Lake Zooplankton Carbon Sources: The Role Of Terrestrial Inputs And The Effects Of Depth And Taxonomic Composition

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

The relative contribution of allochthonous and autochthonous production in zooplankton nutrition has been of interest since the net heterotrophy of lakes was recognised to be common. I measured the ^{13}C signature of epilimnetic CO_2, particulate organic carbon (POC), and zooplankton in 27 north-temperate lakes in late summer and used the relationships between the POC and zooplankton ^{13}C signatures and the CO_2 signature to estimate the autochthonous contribution to these fractions of the plankton. My hypothesis was that POC and zooplankton signature would reflect the $^{13}CO_2$ signature if they were autochthonous. Conversely, increasing allochthonous C would result in a ^{13}C signature of POC or zooplankton that is increasingly influenced by the allochthonous ^{13}C signature (-28‰) and decreasingly dependent on the CO_2 signature. The average autochthonous contribution to epilimnetic POC was estimated to be between 62 and 75%. Epilimnetic zooplankton were, on average, between 77 and 91% autochthonous, indicating that zooplankton bias their feeding towards the autochthonous fraction of POC. On average, zooplankton were 1.2‰ enriched in ^{13}C relative to POC, but their biased feeding on phytoplankton means that they can be depleted relative to POC in lakes where POC is highly depleted in ^{13}C. The relationship between ^{13}C-POC and $^{13}CO_2$ allowed us to estimate average photosynthetic fraction as -15.9‰. This estimate is independent of how much allochthonous C contributes to POC. Variation in photosynthetic fractionation was not a major contributor to differences among lakes in POC and zooplankton ^{13}C signature. Allochthonous C is an important, although clearly secondary, source of C to zooplankton of these lakes in late summer.

I expanded the above analysis by culling the literature for ^{13}C stable isotope data of lake CO_2, POC, and zooplankton. I found that, similar to the lakes that I had sampled, POC signature showed a strong influence of allochthonous C, and inferred that it was close to 50% allochthonous on average. I calculated an autochthonous fractionation of -14.1‰ for the metadata, which was similar to that of the lakes I sampled. While POC had a considerable allochthonous contribution, zooplankton signatures were strongly related to the CO_2 signatures, suggesting that their carbon was mostly autochthonous. Therefore, while terrestrial inputs form a major portion of POC, zooplankton C, on average, was largely autochthonous.

I also examined the differences in $^{13}C/^{15}N$ among zooplankton taxa, and differences in $^{13}CO_2$, $^{13}C/^{15}N$ of POM, and $^{13}C/^{15}N$ of zooplankton with depth. There were small differences among the ^{15}N of various taxa, and I did not detect differences in ^{13}C amongst taxa. I found vertical heterogeneity was most marked in $^{13}CO_2$ signatures, which generally depleted appreciably with increasing lake depth. The signatures of ^{13}C-POM and ^{13}C-zooplankton also generally depleted with depth, but much less so than did $^{13}CO_2$. I interpret this as indicating that a large portion of POM and zooplankton C in the metalimnia and hypolimnia of these lakes is derived from C fixed in the epilimnia.

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Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Tansley (1935) defined an ecosystem as a group of interacting organisms along with the abiotic environment with which they also interact. The boundaries between ecosystems are, of course, somewhat arbitrary. In his definition, Tansley notes that, "… the systems we isolate mentally are not only included as parts of larger ones, but they also overlap, interlock and interact with one another. The isolation is partly artificial, but it is the only way in which we can proceed… Some systems are more isolated in nature, more autonomous, than others."

While ecosystem science endeavours to quantify the important interactions among ecosystems, the compartmentalisation of systems risks exclusion of important interactions with elements that are considered to be outside of a defined system. Polis (1997) emphasised the lack of isolation among systems and the importance of nutrient, organic C, and organism transport across systems. One of these potentially important inputs is the subsidy of fixed C from one system to another. Such subsidies can result in an ecosystem being able to support higher levels of secondary production than would have been possible without the subsidy (Polis et al. 1997). Additionally, these subsidies may act to stabilise a system, damping the effects of variations in primary production within the system on secondary production (Wetzel 1995).

In aquatic systems, the magnitude of these allochthonous inputs from adjacent terrestrial ecosystems can be very high. In some headwater streams, virtually all of the organic C can be allochthonous (Fisher and Likens 1973).

Allochthonous inputs to lakes can also be substantial. It has been found that most temperate lakes are supersaturated with CO_2 (Cole et al. 1994; Jonsson et al. 2003; Sobek et al. 2003) and have photosynthesis to respiration ratios of less than one (del Giorgio and Peters 1993), both likely consequences of the respiration of allochthonous C (Karlsson et al. 2007; Lennon 2004). This has led to the inference that the production of respiratory CO_2 from allochthonous inputs means that some of these inputs must also be assimilated. It should be noted, however, that an appreciable amount, and perhaps the majority, of this CO_2 may be produced through the photolysis of dissolved organic carbon (DOC) (Molot and Dillon 1997).

Terrestrial organic matter enters lakes as DOC or as particulate organic C (POC). The most likely route by which zooplankton could access allochthonous C is through bacterial assimilation or 'packaging' of the DOC to a form ingestible by other consumers (Wetzel 1992). Leaf litter is known to be an important resource for littoral benthic invertebrates (Mann 1988; Mancinelli et al. 2007) and terrestrial insects are a significant food for some fish (Polacek et al. 2006; Mehner et al. 2005; Saksgard and Hesthagen 2004). The terrestrial POC reaching the pelagic region, however, is generally highly processed and generally very recalcitrant (Moore, 2004; Wetzel, 1995), though models from isotope-addition experiments have been used to suggest that filter-feeding zooplankton can directly access detrital POC (Cole et al. 2006). While it has been found that some protists are capable of direct absorption of organic compounds (Sherr 1988), this is probably a far less important pathway than through bacterial intermediates converting DOC to POC.

Thus, bacteria and their consumers, organisms forming the so-called 'microbial loop', are likely the most significant pathway from allochthonous DOC to higher trophic levels (Azam et al. 1983; Pomeroy 1974). A potential problem with this scenario, however, is that respiratory losses as C is

transferred to protists large enough for zooplankton to consume would cause most of the C to be lost to respiration. It is also possible that filter-feeding zooplankton, such as *Cladocera*, are capable of feeding directly on bacteria (Geller and Muller 1981).

These observations, that allochthonous inputs to lakes are high, CO_2 supersaturation of lakes is common, and the potential that the microbial loop could be a pathway for these allochthonous inputs to higher trophic levels, have led to the hypothesis that lake food webs may be receiving an appreciable subsidy of organic C from terrestrial ecosystems. Several studies, perhaps most notably isotope-addition experiments done on a small number of Wisconsin lakes, have led to the conclusion that zooplankton in smaller lakes may not only be appreciably subsidised by allochthonous C, but they may acquire a majority of their nutrition from terrestrial inputs (Carpenter et al. 2005; Carpenter et al. 2007; Cole et al. 2002; Pace et al. 2004). Interestingly, in many stream studies, recent work has been making very different findings. As mentioned, allochthonous inputs to streams, especially headwater streams, are very high relative to autochthonous production. Additionally, the terrestrial organic C entering streams would often be more labile than that entering lakes. Recent studies examining the balance of allochthony and autochthony in streams, however, have found that stream invertebrates variably rely on allochthonous inputs (Hicks 1997; Junger and Planas 1994; Rounick et al. 1982; Salas and Dudgeon 2001), or rely overwhelmingly on the relatively small autochthonous production (Brito et al. 2006; Lau et al. 2008; March and Pringle 2003; Martineau et al. 2004; Sobczak et al. 2005; Thorp and Delong 2002). On the other side of the river continuum, estuarine studies have made the similar finding that food webs in these systems rely on autochthonous production, even though, like headwater streams, allochthonous inputs overwhelmingly dominate the organic C in the system.

In this work, I investigate the contribution of allochthonous inputs to zooplankton nutrition. In chapter 2, I use a novel cross-system analysis to examine several oligo-mesotrophic north-temperate lakes. Lakes of this type, because of their low autochthonous productivity, have been identified as the most likely type of lake to have food webs reliant on allochthonous production (France et al. 1997). In Chapter 3, I use the analysis developed in the first chapter in a meta-analysis of a wider array of temperate to subarctic lakes. These two chapters concern epilimnetic food webs. In the next chapter, I examine the possible implications of lake stratification and food web structure in a subset of the lakes examined in the first chapter. Finally, I review the findings of this work and suggest future directions.

Chapter 2
Relative contribution of autochthonous and allochthonous carbon to limnetic zooplankton: A new cross-system approach.

2.1 Introduction

Alternative sources of energy to the base of food webs may be important to ecosystem stability (Rooney et al. 2006) and production (Pace et al. 2004). In spite of this, lakes are typically studied as closed systems wherein only autochthonous production by autotrophs, such as phytoplankton, form the base of food webs. Many lakes receive sufficient allochthonous organic C from their drainage basins such that CO_2 production exceeds C-fixation and there is net CO_2 evasion (Cole et al. 1994; Jonsson et al. 2003; Sobek et al. 2003), presenting the possibility that these allochthonous contributions are important to lake food webs (Karlsson et al. 2007; Lennon 2004). However, abiotic processes as well as respiration remineralise that allochthonous C (Bertilsson and Tranvik 2000, del Giorgio et al. 1997, Graneli et al. 1996) so that CO_2 supersaturation alone does not provide evidence of the importance of allochthonous C to the food web. Measuring the quantitative significance of allochthonous C to lake food webs has proven difficult, however. Carbon stable isotopes have been used extensively in an attempt to address this (Bade et al. 2006, Carpenter et al. 2005, Cole et al. 2002, Karlsson et al. 2003, Pace et al. 2004). The $^{13}C/^{12}C$ ratio (the ^{13}C signature) of consumers reflects the ^{13}C signature of their food sources. Therefore, if the ^{13}C signature of potential food sources (allochthonous vs. autochthonous in this case) is known, the signature in consumers will indicate what the relative contribution of the potential food sources are to their nutrition (Peterson and Fry 1987). In lakes, obtaining the

autochthonous ^{13}C signature at the base of the planktonic food web has been problematic, since isolation of the autotrophic microbes from allochthonous POC is generally not possible. The most definitive studies have used whole-lake additions of inorganic ^{13}C to trace autochthonous C fixation, and have therefore involved a limited number of small lakes (Carpenter et al. 2005, Cole et al. 2002, Pace et al. 2004). Other approaches have depended on predicting or estimating the photosynthetic enrichment of ^{13}C by phytoplankton (Bade et al. 2006, Karlsson et al. 2003).

While allochthonous C can enter lake food webs in a number of ways; the main pathway to the planktonic food web is thought to be heterotrophic bacteria consuming allochthonous dissolved organic C (DOC). These bacteria, in turn, may be consumed by heterotrophic and mixotrophic protists. Zooplankton might access allochthonous energy sources by consuming protists, or through direct feeding on bacteria. Another potential pathway is direct consumption of particulate detritus by zooplankton (Cole et al. 2006), though the importance of this pathway has yet to be established.

The magnitude of allochthonous inputs can be high. However, some of the allochthonous DOC and particulate organic C (POC) entering lakes is refractory (Tranvik 1988, Tranvik and Höfle 1987). Bacterial assimilation of allochthonous material may be very inefficient, with much of the C respired rather than assimilated (Kritzberg et al. 2005). Additionally, the number of trophic steps between bacteria and consumers may result in a very small proportion of energy from allochthonous DOC and POC reaching zooplankton. The significance of allochthonous C to lake food webs has therefore remained an important, yet elusive, problem in aquatic ecology.

Here, I use a novel cross-system analysis to determine the contribution of allochthonous and autochthonous C to POC and zooplankton of several

lakes. Additionally, I derive an estimate for the mean autochthonous ^{13}C fractionation across these systems.

2.2 Materials and Methods

Lake sampling

Twenty-seven lakes in central Ontario were sampled in mid to late August of 2004 (Table 2.1). Situated in the Canadian Shield, the study lakes are small, ranging in area from 2 to 213 ha. They range from ultra-oligotrophic to meso-eutrophic, with total phosphorus (TP) concentrations of 2.7 to 25.9 $\mu g\ L^{-1}$ (mean = 8.2 $\mu g\ l^{-1}$). DOC concentrations range from 1.9 to 13 $mg\ L^{-1}$ (mean = 5.5 $mg\ L^{-1}$), with most lakes below 7 $mg\ L^{-1}$ (Ontario Ministry of the Environment, unpublished data).

All of the lakes were sampled once during 4 August to 27 August 2004, while sampling of 17 of these was repeated approximately 10 d after the first sampling to examine the temporal stability of the measurements. Samples were taken at the deepest point in each lake. Dissolved inorganic carbon (DIC) concentration, ^{13}C-DIC, CO_2 partial pressure (PCO_2), ^{13}C-POC, and chlorophyll *a* (chl *a*) samples were collected at mid-epilimnion using a peristaltic pump sampler, while zooplankton samples were collected using vertical net hauls through the epilimnion. Epilimnetic depth was determined using a YSI temperature/dissolved O_2 meter. Samples for chl *a* were collected on 25-mm Whatman GF/F filters and measured using fluorometry by the method of Strickland and Parsons (1977).

Table 2.1: Characteristics of the study lakes. Depth, area, pH, [TP], and Secchi depth data are from the Ministry of the Environment of Ontario. For lakes that were sampled twice, the average of the two measures is reported.

Lake	Lat.	Long.	Z mean (m)	Z max (m)	Area (ha)	pH	[TP] (μg L^{-1})	Secchi depth (m)	[chl *a*] (μg L^{-1})	[DIC] (μ mol L^{-1})	[DOC] (mg L^{-1})	PCO_2 (μatm)
Basshaunt	45° 07′ N	78° 28′ W	8	24	47	7.1	5.0	4.3	3.0	159	5.3	322
Bat	45° 35′ N	78° 31′ W	3	8	2	4.9	15.8	2.2	3.0	42	6.9	966
Bigwind	45° 03′ N	79° 03′ W	11	32	111	6.7	4.6	5.4	2.0	224	3.9	455
Blue Chalk	45° 12′ N	78° 56′ W	9	23	52	7.1	3.0	6.2	0.9	108	2.6	493
Brandy	45° 06′ N	79° 31′ W	4	8	108	7.1	24.4	1.3	11.9	190	13.0	1187
Buck	45° 23′ N	78° 60′ W	11	30	40	7.1	3.4	7.3	1.2	168	2.8	306
Chub	45° 13′ N	78° 59′ W	9	27	32	6.0	4.9	2.5	1.6	44	6.8	806
Crown	45° 26′ N	78° 40′ W	8	30	136	6.5	4.2	5.0	1.5	125	3.2	378
Devine	45° 12′ N	79° 14′ W	4	9	40	6.2	8.7	3.5	2.1	80	10.5	782
Dickie	45° 09′ N	79° 05′ W	5	12	93	6.2	8.3	3.0	4.2	126	6.5	743
Fawn	45° 10′ N	79° 15′ W	4	8	86	6.7	19.5	1.6	4.6	79	9.3	1234
Glen	45° 08′ N	78° 30′ W	7	15	16	8.4	5.6	4.8	1.2	932	4.4	202
Hamer	45° 14′ N	79° 48′ W	3	9	35	6.0	25.9	2.4	1.8	74	8.8	1166
Harp	45° 23′ N	79° 07′ W	12	40	67	6.3	7.2			99	3.6	211
Healey	45° 05′ N	79° 11′ W	3	7	122	6.4	11.1	1.7	2.4	84	6.4	755
Kimball	45° 21′ N	78° 41′ W	22	61	213	6.0	4.8	5.5	3.2	49	3.6	467
Leech	45° 03′ N	79° 06′ W	6	14	82	6.7	7.6	3.4	3.2	91	5.4	508
Leonard	45° 04′ N	79° 27′ W	7	15	195	6.7	5.4	5.4	1.8	60	4.8	392
Little Clear	45° 24′ N	79° 00′ W	8	25	11	7.0	4.2	4.5	2.0	120	4.1	553
McKay	45° 03′ N	79° 10′ W	5	20	122	6.7	8.1	3.0	2.7	97	5.3	732
Moot	45° 09′ N	79° 10′ W	3	8	46	6.2	13.0	1.7	2.7	64	7.2	826
Red Chalk	45° 11′ N	78° 56′ W	17	38	44	6.3	5.0			72	2.4	301
Saw	45° 03′ N	79° 02′ W	5	13	28	6.2	7.5	1.9	2.5	92	8.3	1365
Solitaire	45° 22′ N	79° 00′ W	13	31	124	7.1	3.9	7.7	0.7	95	2.7	381
Walker	45° 24′ N	79° 05′ W	6	17	68	7.1	3.8	5.0	2.0	106	4.3	532
Westward	45° 29′ N	78° 47′ W	21	44	63	6.8	2.7	5.3	2.8	56	1.9	237
Young	45° 13′ N	79° 33′ W	12	21	106	7.1	4.1	7.2	0.9	117	3.5	476

For DIC concentration, duplicate 20-mL samples were collected without headspace in rubber-stoppered vials, preserved with 0.05 mL of a saturated solution of $HgCl_2$, and then refrigerated in the dark until analysis. For analysis, a 5-mL headspace of He was created in each sample vial, then acidified with 0.1 mL of 85% H_3PO_4 to convert all DIC to CO_2. After equilibration, the headspace was analysed for CO_2 concentration using a Shimadzu 8A gas chromatograph (Stainton 1973).

Duplicate samples for PCO_2 were collected in 60-mL bottles. The bottles were prepared by adding 3.5 g KCl to each bottle, evacuating, purging with He, and re-evacuating. They were filled with sample water without introducing air by piercing the septum of the bottle with a syringe needle that was connected to a sampling pump. Samples were stored refrigerated in the dark until analysis. PCO_2 was analysed by creating a 5 mL headspace of He, allowing it to equilibrate with the sample, then analysing the headspace for CO_2 concentration with a Shimadzu 8A gas chromatograph (Stainton 1973).

At the pH range of the study lakes (4.9 to 8.4), carbonate comprises a negligible portion of the total dissolved inorganic C concentration, so can be ignored when calculating the relative concentrations of inorganic carbon species in the lakes. Concentrations of $CO_{2(aq)}$ and $HCO_3^-{}_{(aq)}$ were determined from in situ temperature, DIC, and PCO_2 (Harned and Davis 1943, Harned and Scholes 1941).

Duplicate samples for $\delta^{13}C$- DIC were collected without headspace in 125-mL bottles, preserved with 0.1 mL of a saturated solution of $HgCl_2$, and then refrigerated in the dark until analysis. For analysis, the sample was acidified with H_3PO_4 to convert all DIC into CO_2. The CO_2 was then captured by freezing in a liquid N_2 cold-trap and collected in evacuated breakseals. The collected gas was analysed using a VG Prism Series 2 dual inlet stable isotope

mass spectrometer. In situ $\delta^{13}C$ of CO_2 was calculated from in situ temperature, PCO_2, DIC concentration, and ^{13}C-DIC (Mook et al. 1974).

Three replicate POC samples for $\delta^{13}C$ were collected by pre-filtering approximately one litre of water through a 48-μm Nitex sieve (to exclude zooplankton), then filtering through a pre-combusted quartz-fibre filter (nominal pore size of 1.2 μm). Five mL of 10% HCl were added to filters to remove any inorganic C, then rinsed with 3 x 5 mL of Milli-Q water. Filters were stored frozen, then dried in a dessicator before analysis. Portions of filters were cut and analysed for $\delta^{13}C$ using a Finnegan Delta Plus continuous flow isotope ratio mass spectrometer with a Carlo Erba NA 1500 elemental (nitrogen) analyser. Analytical precision for ^{13}C and ^{15}N was 0.1‰ and 0.3‰, respectively.

Zooplankton were collected with vertical hauls through the epilimnion using a 50-cm diameter plankton net with a mesh size of 153 μm. Three replicate hauls were collected and preserved in approximately 70% (final conc.) ethanol. Samples were observed microscopically to ensure that they did not contain an appreciable amount of phytoplankton. I confirmed that ethanol preservation did not alter the ^{13}C signature of zooplankton by comparing the signatures of ethanol-preserved to unpreserved (dried immediately) samples, finding that their ^{13}C signatures did not differ.

For analysis, zooplankton were collected on a 153-μm nylon mesh, rinsed with 5 mL of 10% HCl to remove inorganic C, then with 3x5 mL of Milli-Q water. The collected zooplankton were placed in pre-combusted vials and dried at 55°C. After drying, zooplankton were ground into a fine powder, and analysed for $\delta^{13}C$ using a Micromass Isochrom continuous flow isotope ratio mass spectrometer with a Carlo Erba 1108 CNHS-O elemental analyser.

Data analyses

My hypotheses concern the slopes of relationships, but use data measured with error. Therefore, I used several regression methods depending on circumstances. Model I regression was used to determine r^2, as this is not biased by error variance in the independent variable. When dependent and independent variables were measured with similar error, a model II regression was used (Sokal and Rohlf 1981). However, when the error in the independent variable was likely to be the greater of the two, model II regression will still produce an underestimate of the true slope, as it considers the error in the two variables to be equal (Sokal and Rohlf 1981). In this case, I calculated an overestimate of the slope by performing a model 1 regression of the independent (x) variable on the dependent (y) variable, then calculating the inverse of the resulting slope (Prairie et al. 1995). Thus, the slope using this 'inverse regression' provides an overestimate of the true slope, while the model II result provides an underestimate. I performed statistical analyses using Systat version 10.

2.3 Results

Lake chl *a* concentrations ranged from 1.4 to 7.4 μg L^{-1} (mean = 2.6 μg L^{-1}). Most of the lakes were CO_2 supersaturated, or close to atmospheric saturation (approximately 377μatm; Keeling and Whorf 2005), ranging from CO_2 partial pressures of 202 to 1365 μatm, with a mean of 621 μatm (Table 2.1).

Mixing model

Providing that CO_2 was the primary source of inorganic C to phototrophs in these circum-neutral lakes (see "CO_2 availability" below), the ^{13}C signature of phototrophs should vary with that of CO_2. Therefore, if POC or zooplankton were entirely autochthonous, they would be influenced only by

the CO_2 signature and the variation in fractionation during C-fixation. Unless fractionation varied systematically with CO_2 signature, the result would be a relationship with slope = 1 between ^{13}C-POC or ^{13}C-zooplankton and $^{13}CO_2$ (Figure 2.1). If, however, they were entirely allochthonous, they would be influenced only by the allochthonous ^{13}C signature, regardless of the CO_2 signature, resulting in no relationship (i.e., slope = 0) between the ^{13}C of POC or ^{13}C-zooplankton and CO_2. Therefore, the slope of the relationship between ^{13}C-POC or zooplankton to $^{13}CO_2$ reflects the autochthonous fraction of their carbon content. The ^{13}C signature of terrestrial C3 plants is usually near -28‰, which has been confirmed for tree leaves near the study area (Aravena et al. 1992).

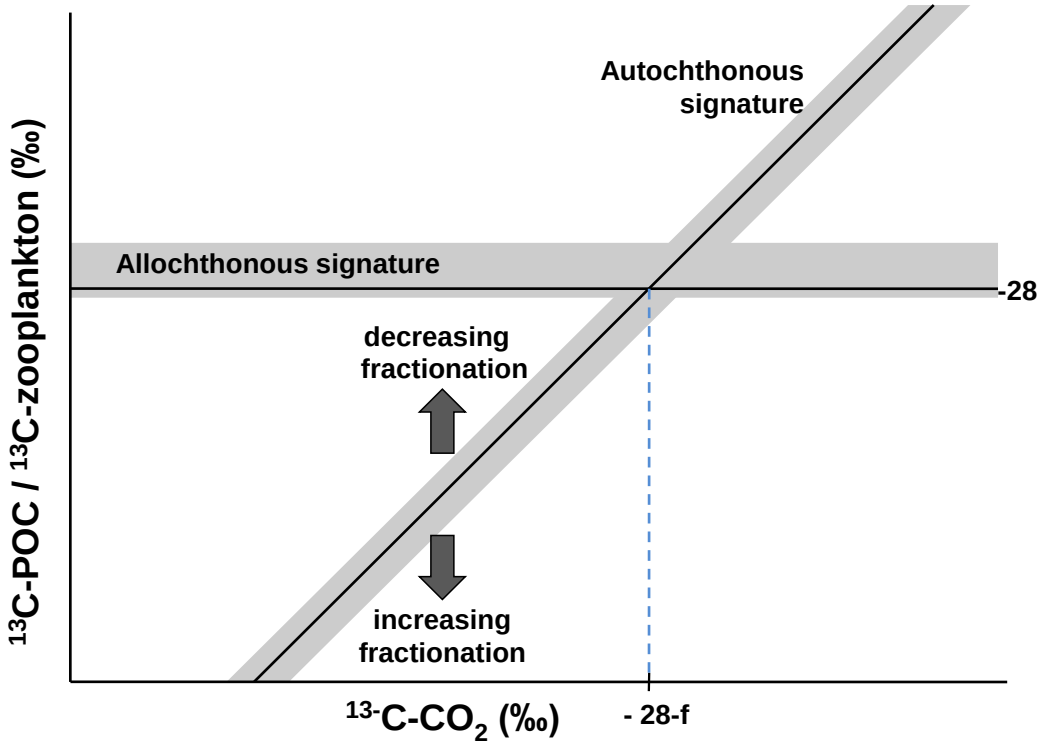

Figure 2.1: Conceptual diagram of the relationship between ^{13}C-POC and ^{13}C-zooplankton with varying $^{13}CO_2$ and the effect of varying proportion allochthonous on the relationship.

Photoautotrophs assimilate CO_2 with a bias against $^{13}CO_2$. Autochthonous POC is therefore depleted in ^{13}C compared to $^{13}CO_2$. I used the relationship between ^{13}C-POC and $^{13}CO_2$ to determine the average POC C-fractionation across the study lakes. I then used this value to calculate the proportion of allochthonous and autochthonous contribution to POC on a lake-by-lake basis using a simple mixing-model:

$$^{13}C\text{-POC} = a(^{13}CO_2 + f) - 28(1-a) \quad \text{(eq. 2.1)}$$

$$^{13}C\text{-zooplankton} = [a(^{13}CO_2 + f) - 28(1-a)\ ] + 1 \quad \text{(eq. 2.2)}$$

where a = the fraction of autochthonous contribution, -28 = the allochthonous signature (‰) (Lajtha & Marshall 1994), f = POC ^{13}C fractionation (‰), and 1 is the trophic fractionation between zooplankton and POC (‰) (DeNiro and Epstein 1978).

Autochthonous contribution to POC and zooplankton

Using a linear fit to the ^{13}C-POC vs. $^{13}CO_2$ relationship (which assumes the proportion allochthonous is constant with $^{13}CO_2$), results in a strong positive relationship ($P < 0.001$) between ^{13}C-POC and $^{13}CO_2$, ($r^2 = 0.75$; Figure 2.2a). The model II slope of this relationship was 0.62. However, since the two variables were measured in different ways, it is unlikely that the errors would be equal in magnitude. A comparison of the correlation of $^{13}CO_2$ at the first sampling time (T_1) vs. the second sampling time (T_2) is weaker ($r = 0.82$) than ^{13}C-POC at T_1 vs. T_2 ($r = 0.91$) suggesting that the variation due to error and/or temporal variation in $^{13}CO_2$ is likely to be greater than for ^{13}C-POC. Model II regression would underestimate the slope in this case. The slope calculated using inverse regression was 0.75. The mean proportion of autochthonous POC is, therefore, between 62% and 75%.

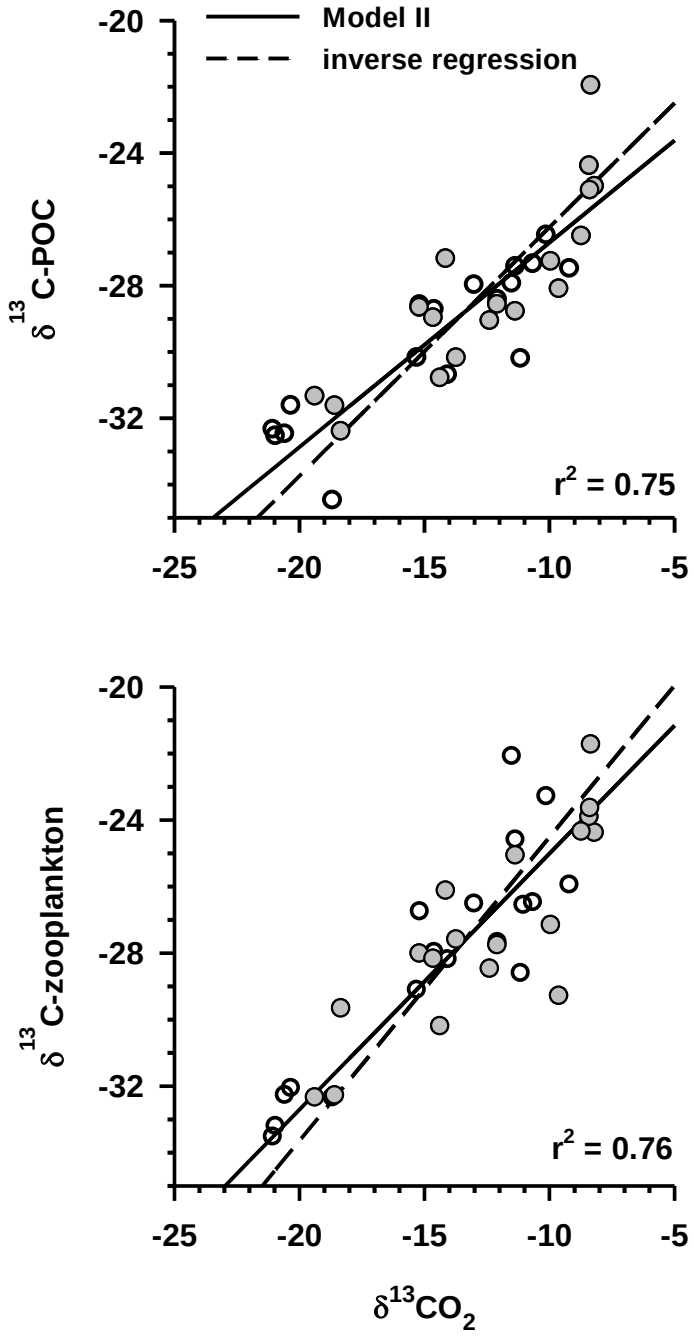

Figure 2.2: (a) δ^{13}C-POC and (b) δ^{13}C-zooplankton vs. $\delta^{13}CO_2$ in 27 lakes on the Canadian Shield in central Ontario in August, 2004. 17 of the lakes were re-sampled 6-11 days afterwards, and these data are included. Grey circles denote samples taken at the first sampling time while open circles denote those taken at the second sampling time. In a), using model II regression, y=0.62x-20.5, while y=0.75x-18.7 using inverse regression. In b), Using model II regression, y=0.77x-18.3. For inverse regression, y=0.91x-15.4.

The significant ($P < 0.001$) relationship between ^{13}C-zooplankton vs. $^{13}CO_2$ is similar to that of ^{13}C-POC vs. $^{13}CO_2$, with $^{13}CO_2$ explaining 76% of the variation in ^{13}C-zooplankton. As with ^{13}C-POC, the correlation between ^{13}C-zooplankton at T_1 and T_2 is higher ($r = 0.90$) than that of $^{13}CO_2$ at T_1 and T_2 ($r = 0.82$), suggesting that the error in $^{13}CO_2$ is greater than that of ^{13}C-zooplankton. As a result, the slope of the model II regression of 0.77 is likely an underestimate, while the slope of the inverse regression of 0.91 is likely an overestimate. Thus, the mean autochthonous contribution to zooplankton is between 77% and 91%.

CO_2 availability

Respiration produces ^{13}C-depleted CO_2, and increases PCO_2. Hence, depletion of $^{13}CO_2$ relative to the atmosphere should be related to an increase in PCO_2. I assessed this by examining the relationship between $^{13}CO_2$ and PCO_2, finding that PCO_2 is indeed negatively ($P < 0.001$) related to $^{13}CO_2$ ($r^2 = 0.61$; Figure 2.3). The approach used in this study requires that the magnitude of phytoplankton fractionation does not vary systematically with $^{13}CO_2$. Related to this, one might expect the C fractionation by phytoplankton would increase with increasing CO_2 availability. To assess this possibility, I looked for a relationship between the difference between ^{13}C-POC and $^{13}CO_2$ (POC fractionation) and PCO_2. Similarly, I examined the fractionation between ^{13}C-zooplankton and $^{13}CO_2$ (zooplankton fractionation) to determine if an effect of CO_2 availability was transferred to zooplankton. The POC fractionation, however, *decreases* significantly ($P < 0.001$) with an increase in PCO_2 ($r^2 = 0.42$; Figure 2.4). This trend was not observed in zooplankton fractionation with PCO_2 (not shown, $P = 0.07$, $r^2 = 0.10$).

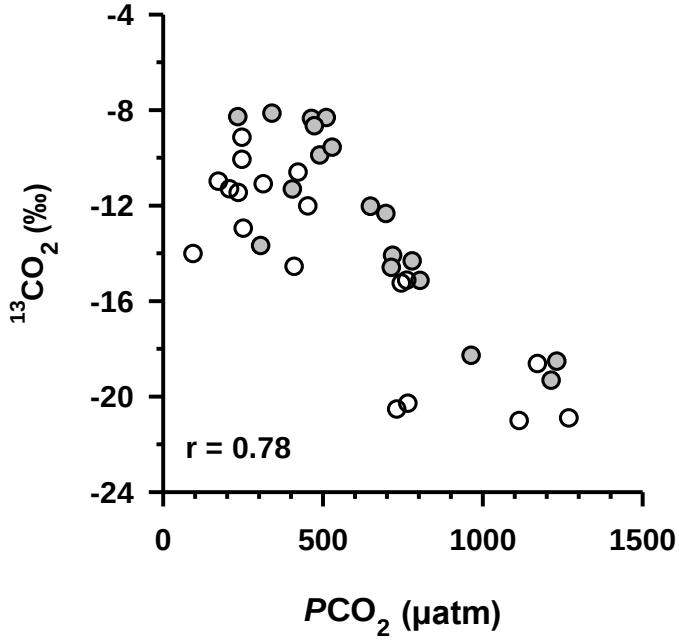

Figure 2.3: $\delta^{13}CO_2$ vs. PCO_2 of the study lakes. Grey circles denote samples taken at the first sampling time while open circles denote those taken at the second sampling time.

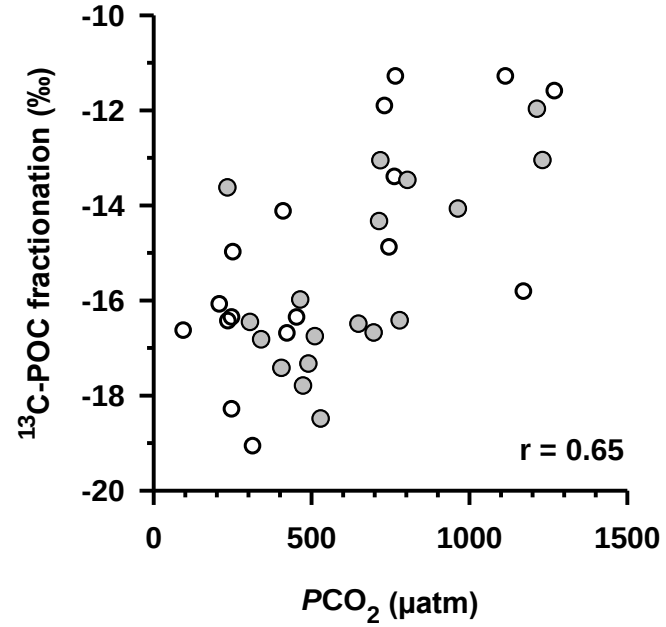

Figure 2.4: POC fractionation of $^{13}CO_2$ vs. PCO_2. Grey circles denote samples taken at the first sampling time while open circles denote those taken at the second sampling time.

Zooplankton-POC relationship

I found a strong, significant relationship between ^{13}C-POC and ^{13}C-zooplankton ($P < 0.001$), with an r^2 of 0.78 (Figure 2.5). Zooplankton ^{13}C is generally slightly enriched compared to ^{13}C-POC, with a mean enrichment of 1.2‰. A line with a slope of one and an intercept of 1‰ (to account for trophic enrichment) is a reasonable approximation of the relationship between ^{13}C-zooplankton and ^{13}C-POC. The model II slope was 1.24, indicating that ^{13}C-zooplankton increases more rapidly with $^{13}CO_2$ than the ^{13}C-POC. Only 7 of 54 points in Figure 5 indicate zooplankton that are depleted relative to POC, and these are in lakes with very depleted POC (-28 to -32‰).

As with the ^{13}C-zooplankton vs. ^{13}C-POC relationship, zooplankton ^{15}N was also enriched compared to ^{15}N-POM (not shown). The relationship was also significant, ($P < 0.001$), though weaker ($r^2 = 0.34$) than the ^{13}C-zooplankton vs. ^{13}C-POC relationship. Mean enrichment of ^{15}N-zooplankton over that of ^{15}N-POM was 3.0‰.

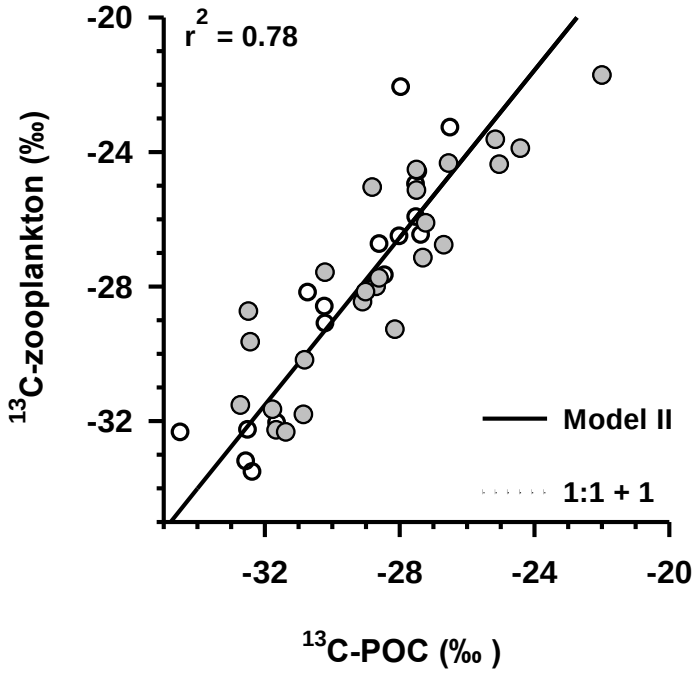

Figure 2.5: δ^{13}C-zooplankton vs. δ^{13}C-POC of the study lakes. For model II regression, y=1.2x+8.3. Grey circles denote samples taken at the first sampling time while open circles denote those taken at the second sampling time.

Autochthonous fractionation

At the point (-12.1‰) where the model II regression line crosses the allochthonous signature (-28‰), the proportion of autochthonous and allochthonous C does not affect the ^{13}C signature of POC; only the fractionation due to photosynthesis affects this value (Figure 2.2a). Therefore, I used the corresponding $^{13}CO_2$ signature at this point to calculate the average autochthonous fractionation in the study lakes. That is, fractionation equals -15.9‰ (-28‰–(-12.1‰)). Using the inverse regression rather than model II produces a very similar value for fractionation (-15.7‰).

2.4 Discussion

Autochthonous contribution to POC and zooplankton

Using a survey of many lakes, I estimate that the average proportion of autochthonous C in POC was between 62 and 75% (Figure 2.2a) while the zooplankton proportion autochthonous was higher, between 77% to 91% (Figure 2.2b). One strength of the approach used here is that because the baseline used is $^{13}CO_2$, rather than POC or some fraction of it, I did not require an estimate of the assimilable fraction of POC. It also is unaffected by mixotrophy, which can be important in oligotrophic lakes (Nygaard and Tobiesen 1993). Another advantage is that the measure of the average proportion autochthonous C in POC and zooplankton requires only the ^{13}C signatures of CO_2, POC, and zooplankton. Further, an estimate of phytoplankton ^{13}C fractionation for the cross-lake autochthonous estimate was not required. I calculated the average phytoplankton fractionation directly from the ^{13}C-POC vs. $^{13}CO_2$ relationship, allowing an estimate of the contribution of autochthonous C to POC and zooplankton on a lake-by-lake basis.

Generally, the measure of the average proportion of autochthonous contribution to POC and zooplankton in the present work is higher than that of other studies. In a study of 15 small lakes (0.01 to 0.27 km^{-2}) in Sweden, Karlsson et al. (2003) estimated that zooplankton were 53% autochthonous. Whole-lake ^{13}C-DIC addition experiments (Carpenter et al. 2005, Pace et al. 2004) have also generally shown higher allochthonous contributions for POC (45 to 60%) and zooplankton (50 to 78%) than the present work. Allochthonous inputs might be more important in smaller lakes. The lakes in those ^{13}C-DIC addition experiments studies ranged from 0.008 to 0.027 km^{-2}, on the lower range of the lakes that I sampled (0.02-2.1 km^{-2}). A ^{13}C-DIC addition

experiment to a larger lake (Crampton Lake, 0.26 km^{-2}) found a dominance of autochthonous production to POC and zooplankton (88% and 92% autochthonous, respectively). However, in these data I did not find a relationship between lake area and the proportion of autochthonous contribution to POC or zooplankton (not shown).

Conversely, a whole-lake ^{13}C-DIC addition experiment to a nutrient-enriched lake (East Long Lake, Wisconsin) found that POC and zooplankton were largely autochthonous (Cole et al. 2002). Similarly, POC and zooplankton in Peter Lake, Wisconsin, were largely allochthonous, but became largely autochthonous after enrichment (Carpenter et al. 2005). Data from the present study do not show a direct relationship between increasing nutrients (TP) and autochthony, due possibly to the correlation between loading of DOC and TP (see below).

Sampling, done in late-summer, may have affected the finding of autochthonous dominance in zooplankton and POC. A seasonal study of Loch Ness by Grey et al. (2001) found mean annual zooplankton C was 60% autochthonous. However, they estimated that filter-feeding *Daphnia hyalina* in late summer were approximately entirely autochthonous, and the herbivorous copepod *Eudiaptomus gracilis* appeared to be close to 100% autochthonous throughout the year. Thus, I may have sampled at a time of year when allochthonous influence was minimal.

Sources of variability in POC and zooplankton signature

Although POC is often used as a surrogate for phytoplankton, POC samples (1.2 to 48 μm) are variable mixtures of autotrophs, heterotrophs that may use both autochthonous or autochthonous DOC, flocculated DOC, and predators that are in the nanoplankton to microplankton size classes. Photosynthetic fractionation may vary between 0 and -22‰. Further, the

signature of the DOC will have been enriched by partial mineralization, so it is not surprising that the ^{13}C signature of this mixture is variable beyond what can be accounted for by the ^{13}C signature of CO_2. Indeed, it is surprising that $^{13}CO_2$ can account for 75% of this variability.

Zooplankton may be a more homogeneous fraction, but there are sources of variability beyond that which can be attributed to POC. Although ^{13}C trophic fractionation is typically assumed to be 1‰ per trophic level, this may vary (DeNiro and Epstein 1978). Also, some zooplankton might be more than one trophic step higher than POC. For example, predatory zooplankton such as cyclopoids should be at a higher trophic level than herbivores such as calanoids (Matthews and Mazumder 2003). Zooplankton feeding directly or indirectly on components of the microbial food web (Perga et al. 2006) may also appear at a higher effective trophic level, introducing variation in the ^{13}C-zooplankton vs. ^{13}C-POC relationship. Nonetheless, these additional sources of variation seem relatively small in the present work as the relationship of zooplankton signature to CO_2 signature is as strong as that of ^{13}C-POC vs. $^{13}CO_2$.

Several sources may have contributed to the 22% of the variation in ^{13}C-zooplankton not explained by variation in ^{13}C-POC (Figure 2.5). Variation in zooplankton trophic fractionation and error due to temporal-spatial variability appear to be small, as discussed above, but a larger source of variation could be a variable portion of inedible or indigestible particles with a different signature from the rest of the POC.

Zooplankton selective feeding on POC

Zooplankton ^{13}C signature was, on average, 1.2‰ enriched compared to POM, while zooplankton ^{15}N was 3‰ enriched to PON on average. These

values are consistent with POM being the major food source for zooplankton (Vander Zanden et al. 2001; Post 2001).

That the slope of the ^{13}C-zooplankton vs. ^{13}C-POC was 1.24, however, suggests some bias in the feeding of herbivorous zooplankton towards the autochthonous fraction of POC. The autochthonous portion of POC at relatively depleted ^{13}C-POC values (left side of Figure 2.5) would have been produced from depleted $^{13}CO_2$, thus producing autochthonous C that is depleted relative to allochthonous C. Therefore, at the more depleted ^{13}C-POC, zooplankton selectively feeding on autochthonous C would be depleted relative to POC. At the enriched end of the POC scale, the opposite would occur. That is, the autochthonous C would be enriched relative to allochthonous C, so that zooplankton selecting autochthonous C would appear overly enriched in relation to POC. This effect is evident in Figure 2.5; the zooplankton that are depleted relative to POC are on the left or depleted end of the ^{13}C-POC scale.

Evidence of selective feeding is also apparent in the ^{13}C –POC vs.$^{13}CO_2$ and the ^{13}C-zooplankton vs. $^{13}CO_2$ relationships (Figure 2.2). The lower slope of the ^{13}C-POC vs. $^{13}CO_2$ relationship compared to that of the ^{13}C zooplankton vs. $^{13}CO_2$ relationship indicates that zooplankton are more autochthonous than is POC. Consequently, zooplankton must be selecting the autochthonous portion from the bulk POC. Arithmetically, the ratio of the slope of ^{13}C-zooplankton vs. CO_2 and ^{13}C-POC vs. $^{13}CO_2$ should be equal to the slope of the ^{13}C-zooplankton vs. ^{13}C-POC relationship. This is approximately what I found (ratios of 1.24 and 1.21, respectively, for model II and inverse regressions), demonstrating that the two analyses are in agreement with each other.

Autochthonous fractionation

I calculated an average POC fractionation of –15.9‰. This estimate represents the average fractionation across lakes, and not that of any one lake. It is likely that fractionation in POC varies among these lakes. Since $^{13}CO_2$ explained 76% of the variation in ^{13}C-POC, however, variable C fractionation among lakes could contribute a maximum of 24% of the variation in the relationship. Other sources of variation, for example in the fraction of allochthonous C in POC and temporal variation in the $^{13}CO_2$ signature, must be included in that 24%. While the maximum fractionation of inorganic C from discrimination by Rubisco ranges from -25 to -28‰ (Goericke et al. 1994), the magnitude of this can be reduced by several factors, including phytoplankton growth rate, CO_2 concentration (Rau et al. 1996), cell size (Popp et al. 1998), light, or nutrient limitation (Burkhardt et al. 1999). Similar to the present work, other lake studies have found lower values for phytoplankton fractionation. Using whole-lake ^{13}C-addition experiments, Cole et al. (2002), and Pace et al. (2004), estimated phytoplankton fractionation ranging from -6‰ to -11.5‰. In a large comparative study, Bade et al. (2006) also found low phytoplankton fractionation, ranging from 0 to 15‰.

The relationship between ^{13}C-POC fractionation and PCO_2 (Figure 2.4) is puzzling. If CO_2 was sufficiently abundant, I would expect no relationship between ^{13}C-POC fractionation and PCO_2. Conversely, if the CO_2 supply was affecting fractionation, then ^{13}C-POC fractionation would decrease at low PCO_2. My finding, however, was that ^{13}C-POC fractionation decreased with increasing PCO_2. One possibility is that higher PCO_2 is related to a higher allochthonous contribution to POC. Since depleted $^{13}CO_2$ is related to high PCO_2 (Figure 2.3), lakes with depleted $^{13}CO_2$ are likely to be lakes that have higher allochthonous inputs. The apparent decrease in fractionation at high PCO_2 may, therefore, be due to an increase in the allochthonous contribution to

POC. Other evidence indicates that zooplankton select autochthonous C from POC. The lack of a significant relationship between zooplankton ^{13}C-fractionation and PCO_2 lends support to the former hypothesis that the relationship observed between ^{13}C-POC and PCO_2 is a result of a varying allochthonous contribution to POC.

Conclusions

This work demonstrates that the relationship between the ^{13}C of POC and zooplankton to $^{13}CO_2$ can be used to determine the autochthonous portion of each on a cross-system average basis. This technique should be applicable to other systems so long as appreciable use of bicarbonate by autotrophs is not occurring. An independent estimate of ^{13}C fractionation by photosynthesis is not required. Rather, this approach generates an average value that can be used to estimate the contribution of autochthonous C to POC and zooplankton in most lakes (i.e., those where allochthonous and autochthonous C signatures are different). Further application of this approach to different sets of lakes at different seasons could improve our understanding of the factors that determine the allochthonous C contribution to lake food webs.

Chapter 3
Terrestrial carbon subsidies to lake planktonic food webs: A meta-analysis

3.1 Introduction

Lakes typically receive appreciable allochthonous inputs (Wetzel 1992). These inputs contribute to lake respiration (Lennon 2004) and net heterotrophy is common in lakes (Cole et al. 1994), observations that have led to the supposition that terrestrially-fixed C entering lakes may be incorporated into lake food webs as well as be respired (del Giorgio and Peters 1993). Quantifying the relative contribution of these allochthonous inputs to aquatic systems has, however, proven a challenging problem.

Stable isotope methods have been the major approach used to elucidate the degree to which lake food webs are fuelled by allochthonous production. Relying on the ability to distinguish organic C from terrestrial and within-lake sources based on their ratio of ^{13}C:^{12}C, this approach, however, has not been without major impediments. The greatest challenge in using stable isotopes to measure the importance of allochthony has been in obtaining an estimate for the autochthonous signature (Post 2002).

Some workers have physically separated allochthonous particulate organic C (POC) from the autochthonous POC (Grey et al. 2001, Jones et al. 1998, Rautio and Vincent 2007), a method that assumes that the separable phytoplankton represent the overall autochthonous signature, which is known to vary among taxa, and with cell size and growth rate (Burkhardt et al. 1999). Because physical separation of phytoplankton is difficult or impossible, the separation and analysis of specific biomarker compounds has also been used. While promising, these methods suffer many of the drawbacks of physical

separation of phytoplankton, with the additional problem that the signature of the extracted compounds might not be representative of the entire phytoplankton cell (Boschker and Middelburg 2002).

Another approach has been to make the autochthonous signature more clearly distinct from the allochthonous through the addition of ^{13}C-labelled bicarbonate, either to entire lakes (Carpenter et al. 2005, Carpenter et al. 2007) or to mesocosms (Taipale et al. 2007). Problems with the application of this technique include the high cost of addition experiments as well as the extended monitoring time (several weeks) required to study a single system.

Another potential problem with the whole-lake addition experiments, as well as some other studies (Karlsson et al. 2003, Pulido-Villena et al. 2005), is that they assume, without testing, that CO_2 is the only source of C used for C-fixation by phytoplankton, which may not be valid under all conditions (Marty and Planas 2007). Variable fractionation of $^{13/12}CO_2$ during photosynthesis may also present a problem in the use of CO_2 as the baseline for autochthonous production. Because phytoplankton favour the light (^{12}C) isotope over ^{13}C, the fixed C produced through photosynthesis will typically be more depleted than the inorganic source. The maximum fractionation, based on the enzyme catalysing the first major step of photosynthesis (Rubisco), of -28 to -25 (Goericke et al. 1994) has often been assumed as the fractionation between CO_2 and lake phytoplankton. Some studies, however, have found fractionation to be weaker than this (Bade et al. 2006, Carpenter et al. 2005, Lennon et al. 2006, Taipale et al. 2007). Thus, for CO_2 to serve as a useful end-member, it would have to be the source of inorganic C for photosynthesis, and fractionation must be predictable (though it need not be maximal).

In Chapter 2, I found that these requirements are met in a set of lakes in the Canadian Shield, finding a high degree of autochthony in POC and

especially in zooplankton in those lakes. In the present work, I culled the literature for suitable data to compare the $\delta^{13}C$ of CO_2, POC, and zooplankton. Applying a similar approach to the previous chapter, I control for the possibility of CO_2-limitation and bicarbonate use, then use a mixing model to quantify the average allochthonous contribution to POC and zooplankton across systems, as well as generate a cross-system estimate of photosynthetic fractionation.

3.2 Methods

Study descriptions

I pooled data ($^{13}CO_2$, ^{13}C-POC, ^{13}C-zooplankton, CO_2 concentration, and PCO_2), from 7 studies from the literature (Table 3.1), using both multiple-lake studies (Bade et al. 2006, Karlsson et al. 2003, Lennon et al. 2006, Marty and Planas 2007), as well as seasonal studies on single lakes (Grey et al. 2001, Gu et al. 2006, Gu et al. 1994, Jones et al. 1999). Lakes ranged from sub-arctic to temperate. In the multiple lake studies where lakes were sampled on more than one occasion, I treated the samples as independent.

Calculations

Where not provided, concentrations of $CO_{2(aq)}$ and $HCO_{3\ (aq)}^-$ were determined from *in situ* temperature, dissolved inorganic carbon (DIC) concentration, and CO_2 partial pressure (PCO_2) (Harned and Davis 1943, Harned and Scholes 1941) or from pH and DIC concentration (Stumm and Morgan 2007). *In situ* $\delta^{13}C$ of CO_2 was calculated from *in situ* temperature, $CO_{2(aq)}$ concentration, DIC concentration, ^{13}C-DIC, and ^{13}C- HCO_3^- (Mook et al. 1974).

A key prerequisite for this approach is that CO_2 is the sole C source for autochthonous production. It is therefore important that phytoplankton were

not limited by CO_2 availability (which would reduce fractionation), nor were they assimilating bicarbonate (which is enriched in ^{13}C relative to CO_2). I tested this by relating the difference between ^{13}C-POC and $^{13}CO_2$ (POC fractionation) to CO_2 concentration to assess the possibility of CO_2-limitation. While POC is not entirely autochthonous (see Results), a pattern of increasing depletion of ^{13}C-POC relative to $^{13}CO_2$ with increasing CO_2 concentration would suggest CO_2-limitation of the autochthonous portion of POC. I omitted samples within a study that showed a pattern of increasing ^{13}C-POC depletion relative to $^{13}CO_2$ with increasing CO_2 concentration (suggesting CO_2-limitation), or if ^{13}C-POC was enriched relative to $^{13}CO_2$ (suggesting bicarbonate use).

Table 3.1: Literature studies used with general lake characteristics. Values in brackets are averages.

Study	No. of lakes	Location	Area (ha)	Mean depth (m)	TP (μg L^{-1})	pH	Chl *a* (μg L^{-1})	[DOC] (mg L^{-1})	[DIC] (mg L^{-1})	PCO_2 (μatm)
Bade et al (2006)	41	Michigan and	0.3-1091 (80.6)	-	4.4-105 (29)	4-9 (6.5)	1.7-57 (12)	1.6-24.6 (9.9)	0.1-20 (5.3)	33-7280 (1214)
Grey et al (2001)	1	Loch Ness, Scotland	5850	132	10*	6-6.2	-	4*	-	688
Gu et al (1994, 1999)	1	Smith L., Alaska	720	2.5	-	6.6	-	40-44	2.1-6.8 (3.9)	986-2686 (1770)
Gu et al (2006)	1	L. Wauberg, Florida	150	3.0	118	6.7-10 (8.1)	92	-	3.3-6.5 (4.1)	0.9-3857 (749)
Karlsson et al (2003)	15	N. Sweden	1-27 (9.2)	0.6-8.6 (3.2)	2.9-11 (6.5)	-	-	1.9-9 (4.7)	0.4-9.2 (1.8)	342-7386 (492)
Lennon et al (2006)	36	N.E. US	-	-	7.1-985 (179)	4.3-8.8 (7.1)	1.4-21 (6.6)	2.7-12 (6.8)	-	-
Marty and Planas (2007)	19	Quebec Shield	30-2.6x10^5 (384)	1.6-61.6 (14.6)	-	6-7.5 (6.7)	0.6-2.8 (1.4)	2.2-12.5 (6.1)	0.4-3.1 (1.2)	398-1234 (682)
Chapter 1	27	Ontario Shield	2.3-213 (77)	2.8-22 (7.8)	3.2-29 (10.2)	5.7-7.8 (6.0)	0.7-12 (2.6)	1.4-11 (4.5)	0.5-11 (1.6)	202-1365 (621)

*listed as typical value in reference

Estimates of POC and zooplankton proportion autochthonous

For those samples where I did not find evidence for CO_2-limitation, the signature of phototrophs should vary with that of CO_2. Therefore, if POC or zooplankton were entirely autochthonous, they would be influenced only by the CO_2 signature, resulting in a relationship with slope = 1 between ^{13}C-POC or ^{13}C-zooplankton and $^{13}CO_2$. If, however, they were entirely allochthonous, they would be influenced only by the allochthonous signature, regardless of the CO_2 signature, resulting in no relationship (i.e., slope = 0) between the ^{13}C of POC or ^{13}C-zooplankton and CO_2. Therefore, the slope of the relationship between ^{13}C-POC or zooplankton to $^{13}CO_2$ reflects the autochthonous fraction of their carbon content.

Ordinary least-squares (OLS) regression assumes that the x-variable ($^{13}CO_2$ in this case) is measured without error. The $^{13}CO_2$ signature would, however, have sampling and analysis error associated with it, similar to that of the error in measuring ^{13}C-POC. In this situation, OLS will underestimate the slope of the relationship. Model 2 regression assumes equal error in each variable, so will produce a more accurate estimate of the true slope. Because of this, I estimated the slope of the ^{13}C-POC vs. $^{13}CO_2$ and ^{13}C-zooplankton vs. $^{13}CO_2$ relationships using model 2 regression.

3.3 Results

$^{13}CO_2$-PCO_2

Lake PCO_2 ranged from 0.9 μatm (Gu et al. 2006) to 7386 μatm (Karlsson et al. 2003) with a mean of 857 μatm. Assuming an atmospheric CO_2 saturation of 377 μatm (Keeling and Whorf 2005), the majority of lakes were supersaturated, spanning a range of 0.002 to 20 times atmospheric saturation (Figure 3.1). Signatures of $^{13}CO_2$ ranged from -7.2‰ (Gu et al. 2006) to -41‰ (Marty and Planas 2007) with the greatest range in $^{13}CO_2$ at low PCO_2. Usually, $^{13}CO_2$ was enriched at low PCO_2, with values > -20‰. However, data from three of the studies did not demonstrate this pattern. In the lakes and reservoirs studied by Marty and Planas (2007) and some of lakes in Bade (2006), systems with low PCO_2 had highly depleted $^{13}CO_2$. In the study of Lake Wauberg by Gu et al. (2006), $^{13}CO_2$ ranged from -7.2‰ to -26.2‰ but was not related to PCO_2.

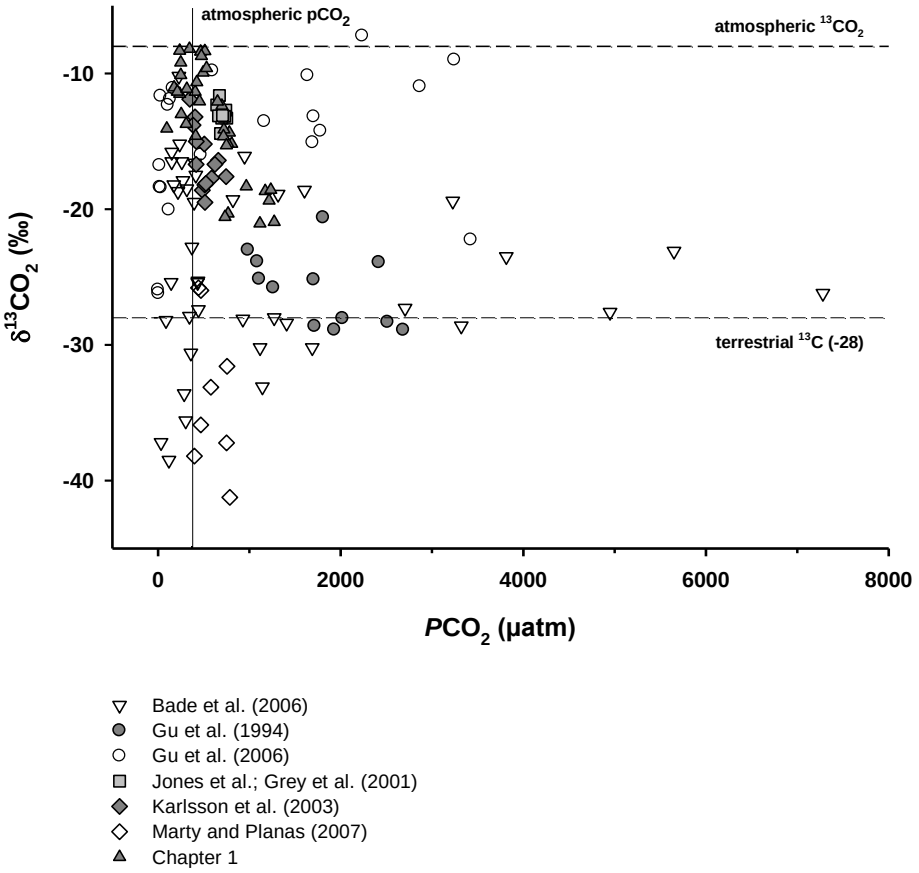

Figure 3.1: Relationship between $^{13}CO_2$ and PCO_2. The vertical line represents approximate PCO_2 at atmospheric saturation while the horizontal line indicates a typical allochthonous ^{13}C signature of -28‰.

CO_2-limitation

As mentioned earlier, an important requirement of this analysis is that autochthonous production was not limited by the availability of CO_2. There was evidence of CO_2 limitation in three of the studies (Figure 3.2). In the study by Gu et al. (2006), ^{13}C-POC – $^{13}CO_2$ declined with increasing CO_2 concentration. For several of the lowest CO_2 concentrations, ^{13}C-POC–$^{13}CO_2$ was positive, suggesting that use of bicarbonate by phytoplankton was occurring. I therefore omitted these data from further analyses. I also omitted the lakes and reservoirs in Marty and Planas (2007) as ^{13}C-POC

ranged from being only slightly depleted relative to $^{13}CO_2$ to being appreciably enriched, which, as with Gu et al. (2006), suggested CO_2-limitation and/or bicarbonate use by phytoplankton. In the study of Bade et al. (2006), some lakes showed a pattern of increasing ^{13}C-POC depletion to $^{13}CO_2$ with increasing CO_2 concentration, as well as some positive POC fractionations at lower CO_2 concentrations. This increase in POC fractionation with increasing CO_2 concentration was only evident at CO_2 concentrations lower than 100 μM. Consequently, I excluded only those samples in Bade et al. (2006) with a CO_2 concentration less than 100 μM, retaining those greater than 100 μM for further analyses.

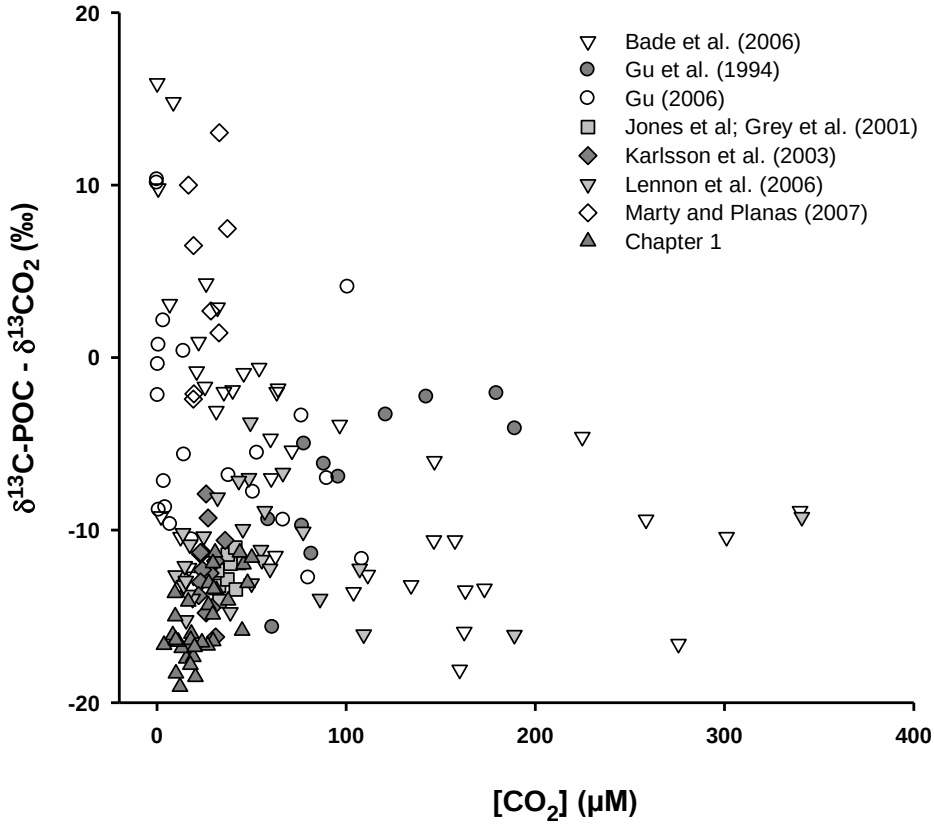

Figure 3.2: Fractionation between ^{13}C signature of POC and $^{13}CO_2$ vs. CO_2 concentration.

In the remaining data, I did not find evidence of CO_2-limitation. Indeed, at lower CO_2 concentrations (~100 μM), POC fractionation tended to increase with increasing CO_2 concentration. Since there is no indication of CO_2-limitation in these studies I used them for further analyses. I will revisit the pattern of decreasing difference between ^{13}C-POC and $^{13}CO_2$ after generating estimates of proportion of POC that is autochthonous and the autochthonous fractionation (see below).

Allochthonous contribution to POC

The ^{13}C-POC signatures after censoring the data ranged from -22 to -35‰. A positive, linear relationship between ^{13}C-POC and $^{13}CO_2$ explained

49% ($P<0.001$) of the variation in ^{13}C-POC (Figure 3.3). The model 2 slope of the relationship is 0.47±0.08 (Wald 95% confidence interval). Thus, the average autochthonous proportion of POC was 47±8%. With the data the I removed due to evidence of bicarbonate use or CO_2-limitation included, a linear relationship between ^{13}C-POC and $^{13}CO_2$, while significant ($P<0.001$) explained only 11% of the variation in ^{13}C-POC. The model 2 slope of the relationship is also lower at 0.24±0.09 (Wald 95% confidence interval).

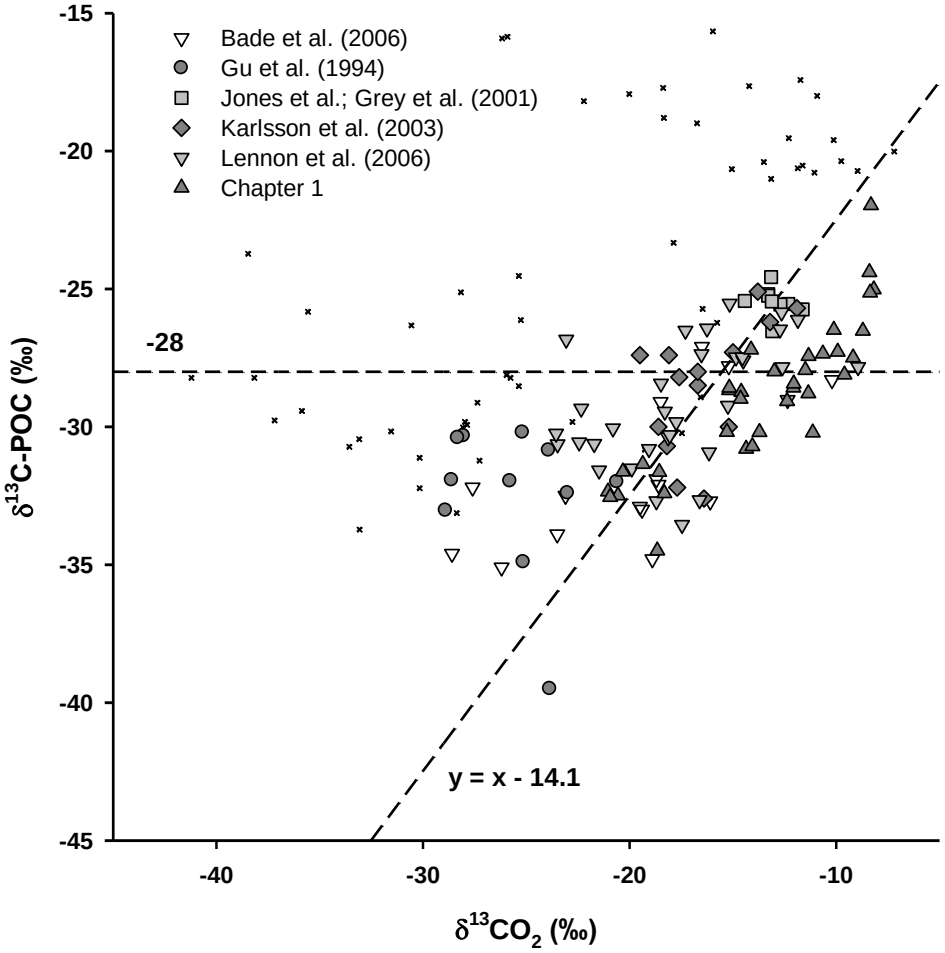

Figure 3.3: Relationship between ^{13}C signature of POC and $^{13}CO_2$. The horizontal line represents the signature of allochthonous C (-28). The model 2 slope of the relationship is 0.47 (r^2=0.49). The 1:1 line is passed through mean x, mean y. Points shown as '×' were omitted from statistical analyses as they showed evidence of CO_2-limitation (see text and Figure 3.2).

Several $^{13}CO_2$ values from the studies done on the subarctic Smith Lake by Gu et al. (1999, 1994) were especially depleted relative to the majority of the data, yet the corresponding ^{13}C-POC were not especially depleted. Because these points were toward the extreme of the $^{13}CO_2$ range, they exert a strong influence on the regression. Removing these data from this study did not change the strength of the relationship (r^2=0.49) and

resulted in a slight increase in the estimate of the autochthonous contribution to POC (52±10%).

Zooplankton proportion autochthonous

Zooplankton ^{13}C signature ranged from -41.1‰ to -21.8‰. As with ^{13}C-POC, zooplankton ^{13}C was positively related to $^{13}CO_2$, with a linear relationship explaining 68% ($P<0.001$) of the variation in ^{13}C of zooplankton (Figure 3.4). The slope of the relationship, using model 2 regression, is 0.87±0.11 (Wald 95% confidence interval). As with the ^{13}C-POC vs. $^{13}CO_2$ relationship, the data from Smith Lake (Gu et al. 1999, Gu et al. 1994) have an especially strong effect on the slope. Removing these data, the model 2 slope increases to 1.07±0.16, with a slight weakening in the strength of the relationship ($r^2=0.64$). Therefore, the estimate of the average proportion of autochthonous C in zooplankton is 75% to 98% autochthonous with the data of Smith L. included, and 91% to 100% with those data excluded. As mentioned previously, I excluded the data of Marty and Planas (2007) due to evidence of bicarbonate use from the above analyses. With these data included, a linear relationship between ^{13}C-zooplankton and $^{13}CO_2$ explains 36% of the variation in ^{13}C-zooplankton ($P<0.001$). The slope of the relationship is also much lower at 0.39±0.09.

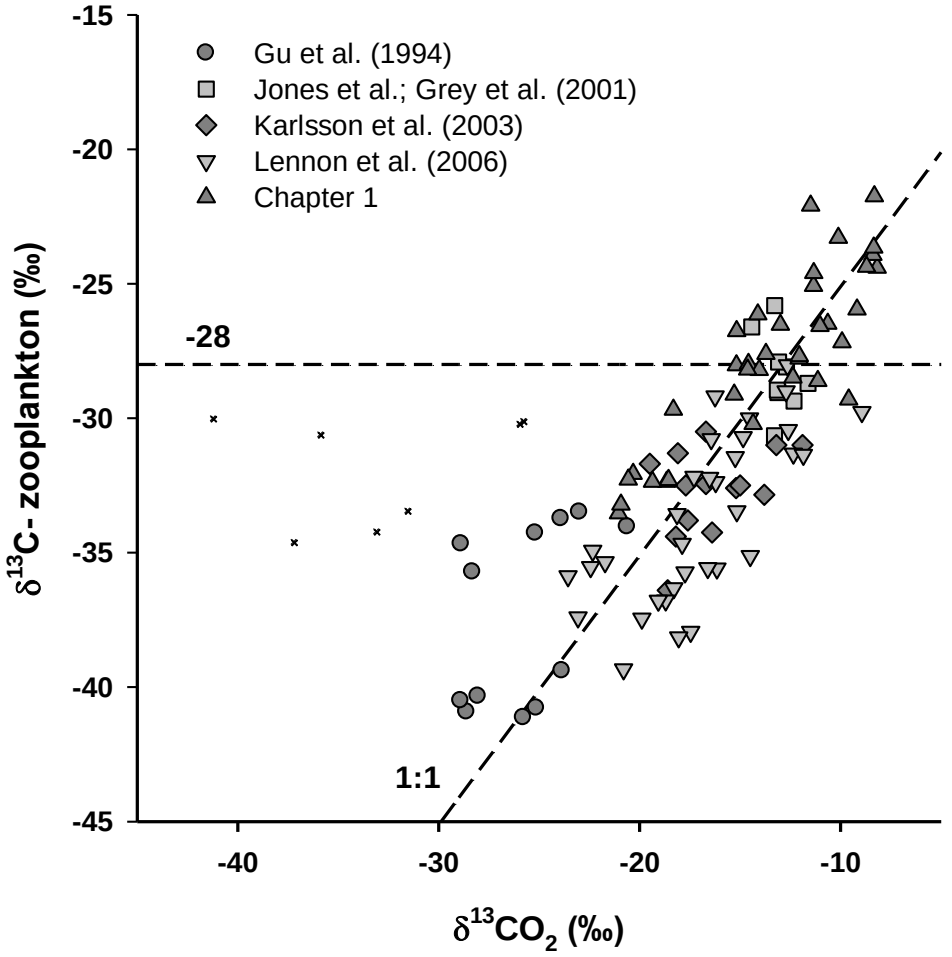

Figure 3.4: Relationship between ^{13}C signature of zooplankton and $^{13}CO_2$. The horizontal line represents the signature of allochthonous C (-28). The model 2 slope of the relationship is 0.87 (r^2=0.68). The 1:1 line is passed through mean x, mean y. Points shown as '×' were omitted from statistical analyses as they showed evidence of CO_2-limitation (see text and Figure 3.2).

Autochthonous fractionation

At the $^{13}CO_2$ signature where the ^{13}C-POC equals that of the allochthonous signature (-28‰), only the autochthonous fractionation affects the ^{13}C signature of the POC. Using the relationship between ^{13}C-POC and $^{13}CO_2$ (Figure 3.3), I estimate this $^{13}CO_2$ signature to be -13.9‰. I used the corresponding $^{13}CO_2$ signature at this point to calculate the average

autochthonous fractionation in across all the lakes and reservoirs. Average autochthonous fractionation, therefore, equals -14.1‰ (*i.e.* -28‰-(-13.9‰)). Similarly, I used the upper and lower limits of the 95% confidence interval of the slope estimate (0.39 and 0.55) to calculate the upper and lower limits of the autochthonous fractionation to be -16.1 to -11.0‰.

POC fractionation and CO_2 concentration

As noted above, there was a decrease in POC fractionation with increasing CO_2 concentration (Figure 3.1). This relationship cannot be explained by the possibility of CO_2 limitation since increasing CO_2 availability should increase fractionation, and cause ^{13}C-POC to become increasingly depleted relative to $^{13}CO_2$. With increasing CO_2 concentration, $^{13}CO_2$ also decreased (r^2=0.48; eq. 3.1), similar to the relationship between $^{13}CO_2$ and *P*CO_2 (Figure 3.1). Since $^{13}CO_2$ and CO_2 concentration covary, the relative influence of allochthonous (which I assume has a constant signature of -28‰) and autochthonous (which I assume varies with $^{13}CO_2$) would also vary with CO_2 concentration. Only if POC was entirely autochthonous would there be no relationship between POC fractionation and CO_2 concentration. The relationship becomes increasingly nonlinear with an increasing proportion of allochthonous material in POC. Therefore, I used the observed relationship between $^{13}CO_2$ and CO_2 concentration:

$$^{13}CO_2 = -3.7\ln[CO_2] - 3.3 \qquad \text{(eq. 3.1)}$$

and the mixing model:

$$^{13}\text{C-POC} = a(^{13}CO_2 + f) - 28(1-a) \qquad \text{(eq. 3.2)}$$

where -28 = the allochthonous signature (‰) (Lajtha and Marshall 1994), f = POC fractionation that I calculated (-14.1‰), and a = the fraction of

autochthonous contribution that I calculated (min. = 0.39, max=0.55) to generate the expected POC fractionation with varying CO_2 concentration.

The predicted relationship between POC fractionation and CO_2 concentration conforms very closely to the best-fit for the relationship (Figure 3.5). Thus, the positive relationship between POC fractionation and CO_2 concentration can be explained by the varying influence of autochthonous contributions to POC with varying CO_2 concentration. This supports the conclusion that, for this subset of lakes, CO_2 was not limiting. Additionally, it suggests that the proportion of allochthonous C in POC does not vary as a function of CO_2 concentration as one might expect.

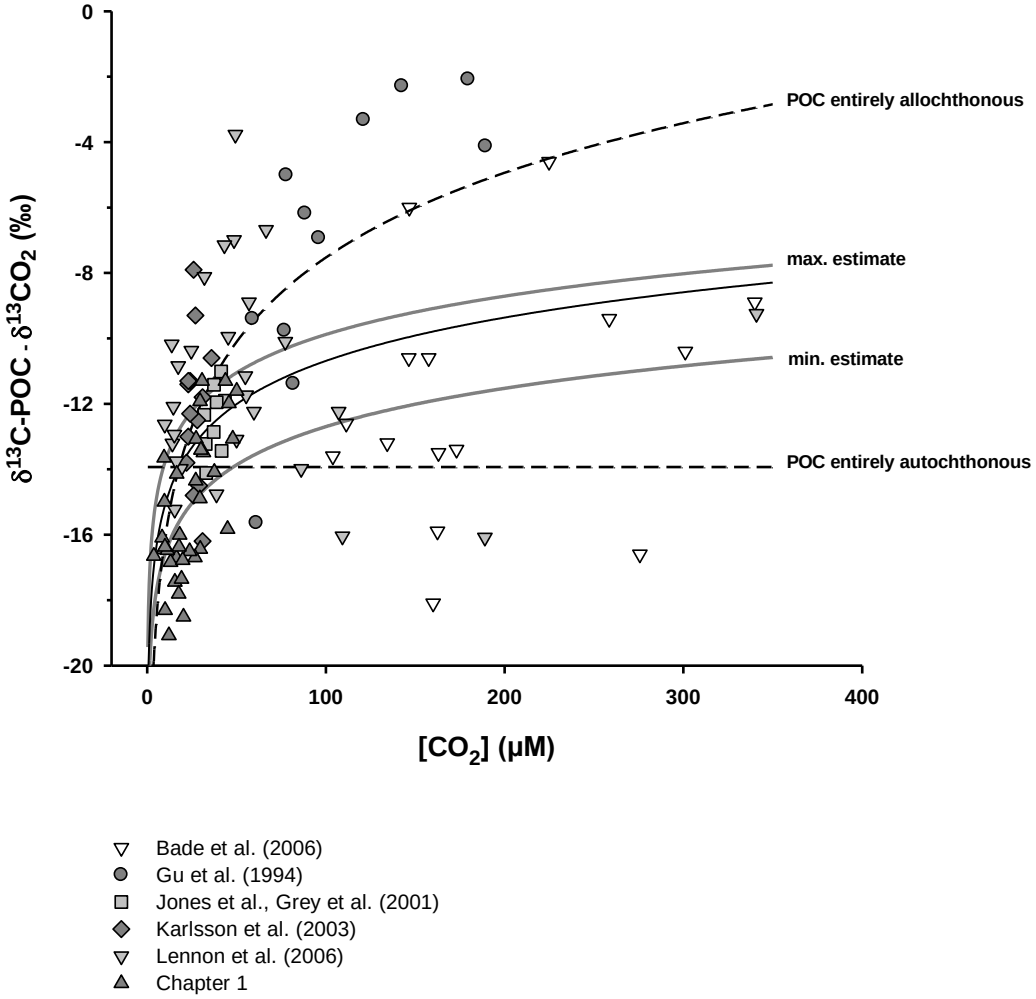

Figure 3.5: Predicted relationship between ^{13}C-POC-CO_2 vs. CO_2 concentration in lakes that did not show evidence of CO_2-limitation. The dashed lines represent the predicted relationships in the absence of variable autochthonous fractionation if POC was entirely allochthonous or entirely autochthonous; grey lines represent the relationships predicted from the minimum and maximum estimates of POC proportion autochthonous and autochthonous fractionation from the relationship between ^{13}C-POC and $^{13}CO_2$ (see text and Figure 3.3). The solid line is the logarithmic best-fit to the data (y=1.9ln(x) - 19.5).

3.4 Discussion

$^{13}CO_2$ versus PCO_2

In the majority of lakes, $^{13}CO_2$ approached the atmospheric $^{13}CO_2$ with decreasing PCO_2. The lakes and reservoirs in the study by Marty and Planas (2007) and some of the lakes in the study by Bade et al. (2006), however, did not fit this pattern. The $^{13}CO_2$ signatures in these lakes and reservoirs were very depleted, many well below a signature expected for terrestrial C (Lajtha and Marshall 1994). This suggests a different source of $^{13}CO_2$, perhaps methanogenic, for these systems. Interestingly, the variability in $^{13}CO_2$ signatures appeared to decrease with increasing PCO_2, approaching a terrestrial ^{13}C signature with increasing PCO_2, suggesting that respiratory CO_2 increasingly dilutes CO_2 from other sources as terrestrial inputs increase. This is similar to the finding by Striegl et al. (2001) in a cross-system study of boreal and north temperate lakes under ice-cover that respiratory CO_2 becomes dominant with increasing PCO_2.

Allochthonous contribution to POC

Across the systems that I studied, allochthonous material was a substantial contribution to POC. On average, close to half (47±8%; 52±10% excluding Gu et al. 1994) of POC was allochthonous. While the distance of each point relative to the 1:1 line and the allochthonous signature indicates the relative contribution of autochthonous and allochthonous C, it is important to note that for points close to the region where the allochthonous and expected autochthonous signatures converge, errors or small differences in fractionation would have a large impact on the estimate of the proportion allochthonous/autochthonous. Thus, it is important to note that the estimates represent the average proportion of allochthonous C in POC across these systems rather than estimates for any individual lake.

While I used a linear relationship between ^{13}C-POC and $^{13}CO_2$ for this estimate of the allochthonous contribution to POC, a nonlinear relationship between these is, however, plausible. As noted above, the depletion of $^{13}CO_2$ with increasing PCO_2 suggests that allochthonous C is respired. Thus, it is conceivable that POC would also become increasingly allochthonous with $^{13}CO_2$ depletion (and therefore increasing terrestrial influence), resulting in a curved, rather than linear, relationship between ^{13}C-POC and $^{13}CO_2$ in which ^{13}C-POC approaches the terrestrial signature with $^{13}CO_2$ depletion. There is, ostensibly, some suggestion of this tendency in some of the data from Gu et al. (1994) and Bade et al. (2006). These points, however, also show weak fractionation between ^{13}C-POC and $^{13}CO_2$, suggesting possible CO_2 limitation (or bicarbonate use; Figure 3.5). Therefore, it is more likely that any apparent tendency toward a terrestrial signature with $^{13}CO_2$ depletion is due to weak fractionation between ^{13}C-POC and $^{13}CO_2$, rather than an increasing allochthonous contribution to POC. Marty and Planas (2007) made a similar conclusion, that the derivation of algal signatures from inorganic C will lead to an overestimation of terrestrial inputs. My results corroborate this, with the addendum that terrestrial inputs will only be overestimated if fractionation is weak due to CO_2-limitation or bicarbonate use by phytoplankton.

When those studies that did show evidence of weak fractionation due to CO_2-limitation or bicarbonate use by phytoplankton were included, the relationship between ^{13}C-POC and $^{13}CO_2$ became much weaker. In each of the studies that we excluded, the authors of those studies found similar evidence of weak fractionation or bicarbonate use, rather than an indication of terrestrial dominance of POC. Gu (2006) determined that in the softwater, eutrophic Lake Wauberg, POC was largely autochthonous, attributing the highly enriched signature of ^{13}C-POC to C-limitation and bicarbonate use.

Using POC: chl *a* ratios, Marty and Planas (2007) estimated that POC in their study averaged 50% autochthonous, ranging from 10% to 100% autochthonous, which is similar to the estimate that we developed in Chapter 1, as well as in the metadata. They concluded that the weak, and apparent positive, fractionations between ^{13}C-POC and $^{13}CO_2$ and the lack of relationship between ^{13}C-POC and $^{13}CO_2$ were due to CO_2-limitation or appreciable bicarbonate use in their systems. Similarly, Bade (2006) found that, in a subset of lakes where the POC was largely autochthonous (based on high chl a: POC ratios), apparent fractionation in several lakes was very weak, or even positive. Bade et al (2006) concluded that this was evidence of CO_2 limitation or bicarbonate use in those lakes. Thus, as with the authors of each of these studies, we excluded those data based on evidence of CO_2-limiation or bicarbonate use.

In two of the studies that I used for this meta-analysis (Jones et al. 2001, Karlsson et al. 2003), the authors also estimated the proportion allochthonous of POC. Karlsson et al. (2003) estimated that the POC was 85% allochthonous, much higher than my estimate. Taken as a group, however, their data do not appear to diverge from the overall pattern in the ^{13}C-POC vs. $^{13}CO_2$ relationship (Figure 3.1). The explanation for the large discrepancy in these estimates may be due to a difference in how autochthonous fractionation was estimated compared to the present work. Karlsson et al. (2003) calculate autochthonous fractionation using a fractionation model based on growth rate, CO_2 concentration, and an estimate of maximum autochthonous fractionation based on laboratory studies, resulting in an estimate of autochthonous fractionation ranging from -26.8 to -18.8 ‰. Since this is greater than is my empirical estimate of -14‰, it would predict a smaller autochthonous proportion to account for a POC signature that is a mix of autochthonous and relatively enriched

allochthonous C than an estimate based on the calculated autochthonous fractionation of the present work.

Jones et al. (2001) concluded that Loch Ness POC (-26.6 to -24.0‰) was primarily allochthonous as its ^{13}C signature was similar to that of the incoming stream POC (-27.1 to -25.9‰) and enriched compared to phytoplankton that were physically separated from the POC (-29.0 to -32.2‰; Jones et al. 1998). In Figure 3.3, however, points from their study appear close to the 1:1 model line (based on an autochthonous fractionation of -14‰). Data from this study are close to the region where the assumed allochthonous signature of -28 coincides with the predicted autochthonous signature, reducing the impact of varying proportions of each endmember on the resulting POC signature. This, and the narrow range of $^{13}CO_2$, relative to the overall range in $^{13}CO_2$ in this study, makes it impossible for us to use the approach developed in the present work to calculate a comparable allochthonous contribution to POC for this study in isolation.

Results from some whole-lake ^{13}C-addition experiments (Carpenter et al. 2005, Pace et al. 2004) have generally estimated similar allochthonous contribution to POC as my estimate, ranging from 29% to 59% allochthonous. The exception was in a fertilised lake (Peter Lake), where POC was found to be close to entirely autochthonous after fertilisation, whereas before fertilisation, it was similar to the other lakes (47% to 50% allochthonous (Carpenter et al. 2005). In a whole-lake addition to a relatively large lake (Crampton Lake) compared to the other lakes in which ^{13}C additions were done, POC was somewhat less allochthonous at 22%. While the lakes used in this meta-analysis encompass the range of lake areas of these whole-lake addition experiments, there was no clear pattern in lake size relative to the 1:1 model line (not shown). Since, however, I did not estimate the proportion of allochthonous POC on a lake-by-lake basis, I

cannot examine directly a relationship between lake area and the proportion of allochthonous contribution to POC.

Allochthonous contribution to zooplankton

While there was an appreciable allochthonous contribution to POC, this was not reflected in zooplankton, which on average, were highly autochthonous. The relationship between ^{13}C-zooplankton and $^{13}CO_2$ appeared to be linear with a slope near 1. As with the ^{13}C-POC vs. $^{13}CO_2$ relationship (see above), this shows that zooplankton do not become more allochthonous with increasing system heterotrophy. This is similar to the conclusion by Lennon et al. (2006) that zooplankton were largely autochthonous and that "the direct transfer of terrestrial DOC inputs to higher trophic levels may be relatively inefficient." Similarly, Karlsson et al. (2007) found in 13 lakes from northern Sweden that, allochthonous inputs were largely respired, with <3% transferred to zooplankton. However, because allochthonous inputs to these systems were so high, they concluded that they still provided an appreciable contribution to zooplankton.

In one of the studies that used in this meta-analysis, (Jones et al. (1999) with Grey et al. (2001)), the authors concluded that there was a seasonal shift in zooplankton nutrition, from being primarily allochthonous from fall to spring, then becoming highly autochthonous toward mid-summer. In the plot from the present study (Figure 3.4), there is some pattern in zooplankton autochthony, with data from spring and fall appearing closer to the allochthonous estimate. Note that while I assumed an allochthonous signature of -28‰, Grey et al. (2001) observed that the ^{13}C signature of incoming streamwater was between -24‰ to -26.6‰. Additionally, the summer values were more depleted than predicted from the cross-system value for autochthonous fractionation, suggesting that autochthonous fractionation in this system was relatively higher in this

system. While the above three studies reached similar conclusions to mine, Karlsson et al. (2003), whose data was also included in this meta-analysis, reached the conclusion that zooplankton were appreciably (47%) allochthonous. As with their higher estimate for the allochthonous contribution to POC (see above), this is probably because they assume a much larger autochthonous fractionation than my estimate. Thus, similar to the situation with POC, reducing their estimate of autochthonous fractionation would also increase their estimate of the proportion of autochthonous contribution to both POC and zooplankton.

Including the data of Marty and Planas (2007) reduced both the slope and the strength of the relationship between ^{13}C-zooplankton and $^{13}CO_2$. The ^{13}C-zooplankton in the study of Marty and Planas (2007) closely reflected that of ^{13}C-POC in that study. As explained above ("Allochthonous contribution to POC"), phytoplankton in that study were likely limited by CO_2 or were accessing bicarbonate (violating a key assumption of the approach used in the present study). Thus, the approach I use here cannot determine from the data of Marty and Planas (2007) whether the ^{13}C-zooplankton vs. $^{13}CO_2$ relationship is due to feeding on allochthonous C, or from the effects of CO_2-limitation/bicarbonate use by phytoplankton.

Autochthonous fractionation

The average autochthonous fractionation value that I calculated (-14.1‰, range -16.1‰ to -11‰) is considerably lower than the maximum fractionation from discrimination by Rubisco of -28‰ to -25‰ (Goericke et al. 1994). Recent *in situ* estimates have also found phytoplankton fractionation to generally be lower than the physiological maximum. In a cross-system study of Wisconsin and Michigan lakes, Bade et al. (2006) found that algal fractionation was often low, ranging from 0‰ to -15‰. Using four different fractionation models, Lennon (2006) predicted

autochthonous fractionation to be from -5.4‰ to -25.1‰. Using ^{13}C-DIC additions to mesocosms from a small humic lake, Taipale et al. (2007) calculated autochthonous fractionation values of -13.1 ± 2.8‰, similar to that of the present work. Whole-lake addition experiments have also found low phytoplankton fractionations, ranging from -5.4‰ (Cole et al. 2002) to -11.5‰ (Pace et al. 2004). While I did calculate fractionation to be lower than the physiological maximum for the set of lakes that did not show evidence of CO_2-limitation, autochthonous fractionation was not highly variable.

Conclusions

I show in this work that across a variety of lakes and reservoirs POC is, on average, approximately half autochthonous. Increasing respiration of allochthonous inputs is not reflected in an increasing allochthonous contribution to POC. This is also true of zooplankton, which remain highly autochthonous regardless of the influence of allochthonous inputs to system respiration.

Chapter 4
Variation in $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter and zooplankton with lake depth and taxonomic differences in zooplankton $\delta^{13}C$ and $\delta^{15}N$: Implications for lake food webs.

4.1 Introduction

The source of organic matter to aquatic food webs has been a topic of current interest. Knowledge of the sources and transformations of organic matter in aquatic systems is important to understanding the structure and function of lake food webs as well as to the interpretation of lake sediment records. Because of their potential to trace sources of organic matter and trophic relationships, stable isotope approaches have been commonly used in this effort. While many studies have focussed on unstratified lakes, epilimnetic processes, or have treated stratified water columns as homogenous, the vertical structure of lakes may have an important influence on the sources and transformations of various components of the food webs of lakes.

While not a food web component, sources and transformations of inorganic C are important to our understanding of food webs because autotrophs assimilate it to create the autochthonous portion of POM. Inorganic C enters epilimnia from atmospheric exchange, streams, and runoff, and is created in the lake from respiration and photolysis of organic matter. Its signature will be altered by the selective assimilation and regeneration of lighter CO_2 by autotrophs. Hypolimnetic inorganic C often has a highly depleted signature. It is isolated from the heavy CO_2 in the atmosphere and respiration of organic matter has been ascribed as the reason for this depleted ^{13}C-DIC signature in lakes (Miyajima et al. 1997; Oana and Deevey 1960; Quay

et al. 1986). More recently, Karlsson et al. (2007) demonstrated that, in unproductive Swedish lakes, the accumulation of depleted ^{13}C-DIC was primarily from the respiration of allochthonous material. In some cases, methanogenesis and methanotrophy may also contribute to highly depleted hypolimnetic signatures (Bastviken et al. 2003; Kankaala et al. 2006; Kankaala et al. 2007). As a zone of transition between the epilimnion and hypolimnion, the metalimnion may have inorganic C signatures influenced by the invasion of CO_2 into the epilimnion and by the production of CO_2 by respiration. Additionally, in clear lakes the metalimnion may also be a site of high phytoplankton biomass and carbon fixation. This carbon fixation can potentially reduce CO_2 concentration and enrich the CO_2 signature.

Particulate organic matter (POM) is a mix of living and dead allochthonous and autochthonous material. For watersheds dominated by C3 plants, the C-signature of the allochthonous material will be close to -28‰ (Peterson and Fry 1987). Since primary production favours ^{12}C to ^{13}C, the autochthonous portion of POM will be depleted relative to the ^{13}C signature of DIC. Additionally, the POM ^{13}C and ^{15}N signature can be altered by diagenesis (Lehmann et al. 2002; Lehmann et al. 2004), while the presence of methanotrophs in POM would deplete the POM ^{13}C signature (Bastviken et al. 2003). Thus, the POM signature in each lake stratum represents autochthonous production at that depth, carbon assimilated into seston from allochthonous dissolved organic matter (DOM), and POM derived from layers above along with diagenetic changes that may have occurred.

Since consumer ^{13}C closely reflects its source, with a slight enrichment of ~0.5‰; (Fry 2007), zooplankton ^{13}C should reflect the particulates on which they feed. Many studies have found, however, that the bulk zooplankton and POM signatures do not closely match (del Giorgio and France 1996; Grey et al. 2000; Jonsson et al. 2003; Karlsson et al. 2003; Lennon et al. 2006). This may be

because zooplankton bias their feeding to a portion of the POM that does not have the same signature as the overall POM. Additionally, some zooplankton are capable of appreciable vertical migrations and may therefore feed on POM that is different from the stratum from which they are sampled. Differences in ^{13}C signature may also occur because of feeding type. Filter-feeding *Cladocera* are thought to be less selective and may therefore more closely reflect the POM ^{13}C signature than other groups. Calanoid copepods are thought to be more selective, preferring phytoplankton, so they may reflect an autochthonous signature more closely than other groups. They could, however, acquire allochthonous C by feeding on mixotrophs or protists (Bonnet and Carlotti 2001; Breteler et al. 1999; Calbet and Landry 1999). While the ^{13}C signature of consumer and source is typically similar, the ^{15}N signature enriches appreciably between consumer and source (McCutchan et al. 2003; Vander Zanden and Rasmussen 2001). Thus, predators such as cyclopoid copepods could be enriched compared to more herbivorous zooplankton.

In this study, I examine vertical differences in $^{13}CO_2$, $^{13}C/^{15}N$ of POM, and $^{13}C/^{15}N$ of zooplankton amongst lake strata in a set of north temperate oligo-mesotrophic lakes to determine the extent to which zooplankton from different layers differ in their signature, and the implications of that for the source of the carbon they assimilate. Additionally, I examine if there is evidence of differences in feeding amongst dominant zooplankton taxa.

4.2 Methods

Site description

Three North Central Ontario lakes were sampled in 2003 and 19 lakes (including the 3 from 2003) from the same region were sampled in 2004 (Table 4.1). Situated in the Canadian Shield, the study lakes are small, ranging in area from 11 to 195 ha. They range from ultra-oligotrophic to meso-eutrophic, with

total phosphorus (TP) concentrations of 3.4 to 25.9 μg L^{-1} (mean = 8.3 μg L^{-1}). DOC concentration ranges from 2.4 to 13 mg L^{-1} (mean = 5.3 mg L^{-1}), with most lakes below 7 mg L^{-1} (Ontario Ministry of the Environment, unpublished data).

I sampled at the deepest point in each lake for inorganic C, POM, and zooplankton. The epilimnia of all lakes were sampled. For the three lakes sampled in 2003, and 8 of the lakes sampled in 2004, I also sampled separately, the meta- and hypolimnia. In situ temperature and relative fluorescence of various phytoplankton pigment groups were measured with a Fluoroprobe (bbe Moldaenke). The fluoroprobe uses fluorescence in response to five light-emitting diodes to diagnose four different pigment groups of algae: greens, cyanophytes, diatoms/chrysophytes, and cryptophytes.

Table 4.1: Characteristics of the study lakes. Depth, area, pH, [TP], and Secchi depth data are from the Ministry of the Environment of Ontario. For lakes that were sampled twice, the average of the two measures is reported. Water chemistry values are from mid-epilimnion.

Lake	Sampling	Lat.	Long.	Z mean (m)	Z max (m)	Area (ha)	pH	[TP] ($\mu g\ L^{-1}$)	Secchi depth (m)	[chl a] ($\mu g\ L^{-1}$)	[DIC] (μ mol L^{-1})	[DOC] (mg L^{-1})	PCO_2 (μatm)
Basshaunt	depth, taxa	45° 07′ N	78° 28′ W	8	24	47	7.1	5.0	4.3	3.0	159	5.3	322
Bigwind	depth, taxa	45° 03′ N	79° 03′ W	11	32	111	6.7	4.6	5.4	2.0	224	3.9	455
Crown	depth, taxa	45° 26′ N	78° 40′ W	8	30	136	6.5	4.2	5.0	1.5	125	3.2	378
Dickie	depth, taxa	45° 09′ N	79° 05′ W	5	12	93	6.2	8.3	3.0	4.2	126	6.5	743
Harp	depth, taxa	45° 23′ N	79° 07′ W	12	40	67	6.3	7.2	n		99	3.6	211
Leech	depth, taxa	45° 03′ N	79° 06′ W	6	14	82	6.7	7.6	3.4	3.2	91	5.4	508
Little Clear	depth, taxa	45° 24′ N	79° 00′ W	8	25	11	7.0	4.2	4.5	2.0	120	4.1	553
Red Chalk	depth, taxa	45° 11′ N	78° 56′ W	17	38	44	6.3	5.0			72	2.4	301
Brandy	taxa	45° 06′ N	79° 31′ W	4	8	108	7.1	24.4	1.3	11.9	190	13.0	1187
Buck	taxa	45° 23′ N	78° 60′ W	11	30	40	7.1	3.4	7.3	1.2	168	2.8	306
Glen	taxa	45° 08′ N	78° 30′ W	7	15	16	8.4	5.6	4.8	1.2	932	4.4	202
Hamer	taxa	45° 14′ N	79° 48′ W	3	9	35	6.0	25.9	2.4	1.8	74	8.8	1166
Healey	taxa	45° 05′ N	79° 11′ W	3	7	122	6.4	11.1	1.7	2.4	84	6.4	755
Leonard	taxa	45° 04′ N	79° 27′ W	7	15	195	6.7	5.4	5.4	1.8	60	4.8	392
McKay	taxa	45° 03′ N	79° 10′ W	5	20	122	6.7	8.1	3.0	2.7	97	5.3	732
Moot	taxa	45° 09′ N	79° 10′ W	3	8	46	6.2	13.0	1.7	2.7	64	7.2	826
Saw	taxa	45° 03′ N	79° 02′ W	5	13	28	6.2	7.5	1.9	2.5	92	8.3	1365
Solitaire	taxa	45° 22′ N	79° 00′ W	13	31	124	7.1	3.9	7.7	0.7	95	2.7	381
Young	taxa	45° 13′ N	79° 33′ W	12	21	106	7.1	4.1	7.2	0.9	117	3.5	476

Inorganic C

Sampling protocol was otherwise similar to that described in Chapter 2, except that in 11 lakes I sampled from the middle of the epilimnion, metalimnion and hypolimnion based on the vertical distribution of temperature. For inorganic C samples, I used a peristaltic pump to collect water from the middle of lake strata. Lake water was pumped directly into sample bottles, preventing any mixing of air with the samples. Duplicate 20-mL samples for dissolved inorganic carbon (DIC) concentration were collected without headspace in rubber-stoppered vials, preserved with 0.05 mL of a saturated solution of $HgCl_2$, then refrigerated in the dark until analysis. For analysis, a 5-mL headspace of He was created in each sample vial, then acidified with 0.1 mL of 85% H_3PO_4 to convert all DIC to CO_2. After equilibration, the headspace was analysed for CO_2 concentration using a Shimadzu 8A gas chromatograph (Stainton 1973).

Duplicate samples for PCO_2 were collected in 60-mL bottles. The bottles were prepared by adding 3.5 g KCl to each bottle, evacuating, purging with He, and re-evacuating. They were filled with sample water without introducing air by piercing the septum of the bottle with a syringe needle that was connected to a sampling pump. Samples were stored refrigerated in the dark until analysis. PCO_2 was analysed by creating a 5-mL headspace of He, allowing it to equilibrate with the sample, then analysing the headspace for CO_2 concentration with a Shimadzu 8A gas chromatograph (Stainton 1973).

At the pH range of the study lakes (4.9 to 8.4), carbonate comprises a negligible portion of the total DIC concentration, so can be ignored when calculating the relative concentrations of inorganic carbon species in the lakes. Concentrations of $CO_{2(aq)}$ and $HCO_{3(aq)}$ were determined from in situ

temperature, DIC concentration, and PCO_2 (Harned and Davis 1943; Harned and Scholes 1941).

Duplicate samples for ^{13}C-DIC were collected without headspace in 125-mL bottles, preserved with 0.1 mL of a saturated solution of $HgCl_2$, then refrigerated in the dark until analysis. For analysis, the sample was acidified with H_3PO_4 to convert all DIC into CO_2. The CO_2 was then captured by freezing in a liquid N_2 cold-trap and collected in evacuated breakseals. The collected gas was analysed using a VG Prism Series 2 dual inlet stable isotope mass spectrometer. In situ $\delta^{13}C$ of CO_2 was calculated from in situ temperature, $CO_{2(aq)}$ concentration, DIC concentration, ^{13}C-DIC, and ^{13}C-HCO_3^- (Mook et al. 1974).

POM

Three replicate POM samples for $\delta^{13}C$ were collected from the middle of each lake stratum using a van Dorn sampler. Approximately one litre of water was pre-filtered through a 48-μm Nitex sieve (to exclude zooplankton), then collected onto a pre-combusted quartz-fibre filter (nominal pore size of 1.2 μm). Five mL of 10% HCl were added to filters to remove any inorganic C, then rinsed with 3 x 5 mL of Milli-Q water. Filters were stored frozen, then dried in a dessicator before analysis. Portions of filters were cut and analysed for $\delta^{13}C$ using a Finnegan Delta Plus continuous flow isotope ratio mass spectrometer with a Carlo Erba NA 1500 elemental (nitrogen) analyser. Analytical precision for ^{13}C and ^{15}N was 0.1‰ and 0.3‰, respectively.

Zooplankton

Zooplankton were collected with vertical hauls using a 50-cm diameter plankton net with a mesh size of 153 μm. For metalimnetic and hypolimnetic sampling, a closing net was used to capture zooplankton from only the stratum of interest. Three replicate hauls were collected and preserved in

approximately 70% (final concentration) ethanol. Samples were observed microscopically to ensure that they did not contain an appreciable amount of phytoplankton. For analysis, zooplankton were collected on a 153-μm Nitex mesh, rinsed with 5 mL of 10% HCl to remove inorganic C, then with 3x5 mL of Milli-Q water. The collected zooplankton were placed in pre-combusted vials and dried at 55°C. After drying, zooplankton were ground into a fine powder and analysed for $\delta^{13}C$ using a Micromass Isochrom continuous flow isotope ratio mass spectrometer with a Carlo Erba 1108 CNHS-O elemental analyser. Where possible, I also sorted zooplankton under a dissecting microscope into major taxa for ^{13}C analyses.

Statistical analyses

Differences in ^{13}C and ^{15}N signatures of POM and zooplankton among strata were analysed using one-way ANOVAs for each lake. One-way ANOVAs were also used on data pooled from all lakes for $^{13}CO_2$, and ^{13}C and ^{15}N of POM and zooplankton. To determine differences in ^{13}C among taxa, I used separate ANCOVAs with $^{13}CO_2$ and ^{13}C POM as covariates. If the interaction terms (taxa x $^{13}CO_2$; taxa x ^{13}C-POM) were non-significant, then an ANCOVA without the interaction term was applied. Systat version 10 was used for statistical analyses.

4.3 Results

Temperature, O_2

Most of the study lakes were well stratified, with epilimnia ranging from approximately 3.5 to 5 m deep (Figure 4.1). Dickie and Leech lakes were relatively shallow, and weakly stratified compared to the other lakes. Dickie Lake in 2003 was the only lake in which the hypolimnion was strongly anoxic. Crown, Bigwind, Red Chalk (2003 and 2004), and Harp (2003 and 2004) Lakes showed metalimnetic peaks in dissolved O_2 concentration which coincided

with peaks in fluorescence. Most lakes, however, showed some increase in phytoplankton fluorescence in the metalimnion even in the absence of a corresponding increase in O_2 concentration. The fluorescence characteristics of these metalimnetic peaks suggest that they were dominated in some lakes by diatoms/ dinoflagellates or, in others, a combination of diatoms/ dinoflagellates and cryptophytes. In addition to a metalimnetic fluorescence peak, Little Clear Lake had a fluorescence peak in the hypolimnion. The fluorescence profile suggested that it was composed of cryptophytes, diatoms/ dinoflagellates, and cyanobacteria.

Inorganic C

In most lakes, metalimnetic DIC concentrations were higher, sometimes by an order of magnitude, than in the epilimnia (Table 4.2). Only in Harp Lake in 2003, Little Clear Lake, and Red Chalk Lake (2003 and 2004) was the metalimnetic DIC concentration not higher in the metalimnion than the epilimnion. DIC concentrations were the highest among the strata in the hypolimnia in all lakes. Lake PCO_2 and CO_2 concentration followed the same pattern as DIC concentration, with the same lakes (Harp Lake in 2003, Little Clear Lake, Red Chalk Lake) the exceptions to the pattern of increasing PCO_2 and CO_2 concentration from the epi- to the metalimnia. Signatures of ^{13}C-DIC and $^{13}CO_2$ became depleted with increasing depth. Little Clear Lake and Red Chalk Lake in 2003 were exceptions to this pattern, with metalimnetic ^{13}C-DIC and $^{13}CO_2$ being enriched compared to those of the epilimnia. I did not have the epilimnetic ^{13}C-DIC or $^{13}CO_2$ for Harp Lake in 2003, but the metalimnetic signatures of these were enriched compared to most of the other lakes. In Red Chalk Lake in 2004, the metalimnetic ^{13}C-DIC and $^{13}CO_2$ was depleted compared to the epilimnion, but the relative depletion was small (4‰) compared to most of the other lakes (10‰ or greater).

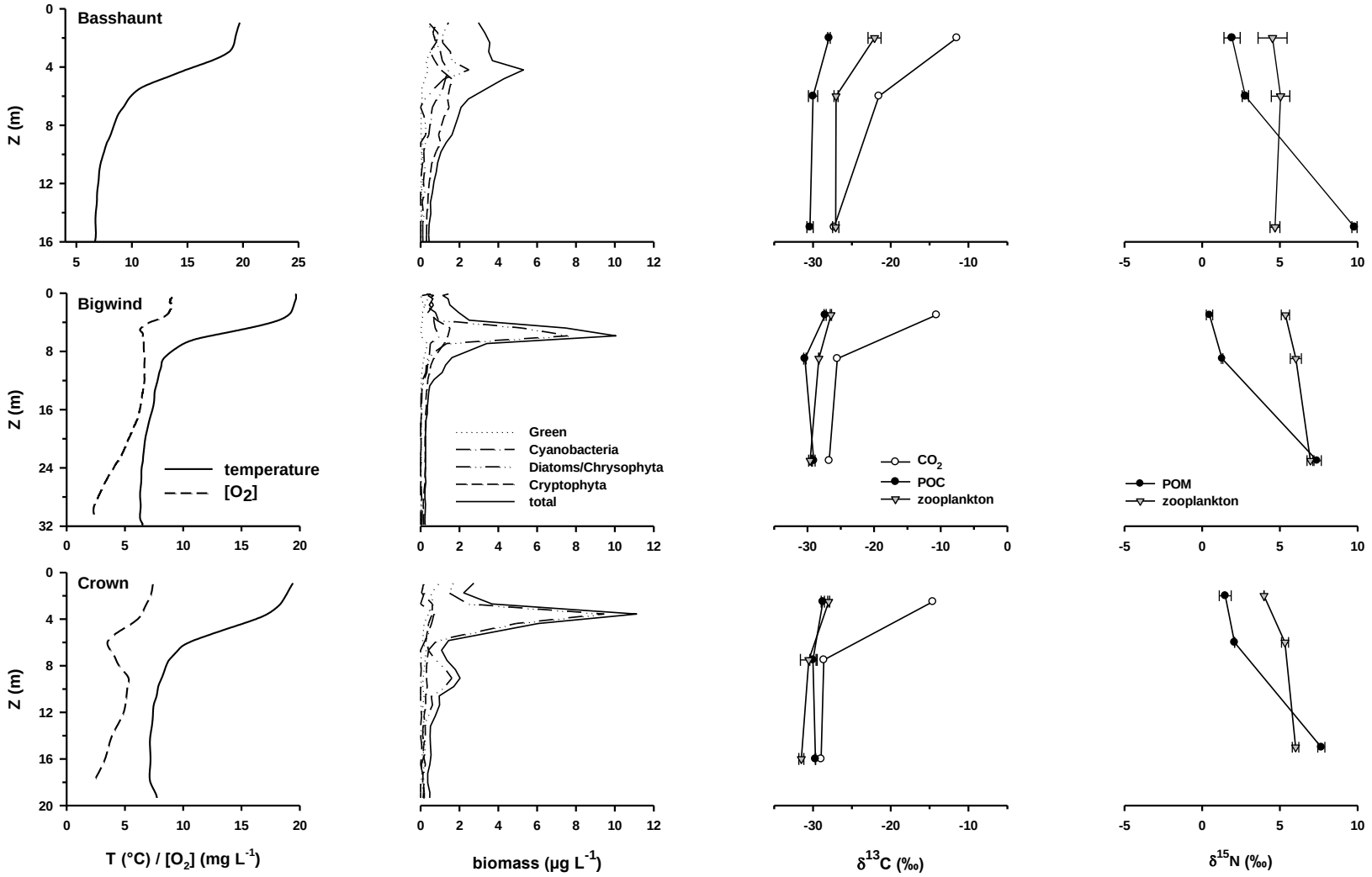

Figure 4.1: Temperature, O_2 concentration, phytoplankton biomass inferred from fluorescence, ^{13}C of CO_2, POM, and zooplankton, and ^{15}N of POM and zooplankton in Basshaunt, Bigwind, and Crown lakes. Error bars are standard error of the mean.

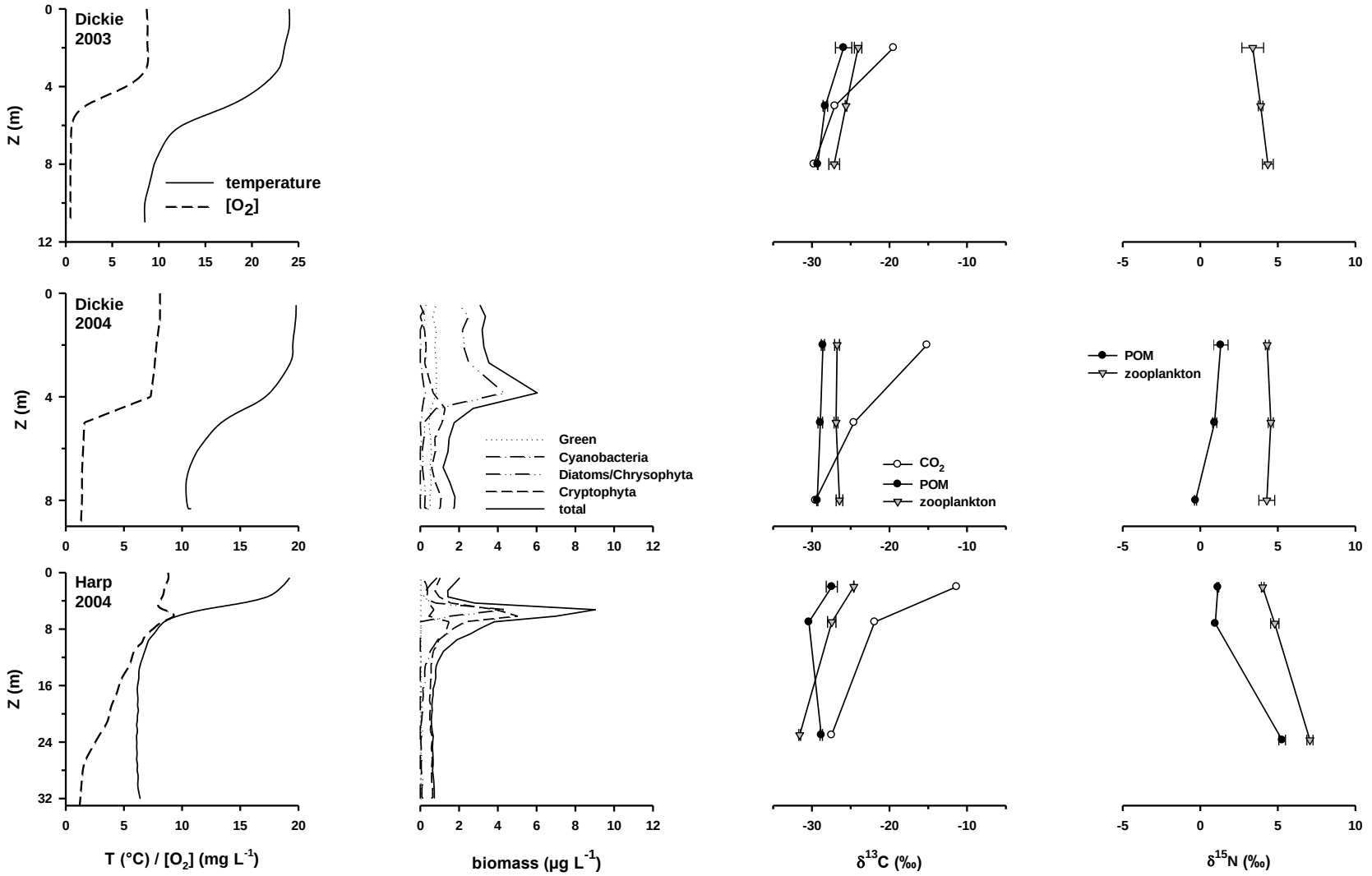

Figure 4.2: Temperature, O_2 concentration, phytoplankton biomass inferred from fluorescence, ^{13}C of CO_2, POM, and zooplankton, and ^{15}N of POM and zooplankton in Dickie (2003 and 2004) and Harp (2004) lakes. Error bars are standard error of the mean.

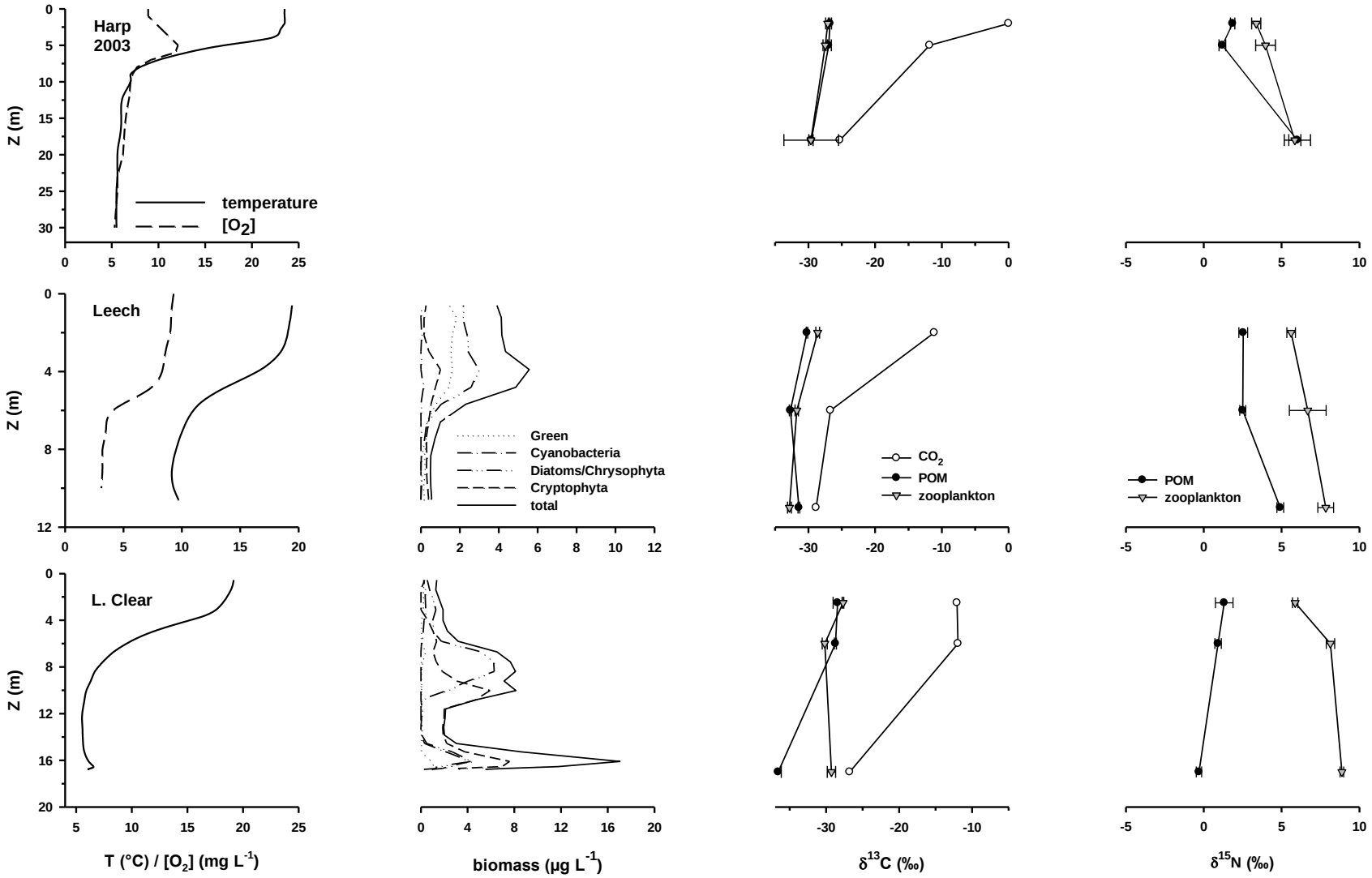

Figure 4.3: Temperature, O_2 concentration, phytoplankton biomass inferred from fluorescence, ^{13}C of CO_2, POM, and zooplankton, and ^{15}N of POM and zooplankton in Harp (2003), Leech, and Little Clear lakes. Error bars are standard error of the mean.

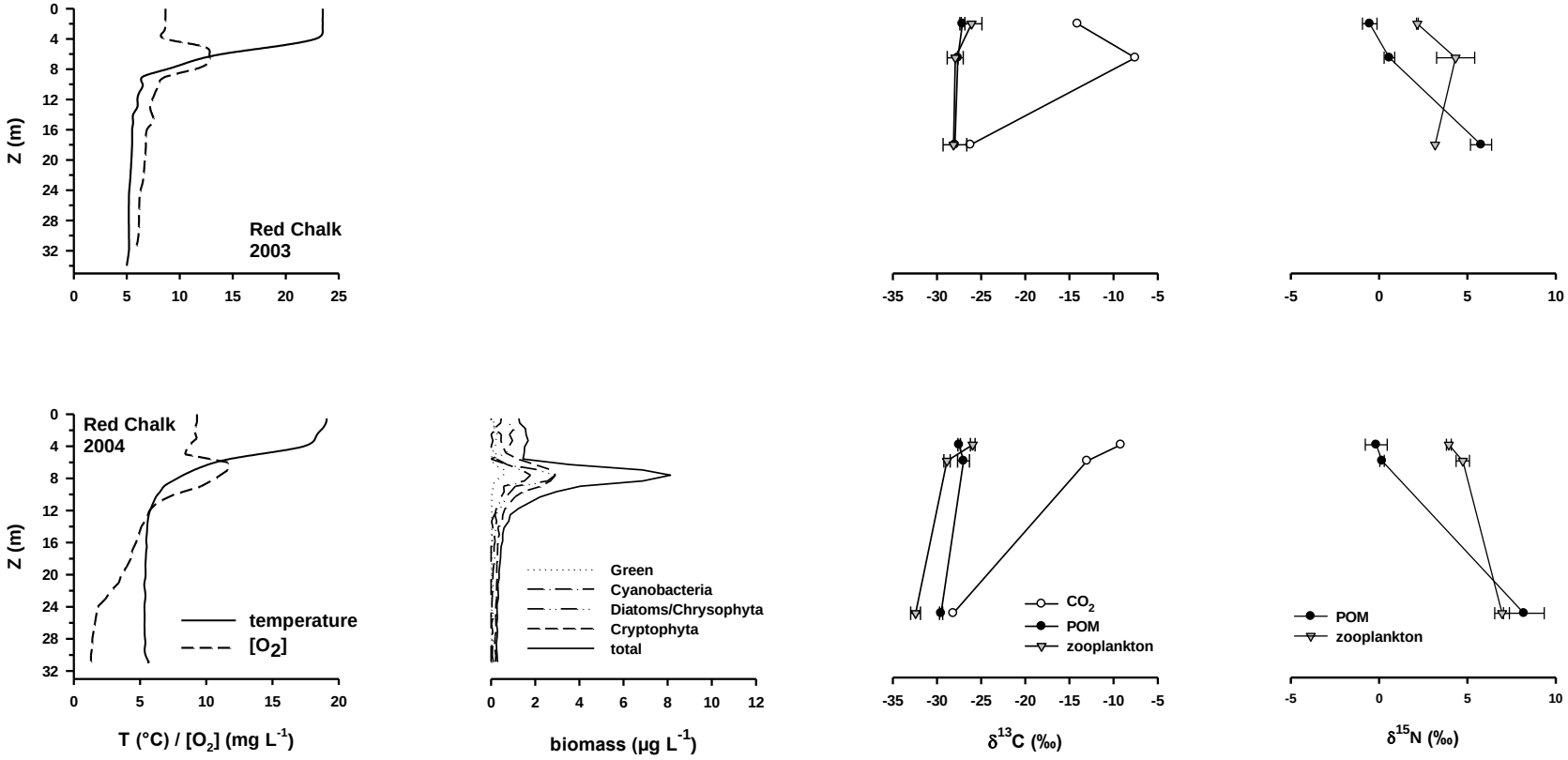

Figure 4.4: Temperature, O_2 concentration, phytoplankton biomass inferred from fluorescence, ^{13}C of CO_2, POM, and zooplankton, and ^{15}N of POM and zooplankton in Red Chalk lake (2003 and 2004). Error bars are standard error of the mean.

Table 4.2: Inorganic C in each stratum of the study lakes. Samples were collected from the middle of each stratum.

Lake	stratum	Z (m)	[DIC] (μM)	PCO_2 (μatm)	$[CO_2]$ (μM)	^{13}C-DIC (‰)	$^{13}CO_2$ (‰)
Basshaunt	epi	2	156	240	9	-2.6	-11.5
	meta	6	331	2311	111	-14.7	-21.5
	hypo	15	383	2938	180	-21.3	-27.3
Bigwind	epi	3	67	428	16	-3.5	-10.6
	meta	9	200	1872	104	-20.3	-25.5
	hypo	23	237	2398	143	-22.4	-26.7
Crown	epi	3	60	416	17	-7.7	-14.6
	meta	8	203	2415	131	-24.8	-28.6
	hypo	16	298	3353	207	-25.5	-29.0
Dickie	epi	2	63	511	18	-13.0	-19.5
(2003)	meta	5	342	5311	224	-23.6	-27.0
	hypo	8	483	5422	296	-25.6	-29.7
Dickie	epi	2	60	771	30	-10.4	-15.2
(2004)	meta	5	327	6405	272	-22.9	-24.6
	hypo	8	426	5401	293	-26.2	-29.5
Harp	epi	2	88	573	20	na	na
(2003)	meta	5	95	944	40	-6.2	-11.8
	hypo	18	233	2909	181	-22.8	-25.3
Harp	epi	2	99	213	8	-2.6	-11.3
(2004)	meta	7	198	2106	113	-17.3	-21.9
	hypo	23	304	2904	185	-23.0	-27.5
Leech	epi	2	90	306	12	-2.8	-11.1
	meta	6	322	3825	180	-22.2	-26.7
	hypo	11	500	4257	248	-23.3	-28.8
Little Clear	epi	3	121	472	18	-4.1	-12.1
	meta	6	150	333	17	-2.5	-11.9
	hypo	17	814	5359	343	-20.2	-26.8
Red Chalk	epi	2	87	370	13	-6.3	-14.1
(2003)	meta	6	70	480	23	0.7	-7.5
	hypo	18	234	2255	142	-21.8	-26.2
Red Chalk	epi	4	73	251	10	-0.9	-9.2
(2004)	meta	6	72	165	8	-3.9	-13.0
	hypo	25	302	2529	163	-22.9	-28.2

Mean $^{13}CO_2$ signature across lake epilimnia was -12.9 ‰, ranging from -19.5‰ to -9.2‰ (Figure 4.1-4.4, Figure 4.5a). The range in metalimnetic $^{13}CO_2$ was large, from -7.5‰ to -28.6‰, with a mean of -20‰. This was significantly ($p= 0.002$) depleted compared to the mean epilimnetic signature (Table 4.2, Table 4.3). The range in hypolimnetic $^{13}CO_2$ was relatively small, from -25.3 to -29.7‰ (mean= -27.7‰), and significantly depleted compared to both the epilimnetic ($p<0.001$) and metalimnetic ($p = 0.001$) signatures. Since $^{13}CO_2$ was calculated from other direct measures (PCO_2, DIC concentration, ^{13}C-DIC), I did not have true replicate samples for $^{13}CO_2$ for each lake. Therefore, I could not make statistical comparisons among $^{13}CO_2$ signatures of lake strata on a lake-by-lake basis.

^{13}C-POM

The strong vertical structure in $^{13}CO_2$ was not as apparent in ^{13}C-POM (Figure 4.1-4.4; Figure 4.5b). In the lake epilimnia, the average ^{13}C-POM was -27.8‰ (range: -25.9 to -30.0‰). The mean metalimnetic ^{13}C-POM signature of -29.2‰ (range: -27.0 to -32.7‰) was not significantly different ($p = 0.07$) from that of the epilimnetic mean. The mean hypolimnetic signature of -29.8‰ (range = -26.4 to -32.8‰) was significantly different ($p = 0.01$) from the mean epilimnetic signature, but not from the mean metalimnetic ^{13}C-POM signature ($p = 0.42$). On a lake-by-lake basis, 7 of the 11 lakes sampled showed significant differences in ^{13}C-POM with lake depth (Table 4.4).

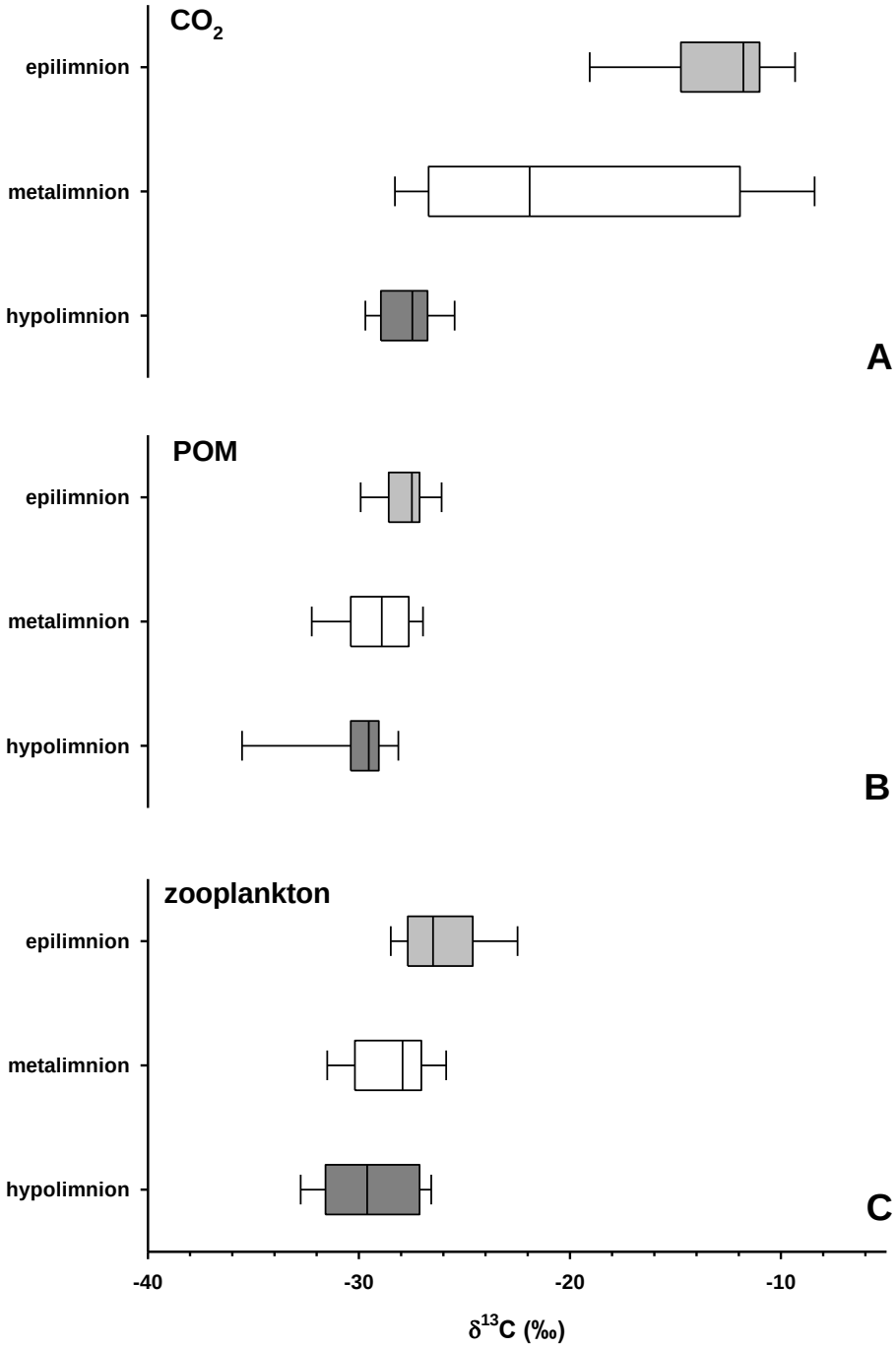

Figure 4.5: ^{13}C of CO_2, POC, and zooplankton in the epi-, meta-, and hypolimnia of the study lakes in which all depths were sampled. Box

boundaries indicate 25th and 75th percentiles, while the line indicates the median. Whiskers indicate the 90th and 10th percentiles.

Table 4.3: Summary of one- way anovas comparing ^{13}C of CO_2, POM, and zooplankton and ^{15}N of POM and zooplankton among lake strata.

	p	**epilimnion vs. metalimnion**	**epilimnion vs. hypolimnion**	**metalimnion vs. hypolimnion**
$^{13}CO_2$	<0.001	0.002	<0.001	0.001
^{13}C-POM	0.032	0.069	0.011	0.418
^{13}C-zooplankton	0.001	0.013	<0.001	0.172
^{15}N-POM	<0.001	0.483	<0.001	<0.001
^{15}N-zooplankton	0.004	0.064	0.001	0.091

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Table 4.4: Summary of one-way ANOVAS performed on a lake-by-lake basis. Epilimnion is denoted "e", metalimnion with "m", and hypolimnion with "h". Strata significantly different from each other ($p<0.05$) are separated by parentheses.

Lake	^{13}C-POM		^{13}C-zooplankton		^{15}N-POM		^{15}N-zooplankton	
	p		*p*		*p*		*p*	
Basshaunt	0.019	(e)(mh)	<0.001	(e)(mh)	<0.001	(em)(h)	0.644	ns
Bigwind	0.001	(e)(m)(h)	<0.001	(e)(m)(h)	<0.001	(e)(m)(h)	0.001	(e)(m)(h)
Crown	0.033	(e)(mh)	0.002	(e)(mh)	<0.001	(em)(h)	<0.001	(e)(m)(h)
Dickie (2004)	0.091	ns	0.323	ns	0.021	(em)(h)	0.587	ns
Dickie (2003)	0.028	(e)(mh)	0.003	(e)(m)(h)	0.103	ns	0.103	ns
Harp(2004)	0.120	ns	<0.001	(e)(m)(h)	<0.001	(em)(h)	<0.001	(e)(m)(h)
Harp(2003)	0.505	ns	<0.001	(em)(h)	0.005	(em)(h)	0.001	(em)(h)
Leech	0.001	(e)(m)(h)	<0.001	(e)(m)(h)	0.003	(em)(h)	0.032	ns
Little Clear	0.001	(em)(h)	0.001	(e)(m)(h)	0.013	(e)(m)(h)	<0.001	(e)(m)(h)
Red Chalk	0.017	(em)(h)	<0.001	(e)(m)(h)	0.003	(em)(h)	<0.001	(e)(m)(h)
Red Chalk(2003)	0.611	ns*	0.221	ns*			0.104	ns*

* no hypolimnetic sample.
Red Chalk 2003 lacked metalimnetic and hypolimnetic ^{15}N-POM samples

^{13}C-zooplankton

Like ^{13}C-POM, ^{13}C-zooplankton signatures did not display the same degree of vertical structure that $^{13}CO_2$ did (Figure 4.1-4.4, Figure 4.5c,). The average epilimnetic ^{13}C-zooplankton signature was -26.1‰ (range = -22.1 to -28.6‰). The metalimnetic signatures, ranging from -25.6‰ to -32.0‰, were depleted on average by 2.3‰ from the epilimnetic average ($p = 0.01$). Hypolimnetic ^{13}C-zooplankton ranged from -26.4‰ to -32.8‰; on average depleted by 3.5 ‰ from the mean epilimnetic signature. The difference between the mean of the hypolimnia and epilimnia was significant ($p<0.001$) while the difference between the hypolimnia and the metalimnia was not ($p = 0.17$). On a lake-by-lake basis, 9 of the 11 lakes showed significant differences in ^{13}C-zooplankton with lake depth.

^{15}N-POM

Signatures of ^{15}N-POM generally enriched between lake metalimnia and hypolimnia. Most of the lakes showed a significant enrichment (average = 5.5 ‰) in ^{15}N in the POM between these depths, though not between the epi- and metalimnia (Table 4.4). Exceptions to this pattern were in Dickie Lake in 2004 (^{15}N-POM for Dickie Lake in 2003 is not available) and Little Clear Lake, in which the hypolimnion was depleted relative to the metalimnion in both years. In Dickie Lake this difference, while significant, was small (0.6‰). In Little Clear Lake, however, the hypolimnetic ^{15}N-POM was 2.6‰ depleted compared to that of the metalimnion.

Across lake epilimnia, mean ^{15}N-POM was 1.2 ‰ ranging from -0.2‰ to 2.5‰ Figure 4.1-4.4; Figure 4.6). The metalimnetic ^{15}N-POM average was similar to that of the epilimnia (1.4‰), ranging from 0.2‰ to 2.8‰ and, across lakes, was not significantly different from epilimnetic ^{15}N-POM

(p= 0.483). Excluding Dickie Lake in 2004 and Little Clear Lake (as they showed an anomalous pattern- see above). There was a significant enrichment in the mean ^{15}N-POM between the metalimnia and hypolimnia of 5.4‰ ($p<0.001$).

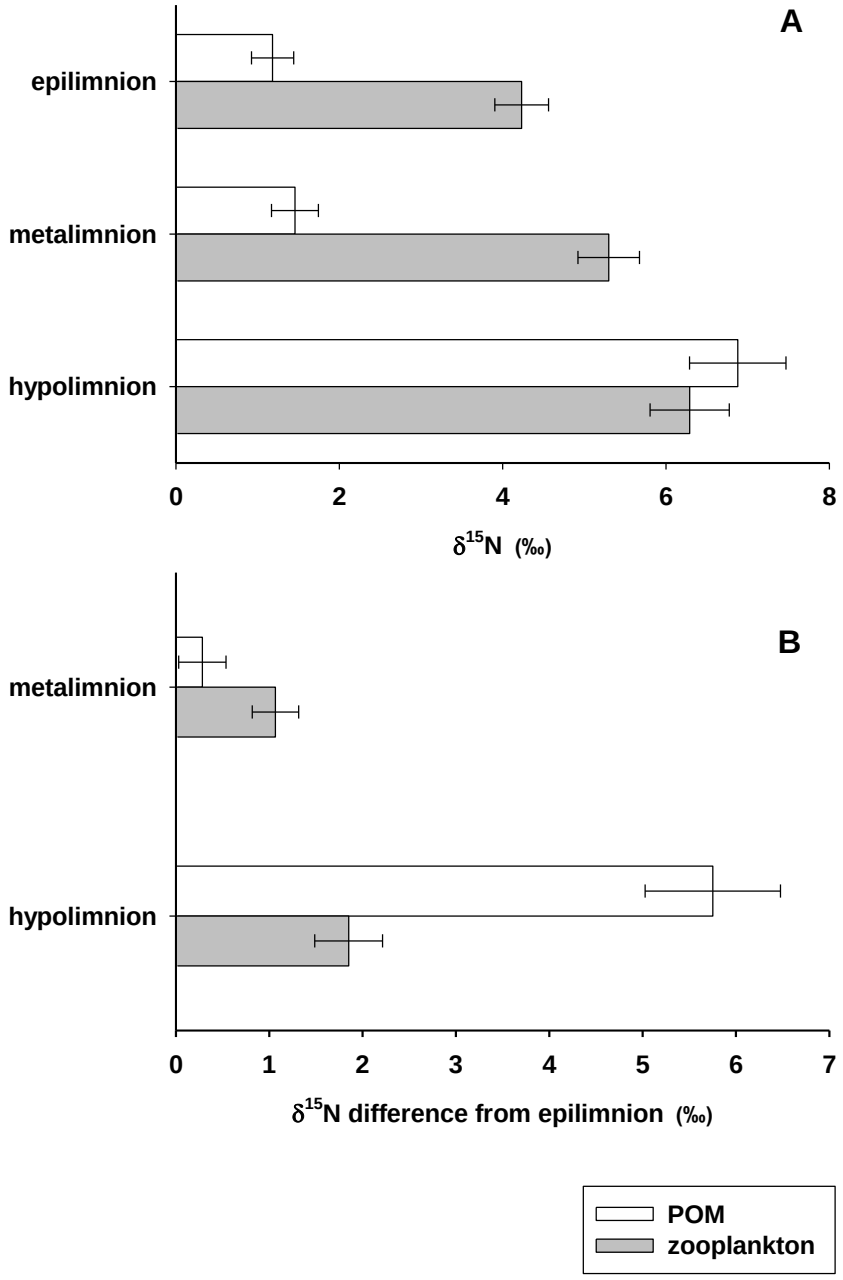

Figure 4.6: a) ^{15}N of POM and zooplankton in each lake stratum, b) difference between ^{15}N of POM and zooplankton from their respective epilimnetic ^{15}N signatures in the meta- and hypolimnia. Error bars are standard error of the mean.

^{15}N-zooplankton

The enrichment of ^{15}N with depth observed in POM was not as apparent in the zooplankton signatures. In 5 of the 11 lakes surveyed, there was a significant enrichment of zooplankton ^{15}N in the metalimnia compared to the epilimnia, while in 6 lakes the hypolimnetic ^{15}N signatures of zooplankton were significantly enriched compared to those of the metalimnia.

Across lakes, the mean ^{15}N-zooplankton from lake epilimnia was 4.2‰, ranging from 2.2‰ to 5.9 ‰ (Figure 4.6). The mean metalimnetic ^{15}N-zooplankton signature, at 5.2‰ (range 3.9‰ to 8.1‰) was not significantly different from the epilimnetic average. The mean hypolimnetic ^{15}N-zooplankton of 6.5‰ (range 4.7‰ to 7.8‰) was significantly enriched compared to the epilimnia ($p=0.001$), but not from the metalimnia ($p=0.09$).

^{13}C-zooplankton versus ^{13}C-POM

To investigate the source of POM consumed by zooplankton, I related the signatures of zooplankton to POM from the same strata where they were collected, and from the epilimnion (Figure 4.7). Epilimnetic ^{13}C-POM explained 30% of the variation in zooplankton ^{13}C, and was almost significant ($p = 0.08$). The slope of the relationship was close to 1 (0.9), and most ^{13}C-zooplankton were enriched relative to ^{13}C-POM. Meta- and hypolimnetic zooplankton were increasingly depleted compared to ^{13}C-POM. Comparing metalimnetic zooplankton ^{13}C to metalimnetic ^{13}C-POM resulted in a relationship closer to the 1:1 line (Figure 4.7b). Similarly, comparing hypolimnetic ^{13}C-zooplankton to hypolimnetic ^{13}C-POM brought the relationship closer to 1:1, though in several lakes (Red Chalk in 2004, Leech,

Harp, and Crown Lakes), ^{13}C-zooplankton was still appreciably depleted compared to ^{13}C-POM.

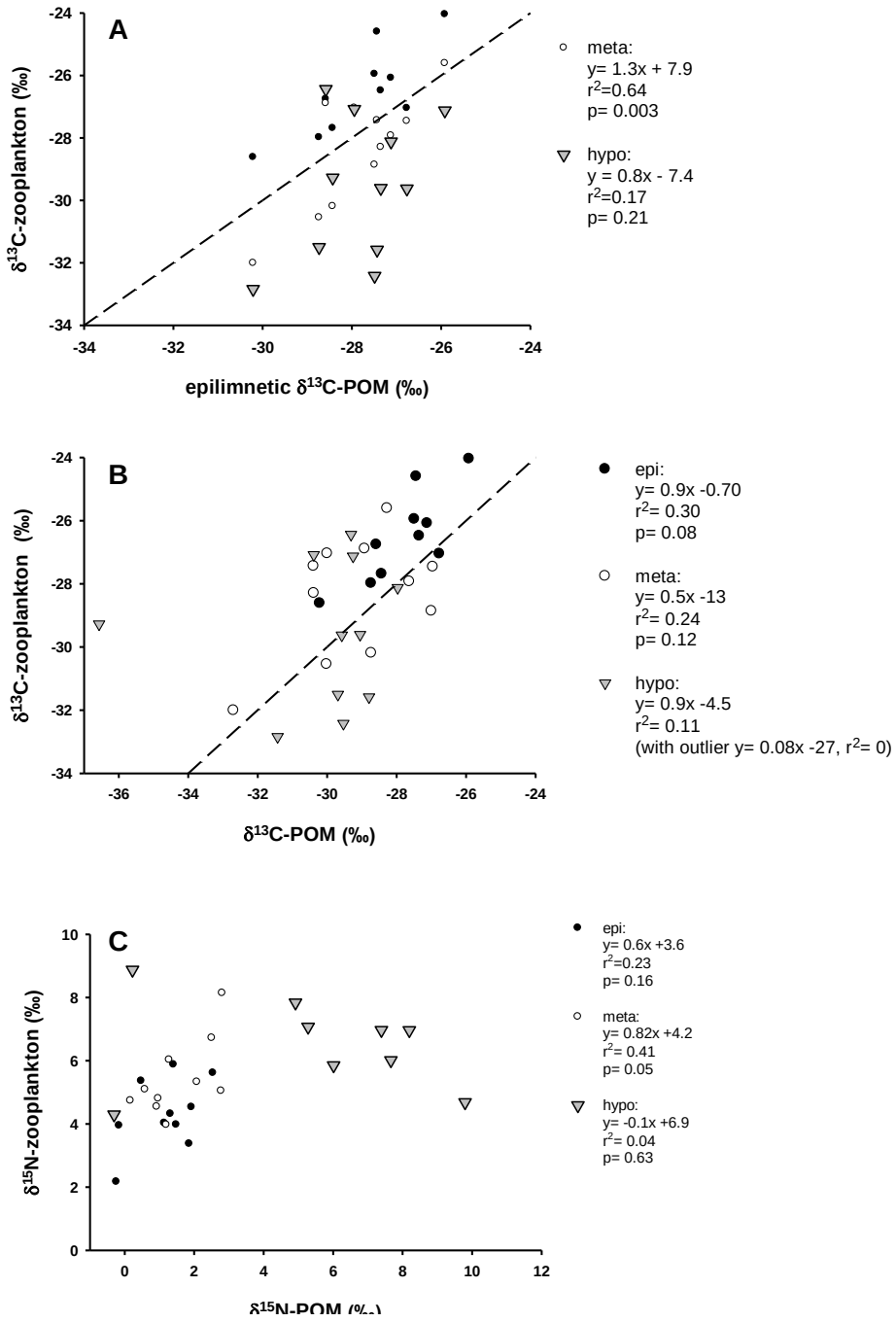
A
δ13C-zooplankton (‰)
epilimnetic δ13C-POM (‰)
meta:
y= 1.3x + 7.9
r2=0.64
p= 0.003
hypo:
y = 0.8x - 7.4
r2=0.17
p= 0.21
B
δ13C-zooplankton (‰)
δ13C-POM (‰)
epi:
y= 0.9x -0.70
r2= 0.30
p= 0.08
meta:
y= 0.5x -13
r2= 0.24
p= 0.12
hypo:
y= 0.9x -4.5
r2= 0.11
(with outlier y= 0.08x -27, r2= 0)
C
δ15N-zooplankton (‰)
epi:
y= 0.6x +3.6
r2=0.23
p= 0.16
meta:
y= 0.82x +4.2
r2= 0.41
p= 0.05
hypo:
y= -0.1x +6.9
r2= 0.04
p= 0.63

Figure 4.7: a) ^{13}C of meta- and hypolimnetic zooplankton vs. epilimnetic ^{13}C-POM. b) ^{13}C of epi-, meta-, and hypolimnetic zooplankton vs. ^{13}C-POM from the same respective stratum. c) ^{15}N of epi-, meta-, and hypolimnetic zooplankton vs. ^{15}N-POM from the same respective stratum.

The relationship between epilimnetic ^{15}N-zooplankton vs. epilimnetic ^{15}N-POM was non-significant ($p= 0.16$) with a slope of 0.6 (Figure 4.7c). The slope of metalimnetic ^{15}N-zooplankton vs. metalimnetic ^{15}N-POM was significant ($p= 0.05$) with a slope of 0.8. In contrast to the epilimnetic and metalimnetic relationships, (Figure 4.7a), the slope of the relationship between hypolimnetic ^{15}N-zooplankton and hypolimnetic ^{15}N-POM was close to zero (-0.1) and non-significant ($p= 0.63$). Comparing metalimnetic ^{15}N-zooplankton with the epilimnetic ^{15}N-POM (not shown) resulted in a non-significant relationship ($p= 0.60$), as did relating hypolimnetic ^{15}N-zooplankton with epilimnetic ^{15}N-POM ($p= 0.68$).

Taxa

There were no taxon-specific differences in C-signature among epilimnetic zooplankton. Using an ANCOVA with $^{13}CO_2$ as a covariate did not help. The $^{13}CO_2$ effect was significant, ($p<0.001$), but the taxon effect was not ($p=0.303$). The taxon and $^{13}CO_2$ interaction was also not significant ($p=0.839$). The result using ^{13}C-POM as a covariate was similar: the ^{13}C-POM effect was significant ($p<0.001$), while the taxon effect was not ($p=0.616$). The interaction was also non-significant ($p=0.616$). Using POM or *Daphnia* as a baseline for ^{13}C signatures, there were no significant differences in taxa ($p=0.804$ and 0.903, respectively). Therefore, I was unable to detect any differences among the epilimnetic zooplankton taxa that sampled.

I also compared ^{15}N signatures of taxa using POM as a baseline, finding some small differences among taxa (Figure 4.8). *Daphnia* ^{15}N signatures were depleted compared to calanoids by 2.0 ‰ ($p=0.001$), cyclopoids by 1.6‰ ($p=0.011$), but were not significantly different from *Holopedium* ($p=0.158$). *Holopedium* were depleted compared to calanoids by 1.3‰ ($p=0.016$). Calanoids, *Holopedium*, and cyclopoids were not significantly different from each other. Similarly, using *Daphnia* as a baseline, calanoids, *Holopedium*, and cyclopoids were not significantly different ($p=0.083$).

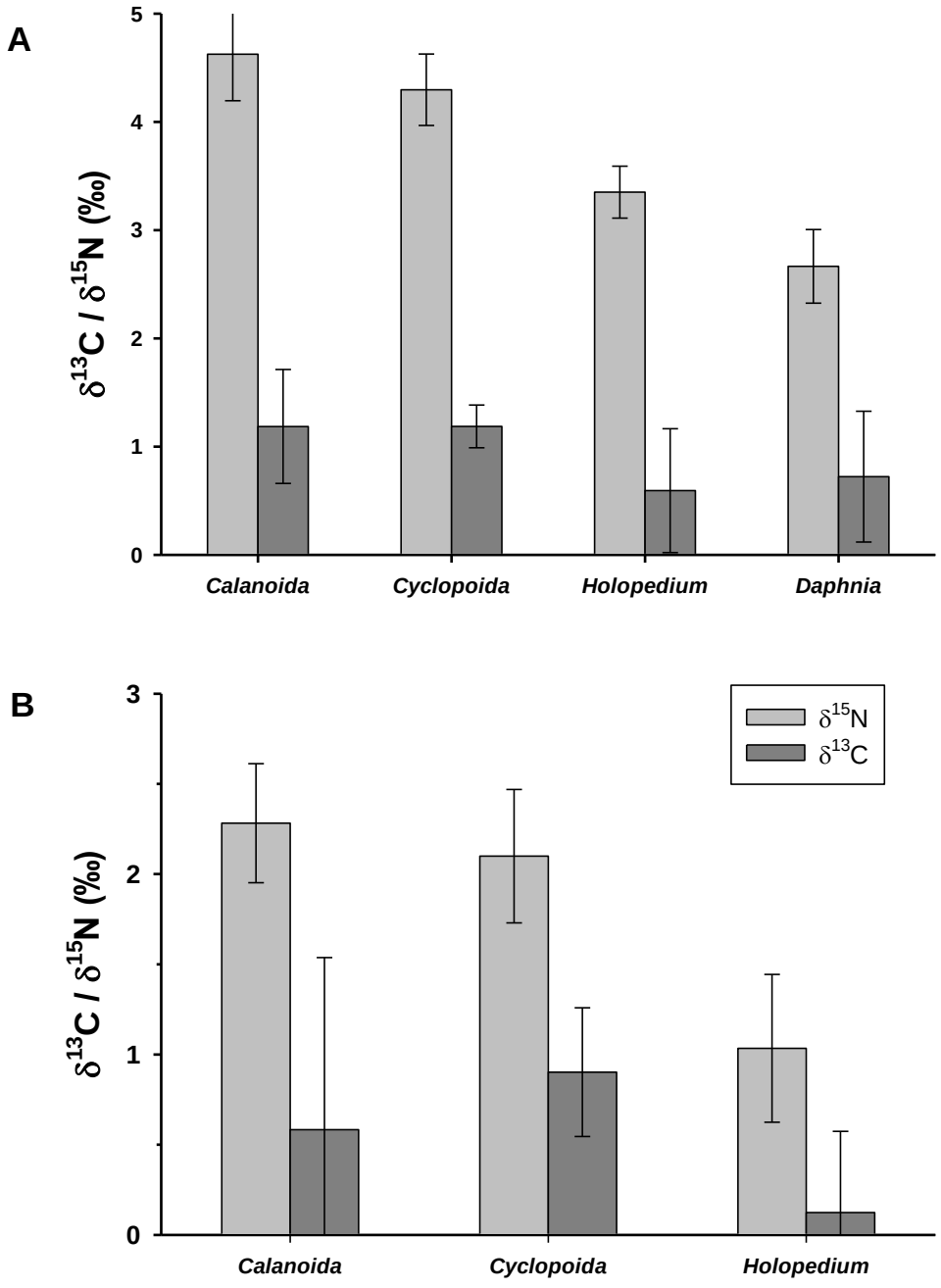

Figure 4.8: ^{13}C and ^{15}N of zooplankton taxa using A) ^{13}C and ^{15}N of POM as a baseline. B) ^{13}C and ^{15}N of *Daphnia* as a baseline. Error bars are standard error of the mean.

4.4 Discussion

$^{13}CO_2$

Epilimnetic $^{13}CO_2$ was always depleted compared to the atmospheric $^{13}CO_2$, even counting for fractionation on dissolution (i.e., less than -7 ‰). Thus, epilimnetic signatures were influenced by in-lake processes as well as atmospheric exchange. In most cases, epilimnia were enriched in $^{13}CO_2$ compared to metalimnia and they were always enriched in ^{13}C compared to hypolimnia.

The wider range in metalimnetic CO_2 signatures was probably due to a combination of effects. A potential source of variability is from processes occurring in the metalimnia. If primary production in the metalimnion was high, it could enrich the CO_2 pool as depleted CO_2 is selectively assimilated. For example, this may be why ^{13}C was enriched in the metalimnion compared to the epilimnion of Red Chalk Lake in 2003. Secondly, because the metalimnion is a zone of rapid transition, affected by both epilimnetic and hypolimnetic processes, slight differences in sampling depths could result in very large differences in the measured $^{13}CO_2$ signature.

In contrast to the variability in the epi- and metalimnia, hypolimnetic $^{13}CO_2$ occurred in only a small range (close to -28‰) of depleted $^{13}CO_2$ signatures. The CO_2 signatures, similar to that of POM, suggests that hypolimnetic CO_2 was primarily respiratory in origin. In general, the CO_2 signatures were not suggestive of methane oxidation, as biogenic methane is highly depleted in ^{13}C (Whiticar et al. 1986) and CO_2 produced from it is therefore also highly depleted. The lowest $^{13}CO_2$ signatures were in Dickie Lake (-29.7 ‰ and -29.5 ‰ in 2003 and 2004, respectively) which did have a

hypolimnion low in O_2. Thus, it is possible that methane oxidation had a slight contribution to these signatures.

^{13}C-POM

While $^{13}CO_2$ demonstrated a marked vertical structure, this was not reflected in the ^{13}C-POM, which typically showed only a slight depletion from the epi- to the hypolimnia. Others have found similar results (del Giorgio and France 1996; Matthews and Mazumder 2006). In Chapter 2, I argued that CO_2 was likely to be the primary C source for autochthonous production in the epilimnia of these lakes. In most of the lakes, PCO_2 in the meta- and hypolimnia was higher than that of the epilimnia (exceptions were in Little Clear and Red Chalk Lakes). Thus, it is reasonable that CO_2 was also the primary C source for meta- and hypolimnetic primary production.

Fixation of CO_2 would produce autochthonous POM that is more depleted than the CO_2 source. Autochthonous POM produced in the meta- and hypolimnion should, therefore, be depleted in ^{13}C compared to CO_2 from the corresponding layer. Generally, POM from the meta and hypolimnia was not as depleted compared to $^{13}CO_2$ from the same stratum as POM from the epilimnion was compared to epilimnetic $^{13}CO_2$. While it is possible that autochthonous fractionation in the meta and hypolimnia was much weaker than in the epilimnia, this seems unlikely. Except possibly in those cases where there was evidence of high metalimnetic production and low CO_2 concentration (Red Chalk Lake in 2004, Little Clear Lake), CO_2 was higher in concentration, light would be lower and fractionation is expected to be stronger. Another potential explanation is that POM was overwhelmingly dominated by allochthonous material, so would be independent of the $^{13}CO_2$

signature. In Chapter 2, however, I found that, across lakes in the region, POM had an appreciable autochthonous component (62 to 75% autochthonous). Therefore, the most likely reason that the POM ^{13}C signatures of the meta- and hypolimnia generally remain similar to the epilimnia is because most of the autochthonous production that contributes to the POM signature occurs in the epilimnia, though the slight depletion of POM at lower strata suggests that some primary production does contribute to POM at depth. An interesting exception to this pattern was found in Little Clear Lake, which uniquely showed a peak in phytoplankton fluorescence in the hypolimnion, and a concomitant depletion in the ^{13}C-POM in this stratum.

Diagenetic changes may also have contributed to the depletion of ^{13}C-POM. Lehmann et al. (2002) found that after approximately 20 d of incubation in the dark under oxic conditions, the ^{13}C of POM of lake water was depleted by 1.6‰. Thus, it is possible that at least some of the depletion of ^{13}C-POM with increasing depth was also due to diagenetic changes in POM.

^{15}N-POM

As with the depletion of ^{13}C-POM with depth, the enrichment of ^{15}N-POM with depth may have been due to diagenetic changes. Enrichment of ^{15}N-POM under oxic (but not anoxic) conditions has been noted in several marine studies (Altabet 1989; Saino and Hattori 1980; Voss et al. 1997; Wada and Hattori 1976). In an experimental study using lake water, Lehmann et al (2002) found that, over 20 d, the ^{15}N of POM enriched by 3‰ under oxic conditions, though it became increasingly depleted after 20 d. Thus, it is possible that POM ^{15}N enrichment occurred as the POM sank through the

water column. I did not observe this enrichment of ^{15}N-POM in two of the study lakes, one of which was Dickie Lake in 2004. This lake, however, had low O_2 concentrations throughout the hypolimnion, so the process of enrichment of ^{15}N-POM may not have occurred in this lake. Unfortunately, I do not have ^{15}N-POM data for Dickie Lake in 2003, which also had low O_2 concentrations throughout the hypolimnion. The other lake that did not have enriched hypolimnetic ^{15}N-POM was Little Clear Lake. While it did not have an anoxic hypolimnion, this lake showed evidence of hypolimnetic primary production. Thus, the hypolimnetic POM may have been dominated by new production that had not been enriched through diagenesis as in the other lakes.

Zooplankton-POM relationship

Zooplankton ^{13}C followed a similar pattern to that of ^{13}C-POM, becoming depleted with depth, suggesting that zooplankton are, at least partially, feeding from the stratum from which they were sampled. For this set of lakes, the relationship between epilimnetic ^{13}C-zooplankton and epilimnetic ^{13}C-POM was weak. In a larger set of lakes with a broader range in ^{13}C-POM, however, I found (Chapter 2) a much stronger relationship between these two.

Epilimnetic ^{13}C-zooplankton were enriched compared to ^{13}C-POM. Also in Chapter 2, I showed that ^{13}C-zooplankton were enriched relative to ^{13}C-POM in this range (-19.5 to -9.2‰, mean: -12.9‰) because the autochthonous portion of POM in most of these lakes would be enriched in ^{13}C compared to the bulk POM. Thus, biased feeding by zooplankton on the

autochthonous portion of POM would produce zooplankton enriched in ^{13}C compared to the bulk ^{13}C-POM signature.

Unlike epilimnetic zooplankton, meta- and hypolimnetic zooplankton were depleted compared to the bulk epilimnetic ^{13}C-POM. This suggests that meta- and hypolimnetic zooplankton were accessing a depleted source of C. The likely explanation of this is that $^{13}CO_2$ in the metalimnia (usually) and hypolimnia (always) were highly depleted compared to the epilimnia. These highly depleted $^{13}CO_2$ values would produce autochthonous POM that is more depleted than that produced in the epilimnia. Comparing meta- and hypolimnetic zooplankton ^{13}C to that of ^{13}C-POM from the same strata results in relationships between the two that are closer to 1:1. However ,when compared in this way, zooplankton of some lakes is appreciably depleted in ^{13}C compared to POM from the same stratum. Again, this reverse relationship with zooplankton depleted to the POM would occur because biased feeding on autochthonous POM in the meta and hypolimnia would produce ^{13}C depleted, rather than enriched (as in the epilimnia), zooplankton compared to the bulk POM.

While it appears that hypolimnetic zooplankton were accessing C produced in the hypolimnion, this does not appear to be true for hypolimnetic N. While the ^{15}N-POM signatures of the hypolimnia were highly enriched compared to that of the epilimnia, hypolimnetic zooplankton ^{15}N did not reflect this enrichment, indicating that they were not accessing this N to a large extent.

Zooplankton Taxa

Cyclopoids and calanoids were enriched by 1.6‰ and 2.0 ‰ compared to *Daphnia*, while calanoids were enriched to *Holopedium* by 1.3‰. Using a trophic enrichment range of 2.2 to 3.4‰ (McCutchan et al. 2003; Vander Zanden and Rasmussen 2001) suggests that calanoids were enriched from approximately half to close to one (0.6 to 0.9) trophic level while cyclopoids were slightly less than this (0.5 to 0.7). Calanoids were also approximately half a trophic level (0.4 to 0.6) above *Holopedium* . These findings are in the range of other studies in which copepods were found to be enriched relative to *Daphnia* and/or *Holopedium* (Gu et al. 1994; Karlsson et al. 2004; Matthews and Mazumder 2003; Rautio and Vincent 2007; Syvaranta et al. 2006; Ventura and Catalan 2008). While the differences in ^{15}N among taxa may be due to the groups feeding at different trophic levels, as discussed by Karlsson et al. (2004), the differences in ^{15}N among taxa may also have been due to variable trophic enrichment among taxa. Zooplankton in these oligo-mesotrophic lakes, which were sampled in late summer, may have been in a highly food-limited condition, during which catabolism could result in preferential excretion of ^{14}N and retention of ^{15}N (Ponsard and Averbuch 1999). Since *Cladocera* are thought to be more starvation-prone than copepods (Rothhaupt 1990), it is possible that their enriched ^{15}Ncompared to copepods contributed to by variable retention of ^{15}N among taxa

While there were differences in ^{15}N among some taxa, I found no differences in ^{13}C among taxa. The range of epilimnetic CO_2 signatures in this set of study lakes would produce an autochthonous signature close to -28‰ (Chapter 2). Thus, it is not possible to interpret the similarity in ^{13}C signatures as being indicative of similar ultimate C source among the groups.

Conclusions

In this work, I found vertical heterogeneity in $^{13}CO_2$, $^{13}C/^{15}N$ of POM, and $^{13}C/^{15}N$ of zooplankton. This vertical structure was most marked in $^{13}CO_2$ signatures which, generally, depleted appreciably with increasing lake depth. The signatures of ^{13}C-POM and ^{13}C-zooplankton also generally depleted with depth, but this was very muted compared to the depletion of $^{13}CO_2$ with depth, suggesting that a large portion of POM and zooplankton C in the meta- and hypolimnia are from the epilimnia. Among taxa, I did not detect differences in C signature, though this may have been masked by the similarity of autochthonous and allochthonous C signatures in this set of lakes. However, I did note small differences in N signature amongst some taxa.

Chapter 5

Conclusions and future directions

In the lakes that I sampled in south-central Ontario near Dorset, POC contained an appreciable terrestrial allochthonous component, ranging from 25% to 38%. Zooplankton, however, apparently favour the autochthonous portion of POC, as they were composed of a smaller fraction of allochthonous material (9 to 23%). As discussed previously, while this is an appreciable contribution, this estimate is much lower than some recent findings (e.g. Carpenter et al. 2005, Karlsson et al. 2003, Pace et al. 2004). One possibility is that the Dorset-area lakes that I studied are atypical or fall within a range of lakes more likely to be autochthonously-driven than most temperate lakes. However, a study on a spectrum of oligotrophic to eutrophic lakes predicted that the more oligotrophic would tend toward having a greater importance of allochthonous inputs to zooplankton nutrition (del Giorgio and France 1996). This is because oligotrophic lakes would have lower autochthonous production available for higher trophic levels. The oligotrophic to mesotrophic Dorset lakes should therefore be ideal candidates for having a significant allochthonous influence on zooplankton. It has also been hypothesised that humic/dystrophic lakes, with their higher allochthonous inputs and darker colour (potentially inhibiting autochthonous photosynthesis), would show a greater trophic importance of allochthonous inputs to zooplankton (Jones 1992). While there were no truly dystrophic lakes in my data set, some lakes did have appreciably high DOC concentrations. Still, a pattern of increasing zooplankton allochthony with increasing DOC or PCO_2 did not emerge. The meta-analysis in Chapter 3 used lakes ranging from temperate to subarctic,

therefore also spans a larger range in lake size, TP, DOC, and PCO_2 than do the Dorset study lakes. Yet they demonstrate a similar pattern in the proportion of allochthonous and autochthonous contribution to POC and zooplankton. POC from the Dorset lakes was somewhat more autochthonous than the lakes used in the meta-analysis (62-75% vs. ~50%). Zooplankton, however, were similarly highly autochthonous in both datasets.

Both the Dorset study lakes as well as the metadata, of which it was a part, lacked some important lake types. Because the analysis I used required photosynthetic fractionation to be independent of CO_2 availability and required that bicarbonate was not a significant source of DIC for photoautotrophs, eutrophic lakes tended to be excluded. As mentioned, however, these lakes are thought to be more autochthonously-driven than are oligotrophic lakes. As discussed in Chapter 3, some of the lakes I excluded from the meta-analysis could have had appreciable methane production derived from allochthonous inputs. I did not, however, find the highly-depleted signatures expected from methanotrophy in the POC or zooplankton. As mentioned, there were no highly dystrophic lakes among the Dorset lakes, or in the metadata. Since dystrophic lakes may be the most likely situation in which allochthonous inputs are important to zooplankton, further work examining this type of lake would yield interesting results as to the potential trophic importance of terrestrial inputs. Reservoirs were also absent from the metadata. Unfortunately, the reservoirs (as well as the lakes) in Marty and Planas (2007) had to be excluded from the meta-analysis in Chapter 3 as they did not meet the fractionation criterion in this study. A major reason that terrestrial C inputs may not be accessible to higher trophic levels is that by the time the terrestrial organic material reaches the pelagic region, it would have been subject to

extensive processing leaving only the most recalcitrant material. In reservoirs, especially ones with fluctuating water levels, it is possible that rising water levels could release highly labile terrestrial material into the pelagic region of the reservoir. Also, reservoirs tend to have shorter water residence times than lakes. Interestingly, in a study of a new reservoir by Embury (2000), *Daphnia* ^{13}C signatures closely tracked ^{13}C-DIC, both before and after flooding of a forest, suggesting that *Daphnia* were not acquiring a terrestrial signature, despite the large, and potentially more labile, input from flooding. There were also no tropical lakes in the present study. With their typically higher autochthonous productivity, however, it would seem less likely that tropical lake food webs are less allochthonously-driven than are temperate ones.

The data from the Dorset study lakes are from late in the stratified season, whereas stream inputs in the area peak at snowmelt. It is therefore likely that this was a period when allochthonous production is high and in summer, though two seasonal studies were included in the meta-analysis. In one, (Grey et al. 2001), the authors concluded that there was a strong seasonal cycle in zooplankton allochthony, with the highest autochthony during summer. Unfortunately, because of the small range in ^{13}C signatures, it was not possible to discern whether there was seasonality in zooplankton allochthony in their dataset using my analysis. While a seasonal pattern was apparent in a study by Gu et al. (1999), zooplankton appeared to remain mostly autochthonous in all seasons. Further work to determine if allochthonous contribution to zooplankton varies seasonally across a spectrum of lake types would be useful.

In this work, I found that zooplankton C was mostly autochthonous. Much recent work, however, has found allochthonous inputs to be the main source of C to pelagic zooplankton nutrition. The importance of allochthonous C to fuelling these 'higher' trophic levels continues to be the primary focus of work attempting to understand the significance of allochthonous inputs to lake ecosystems. It is, however, important that this focus on metazoan nutrition does not result in a failure to appreciate perhaps far more pervasive roles of allochthonous C inputs on other key aspects of lake ecosystem function. Indeed, Wetzel (1992) pointed out that the reason allochthonous energy subsidies had been ignored is because they did not (in his view) fuel metazoan food webs. The more recent work that suggests that allochthonous inputs may fuel these metazoan pathways has brought attention to the potential importance of allochthonous energy subsidies, but perhaps has narrowed our view to this question alone. This 'zoocentric' view, as Wetzel (1992) called it, has placed a disproportionate amount of attention on metazoa, which are a relatively small part of overall energy and nutrient pathways of lake ecosystems compared to that of the microbial/detrital pathways. Of course, for economic and cultural reasons, the metazoan pathways are of most interest to humans. But the production and stability of these pathways may rely, to a great extent, on the much larger microbial/detrital pathways. For example, by providing an alternative to C fixed by phytoplankton for bacteria, allochthonously-supplied energy may damp fluctuations in nutrient cycling by bacterioplankton and therefore fluctuations in phytoplankton production. Another example of an indirect role of allochthonous C is through the alteration of the light environment, which could alter phytoplankton production (Carpenter et al. 1998, West et al. 1999). While more indirect, such

effects of allochthonous energy subsidies may exert a far more profound effect on lake ecosystems than their effect as a direct C source for pelagic metazoan food webs. Allochthonous C may exert its greatest direct influence on metazoan food webs in littoral zones, where fish and benthic invertebrates can directly access particulate detritus and insects (Mann 1988; Mancinelli et al. 2007; Mehner et al. 2005; Polacek et al. 2006; Saksgard and Hesthagen 2004). Additionally, the respiration of allochthonous C, whether from detrital or metazoan pathways, has significance to our understanding of C cycling. While I found, in this work, that zooplankton largely access autochthonously produced C, allochthonous C may exert a more indirect, but perhaps more profound, suite of effects on lake ecosystems.

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