-40
-44
-48
δ13C
Terrestrial δ13C
POM 2002
POM 2003
Algal δ13C
2002 2003
Holopedium
Epischura
Chaoborus
Leptodiaptomus
Daphnia >1.8mm
Daphnia <1.8mm
0 2 4 6 8 10 12 14 16 18
Depth at maximum zooplankton density (m)

Chapter 8: Unresolved questions and fundamental conclusions

Research Objectives

The objective of this thesis was to study the complexity of zooplankton feeding behaviours by measuring the isotopic heterogeneity of zooplankton communities. Biogeochemical processes lead to large variation in nitrogen and carbon cycling among lakes, and within a lake over time (Quay et al. 1986; Leggett et al. 1999; Leggett et al. 2000; Lehmann et al. 2004). Time series of zooplankton $\delta^{13}C$ and $\delta^{15}N$ provide a record of carbon and nitrogen cycling over the season. Although this "baseline" variation holds valuable information about carbon and nitrogen cycling in lakes (Lehmann et al. 2004), it confounds our ability to extract information about zooplankton feeding behaviours. Below I summarize the future directions and unresolved questions that arose from my thesis, and then outline the fundamental conclusions and implications of each chapter.

Unresolved questions and future directions

My thesis work has led to many questions that I have been unable to resolve in any of my thesis chapters. I address these questions in the following section in order to stimulate future research in this area.

Dietary lipids and the Daphnia lipid conundrum

The $\delta^{13}C$ of *Daphnia* lipid in Figure 6.3 was surprisingly high based on the observed relationship between C:N and $\delta^{13}C$ in Figures 5.5 and 6.4. This resulted in a higher than predicted $\delta^{13}C_{LE}$ for *Daphnia* (Fig. 6.7). The slope of the relationship between C:N and $\delta^{13}C$ (Figs. 5.5, 6.4) is steeper for *Daphnia* than for *L. tyrelli*. Based on this, my stoichiometric model predicts that the $\delta^{13}C$ of *Daphnia* lipid should be lower than I observed (Fig. 6.3) and should be lower than the $\delta^{13}C$ of lipid in *L. tyrelli*. In

Chapter 6, I was unable to reconcile this discrepancy (Fig. 6.7). I tried modifying the model to include isotopic differences between proteins and carbohydrates. I could explain the high $\delta^{13}C$ of *Daphnia* if I included a ~2‰ difference between carbohydrates and proteins. Further research on the isotopic composition of lipid extracted zooplankton tissue would improve the accuracy of this model.

Lipid variation in oligotrophic and eutrophic systems

One of the most intriguing aspects of my annual time series from SOL was the large seasonal variation in zooplankton C:N (Fig. 6.1A). These patterns were taxa specific (Fig. 5.2), and the general pattern for each taxon was similar in both years for SOL (Fig. 5.2, Fig. 6.1) and COL (data not shown). The patterns are divergent for zooplankton in the two oligotrophic lakes SOL and COL, but temporally coherent in the eutrophic Pluβsee. This matches the results of Arts et al. (1992, 1993), who found seasonally correlated patterns of lipid accumulation in zooplankton from a hyper-eutrophic lake with high food quantity (Arts et al. 1992), and seasonally divergent patterns in a saline lake with low food quantity (Arts et al. 1993). If this is a general pattern, it may indicate that lipid accumulation and life history patterns diverge among similar species in low productivity environments. This could indicate one aspect of the behavioural flexibility of zooplankton that may allow coexistence of multiple species in lakes of low productivity. Future studies could examine the synchrony of lipid storage in zooplankton among species in lakes of varying productivity.

Trophic fractionation of zooplankton

There has been almost no work done on the trophic enrichment of ^{15}N in freshwater zooplankton. This huge research gap hinders all attempts at using $\delta^{15}N$ to study trophic variation in zooplankton communities. Vanderklift and Ponsard (2003) recently did a meta-analysis of trophic enrichment in consumers, and reported only one estimate for a freshwater zooplankton (*Daphnia magna*). To date, two studies have estimated the trophic enrichment of zooplankton (Adams and Sterner 2000; Power et al. 2003), and both used *Daphnia magna*. Adams and Sterner (2000) found that enrichment depends on the C:N of the food source (Adams and Sterner 2003), whereas Power et al. (2003) found that enrichment depends on water temperature. Clearly, there is a dire need for more studies on the trophic enrichment of zooplankton.

In chapter 2, I found that the $\delta^{15}N$ of copepods was higher than *Daphnia*, and argued that this could indicate trophic variation. Alternatively, a high trophic enrichment of ^{15}N in copepods could explain their high $\delta^{15}N$ compared to *Daphnia*. However, there are three reasons why I do not believe that differences in trophic enrichment can fully explain among lake patterns in the $\delta^{15}N$ of *Daphnia* and copepods. First, copepods are omnivores so a higher $\delta^{15}N$ is expected *a priori*. Second, the $\delta^{15}N$ of copepods is more similar to invertebrate predators in lakes where they co-occur (Chapter 4, unpublished data). Third, the variation in trophic fractionation among consumers is small compared to observed differences in $\delta^{15}N$ between copepods and *Daphnia*. Post (2002) found that the variation in the trophic enrichment among consumers was 3.4‰ (SD= 0.98, N= 56). Given this empirical distribution of trophic enrichments (Post 2002), it is unlikely (P= 0.024, N= 1000 randomized comparisons) that the difference between two randomly

chosen taxa would have a difference as large or bigger than 3.4‰ (which is best estimate of the difference between calanoids and *Daphnia*). Based on these three arguments, differences in $\delta^{15}N$ between *Daphnia* and copepods likely indicate trophic variation within zooplankton communities.

Baseline seasonal variation in zooplankton $\delta^{15}N$

In marine systems, the $\delta^{15}N$ of plankton is commonly used to infer changes in the source (N-fixation, NH_4^+ vs. NO_3^-) of nitrogen (Montoya et al. 2002), and changes in the amount of nitrogen "lost" (e.g. by algal uptake, or denitrification) from a closed system (Altabet et al. 1995). Only a few recent studies use the $\delta^{15}N$ of marine zooplankton to estimate zooplankton trophic position (Schmidt et al. 2003, Sommer et al. 2005). In comparison, the $\delta^{15}N$ of zooplankton in lakes is primarily used to estimate the trophic position of fish (Cabana and Rasmussen 1994), and detect changes in external sources of nitrogen (Savage et al. 2004). Few lake studies have looked at how time series of zooplankton $\delta^{15}N$ can indicate the amount of nitrate utilization over a season, or changes in the internal sources of nitrogen. For example, the seasonal increase in the $\delta^{15}N$ of zooplankton in Council Lake, Sooke Reservoir, Shawnigan Lake, and Elk Lake (Figure 3.2, 4.1), may result from algal uptake of nitrogen over the season leading to an increasingly enriched (higher $\delta^{15}N$) pool of nitrate.

Perga and Gerdaux (2005) found that the seasonal pattern of zooplankton $\delta^{15}N$ is repeatable among years. More recently, J.A. Rusak found a consistent seasonal pattern of bulk zooplankton $\delta^{15}N$ in two lakes over the past 25 years (pers. comm.). Along with my data (Chapter 3, and 4), this suggests that lakes have characteristic patterns of seasonal

change in zooplankton $\delta^{15}N$ (in terms of annual amplitude and timing of maxima) that are related to seasonal changes in nitrogen cycling. Perga et al. (unpublished manuscript) found that the $\delta^{15}N$ of zooplankton was positively correlated with levels of NH_4^+. This suggests that the $\delta^{15}N$ of zooplankton might track changes in the dominant internal sources of nitrogen that are used by algae. For example, high zooplankton $\delta^{15}N$ is common during the winter of oligotrophic lakes; however, no study has proposed an explanation for this pattern. Algae in the winter may use a recycled form of nitrogen, or may access a different source of nitrogen that is not available in the summer. The biogeochemical processes that lead to seasonal variation in zooplankton $\delta^{15}N$ are virtually unstudied in lakes, even though they are critical for distinguishing trophic from isotopic variation (Chapter 4).

Temporal coherence of zooplankton $\delta^{15}N$

One of my unaccomplished goals for this thesis was to examine the temporal coherence of time series of zooplankton $\delta^{15}N$. Rusak et al. (1999) examined temporal coherence of zooplankton population abundance and found that extrinsic and intrinsic factors regulate the population dynamics of zooplankton communities. This approach could separate regional coherence of baseline variation in $\delta^{15}N$ among lakes (extrinsic factor) from within lake variation of multiple zooplankton taxa (intrinsic factors). If intrinsic factors explained most of the variation in zooplankton $\delta^{15}N$, this could provide evidence for regional differences in the seasonal patterns of food web structure. For example, compare the seasonal $\delta^{15}N$ patterns of Council Lake (Fig. 4.1) with Sooke Reservoir (SOL) and Shawnigan Lake (SHL) (Fig. 3.2). Trophic variation is more likely

present in the Council Lake zooplankton community, than in SOL and SHL, because $\delta^{15}N$ patterns are different among taxa. In SOL and SHL, the seasonal patterns of zooplankton $\delta^{15}N$ are regionally coherent and possibly respond to similar extrinsic factors. In this case, it is more likely that seasonal patterns of food web structure are similar between SOL and SHL. Unfortunately, there is not enough data in this thesis or in the literature to adequately address these questions.

Distinguishing between microbial and algal food chains

The pelagic lake habitat has multiple interwined food chains (Porter 1996). The $\delta^{15}N$ of zooplankton might enable limnologists to distinguish between how algal and bacterial based food chains support zooplankton production. One explanation for the high $\delta^{15}N$ of copepods is their reliance on the microbial food chain, which may be longer than an algal based food chain. To my knowledge, this hypothesis for the high $\delta^{15}N$ of copepods has only been discussed twice (Karlsson et al. 2004; Feuchtmayr et al. 2004), and neither study measured the $\delta^{15}N$ of bacteria or the enrichment of ^{15}N through the microbial food web. The strength of microbial and algal based food chains varies over the season (Porter 1996), and zooplankton taxa differentially rely on these two food chains. In Chapter 4, I speculated that the seasonal patterns of zooplankton $\delta^{15}N$ indicate multiple food chains. However, a direct test of this hypothesis is not possible without more lab-based studies of isotopic enrichment in zooplankton and in the microbial food chain.

Individual specialization in omnivory

The intrapopulation variance in $\delta^{15}N$ can reflect the level of individual variation in omnivory in a population of consumers. This idea has been mentioned in two separate

instances (Ponsard and Arditi 2001; Williams and Martinez 2004), but no one has explicitly related isotopic variance with omnivory. In a recent paper, Matthews and Mazumder (2004) outlined a modeling approach to link intrapopulation variation in $\delta^{13}C$ with individual specialization in resource use. In the same way, $\delta^{15}N$ could be particularly useful for examining patterns of omnivory in food webs. For example, does the level of individual specialization in omnivory increase up the food chain? If so, differences in $\delta^{15}N$ among individual consumers would increase up the food chain, and variance in $\delta^{15}N$ would be positively correlated with trophic position. This hypothesis could be tested using a combination of modeling approaches from Matthews and Mazumder (2004) and Matthews and Mazumder (2005b).

Isotopic variance as an indicator of trait variance

At the beginning of this thesis, I set out a theoretical basis for studying temporal variation in the food web structure of zooplankton. I then used isotopic variation to measure the structure of zooplankton communities. However, I did not directly link the trait variance of a plankton community with its isotopic variance. Trait variance describes inter and intraspecific variation in feeding behaviour, and is proportional to the capacity of a community to respond to external selective forces (Norberg 2004). For example, the size distribution of plankton is proportional to the resilience of a plankton community to nutrient loading (Cottingham and Schindler 2000). Previously, I have suggested that isotopic variance can indicate intrapopulation variation in the feeding behaviour of consumers (Matthews and Mazumder 2004). Likewise, the temporal isotopic variance of a plankton community could indicate seasonal variation in the food web structure of the community. In a way, temporal isotopic variance could then estimate a behavioural

component of a zooplankton community's trait variance (Norberg 2004). Seasonal changes in the $\delta^{15}N$ and $\delta^{13}C$ of zooplankton could measure the capacity of a particular community to respond to seasonal and spatial changes in resources. The continuing challenge is to isolate the component of seasonal variation in zooplankton $\delta^{15}N$ that is related to variation in feeding behaviour.

There are several examples from my thesis where stable isotopes helped describe the trait variance of a community. In Council Lake, for example, seasonal $\delta^{15}N$ patterns of zooplankton are taxa specific (Fig. 4.1). In other lakes, a similar suite of species have temporally coherent $\delta^{15}N$ patterns (Perga and Gerdeaux 2005). Therefore, the Council Lake zooplankton community exhibits temporal variation in its feeding behaviour that matches the temporal and spatial distribution of resources. In Chapter 7, I found that some components of the zooplankton community were able to exploit hypolimnetic resources. This adaptive capacity of the Council Lake community was not as evident in Sooke Reservoir, even though the vertical distribution of chlorophyll *a* is similar between SOL and COL (Davies 2004). I suspect that the zooplankton community in SOL is unable to exploit deep water production to the same degree as in COL. In general, the exploitation efficiency of zooplankton communities depends on the species composition and the feeding behaviours of those species. This thesis has made some contribution towards using $\delta^{13}C$ and $\delta^{15}N$ to describe the trait variance of zooplankton communities. However, there is still great promise in using stable isotopes to study the diversity of zooplankton feeding behaviours in lakes that have seasonally and spatially variable resources.

Fundamental contributions towards understanding the food web structure of zooplankton communities

In Chapter 2, I found that the $\delta^{15}N$ of copepods was higher than the $\delta^{15}N$ of *Daphnia* and *Holopedium*. This is a robust pattern for coastal and interior lakes of British Columbia (unpublished data). In a large survey of British Columbia lakes, the $\delta^{15}N$ of calanoid copepods was higher than *Daphnia* in 44 out of 47 lakes. The $\delta^{15}N$ of calanoid copepods was on average 3.4‰ higher than *Daphnia* where they co-occurred (SD= 1.74, N= 47). This indicates that there is significant trophic variation within these zooplankton communities, and not all species, life stage, and sizes of zooplankton are at the same trophic level. Models of food web structure in lakes should therefore include more continuous trophic variation within the zooplankton "trophic level".

In Chapter 3, I examined how baseline variation in *Daphnia* $\delta^{15}N$ affects the estimation of fish trophic position. I found that a seasonal average model (SAM) was as effective as a temporal integration model (TIM) for estimating the trophic position of fish. However, the TIM model was more useful for determining how much of the isotopic variation among individual fish is related to trophic variation. Therefore, the TIM is the first step towards using the intrapopulation variance in $\delta^{15}N$ to indicate individual specialization with respect to trophic position (Matthews and Mazumder 2004). This study is the first to explicitly propose mechanisms for individual differences in $\delta^{15}N$ that are not necessarily related to trophic position, assuming that all individuals have the same trophic enrichment of ^{15}N. In the future, we should be able to use this type of approach to measure individual specialization of consumers in a wide variety of ecosytems, and

explore the consequences of intrapopulation variation in trophic position for food web dynamics (Polis and Strong 1996).

In Chapter 4, I found that large seasonal variation in the zooplankton $\delta^{15}N$ did not match my expectations about seasonal changes in zooplankton feeding behaviour. The $\delta^{15}N$ of the more herbivorous taxa (*Daphnia and Holopedium*) increased and exceeded the $\delta^{15}N$ of the more predacious taxa (*Epischura* and *Chaoborus*) at the end of July. If all these zooplankton taxi were on a single linear food chain, I would expect a synchronous response of each taxon (with some damping and time lag) to variation in $\delta^{15}N$ at the bottom of the food chain. Since this was not the case, I suspect there are multiple food chains (i.e. multiple pathways of nitrogen) that lead to various members of the zooplankton community. This approach has intriguing implications for using long time series of zooplankton $\delta^{15}N$ to determine the relative strength of microbial versus grazing food chains in lakes of differing productivity, or within a lake in different seasons.

In Chapters 5 and 6, I argued that current interpretations of zooplankton $\delta^{13}C$ neglect zooplankton lipids. Lipids are primarily dietary in freshwater zooplankton, so we should consider lipids (and their $\delta^{13}C$) as a carbon source for zooplankton. I developed a stoichiometry model that makes clear predictions about the relationship between zooplankton lipid content and C:N, and the relationship between C:N and $\delta^{13}C$. It also explained why the C:N of *Daphnia* is not highly variable (Sterner and Hessen 1994). *Daphnia* can regulate its body composition and change lipid storage by upwards of 30%, but this does not affect its C:N because *Daphnia* has a low lipid content. This is because of the non linear relationship between lipid content and C:N (Fig. 6.2). The implication of these two chapters is that the isotopic composition of the lipid and non-lipid fraction of

zooplankton can reveal the source and pathway of carbon transfer between resources to consumers. The isotopic complexity within and among taxa of zooplankton suggests that there are complex pathways through the zooplankton food web that connect primary producers with upper trophic levels. This suggests that understanding the link between phytoplankton, bacteria, and zooplankton is critical for determining the structure and function of lake food webs.

In Chapter 7, I concluded that *Holopedium*, *Epischura,* and *Chaoborus* relied more on allochthonous carbon sources than *Daphnia* and *Leptodiaptomus tyrelli*. Based on the vertical distribution of resources in Council Lake, I argued that the presence of allochthonous carbon modifies the temperature food trade-off of zooplankton (Lampert et al. 2003). This argument assumes that allochthonous and autochthonous carbon vary in their food quality. If so, then the habitat specialization of zooplankton in Council Lake results from the trade-off between high food quality and low minimum resource requirements (Tessier and Woodruff 2002b). *Holopedium* can persist in the epilimnion because they have a low minimum resource requirement and can exploit low quality food that has a large contribution of allochthonous carbon. Taxon-specific differential exploitation of allochthonous carbon has significant implications for the extent to which terrestrial subsidies are exploited by zooplankton communities. Currently, studies have only considered a very narrow range of zooplankton communities (Grey et al. 2001; Pace et al. 2004). Our results clearly demonstrate that community wide exploitation of allochthonous carbon intimately depends on the species composition of the zooplankton community, the feeding behaviour of the community's constituent parts, and the spatial and temporal structure of primary resources.

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