Food-Web Dynamics of Lake Ontario as Determined by Carbon and Nitrogen Stable Isotope Analysis

by

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Abstract

Carbon and nitrogen stable isotopes were applied as naturally occurring tracers in determining the trophic relationships of different organisms in the pelagic food web of Lake Ontario. Particular attention was given to understanding how biogeochemical processes influenced the isotope signatures of primary producers and consumers at the base of the food chain and how isotope signatures at the baseline varied seasonally.

The $\delta^{13}C$ of particulate organic matter was observed to shift by 10 ‰ from spring to mid summer. This reflected the combined influences of primary production, temperature, changing pH and the system inputs on the concentration and $\delta^{13}C$ of the dissolved CO_2 used as a substrate for primary production. The seasonal range in $\delta^{15}N$ at the base of the food web, was similarily determined to be a function of the $\delta^{15}N$ of the organic and inorganic sources of nitrogen available seasonally and algal preferences for the different nitrogen sources. The range in the $\delta^{15}N$ of base of the food web, was 16 ‰. The seasonal pattern of fluctuation in carbon and nitrogen isotope signatures at the base of the food web was exploited in the dietary analysis of an omnivorous crustacean (*Mysis relicta*), smelt (*Osmerus mordax*), alewife (*Alosa pseudoharengus*) and slimy sculpin (*Cottus cognatus*). Inferences were made as to the relative importance of benthic and pelagic sources in contributing to the contaminant loading of Lake Ontario lake trout on the basis of stable isotope analysis.

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Chapters 3 to 6 either have been submitted, or will be submitted with revision, to professional journals as co-authored papers for peer review. The authorship on the papers will read as follows:

Biogeochemical Influences on the Carbon Isotope Signatures of Lake Ontario Biota

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The Influence of Inorganic Nitrogen Cycling on the $\delta^{15}N$ of Lake Ontario Biota

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A quantitative determination of the diet of *Mysis relicta* in Lake Ontario

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A Stable Isotope Analysis of Food Sources of Lake Ontario Forage Fish

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Introduction

The uptake of hydrophobic organic contaminants (HOCs) is closely tied to the flow of energy in aquatic systems (Rasmussen *et al.* 1990). Most of the available HOCs are adsorbed to particulate in the water column or they are absorbed by biota in the system (Harding and Philips 1978; Voice 1983; Mackay and Diamond 1989). The HOCs are subsequently transferred up the food chain and 'biomagnify', which means that organisms at higher trophic levels have higher body burdens of HOCs per unit mass (Oliver and Niimi 1988; Rasmussen *et al.* 1990). By understanding the trophic dynamics of a system inferences can be made with respect to the relative importance of different routes of contaminant uptake by pelagic predators. This was the primary objective in the initiation of this study, to map trophic relationships within the pelagic food web of Lake Ontario using carbon and nitrogen stable isotopes, and to use the information obtained to make inferences on the routes of contaminant transfer within the system. A better understanding of food web dynamics may also be applied in more effective lake management practices. A quantitative assessment of trophic relationships would lead to enhanced predictive ability. The norm must be understood before we can assess the impacts of anthropogenic and environmental stresses.

The second objective in this thesis was to investigate the efficacy in using carbon and nitrogen stable isotopes as a tool in elucidating food web structure. The focus here was

on development of the relationship between system biogeochemistry and the observed isotope signatures.

Background Ecology

Much of the research in theoretical and applied ecology, especially over the past three decades, has been done with a view toward predicting how a particular system will react under conditions of anthropogenic stress. Ecosystem stress may be defined as a disorganizing influence (Odum *et al.* 1979; Pratt 1990). All inputs which produce deviations from the nominal state of the system are therefore sources of stress (Odum *et al.* 1979; Pratt 1990). The problem for ecologists becomes defining the nominal state of the system. Typically this is done in terms of the expected biological structure within an area, given the geography, climate and resources available.

An ecosystem is defined as a whole system, including the organism-complex and the environment in which they exist (Godwin 1977). The physics, chemistry, biochemistry, geochemistry and biology of the system components co-act to determine the dynamic ecological setting in which species exist. The biological component within an ecosystem may be separated into successive levels of organization: individuals, populations, communities. Populations and communities interact within food webs and are influenced by inorganic and organic system inputs. Individual organisms faced with environmental change will either resist the change or compensate for it (Pratt 1990). When this ability is overly taxed, in either duration or magnitude, the organism is said to be stressed and

becomes less competitive. Stress induces changes in populations and communities. Community structure is then viewed as the result of long-term, on-going, adaptation by individual organisms faced with a changing environment. The community structure will only be altered when changes faced by individual organisms become stressful (Pratt 1990).

That alteration in populations stems from the response of individuals is the rationale behind research into the biochemistry of stress response in individual organisms as a determinant of toxicant impact. Before impacts are observed in individuals, biochemical coping mechanisms are likely to be induced as a response to stress. Once characterized, biochemical responses may serve as "biomarkers" or sentinels in monitoring stress due to the presence of toxic substances (Huggett *et al.* 1992). An "index of biotic integrity" or diversity index has been similarly utilized at the community level as an assessment of whether or not the community composition is representative of a "healthy" system (Abel 1989). In both measures, problems exist in making a cause and effect linkage between levels of organization (Munkittrick and McCarty 1995). The presence of effects at the molecular level cannot in itself be characterized as an impact since the competitive ability of the individual is not necessarily compromised (Munkittrick and McCarty 1995). While an altered community structure definitely demonstrates an impact, demonstrating causality requires extrapolation down to the level of individual response (Munkittrick and McCarty 1995). Investigation may be made from a "top-down" perspective, from communities to individuals and below, or "bottom-up", in the reverse direction. Either

way, in order to make inferences about causality or predict the effects induced by a particular form of stress, an understanding of the structure and function of the ecosystem is necessary.

Theory in Ecosystem Structure and Function

The concepts of ecosystems and trophic levels as they are currently understood were defined in early studies of the effects of energy flow on trophic structure (Lindeman 1942; Odum and Odum 1955). Early theories relating energy to ecosystem structure held that the height of a trophic pyramid was a function of the amount of available energy and efficiency of energy transfer between trophic levels (Hutchinson 1959; Slobodkin 1960, 1962). A sustainable source of sufficient energy must be available in order for an additional trophic level exploiting that source to become established (Oksanen 1988). Additionally, the amount of biomass at each trophic level will be constrained by resource supply since thermodynamics dictates that the energy necessary for growth and reproduction cannot be generated within a trophic level. This principle continues to be applied in estimates of maximum productive capacity of ecosystems (Beddington 1995). However, field studies have demonstrated that the availability of energy does not generally translate into extended food chains (Pimm and Lawton 1977; Pimm and Kitching 1987).

There are other forces active as determinants of food web structure besides the input of energy to a system. Alternative hypotheses have been postulated which incorporate

energetic constraints with other physical and biological limitations. The dynamic constraints hypothesis suggests that longer food chains are more susceptible to collapse following a natural disturbance resulting in a reduction in the resources available to species at the top of a food pyramid (Pimm and Lawton 1977; Pimm 1982). Species on the end of long food chains will be unable to adapt quickly enough to changes in resource supply and will therefore be eliminated (Pimm and Lawton 1977; Pimm 1982). This suggests that food chains should be shorter in more hostile environments, which is not generally observed (Polis 1991). Omnivory has alternately been described as both a destabilizing and stabilizing influence on food web structure (Pimm and Lawton 1977; Yodzis 1980, 1984; Briand 1983; Sprules and Bowerman 1988; Stein *et al.* 1995).

Omnivory requires organisms to both prey on, and compete with, co-evolved species, a situation which, in theory, should be unstable (Pimm 1982). By extension, it may be argued that omnivory should be rare in that it requires the evolution of species able to competitively feed at two trophic levels simultaneously. Empirical studies and further modeling efforts have demonstrated that omnivory in aquatic systems is common and not necessarily an unstable situation (Briand 1983; Sprules and Bowerman 1988; Yodzis 1984; Stein *et al.* 1995). Omnivorous organisms are expected to garner most of their energy from shorter food chains where energy is less dilute (Yodzis 1980; Hastings 1988). In so doing, omnivores effectively limit food chain length (Yodzis 1980; Hastings 1988).

The trophic cascade is a hypothesis integrating food web structure and function (Carpenter *et al.* 1985). The trophic cascade is predicated on interspecific interactions within ecosystems combined with food web ecology applied in explaining relationships commonly observed in natural systems (Hairston and Hairston 1993). The premise of the theory is that any organism using a resource as a source of energy is likely to have the ability to do so efficiently enough to eliminate it (Hairston *et al.* 1960). The earth is green and therefore grazers are not food-limited and must be predator-limited (Hairston *et al.* 1960; Fretwell 1987). If grazers are predator-limited their predators must be food-limited (Rosenzweig 1973). Given this scenario, changes in the population density or overall biomass at one trophic level will be likely to influence the same parameters in the next level. Trophic cascade therefore suggests that community structure is determined by the interactions between different trophic levels. A change in biomass at one level will impact all other levels in the food chain, i.e. there will be a cascading response. Field investigations of trophic cascade hypotheses, through experimental manipulation of aquatic systems or observations made in the wake of disturbances to a particular population within the system, have recorded a wide range of ecosystem responses (Hrbácek *et al.* 1961; Lynch and Shapiro 1981; Carpenter *et al.* 1985, 1987; McQueen *et al.* 1986; Persson *et al.* 1988, 1992). The changes in community structure witnessed were often not the direct result expected. Behavioral changes in predator-prey interaction, or altered interspecific competition within a trophic level (due to increased or decreased predation pressure on a specific population), can also produce changes in community structure. It is recognized that the biomass and size structure of individual

system components may be regulated by top-down or bottom-up forces (McQueen *et al.* 1986). Top-down forces come in the form of cascading predation pressure (Hrbácek *et al.* 1961; Lynch and Shapiro 1981; Carpenter *et al.*1985; McQueen *et al.* 1986). Bottom-up forces are abiotic physicochemical factors which have been demonstrated to influence lake productivity (the total annual carbon fixation by phytoplankton) (Schindler 1971; Schindler and Fee 1973; McQueen *et al.* 1986).

Stoichiometric relationships between zooplankton, fish and primary producers may play a role in determining community structure and limiting/enhancing rates of primary productivity (Sterner and Hessen 1992; Hessen 1997). In order to develop a predictive ability, the relative magnitude of influence of top-down *vs.* bottom-up forces in different situations and under different environmental conditions needs to be understood. The integration of biogeochemistry as a component of food web dynamics adds a degree of complexity but may serve in explaining why the response of some systems to perturbation is at odds with current theory (Sterner and Hessen 1992; Hessen 1997). Thus, the trophic cascade hypothesis is evolving with the recognition and integration of alternative influences of predation pressures and thermodynamic constraints as being factors determining ecosystem structure.

Food Webs

In studies of the structure and function of whole ecosystems, discussions of interactions are usually restricted to trophic levels (Hairston and Hairston 1993). There are a number

of features of food webs which make it difficult to apply food web theory in developing generalizations applicable to whole systems (Hairston and Hairston 1993). There is no convention for the selection of appropriate spatial and temporal scales or the level of detail to be included in a food web which makes comparison difficult (Pimm 1982; Pimm and Kitching 1988; Paine 1988; Peters, 1988). Ontogenetic changes in trophic position of species are difficult to represent in static food web structures (Cohen and Newman 1988; Paine 1988). Uncertainty in determining relative interaction strengths is often circumvented in food webs by giving equal weighting to all possible interactions between species (Pimm 1982; Paine 1988; Pimm and Kitching 1988; Peters 1988). It is unrealistic to assume that all energetic pathways are of equal importance. Without a means of weighting the relative importance of linkages, the value of food webs in the development of a predictive capability is diminished. Developing food webs has been a very subjective exercise of providing structure through mapping collections of qualitative observations (Briand and Cohen 1984; Peters 1988). Without a quantitative or consistent foundation for interpretation of interactions within food webs, it is difficult to derive generalities between published food webs which may be used in making inferences on ecosystem structure (Peters 1988).

There are advantages to using food webs as a foundation for studies of ecosystems. Food webs have the inherent ability to deal with omnivory as an integral component of the biological structure of an ecosystem (Sprules and Bowermann 1988). Food webs also have the necessary detail to enable inferences on the interactions between each organism

and their environment as components of an ecosystem. The development of a functional understanding of ecosystems with predictive power is going to require investigation that deals with ecosystem complexity in its entirety. This will ultimately require holistic studies in the form of ecosystem manipulations where a form of stress is applied and the changes induced are observed. The development of technologies capable of measuring more subtle structural changes in food webs will aid in interpreting the observed response of a system to an applied form of stress.

The quantitative determination of the relative strength of linkages within food webs offers a great deal in terms of sorting out energetic pathways and establishing an understanding of the mechanisms controlling the flow of energy. The flow of energy is inextricably linked to the flow of carbon. Carbon-based mass balance models have used biomass estimates, converted to carbon or a caloric content, coupled with a qualitative understanding of the system under investigation, to move toward quantifying interaction strengths (Flint 1986). The foundation for such models is empirical evidence of interaction and extensive knowledge of the system with respect to the relative magnitude of populations. The ability to measure the relative strength of interactions without population estimates would be an asset in terms of the work required to characterize a system and to observe a change in the food web dynamics.

Stable Isotopes

Stable isotope analysis has received much attention recently in the field of ecology for its potential as a tracer of energy in ecological systems. Individual atoms of elements do not have a uniform mass. Elements are a collection of isotopes, which are atoms with similar chemical properties but differing in nuclear structure. A small percentage, typically less than 1%, of the atoms of any element in nature will have one or more neutrons than the majority of the atoms making up the bulk of the total supply of the element. It was initially believed that the ratio of heavy to light isotopes in materials, for example ^{12}C and ^{13}C, remained constant (Bigeleisen 1947; Bigeleisen and Wolfsbeerg 1958). Investigations of the relative proportions of heavy and light isotopes contained in materials formed through different processes demonstrated changes in the relative proportions of heavy and light isotopes compared to source materials (Bigeleisen and Wolfsbeerg 1958). Subsequent investigation as to how and why these changes occur has led to advancements in understanding the differences in behavior of heavy and light isotopes with respect to chemical kinetics and bond energies. Ratios of heavy to light isotopes are expressed as δ values or 'signatures', in units 'per mil'(‰), which is the parts per thousand difference from a standard. An example of the calculation for reporting the relative ratios of carbon stable isotopes is given here:

$$\delta^{13}C = [(^{13}C/^{12}C_{\text{sample}})/(^{13}C/^{12}C_{\text{standard}}) - 1] \times 10^{3}$$

If the ^{13}C to ^{12}C ratio in the sample is lower than the ratio in the standard, the δ value will be negative. In comparing two samples, a more positive δ value indicates heavy isotope enrichment.

The level of understanding as to how the ratio of naturally occurring stable isotope pairs will be influenced by various chemical or biological processes has continued to grow. As this knowledge has increased, and the analytical ability to precisely measure the relative proportions of isotope pairs at trace levels has developed, the potential for the application of stable isotope pairs as naturally occurring tracers in the environment has also increased. For an ecosystem scientist, possession of the ability to infer the roots of origin of a material or trace the flow of an element through a number of chemical pathways in an ecosystem is an asset. There are currently a number of books and review papers available which describe how stable isotope analysis, particularly of carbon and nitrogen isotope pairs, has been applied in ecology and to ecosystem studies at several different levels (Peterson and Fry 1987; Lajtha and Michener 1994). The number of research papers published in which stable isotope analysis has been employed continues to grow. Unfortunately, in a large proportion of this literature only a portion of the basic theory of isotope analysis is applied. Literature demonstrating that isotope signatures can be temporally and spatially heterogeneous, especially at the level of nutrients and primary producers, is often ignored in food web studies. Theoretical constructs, or observed generalities through continued application, often become axiomatic. Such has been the case with the use of stable isotopes in ecosystem science. The application of the theory

in interpretation of stable isotope data is often done without due consideration of ecological and biogeochemical processes as features of the system under investigation which has the potential to lead to erroneous conclusions.

Stable Isotopes in Ecosystem Studies

Stable isotopes have been used in the study of trophic relationships as tracers of energy source (Fry 1991). There are two underlying principles upon which most of the previously published work done has been predicated. The first assumption is that there is very little fractionation between ^{13}C and ^{12}C in trophic transfer so that the carbon isotope signature ($\delta^{13}C$) is consistent between a predator and its prey. The second assumption is that there is a consistent level of enrichment of the heavier isotope of nitrogen in successive trophic levels. A predator on average will have a nitrogen isotope signature ($\delta^{15}N$) approximately 2.5 to 3.0 ‰ larger than its prey (Owens 1987; Peterson and Fry 1987). Since the $\delta^{13}C$ remains relatively constant in trophic transfer, it has often been used as an indicator of food source. The $\delta^{15}N$ has been exploited as an indicator of trophic position. By plotting the $\delta^{13}C$ *vs.* $\delta^{15}N$ for the various species inhabiting an ecosystem, a food web can presumably be mapped. The trophic status of each organism can be inferred on the basis of where it sits on this plot in relation to the other organisms plotted (Fry 1991). The assumption of consistency in the level of isotope enrichment in nitrogen between and among species may be questioned. Laboratory studies measuring the isotope composition of organisms relative to their diet are rare (DeNiro and Epstein 1981; Macko *et al.* 1982; Toda and Wada 1990; Hesslein *et al.* 1993). Those that exist,

demonstrate that there is a great deal of variability in the observed levels of nitrogen enrichment both within and among species (DeNiro and Epstein 1981).

Field studies in which stable isotopes have been applied to discern trophic relationships have demonstrated a range in trophic fractionation from approximately 4 to 2 ‰ (Minigawa and Wada 1984; Owens 1987). Virtually all studies have asserted that there is a consistent level of fractionation between trophic levels. Differences are often attributed to omnivory or migration (Hobson 1993; Kivi *et al.* 1996; Vander Zanden and Rasmussen 1996; Hansson *et al.* 1997). Levels of variability within species are difficult to discern from the literature since the values reported are often averages and most stable isotope studies are characterized by a lack of replication in analysis often justified by the analytical precision. Studies which have reported measurements of many replicates have demonstrated a broad range of variability (France 1995a, 1995b; Kiriluk *et al.* 1995; Yoshioka and Wada 1994).

Another assumption made in food web studies using isotopes is that the baseline isotope signature remains constant or, at a minimum, is accounted for in some manner. The interpretation of stable isotope data is not divorced from the temporal problems in interpretation inherent in all ecosystem investigation (Goering 1990; Yoshioka and Wada 1994; Cabana and Rasmussen 1996). The difference in the time scale of response to perturbation of various ecosystem components is a problem to be addressed in the

investigation of whole lake ecosystems (Carpenter and Kitchell 1988). The speed with which a population of a given species is able to respond to environmental perturbation is loosely correlated with the average mass of the individual organisms. For example, phytoplankton are able to respond quite quickly to changes in nutrient input whereas the population dynamics of large fishes may shift on a time scale of decades (Carpenter and Kitchell 1988). Therefore in comparing the relative response of different trophic levels to a given perturbation in ecosystem studies, the duration and frequency of data collection are extremely important considerations (Carpenter and Kitchell 1988). The same is true in studies using carbon and nitrogen stable isotopes. The isotope signature of an organism is dependent on the isotope signature of its diet, past and present, which may or may not reflect current conditions. An organism's isotope signature is the integrated sum of all of the element assimilated in the period of time required for the organism to completely turn-over the entire pool of that element in its body. The scale of response to change of the isotope signature of an organism is likely to be similar to the scale of response in the population dynamics to a perturbation affecting reproductive ability. The further an organism is along the food chain, the greater the lag time between the observed signature and the baseline signature from which it originated. A shift in feeding of a top predator will gradually show up in its isotope signature over a period of years. A shift in the isotope signature of nutrient in an aquatic system may be reflected in the isotope signature of phytoplankton utilizing that nutrient source within days.

Variability in $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web suggests differences in the relationship between primary producers, dissolved inorganic carbon (DIC), and dissolved inorganic nitrogen (DIN) with respect to isotopes (McCabe 1985; Goering 1990; Gu and Anderson 1993; Yoshioka and Wada 1994; Cabana and Rasmussen 1996). Isotope data from Lake Ontario may be used as an example; Kiriluk *et al.* (1995), used stable isotopes as a measure of the source ($\delta^{13}C$) and trophic status ($\delta^{15}N$) of biota in Lake Ontario to demonstrate that there is a trophic enrichment of selected organic contaminant concentrations as you move up the food chain. Plotting the $\delta^{13}C$ *vs.* the $\delta^{15}N$, source *vs.* trophic position, provided a vague assessment of lake trout diet and trophic position of biota in Lake Ontario. As might be expected given the theory of isotope signatures described and what is known of the feeding of lake trout in Lake Ontario, the $\delta^{13}C$ of lake trout was consistent with a diet of sculpin, alewife and smelt and the $\delta^{15}N$ of lake trout was, on average, higher than the forage fish. Also observed was a wide range in $\delta^{13}C$ values, approximately 8 ‰, in a system where all of the carbon was ostensibly from the same, and therefore isotopically consistent, autochthonous origin. The range in $\delta^{15}N$ of lake trout was found to be in the order of 6 to 8 ‰ and there was an observed 8 ‰ difference in $\delta^{15}N$ between spring and fall net plankton samples (Kiriluk *et al.* 1995). It seems unlikely that individual lake trout would differ in their relative trophic status by as much as 2 trophic levels in the same system. It is also unlikely that the trophic position of net plankton changed substantially. These results pose a number of questions with implications for the interpretation of the study results. It may be asked what was responsible for the observed variation and range in signatures.

The biogeochemistry of the system determines the isotope signatures of primary producers. Temporal studies of the $\delta^{13}C$ of particulate organic matter in relation to inorganic carbon suggest that the carbon isotope composition is a predictable function of system biogeochemistry (McCabe 1985). Marine/estuarine studies indicate that the isotope composition of the inorganic nitrogen pools can vary on a time scale of days (Horrigan 1990). Carbon isotopes have been used by plant physiologists in developing an understanding of the mechanisms of carbon uptake by algae. As a result, the information available on the subject is detailed and complex. Relative to studies of carbon, very little work has been done looking at temporal fluctuations of $\delta^{15}N$ of primary producers in relation to inorganic nitrogen in freshwaters (Montoya 1990). A review of the current understanding of the processes influencing the isotope signatures of dissolved inorganic nitrogen (DIN) and carbon (DIC), as well as the role nutrient isotope compositions may have in determining $\delta^{13}C$ and $\delta^{15}N$ at the base of the food-chain is presented in chapter 2 of this thesis. It is critical to recognize that the concentration and isotope signature of available DIN and DIC determine the $\delta^{13}C$ and $\delta^{15}N$ of primary producers and organisms feeding on those primary producers. Changes in a systems biogeochemistry will be reflected in the isotope signatures of organisms at the base of the food web.

Implications for Isotope Studies of Food Webs

Primary producers will respond quickly to changes in the isotope signatures of nutrients at the base of the food web. Zooplankton would be expected to have a similar scale of variability in their isotope signatures as the primary producers on which they are feeding. A lag in response associated with the life-cycle and tissue turnover of the species analyzed may be expected, particularly if the species is carnivorous. Changes in the isotope composition of phytoplankton and zooplankton in a system, due to seasonal shifts in the biogeochemistry, occur on the scale of days. Trends should be readily observable with a weekly or bi-weekly sampling regime. As you move up the food-chain, the fluctuation due to nutrient cycling would be expected to be dampened until it is no longer discernible. That is because tissue turnover time in fish is slow compared to algae or zooplankton. In a fish, the seasonal fluctuation in the food source would be integrated into an organism with a biomass sufficiently large that only a sustained change in the isotope composition of the food source would be expected to have any influence.

Seasonal variation in isotope signatures at the baseline has the potential to be an extremely useful property. By monitoring the isotope signatures of aquatic organisms on a time scale related to their rate of tissue turnover, it may be possible to elucidate not only who's eating what, or where, as in previous studies, but also when (Chapter 6). Seasonal variation does however create problems in the interpretation of stable isotope data collected as an integrative snapshot of dietary interactions within a system, since the

dynamism of the system is not likely to be accounted for in this approach. In the study by Kiriluk *et al.* (1995), very different interpretations could be made depending on whether sampling was done in the spring or fall. If 2.5 to 3.0 ‰ is accepted as the enrichment in ^{15}N expected between successive trophic levels, and net plankton is chosen as a baseline, the length of the food chain between net plankton and lake trout could be alternatively interpreted as 5 trophic levels or 2 depending on the time of year the plankton sample was collected. It has previously been pointed out that increased variability in isotope signatures exists within the lower trophic levels (Cabana and Rasmussen 1996, France 1995a). However, studies of food webs using isotopes which consider time-scales of organism response to dietary change and the potential for fluctuations in the baseline are still rare.

A method proposed to side-step the problems with baseline fluctuations in aquatic systems is to use the isotope signature of mollusks or similar long-lived filter feeding organisms as a surrogate for the "average" zooplankton signature or the signatures of other short-lived organisms (Cabana and Rasmussen 1996). Mollusks will integrate carbon and nitrogen from particulate organic matter on a time-frame of several months to a year depending on their rate of tissue turnover. The mollusk isotope signature, or baseline signature, is then used as a system specific correction to standardize isotope signatures of fish across systems enabling comparison of food chain lengths using nitrogen isotopes. This has proven to be an effective technique in studies of northern Ontario lakes (Cabana and Rasmussen 1996). It should be noted that this correction is

application-specific and there are considerations to be made before such techniques should be universally employed. Selectivity in feeding may potentially bias the results. Species-specific differences in nutrient uptake and preference may result in substantially different isotope signatures between coexisting primary producers (Wada and Hattori 1978; Montoya 1990). Not all zooplankton feed on all available species of phytoplankton. Selection for or against different species of algae by secondary producers may result in variation from the 'average' signature, obtained from mollusks. Mollusks may also feed selectively which could similarly distort interpretation.

The source of particulate organic matter (POM) may differ between habitats and the comparisons and interpretation. Consistency in the source doesn't necessarily mean consistency in the isotope signature. The $\delta^{15}N$ of POM available in the epilimnion entering the pelagic food web and the POM available at the sediment surface may be different even though all of the POM is autochthonous. In Lake Ontario, a spring bloom of diatoms is not grazed extensively in the water column and as a result, makes up the bulk of the sedimented organic carbon deposited annually (Shelske and Hodell 1991). The majority of zooplankton grazing on phytoplankton takes place during the summer. More carbon and nitrogen from summer primary production enters the pelagic food chains and therefore is removed before it reaches the sediment surface. Thus the material incorporated into the isotope signature of mollusks at the sediment surface is not necessarily indicative of the baseline signature for all organisms. The objective in baseline correction is to remove variation between organisms attributable to differences

in system biogeochemistry so that differences may be interpreted on the basis of dietary interactions. It makes no sense to apply a correction factor established as a seasonal average to the signature of a species whose tissue turnover time is shorter than a season. It may also be inappropriate to use the signature of a species whose turnover time is shorter than a season as a basis for correction unless it is known, with some certainty, that there is no fluctuation in the isotope signature of primary producers on a seasonal basis. An exception may be that the baseline signature observed corresponds with the period of peak grazing by a primary consumer and so more closely reflects the true baseline than a seasonal average (Chapter 6).

A seasonal fluctuation in the $\delta^{15}N$ of various components of a food web also has implications for the use of isotopes as a means of quantifying the degree of omnivory. Assuming the $\delta^{15}N$ of the organisms in a food web are constant, then $\delta^{15}N$ may be used as a means of quantifying the degree of omnivory exhibited by any individual (Cabana and Rasmussen 1994). By creating a matrix of $\delta^{15}N$ values indicating all possible feeding relationships, the degree of omnivory required to obtain an observed signature may be calculated (Cabana and Rasmussen 1994). However, if the $\delta^{15}N$ of the organisms vary over time and space, using matrix algebra to quantify the degree of omnivory becomes a challenging exercise. With respect to an assessment of dietary interactions, variation in the integration of an isotope signature is another consideration. The isotope composition of a fish is likely to be dominated by the food obtained while it is growing rapidly. Metabolic turnover times in feeding studies of rapidly growing fish were slow, most of

the observed change was determined to be a function of growth (Hesslein *et al.* 1993). Therefore, the signature of an organism sampled is likely to include a carry-over from feeding during earlier stages in the organisms development. This should be considered when trying to obtain a quantitative assessment of the relative proportions of an organisms diet from stable isotopes. The growth of the organism and the isotope composition of the food available during different periods of the organisms development may be factors influencing the isotope signature observed. Given these considerations the use of matrix models to assess the degree of omnivory in an organism's diet becomes more complex. It is feasible. However, it may require more work and a more detailed understanding of the system dynamics than a researcher might be led to believe from a survey of available literature.

The Use of Stable Isotopes as a Tool in Ecological Monitoring

Long-term ecological monitoring data as a foundation for environmental impact assessment is often unavailable. When long-term data is available, it is seldom complete (Schindler 1987; McIntosh 1985). Without long-term trend data, it is difficult to determine if an observed change in ecosystem structure is a consequence of a natural cycle of systemic change or if it is due to some perturbation. With scant historical data available, data collection to establish a baseline for future reference is often undertaken as a component of impact assessment. The differences in ecological time scales mentioned previously should figure prominently in baseline studies. However, most impact studies are conducted under strict time constraints and do not have the time or the resources

necessary to fully determine the scope for variability in the system being studied. The development of paleoecology is important as a means of characterizing systemic change where long-term ecological monitoring data is lacking. Paleoecology is advanced through the use of tools that may be used as time-integrative measures of change, such as stable isotopes.

Paleolimnological investigations of sediments have been conducted in Lake Ontario and Lake Erie and changes in productivity and climate have been inferred from the $\delta^{13}C$ of organic carbon and calcite in sediment cores (Schelske and Hodell 1991; 1995). The isotope fractionation that occurs in photosynthetic uptake of carbon establishes the basis for these inferences. In theory, ^{12}C is taken up preferentially as a photosynthetic substrate leaving a DIC pool enriched in ^{13}C (Schelske and Hodell 1991). As the $\delta^{13}C$ of the DIC increases so do the materials synthesized from the available DIC (Schelske and Hodell 1991). Therefore, higher volumetric levels of productivity result in an elevated $\delta^{13}C$ in calcite and organic carbon produced in the epilimnion (Schelske and Hodell 1991). Therefore, cores from more eutrophic systems would be expected to contain organic carbon which is more enriched in ^{13}C. Studies of lake systems have demonstrated a range in correlation between concentrations of chlorophyll *a* in the water column, a measurement of the standing crop of primary producers, and the $\delta^{13}C$ of particulate organic matter collected at the same time (McCabe 1985; McKenzie 1985; Gu et al 1996). A survey of the literature on the relationship between photosynthetic uptake, $\delta^{13}C$ and dissolved inorganic carbon (DIC), indicates that there may be circumstances where

this relationship would break down (, McCabe 1985; Sharkey and Berry 1985; Quay *et al.* 1986; Falkowski 1987; Takahashi *et al.* 1990). The influence of photosynthetic uptake is not the only determinant of the isotope composition of the DIC. The $\delta^{13}C$ of fixed carbon will not always be a function of the rate of photosynthesis. The carbonate chemistry, the temperature profile, pH, atmospheric chemistry and biology of the lake will interplay on a temporal and system specific basis to determine the $\delta^{13}C$ of both the DIC and the POC produced. The use of the $\delta^{13}C$-productivity relationship to infer past environmental conditions also assumes that the level of photosynthesis is regulated strictly through physical or biogeochemical factors such as temperature changes or nutrient inputs to the system. However, levels of photosynthesis may also be regulated biologically through the level of grazing (Carpenter and Kitchell, 1988). More work needs to be done looking at the underlying processes influencing the $\delta^{13}C$ of sediment organic carbon across systems with a range of trophic status and community structures on a time scale consistent with environmental change.

Summary

Stable isotope chemistry cannot effectively be applied in ecology without due consideration of all factors potentially influencing isotope fractionation. There has been tremendous potential to broaden the scope of the research to encompass physical and organic chemistry, biogeochemistry, plant and fish physiology, ecology, ecotoxicology and bioenergetics. This thesis addresses some of the broader issues while developing a

quantitative assessment of trophic relationships within the pelagic system of Lake Ontario.

Chapter 2 is a review of the processes that determine the stable isotope signatures of primary producers which establish the baseline isotope signatures of any system. Chapters 3 and 4 detail which particular influences are prominent in determining the isotope baselines in Lake Ontario. Chapter 5 uses the information garnered in chapters 3 and 4 as a foundation for the apportionment of the diet of *Mysis relicta* . Chapter 6 is an interpretation of the temporal and spatial patterns of $\delta^{13}C$ and $\delta^{15}N$ in forage fish in Lake Ontario and an assessment of their efficacy as trophic indicators. Chapter 7 reviews some of the conclusions drawn in the previous chapters and draws upon those conclusions to make inferences with respect to the transfer of organic contaminants within the Lake Ontario pelagic food web. Appendix 1 describes the influence of lipid on isotope signatures and a method developed for lipid normalization of carbon isotope data. Appendix 2 details the lack of influence acid addition has on isotope signatures. Appendix 3 is 'Nitrofish', a modification of the 'Fish' fugacity model, designed to predict concentrations of hydrophobic organic contaminants. The 'Fish' model is altered to determine the level of isotope fractionation that can be expected in an organism based on studies of differences between nitrogen isotopes in the kinetics of amino acid metabolism.

Chapter 2

Nutrient Isotope Composition

The concentration and isotope signature of available dissolved inorganic nitrogen (DIN) and carbon (DIC) determine the $\delta^{13}C$ and $\delta^{15}N$ of primary producers and organisms feeding on those primary producers. This chapter reviews the literature in which linkages between biogeochemistry and the isotope signatures of inorganic nutrients and primary producers have been investigated.

Biogeochemical Processes Influencing the $\delta^{13}C$ of DIC

The amount and composition of available DIC varies between systems as a function of system inputs and is influenced by biological processes of respiration and photosynthesis as well as equilibrium kinetics and atmospheric gas exchange. DIC is the sum of, bicarbonate (HCO_3^-), dissolved carbon-dioxide ($CO_{2(aq)}$), which is approximately equal to H_2CO_3, and carbonate (CO_3^{2-}) in the system. These different components of the DIC pool of aquatic systems will move toward a state of equilibrium with each other and with atmospheric CO_2 (Stumm and Morgan 1981). The carbon isotope signature, $\delta^{13}C$, of DIC is determined by the relative magnitudes of the forms of DIC and the processes influencing the chemical and isotope equilibrium between components of the lake carbonate system.

Gas exchange across the air water interface will influence the $\delta^{13}C$ of $CO_{2(aq)}$ (Weiler 1978). A study of lakes world-wide found that less than 10 % of lakes surveyed were within $\pm$ 20 % of equilibrium with the atmosphere (Cole *et al.* 1994). At any point in time there is usually some degree of CO_2 exchange between the atmosphere and any aquatic system as it attempts to move toward equilibrium between the dissolved CO_2 in the water and atmospheric CO_2. Under conditions of atmospheric equilibrium, HCO_3^- will have a characteristic $\delta^{13}C$ signature of 0 to 1 ‰. There are equilibrium fractionation effects associated with $CO_{2(aq)}$ diffusion / $CO_{2(aq)}$ gas dissolution (ϵ_k and $\epsilon_{aq\text{-}g}$) across the air-water interface (Zhang *et al.* 1995). The level of fractionation associated with these processes is temperature dependent. The fractionation associated with gas dissolution was determined to be approximately:

$-(0.0049 \pm 0.003)T(°C) - (1.31 \pm 0.06)‰$.

The kinetic effect associated with diffusion produced a fractionation of :

-0.81 ± 0.16‰ at 21 °C and - 0.95 ± 0.20‰ at 5 °C (Zhang *et al.* 1995).

The relative levels of HCO_3^- and $CO_{2(aq)}$ will depend on the pH of the system, the temperature, and the systems alkalinity (Stumm and Morgan 1981). Components of the lake carbonate system seeking to attain a state of chemical equilibrium also move toward a state of isotope equilibrium (Weiler 1978). The rate constants for the dehydration of bicarbonate or hydration of carbon dioxide differ depending on whether the carbon reacting is ^{13}C or ^{12}C (Marlier and O' Leary 1984) . Carbon isotope

fractionation in the formation of dissolved CO_2 from HCO_3^- have been determined to fall in the range of -12.00 ‰ at 0°C to -8.42 ‰ at 25°C (Mook 1974). As described previously, the reaction kinetics between HCO_3^- and $CO_{2(aq)}$ are relatively slow (Miller and Coleman 1980). Since isotope equilibration is a function of discrimination during chemical equilibration, the rate at which isotope equilibrium might be attained kinetically is at least as slow or slower than the rate at which chemical equilibrium can be attained. As a result, under conditions where the rate of $CO_{2(aq)}$ removal is greater than the rate of HCO_3^- dissociation, the chemical equilibrium may be shifted producing a subsequent shift in the isotope composition of the DIC (Goericke *et al.* 1994).

Primary producers will draw upon the available $CO_{2(aq)}$ as a source of carbon for photosynthesis. Photosynthesis will tend to drive the isotope signature of DIC higher through the removal of isotopically light carbon from the DIC pool. If the level of photosynthesis is large relative to the DIC pool being drawn upon, the DIC will be enriched in ^{13}C and subsequently the $\delta^{13}C$ will increase. If the pool of available DIC is large, carbon removal by primary producers may not have much of an impact, thus the increase in $\delta^{13}C$ will be slight or unrecognizable.

Remineralized CO_2, a product of respiration within the system, will be added to the $CO_{2(aq)}$ pool. Respired CO_2 has an $\delta^{13}C$ similar to the source of carbon metabolized

(Jacobsen *et al.* 1970). In systems where respiration provides a significant contribution to the overall DIC pool the $\delta^{13}C$ of the DIC will be lower relative to equilibrium values.

Determination of $\delta^{13}C$ of Primary Producers

The $\delta^{13}C$ of fixed carbon will depend at least somewhat on the $\delta^{13}C$ of the DIC. The $\delta^{13}C$ of primary producers will also depend on the concentration of $CO_{2(aq)}$ and the algal growth rate.

The $\delta^{13}C$ of *Phaeodactylum tricornutum*, $\delta^{13}C_p$, in culture was measured over a series of growth rates under conditions where both the $\delta^{13}C$ and concentration of $CO_{2(aq)}$, $[CO_{2(aq)}]$, were monitored in a chemostat (Laws *et al.* 1996). The $[CO_{2(aq)}]$ was negatively correlated with the growth rate of the diatom. The $[CO_{2(aq)}]$ also had a linear relationship with the biological fractionation associated with carbon fixation expressed as:

$$\epsilon_p \ ((1000(\delta^{13}C_{CO2} - \delta^{13}C_p) / (1000 + \delta^{13}C_p)) .$$

Using this relationship, growth rates for phytoplankton in the equatorial Pacific were estimated from measurements of $\delta^{13}C$ of chlorophyll *a* isolated from POM. These estimates were found to be in good agreement with other techniques used in estimating rates of phytoplankton growth (Laws *et al.* 1996).

The observed level of isotope fractionation in primary producers is a function of the amount of carbon available at the site of activity of the enzyme binding the carbon substrate for photosynthesis. It is also dependent on the level of isotope discrimination characteristic of the particular enzyme system utilized by the cell in carbon fixation. Carbon fixation in algae is almost exclusively the product of 1,5-bisphosphate carboxylase-oxygenase (RUBISCO) activity. Under conditions of CO_2 saturation, this enzyme pathway has been shown to exhibit discrimination against ^{13}C, resulting in isotope fractionation in the order of -29.4‰ (Guy *et al.* 1987). If the $CO_{2(aq)}$ in the water column is in isotope equilibrium with the atmosphere as described above, and $CO_{2(aq)}$ uptake is the manner in which primary producers attain carbon for photosynthesis, a product with signature -38 ‰ might be expected (Fogel and Cifuentes 1993). However, the maximum level of fractionation associated with photosynthesis in aquatic systems is normally observed to be -20 to -22 per mil (Fogel and Cifuentes 1993). The discrepancy between the level of fractionation associated with the enzyme pathway and that observed has been explained as a function of the amount of carbon actually available to the enzyme pathway. Sharkey and Berry (1985) developed the following model describing fractionation. It is a modification of a similar model formulated by Farquhar *et al.* (1982):

$$\delta = d + b_3 * (F_3/F_1)$$

Where d is the equilibrium isotope effect between CO_2 and HCO_3^-, b_3 is the isotope fractionation associated with carboxylation and F_3/F_1 is the ratio of carbon dioxide leaking out of the cell to the amount entering the cell. The underlying theory is that when

DIC is scarce, more of the carbon entering the cell will be utilized in photosynthesis and there will be less opportunity for discrimination between isotopes since most of the carbon would be used before it could leak out of the cell. Thus the isotope discrimination observed in photosynthesis may be virtually eliminated to the point where observed $\delta^{13}C$ is probably associated with discrimination due to diffusive resistance causing the fixed carbon produced to have a signature very near that of the source (Sharkey and Berry 1985).

Alternatively, β-carboxylation may be responsible for a portion of the carbon fixation occurring in a system especially if the $CO_{2(aq)}$ concentrations are reduced. In a β-carboxylation photosynthetic pathway, phosphoenol pyruvate-carboxylase uses HCO_3^- as an inorganic substrate and has a kinetic fractionation factor of only 2 ‰ (Goericke *et al.* 1994). Active uptake of bicarbonate and increased β-carboxylation activity have been associated with elevated $\delta^{13}C$ signatures. Carbon isotope discriminations of 2 ‰ to 20 ‰ relative to $CO_{2(aq)}$ have been observed in cultures with high rates of β-carboxylation (Guy *et al.* 1987). In laboratory experiments with different species of marine phytoplankton, under conditions where the free CO_2 concentration was kept at 11.5 μM and all other physical mitigating factors were kept constant, the observed range of isotope signatures was -5.5 ‰ to -29.7 ‰ (Falkowski 1991). Differences were attributed to variations in β-carboxylation capacities between species (Falkowski 1991).

When available in sufficient quantities, $CO_{2(aq)}$ is normally utilized as the source of carbon for photosynthesis. This is because active transport of HCO_3^- into algal cells is likely necessary if HCO_3^- is to be used as a source of carbon for carbon fixation. There is an electric potential difference across the plasmalemma of algal cells that must be overcome in order for HCO_3^- to enter (Beardall 1985, Raven and Lucas 1985). The active transport of HCO_3^- into algal cells therefore has an associated energetic cost (Raven and Lucas 1985). The advantages of active uptake include increased internal carbon concentrations for photosynthesis, decreased photorespiratory losses and a decrease in required RUBISCO levels. In order for active transport to be feasible, these gains must outweigh the energy expended in active HCO_3^- uptake (Raven and Lucas, 1985).

Due to the associated energy expenditure, the use of HCO_3^- as a photosynthetic substrate would not normally be expected under conditions where concentrations of $CO_{2(aq)}$ were not limiting. However, the impetus triggering the active uptake is not $CO_{2(aq)}$ limitation *per se* but an increase in the ratio of the concentrations of $CO_{2(aq)}$ to O_2 (Kaplan 1985). A drop in the ambient concentration of $CO_{2(aq)}$ creates difficulty for cells depending on CO_2 as a source of carbon for photosynthesis. The diffusion rate of CO_2 and O_2 in water is about 10 000 times less than in air (Prins and Elzenga 1989). With CO_2 as sole DIC source, under conditions of net photosynthesis, the slow diffusion rate can result in low tissue concentrations of CO_2 and high concentrations of O_2 (Prins and Elzenga 1989). This effect may be exacerbated by the boundary layer which exists adjacent to the plant

cell surface (Smith 1985; Prins and Elzenga 1989). The boundary layer is the term used to describe the unstirred layer of solution directly adjacent the cell surface through which turbulent transfer of solutes to the cell surface is prevented (Smith 1985). The size of the boundary layer will vary depending on the species of phytoplankton considered and the environment in which it exists (Smith 1985; Hecky and Hesslein 1995). At a minimum, this layer exists at a thickness of 10 μm for microalgae under turbulent conditions (Smith 1985). With bulkier cells, or aggregates of cells in high densities, such as is often found with benthic algae, thicker boundary layers might be expected (Smith 1985; Hecky and Hesslein 1995). Transport of DIC through the boundary layer occurs only by diffusion (Smith 1985; Prins and Elzenga 1989). This can make it difficult for algal cells to obtain enough carbon substrate to maintain elevated rates of photosynthesis (Smith 1985; Prins and Elzenga 1989). Difficulty in diffusion of $CO_{2(aq)}$ through the boundary layer may create localized limitation at the surface of the cell. Algal cells with a larger boundary layer may have more of a problem obtaining enough $CO_{2(aq)}$ to meet photosynthetic demand and so active HCO_3^- transport may be induced.

Concentrating CO_2 within the cell at a level above that required for photosynthetic activity may be a mechanism for controlling other metabolic processes. Nitrogen limitation has also been demonstrated to initiate the activate uptake of $CO_{2(aq)}$ regardless of the DIC concentration (Beardall *et al.* 1982). The levels of β-carboxylation activity have been found to exceed RUBISCO activity when NH_4^+ is being assimilated (Guy

1989). The implication is that alternative methods of carbon uptake may be induced under conditions where the $CO_{2(aq)}$ exists at levels above those that would normally be considered limiting.

In addition to the potential to induce active uptake of HCO_3^-, the boundary layer may produce species specific differences in isotope fractionation among species relying on the diffusion of $CO_{2(aq)}$ to meet their photosynthetic carbon requirements. Under conditions of low ambient $CO_{2(aq)}$ concentrations, species with a thinner boundary layer might be expected to exhibit a greater level of isotope fractionation than cells with a larger boundary layer since they could presumably be able to obtain $CO_{2(aq)}$ more easily. This effect is the opposite of what has been observed under conditions of elevated $CO_{2(aq)}$ concentrations where the kinetic fractionation associated with diffusion through the boundary layer resulted in lower $\delta^{13}C$ values in algae with larger boundary layers (Hecky and Hesslein 1995).

Algal cells have a much lower diffusive resistance to CO_2 than they do to HCO_3^- (Beardall 1985). When the pH of the system is above 7, HCO_3^- may be much more readily available at the cell surface. Algae may be able to use this to their advantage through the facilitated conversion of HCO_3^- to $CO_{2\,(aq)}$ in order to increase the levels of $CO_{2\,(aq)}$ in the microenvironment of the cell. Carbonic anhydrase, potentially located on the surface of algal cells, catalyzes the dehydration-hydration reactions in the

equilibration of HCO_3^- and $CO_{2\,(aq)}$ (Tsuzuki and Miyachi 1989). The equilibrium isotope effect between HCO_3^- and $CO_{2\,(aq)}$ is conserved in the action of carbonic anhydrase (Paneth and O'Leary 1985). Under conditions of $CO_{2\,(aq)}$ limitation, extracellular carbonic anhydrase may catalyze the formation of enough $CO_{2(aq)}$ in the microenvironment of the cell for the cell to obtain sufficient CO_2 via diffusion to meet its needs. The extrusion of H^+ into the microenvironment surrounding the cell would also cause the dehydration of HCO_3^- and increase the concentration of $CO_{2(aq)}$ available to the cell. The potential for algae to modify the level of $CO_{2(aq)}$ in their immediate surroundings depends on the species specific ability to catalyze the hydrolysis of bicarbonate and the availability of HCO_3^-. From an isotope perspective, the facilitated conversion of HCO_3^- to $CO_{2\,(aq)}$ results in a lack of correlation between the ambient concentrations of $CO_{2(aq)}$ and the isotope composition of primary producers

Field studies of the $\delta^{13}C$ of DIC and POM

There are a number of studies which have made determinations of the $\delta^{13}C$ of POM and/or DIC on spatial, temporal or physical-chemical gradients. Using the laboratory studies described as a backdrop for interpretation, these studies provide a great deal of insight into the relationship between $\delta^{13}C$ of POM and inorganic carbon cycling.

The pCO_2 (and therefore $CO_{2(aq)}$ levels), latitude/temperature and the $\delta^{13}C$ of phytoplankton in marine waters were found to be correlated (Rau *et al.* 1989). The

equilibrium between $CO_{2(aq)}$ and HCO_3^- is shifted toward $CO_{2(aq)}$ in colder waters where CO_2 is more soluble (Rau *et al.* 1989). It was hypothesized that the $\delta^{13}C$ of plankton in marine waters toward the poles is more negative than in equatorial regions since there is less demand on a larger available pool of $CO_{2(aq)}$ due to lower levels of open-water primary production (Rau *et al.* 1989). In investigating the influence of pH and temperature on the $\delta^{13}C$ of algae growing in hydrothermal springs in Yellowstone National Park, it was discovered that under conditions favoring elevated concentrations of $CO_{2(aq)}$, the $\delta^{13}C$ of the algae were more negative (Estep 1984). Seasonal studies of the $\delta^{13}C$ of particulate organic matter (POM) and DIC from the Delaware estuary found an opposite effect to the temperature and concentration relationship observed by Rau (Fogel *et al.* 1992). Concentrations of $CO_{2(aq)}$ and the $\delta^{13}C$ of DIC changed very little from March of 1987 to June 1987. The $CO_{2(aq)}$ concentrations increased 3.3 μM, $\delta^{13}C$ dropped from -1.2 to -2.5 ‰ (Fogel *et al.* 1992). The $\delta^{13}C$ of the POM over the same time period dropped 7.8‰ (Fogel *et al.* 1992). One potential explanation is that respired CO_2 was a significant contributor to the DIC used by primary producers in photosynthesis in this system during the summer and the importance of heterotrophic processes (Fogel *et al.* 1992). The lack of corresponding change in the DIC is then a function of the pH and alkalinity of the system. A pH range of 7.0 to 8.4 was reported for the system over the course of the study (Fogel *et al.* 1992). Within the pH range of the Delaware study, the influence of $CO_{2(aq)}$ in the estuarine system is likely overwhelmed by the amount of HCO_3^- in the system, which is approximately 2.0 meq L^{-1} in marine environments. Subsequently, a large shift in the $\delta^{13}C$ of $CO_{2(aq)}$ will produce a substantially smaller

shift in the $\delta^{13}C$ of the DIC. It was determined that it was impossible to correlate productivity to changes in $\delta^{13}C$ of POM (Fogel *et al.* 1992). They also found that neither temperature or $CO_{2(aq)}$ had a direct relationship to $\delta^{13}C$ of POM, and concluded that the uptake and fractionation of carbon by phytoplankton is very complex and not simply related to any one factor (Fogel *et al.* 1992). A similar conclusion was reached in a seasonal study of the $\delta^{13}C$ of DIC and POM in a shallow eutrophic lake (Takahashi *et al.* 1990). The $\delta^{13}C$ of POM increased from -25 ‰ to -16.7 ‰, the $\delta^{13}C$ of DIC over the same time period dropped from -5.9 ‰ to -16.7 ‰ (Takahashi *et al.* 1990). The drop in the $\delta^{13}C$ of DIC was attributed jointly to chemical enhancement of atmospheric CO_2 invasion and supply of CO_2 from the decomposition of sedimentary organic matter. The increase in POM was concluded to be a function of a complete lack of photosynthetic fractionation under conditions of DIC depletion related to many factors in addition to productivity (Takahashi *et al.* 1990).

These studies may be contrasted with the observed changes in $\delta^{13}C$ of DIC during a detailed study of a lake with comparatively low alkalinity (142 μeq/L) where the concentration of $CO_{2(aq)}$ represented approximately 10 % of the total DIC (Hesslein *et al.* in press). The concentration of the DIC (155 μM/L·mid-summer) fluctuated seasonally in the same order as the factor of 3 change in $CO_{2(aq)}$ concentration. The DIC concentration and $\delta^{13}C$ of DIC were controlled by rates of respiration, photosynthesis and gas exchange, and the distribution of the effect of these processes as a result of

stratification (Hesslein *et al.* in press). The $\delta^{13}C$ of DIC shifted from a spring minimum between -12 ‰ and -13 ‰ to -6 ‰ in late August before falling to -14 ‰ the following spring (Hesslein *et al.* in press). The $\delta^{13}C$ of $CO_{2(aq)}$ was determined seasonally at different depths to vary in a range from -28 ‰ to -54 ‰ (Hesslein *et al.* in press). Similar studies on small lakes produced the same resulting trends in $\delta^{13}C$ of DIC (Quay *et al.* 1986; Herczeg and Fairbanks 1987; Aravena *et al.* 1992).

There was little seasonal change in the $\delta^{13}C$ of phytoplankton and zooplankton (< 1 ‰) collected from different locations in Narragansett Bay (Gearing *et al.* 1984). Distinctions could however be made between different locations and species of primary producers on the basis of $\delta^{13}C$ (Gearing *et al.* 1984). Larger cells (diatoms) were enriched relative to smaller cells (< 10 μm microflagellates and ultraplankton) collected later in the season. In a seasonal study of Smith Lake in Alaska, the $\delta^{13}C$ of POM increased approximately 1 ‰ between May and August before dropping 8 to 10 ‰ in September (Gu *et al.* 1994).

A detailed seasonal study of the inter-relationship between the $\delta^{13}C$ of POM, the $\delta^{13}C$ of DIC, the $\delta^{13}C$ of sediment organic carbon, and system biogeochemistry was undertaken in the Hamilton lakes basin in New Zealand (McCabe 1985). A pond culture and 6 lakes were studied ranging in trophic status from oligotrophic to hypereutrophic in volcanic and peat catchments with low alkalinity. Large fluxes were observed in the $\delta^{13}C$ of $CO_{2(aq)}$ and POM (8 ‰) and in concentrations of DIC monitored seasonally. The $\delta^{13}C$ of

POM varied from - 35 ‰ to -19 ‰ (McCabe 1985). Variation in $CO_{2(aq)}$ concentrations between 1 μM and 90 μM accounted for 30 % of the variation in $\delta^{13}C$ observed in POM in pond cultures. In the lakes studied, 8 % of the variation in $\delta^{13}C$ of DIC was a function of the pH. There was a positive linear correlation between the logarithm concentration of POM (log[POM]) and $\delta^{13}C$ of POM in the 4 most eutrophic lakes surveyed. There was no correlation between log[POM] and $\delta^{13}C$ of POM in the 2 more oligotrophic lakes (McCabe 1985).

The $\delta^{15}N$ of DIN and POM

Dissolved inorganic nitrogen (DIN) available to primary producers in fresh waters occurs as dissolved N_2, NH_4^+, NO_3^-, NO_2^-. As with carbon, fractionation between source DIN (N_2, NO_3^-, NO_2^-, NH_4^+) and organic nitrogen depends on the form of nitrogen used, the amount which is available, and the subsequent ratio of the flux of nitrogen entering and exiting the cell (Handley and Raven 1992). All enzymes except nitrogenase show some level of discrimination against ^{15}N. It is this factor that produces the patterns in $\delta^{15}N$ which make it such a useful tracer (Handley and Raven 1992). Biologically mediated transformations of nitrogen include assimilation by primary producers, nitrification, denitrification, and heterotrophic remineralization of organic matter. Each of these processes affect the $\delta^{15}N$ ratio in the dissolved nitrogen pools (Horrigan *et al.* 1990).

The fractionation factor, α, associated with the process of nitrification of ammonium to nitrite by *Nitrosomonas europa* was determined to be approximately 1.03, which produces a -30 ‰ difference between product and substrate (Mariotti *et al.* 1981; Yoshida *et al.* 1989). A very large fractionation factor (1.0653) was determined for the nitrification of ammonium to N_2O (Yoshida 1988). The denitrification of nitrate to N_2 was determined to be temperature dependent and produced a fractionation with a factor of 1.0294 or a -29.4 (2.4 ‰ difference at 20 °C and 1.0246 or -24.6 (0.9 ‰ at 30 °C (Mariotti *et al.* 1981). Wada and Hattori also measured (associated with denitrification of

nitrate to N_2 and determined it to be 1.03 (1978). Their experiments suggested that the fractionation in the process occurs during N-O bond breakage (Wada and Hattori 1978).

The value of any given form of nitrogen as a substrate for assimilation by algae depends on species-specific adaptation to utilization of that particular form, and its concentration relative to the concentration of other forms of nitrogen in the surrounding media. In comparison to the processes of nitrification and denitrification, fractionation associated with nitrogen fixation is small (α= 1.002 to 1.003). Nitrogen fixing algae, such as blue greens, typically have a very low $\delta^{15}N$ similar to atmospheric N_2. Experimental determination of fractionation factors of different algal species in the uptake of nitrate in batch culture suggest that significant fractionation occurs during the initial transport of NO_3^- (Montoya 1990). The magnitude of fractionation was determined to vary between algal species (from 12.1 ‰ to 0.9 ‰). Differences among species may be a function of the size of the boundary layer adjacent to the algal cells. Variation in the boundary layer thickness could provide different levels of diffusive resistance (Goericke *et al.* 1994). Fractionation associated with NH_4^+ uptake has been estimated to be between 6.5 and 9.1 ‰ (Cifuentes *et al.* 1989; Montoya 1990). Positive fractionation of 9.7 ‰ and 5.3 ‰ have been measured in batch culture experiments (Wada and Hattori 1978). Where concentrations are not limiting, assimilation of NH_4^+, NO_2^-, or NO_3^- by phytoplankton or bacteria should leave the substrate pool enriched in ^{15}N (Horrigan *et al.* 1990).

There is a broad range of measured isotope signatures for dissolved inorganic and organic nitrogen in aquatic systems. Particulate nitrogen (PN) in the oceans and in estuaries studied range from -2.0‰ to + 40‰, $\delta^{15}N$ of marine nitrate range from + 3‰ to + 19‰ (Montoya 1990). Marine NH_4^+ has been measured at -3.5 ‰, 6.5 ‰ and 7.5 ‰ (Miyake and Wada 1967). In Chesapeake Bay, spring $\delta^{15}N$ levels of NH_4^+ were found to vary by almost 9 ‰ (11.5 ‰ to 20.2 ‰) at the same point on successive days (Horrigan *et al.* 1990). The $\delta^{15}N$ for NH_4^+ for the system was observed to increase seasonally (Horrigan *et al.* 1990). Combined NO_3^- and NO_2^- ranged from 6 ‰ to approximately 20 ‰ at different sampling stations at different times. No seasonal pattern in the $\delta^{15}N$ of ($NO_3^- + NO_2^-$) was observed (Horrigan *et al.* 1990).

There is very limited data on the $\delta^{15}N$ of DIN and particulate nitrogen (PN) in freshwaters. The $\delta^{15}N$ of NH_4^+ in Lake Superior was measured at 5.3 ‰ to 6.7 ‰ (Pang and Nriagu 1977). A seasonal range of +1.2 ‰ to +7.0 ‰ was found for blue-green algae in the Lahonton Lake System in Nevada (Estep and Vigg 1985). The $\delta^{15}N$ of DIN was measured at +1.1 ‰ in the same system (Estep and Vigg 1985). A shift in $\delta^{15}N$ of POM from 3.3 ‰ prior to a spring bloom to 6.9 ‰ following the bloom peak was observed in seasonal study of Auke Bay Alaska (Goering *et al.* 1990). It has been suggested that $\delta^{15}N$ of POM increases with increasing productivity of the lake basin, i.e., $\delta^{15}N$ of POM in oligotrophic lakes is lower than the $\delta^{15}N$ of POM in eutrophic lakes (Gu *et al.* 1996). In theory, increased recycling of remineralized nitrogen in eutrophic lakes leads to elevated nitrogen isotope signatures. However, this relationship has not been demonstrated and is

potentially confounded by the influence of N_2-fixing algae in nitrogen limited systems (Gu *et al.* 1996; Yoshioka and Wada 1994; Graham 1997). Information on algal community structure and nutrient supplies are essential for effective interpretation of $\delta^{15}N$ data at the base of the food chain (Gu *et al.* 1996). A seasonal study of zooplankton and POM in prairie lakes demonstrated that *Diacyclops thomasi* actively selected for N_2-fixing cyanobacteria late in the season which dropped its $\delta^{15}N$ signature below that of *Daphnia sp.* feeding on other primary producers in the same lake (Graham 1997). The observed $\delta^{15}N$ of organisms feeding on primary producers has also been correlated with the population density surrounding any given lake (Cabana and Rasmussen 1996). The rationale is that DIN from sewage sources will have an elevated $\delta^{15}N$. Therefore, differences in baseline levels of $\delta^{15}N$ between lakes will be a reflection of the relative level of influence of sewage nitrogen as a source of DIN to primary producers. Population density accounted for 68 % of the variation in $\delta^{15}N$ of the primary consumers surveyed (Cabana and Rasmussen 1996).

Chapter 3

Biogeochemical Influences on the Carbon Isotope Signatures of Lake Ontario Biota

Introduction

Stable isotopes of carbon have often been used as tracers of carbon in studies of aquatic systems. The use of stable isotopes as tracers is based on an understanding that changes in the proportion of ^{13}C and ^{12}C in compounds will produce slightly different bond energies and alter their reaction kinetics. The relative ratio of heavy to lighter isotopes will vary in different substrates as a function of the processes involved in their formation. Therefore, insight into the dynamics of a system may be garnered by measuring the ratio of isotopes in those substrates. Isotope ratios of carbon are described as carbon signatures, expressed as $\delta^{13}C$ values in units per 'mil'. Carbon signatures are the parts per thousand difference between the ratio of isotopes in a sample and the ratio in a standard of known isotope composition. Larger $\delta^{13}C$ values reflect a greater proportion of ^{13}C in the substrate analyzed. While there are many studies where this tool has effectively been employed, inferences are often made without due consideration of all factors with the potential to influence carbon signatures.

Changes in productivity have been inferred in Paleolimnological investigations from the $\delta^{13}C$ of organic carbon and calcite in sediment cores (McKenzie 1982; 1985; Schelske and Hodell 1991). The foundation for these inferences are the isotope fractionation, or

level of isotope discrimination, that occurs in photosynthetic uptake of carbon in aquatic systems. In photosynthesis, ^{12}C is used by algal cells preferentially, leaving a dissolved inorganic carbon (DIC) pool enriched in ^{13}C. In theory, higher productivity is reflected in elevated $\delta^{13}C$ in calcite and organic carbon produced in the epilimnion (Schelske and Hodell 1991). A seasonal fluctuation in the $\delta^{13}C$ of DIC and organic carbon, reflecting seasonal changes in the magnitude of primary production is predicted (Schelske and Hodell 1991). There should also be a linear relationship between concentrations of chlorophyll *a* and/or areal rates of productivity and the $\delta^{13}C$ of primary producers. The assumption is made that the uptake of carbon by primary producers is always the predominant influence on the $\delta^{13}C$ of DIC and therefore the organic carbon produced using DIC as a substrate for photosynthesis. It is further assumed that isotope fractionation, the discrimination between ^{13}C and ^{12}C, remains constant in photosynthesis.

In contrast to paleolimnological studies inferring the past trophic status of a lake, inferences on trophic relationships based on $\delta^{13}C$ often assume seasonal stability in the $\delta^{13}C$ of potential food sources, even at lower trophic levels. The use of carbon isotopes as a tracer in food web studies is based on the observation that there is little difference in the carbon signatures between an organism and its food source. That there is likely to be some temporal dynamism in the baseline $\delta^{13}C$ within a system is often overlooked.

The $\delta^{13}C$ of primary producers is determined by the concentration and $\delta^{13}C$ of DIC. The amount and composition of available DIC varies between systems and is influenced by biological processes of respiration and photosynthesis as well as equilibrium kinetics and atmospheric gas exchange (Quay *et al.* 1986; Herczeg and Fairbanks 1987; Aravena *et al.* 1992; Hesslein *et al.* 1997). The relative magnitudes of the forms of DIC and the processes influencing the equilibrium between components of the lake carbonate system influence the carbon isotope signatures of particulate organic matter in two ways. First, the components of the lake carbonate system seeking to attain a state of chemical equilibrium also move toward a state of isotopic equilibrium (Weiler 1978; Hesslein *et al.* 1997). Many of the processes influencing the composition of the DIC pool also serve to alter the $\delta^{13}C$ of individual carbon species which are part of the total DIC of the system. The $\delta^{13}C$ of fixed carbon will be determined to some degree by the $\delta^{13}C$ of the inorganic carbon used in photosynthesis (Estep 1984; Rau *et al.* 1989; Fogel *et al.* 1992). Second, the observed level of isotope fractionation in primary producers is a function of the amount of carbon available at the site of activity of the enzyme binding the carbon substrate in carbon fixation (Farquhar *et al.* 1982; Fogel *et al.* 1992; Fogel and Cifuentes 1993). It is also dependent on the level of isotope discrimination characteristic of the particular enzyme system used by the cell in carbon fixation (Falkowski 1991; Goericke *et al.* 1994).

If a seasonal fluctuation in the $\delta^{13}C$ of primary producers exists, then the zooplankton feeding on primary producers should have a $\delta^{13}C$ which reflects that fluctuation, possibly with a time-lag corresponding to the length of time required for the carbon fixed in photosynthesis to enter the pelagic food web Larger herbivorous zooplankton and carnivores should subsequently take longer to reflect a change in their carbon source than smaller more rapidly growing species (Tieszan 1983). The recycling of carbon at the base of the food web through the microbial loop may also alter the temporal pattern in the $\delta^{13}C$ of primary consumers.

In this study, the linkages between the $\delta^{13}C$ of particulate organic matter (POM), zooplankton, and DIC were investigated. The relationship between seasonal concentrations of chlorophyll *a*, the level of photosynthetic production, and $\delta^{13}C$ of POM was also examined. Two sampling sites in Lake Ontario of the Laurentian Great Lakes were chosen for comparison and a two year seasonal study was conducted. There is a past record of isotope analysis from the sediment, the water column, and biota of the system (Weiler and Nriagu 1978; Schelske and Hodell 1991; Kiriluk *et al.* 1995). One site, Station 41, was located mid-lake 28 km off-shore at approximately 120 m depth. The second site, Station 81, was located in the east-basin at approximately 30 m depth (Fig. 1). Terrestrial and detrital inputs to the POM were expected to be negligible at both sites. Lake Ontario is a mesotrophic system, with an average pH in the range of 7.8 to 8.6 and high ambient concentrations of DIC (~1800 μM). The lake stratifies in mid-June

and biologically induced precipitation of $CaCO_3$ occurs seasonally at the end of July or early August (Lean *et al.* 1987). A model of $\delta^{13}C$ cycling through the DIC and organic carbon pools is used as a means of summarizing our findings. Seasonal trends in the $\delta^{13}C$ of zooplankton are examined in the context of a current understanding of trophic relationships at the base of the Lake Ontario pelagic food web. The implications of this work with respect to the development of a greater understanding of processes determining the carbon isotope baseline within aquatic systems are also discussed.

Methods

All of the sampling for this project was done in conjunction with the Lake Ontario Biomonitoring program (Department of Fisheries and Oceans, Burlington). Samples were collected every two weeks from April 20 to September 27, 1994, and between May 11 and October 31, 1995, at Station 41 and Station 81 (Fig. 3.1).

The photosynthetic rates reported here were determined utilizing ^{14}C-uptake under conditions of optimal irradiance in ship-board incubators as described in Millard *et al.* (1996). All of the analysis was done onboard the *CSS Lauzier* as part of the Bioindex biomonitoring program (Department of Fisheries and Oceans, Burlington).

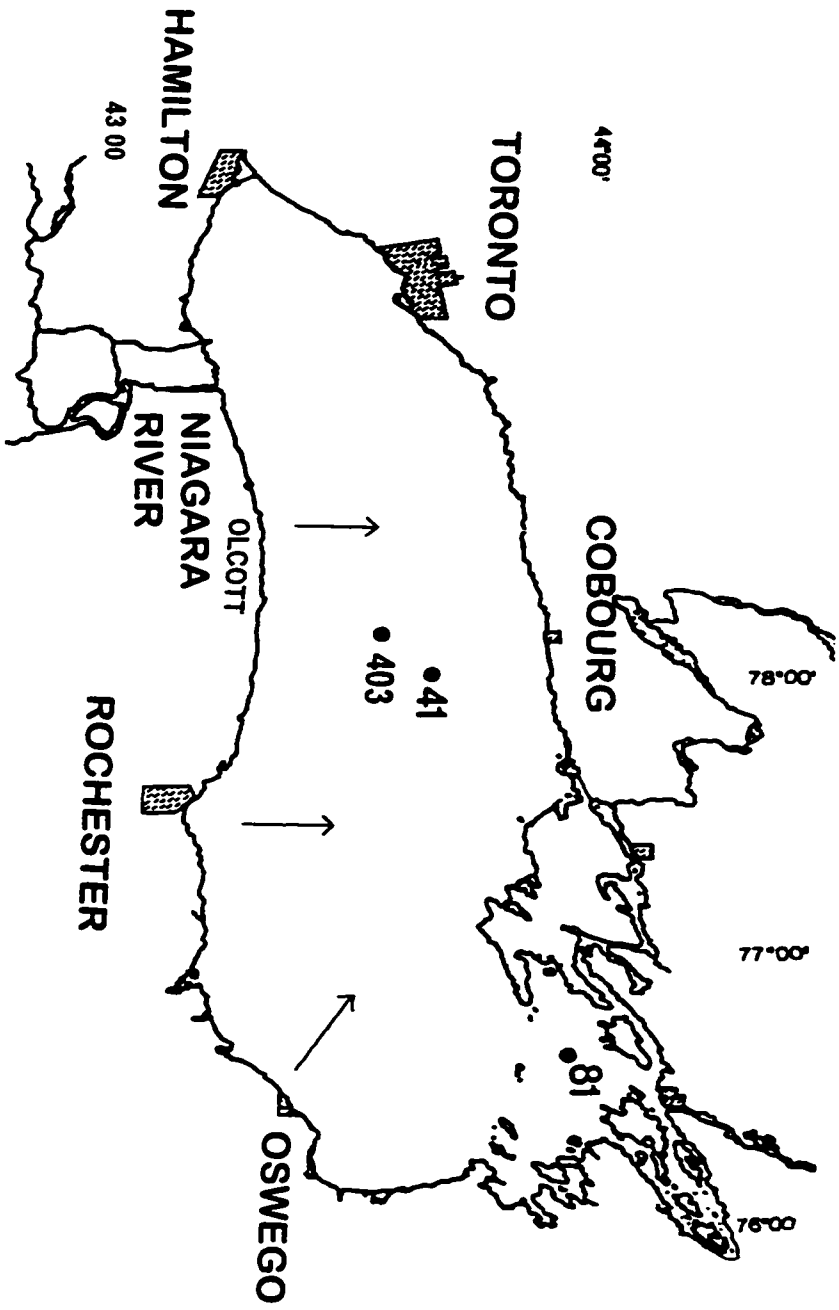


Figure 3.1 A map of Lake Ontario indicating the locations of the sites of collection for the samples used in this thesis. Arrows indicate transects of forage fish collections.

The relative concentrations of dissolved free CO_2 ($CO_{2(aq)}$) and bicarbonate (HCO_3^-) were calculated using equations describing equilibration of the carbonate system in freshwater (Stumm and Morgan 1981). Input parameters included the DIC concentration, pH, temperature and conductivity measured at the depth corresponding to that from which the POC sample and DIC for $\delta^{13}C$ and pCO_2 were obtained. Mineral concentrations were obtained from seasonal surveys of Lake Ontario conducted in 1993. Alkalinity was calculated from the measured concentration of DIC. The partial pressure of CO_2, (pCO_2) was calculated using Lyman's constants as per the method described in Weiler (1974). Calculated concentrations were compared with measurements obtained from headspace analysis of the DIC samples collected in evacuated bottles for $\delta^{13}C$ analysis. Calculations of the rate of equilibration between HCO_3^- and $CO_{2(aq)}$ in the system, or the supply rate of $CO_{2(aq)}$ from HCO_3^-, were made using the equations detailed in Miller and Coleman (1980).

Water samples

Water samples for nutrient analysis and determination of both the $\delta^{13}C$ of dissolved inorganic carbon (DIC) and $CO_{2\,(aq)}$ concentration were obtained from discrete 10 L water samples collected with Nisken bottles. Samples were taken at 10 m when the lake was thermally homogenous and from the mid-epilimnion once the lake had stratified. Mixing depth was determined from temperature profiles obtained using an electronic bathythermograph. Analyses were augmented by measurements of the pH, and

conductivity made at several depths *in situ* using a Hydrolab™ water chemical analyzer. Subsamples of water from the Nisken bottles were collected in evacuated 250 mL bottles containing potassium chloride as a preservative for carbon isotope and $CO_{2(aq)}$ analyses. The headspace gas within the evacuated container was later analyzed to determine the partial pressure of dissolved carbon dioxide (pCO_2). The $\delta^{13}C$ of DIC was determined as per the method described in Hesslein *et al.* (1997). Analysis of these samples was conducted using a VG 602 micromass dual-inlet mass spectrometer. On August 2, 1995 duplicate discrete samples were collected hourly from 3 m depth between 6:00 and 22:00 hrs to determine if there was any diurnal fluctuation in the $\delta^{13}C$ of DIC. DIC samples were also collected on August 2, 1995 at depths of 7.5, 12.5, 25.0, 75.0, and 120.0 m. The mixing depth August 2 was 6.5 m.

Water for nutrient analysis was filtered through a 0.45 μm Sartorius filter and stored at 4 °C. Nutrient analyses were conducted by the National Laboratory for Environmental Testing, in Burlington, Ontario, Canada (Environment Canada 1979).

Particulate Organic Matter

Particulate organic matter (POM) was collected and size-fractionated using a number of different techniques in 1995. This was done for comparison of size fractions and also to determine the relative efficacy of different sample collection methods.

Samples of POM, were obtained from 200 L of water pumped from the mid-epilimnion and filtered through a 44 μm mesh screen into storage containers. Most of these samples were then sequentially filtered through 20 μm mesh screen (POM_{20}) and precombusted (500 °C) 0.7 μm nominal pore size, Whatman GF/F glass fibre filters (POM_{GFF}). The exception was the sample collected on a GF/F filter May 10, 1995 at station 81 which was not pre-filtered through a 20 μm mesh screen. The intensive prefiltering effectively removed any detritus or zooplankton which could confound interpretation of results. The POM_{20} and POM_{GFF} samples collected (when available in sufficient mass) had 2N H_2SO_4 added to them to remove residual carbonate and were then dried at 60 °C prior to analysis. In May and June of 1995, at both sites, additional samples were obtained using a Westfalia continuous-flow centrifuge POM_W (44 to 0.45 μm) to separate particulate from 200 L samples collected as described above. POM obtained from the centrifuge was dried at 60 °C prior to analysis.

In August and October of 1995, tangential flow filtration, as described in Barthel *et al.* (1989), was used to capture particulate approximately 0.2 to 1 μm (POM_{TFF}) in diameter from 200 L samples filtered through GF/F filters. The sample was continuously passed through a Millipore Pelicon cassette system fitted with a microporous membrane cassette with a rated pore size of 0.2 μm. The filtrate, except for 3 L retained for dissolved inorganic nitrogen extraction, was discarded. The remaining sample retentate was

recirculated until the sample volume was reduced from 200 L to less than 500 mL. The POM_{TAN} (tangential flow retentate) was subsampled for examination using florescence microscopy in order to make a rough assessment of the composition of the sample and determine the integrity of the glass fibre filtration. No further analysis was done to determine how much of the sample may have been composed of colloidal material or organic material other than whole cells that were larger than 0.2 μm. The bulk of the retentate was freeze-dried to obtain the POM_{TAN} which was analyzed to determine its isotope signature.

Zooplankton samples were collected in 1994 and 1995 using 64 μm mesh Nitex™ nets in vertical tows, from 20 m to the surface prior to stratification, and from the thermocline to the surface when the lake was stratified. Samples were sequentially filtered through >250 μm, >210 μm, >110 μm and >64 μm mesh Nitex™ screens and preserved in ethanol. The >64 μm fraction was not retained in 1995. The POM_{110} or POM_{64} samples consisted of material collected on either 110 μm or 64 μm mesh filters after passing through 295 μm and 210 μm mesh filters. All the POM_{110} or POM_{64} samples were flushed with distilled water to remove as much algae as possible in order to obtain an assessment of the $\delta^{13}C$ of the rotifers, small cladocerans, nauplii and copepodites retained. Algal removal was accomplished with varying levels of success. These samples were then viewed under a microscope to roughly determine their content. On separate occasions the material flushed through the 64 μm mesh filters was collected on a 20 μm mesh filter for

analysis (POM_{20}). Rotifers began to represent a substantial portion of the biomass of the >64 μm samples by June 28. Prior to this, samples contained largely algae, nauplii and copepod eggs. Selected subsamples were removed from the larger size fractions (>210 μm and >295 μm) and were also viewed under a microscope to determine their content. These subsamples had all debris removed from the zooplankton prior to analysis. Individual species were obtained through separation under a microscope. Composite samples of many hundreds of individuals were then rinsed with distilled deionized water and dried prior to analysis. The only species available in sufficient quantity over the entire period studied to facilitate separation for stable isotope analysis was *Diacyclops thomasi*. Samples of other species were obtained at points during the season when they similarly became available in sufficient numbers to enable enough individuals to be picked for analysis. Calanoid copepods, mostly diaptomids, were pooled to provide sufficient mass for analysis. Samples were made up predominantly of *Leptodiaptomus sicilis* and *Skistodiaptomus organensis*. One sample of *Bythotrephes* sp. was obtained July 15, 1995, mid-lake.

The $\delta^{13}C$ values for all particulate and zooplankton samples were obtained using a VG Optima continuous-flow isotope-ratio mass-spectrometer (CF-IRMS). Instrument accuracy, precision and range of linearity were monitored with sets of National Institute of Standards and Technology (NIST) -USA standards of known concentration and carbon isotope composition. Duplicate standards of identical mass within the linear range

of operation consistently had measured values within 0.25 ‰ of each other. GF/F filters loaded with POM were subsampled and duplicate samples were either treated with the addition of 2 N HCl and redried at 60°C to remove residual carbonate, or were analyzed without acid addition (Fig. 3.2). The POM collected using; Nitex screens, the Westfalia centrifuge or tangential flow filtration was treated with acid (2N HCl) and redried prior to carbon isotope analysis. The tangential flow samples were treated with acid 2 -3 times to remove all traces of $CaCO_3$.

Results

The $\delta^{13}C$ of DIC was observed to increase approximately 2.0 ‰ between April and October at both sites in each of the years investigated (Fig. 3.3), ranging from -2.0 ‰ to 0.1 ‰ in the East Basin and from -2.5 ‰ to 0.1 ‰ mid-lake. The average difference between duplicate values was 0.3 ‰ and 0.4 ‰ at the Stations 81 and 41 respectively.

The values obtained for the $\delta^{13}C$ of DIC are 1.5 to 2.0 ‰ more negative than those obtained in 1993 by Weiler and Nriagu (1978). There was no discernible diurnal fluctuation in the $\delta^{13}C$ of DIC. Duplicate samples collected at increasing depths indicated that the $\delta^{13}C$ of hypolimnetic DIC was approximately 1.5 ‰ lower than that found in the epilimnion (Table 3.1).

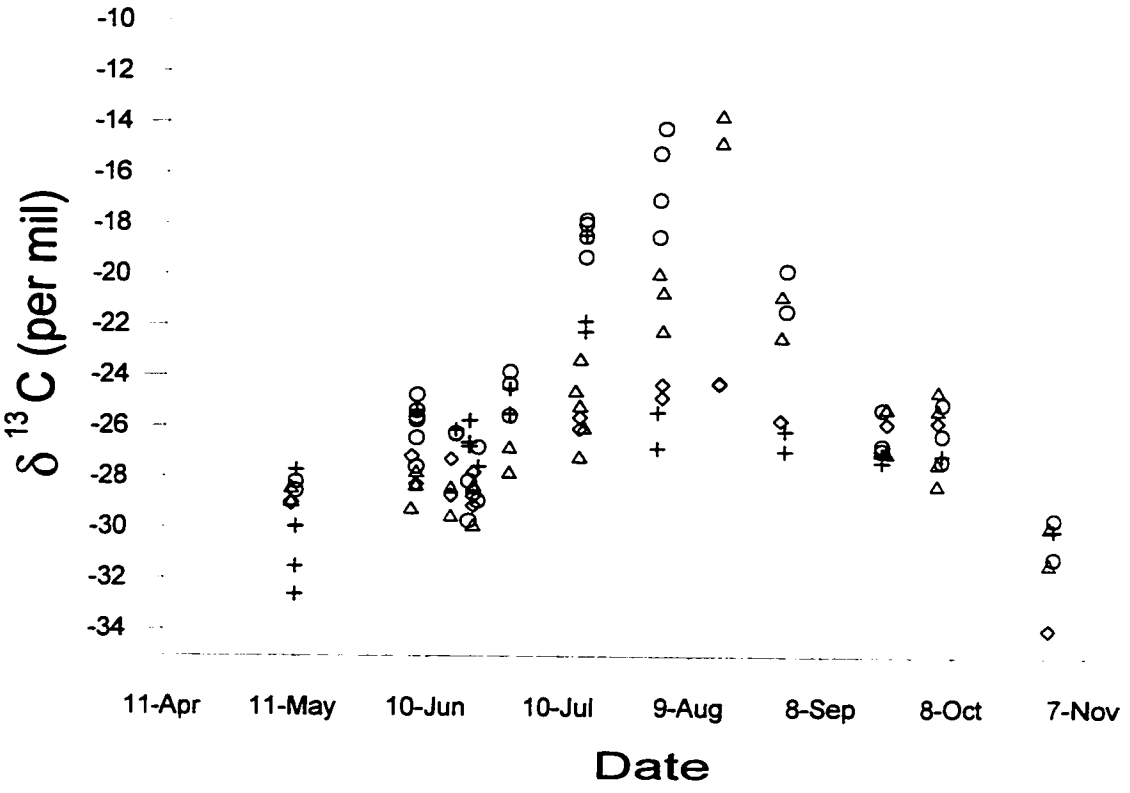


Figure 3.2. The seasonal change in $\delta^{13}C$ of particulate organic matter (POM, 20 to 1 μm). The ⁻⁻ indicates POM treated with acid from Station 41,O, is untreated POM from Station 41, ·', is POM treated with acid from Station 81, —, is untreated POM from Station 81.

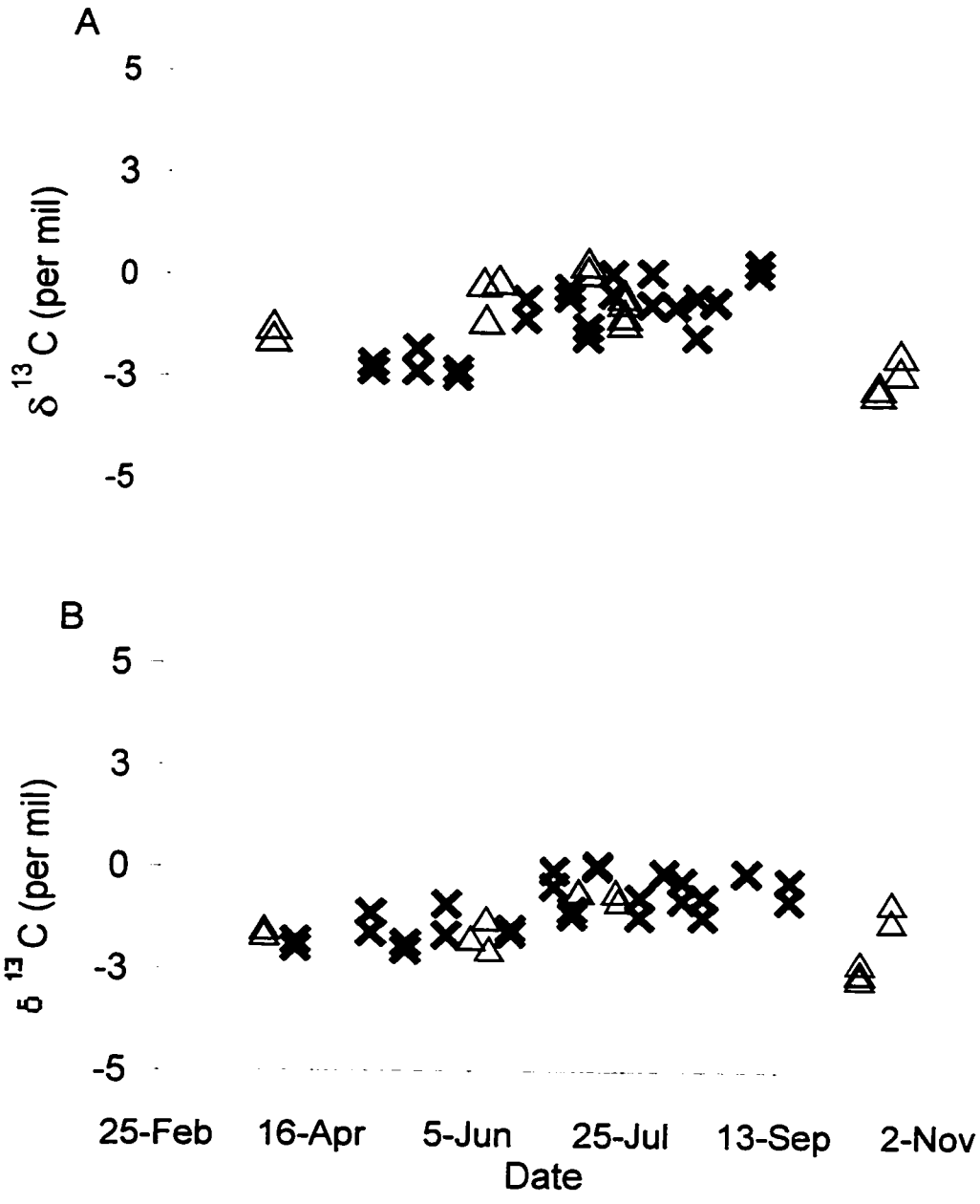


Figure 3.3. The seasonal change in the $\delta^{13}C$ of epilimnetic dissolved inorganic carbon at Station 41 (A) and Station 81 (B). (✖) Represents samples collected in 1994; (Δ) represents samples collected in 1995.

Table 3.1. The $\delta^{13}C$ of DIC in water samples collected at various depths on August 2, 1995. The mixing depth (Zm) was 6 metres.

Depth (m)	$\delta^{13}C$
3.0	-0.82
7.5	-0.51
12.5	-0.92
25.0	-2.11
75.0	-2.50
120.0	-2.28

Calculations of the relative proportions of HCO_3^- and $CO_{2(aq)}$ indicate that the ambient levels of bicarbonate are far greater than the levels of dissolved carbon dioxide (Table 3.2; 3.3). Given the relative levels of the two forms of DIC, even though the equilibration between HCO_3^- and $CO_{2(aq)}$ is a slow process, the magnitude of the available bicarbonate means that the rate in which the system is able to supply $CO_{2(aq)}$ is fairly rapid (Table 3.2; 3.3).

Florescence microscopy was used to examine the tangential flow retentate to assess the composition of the sample and determine the integrity of the glass fibre filtration. A few cells estimated to be between 1 and 3 μm in diameter were found in both the August and October retentate subsamples. Most of the sample was composed of cells which were < 1 μm in diameter. The concentration of colloidal material or organic material, other than whole cells larger than 0.2 μm, was not determined. The $\delta^{13}C$ of the freeze-dried retentate (POM_{TFF}) was 2.0 ‰ to 3.0 ‰ more enriched than the POM_{GFF} (1 to 20 μm) at Station 81, August 2 and October 31 (Table 3.4). The POM_{TFF} fraction collected at Station 41, October 30 had a $\delta^{13}C$ of -21.5 ‰, similar to Station 81 (Table 3.4).

A seasonal shift was observed in the isotope signatures of POM_{GFF} samples collected. In late May through mid-June, the $\delta^{13}C$ of all size classes of POM at both sites remained in the -30 to -27 ‰ range, with the exception of the POM_{20} fraction collected June 7, which was -23 ‰ (Fig. 3.6, 3.7, Table 3.4). The $\delta^{13}C$ of POM_{GFF} collected at Stations 41 and 81

Table 3.2. Stn 41. Seasonal changes in the carbonate chemistry of Lake Ontario at Station 41, 1995. **DIC**; measured concentrations of dissolved inorganic carbon from the epilimnion at Station 41. **$cHCO_3^-$**, **cCO_3^-**, and **$cCO_{2(aq)}$**, all represent calculated concentrations of th different DIC pools. **$mCO_{2(aq)}$**, is the measured concentration of $CO_{2(aq)}$ determined from headspace CO_2 analysis. **$d[CO_2]/dt$** is the rate at which CO_2 removed from the system could be regenerated given HCO_3 as the source of CO_2 calculated using the kinetics and the equations in Miller and Coleman (1980). **Popt.**, is the maximum observable rate of photosynthesis under conditions of optimal incident irradiance. **ci**, is the calculated concentration of CO_2 inside an algal cell to produce a $\delta^{13}C$ identical to that observed in the POM. **pCO_2**, are calculated values for the partial pressure of dissolved CO_2 using Lyman's constants.

Date	DIC µmol C/l	$cHCO_3$ (µmol/L)	cCO_3 (µmol/L)	cCO_{2aq} (µmol/L)	$mCO_{2\,(aq)}$ (µmol/L)	$d[CO_2]/dt$ (µmol/l*s)	P opt (µmol C/l/h)	ci (µmol)	pCO_2 atm x 10^4
11-Apr	1862.50	1760.66	3.31	98.43	50.61	2.18			16.00
19-Apr	1870.83	1828.25	11.24	31.32		0.95			5.40
25-Apr	1933.33	1890.19	12.43	30.68		0.98			5.58
2-May	1929.17	1887.01	12.97	29.16		0.94	0.50		5.39
10-May	1891.67	1846.49	10.46	34.68		1.05	0.43	26.66	6.34
18-May	1887.50	1822.46	5.97	59.01		1.50	0.65		10.57
24-May	1837.50	1783.49	7.39	46.56		1.33	0.54		8.88
31-May	1804.17	1766.98	15.32	21.84		0.79	0.67		3.88
7-Jun	1912.50	1869.18	30.89	12.41		0.64	0.92	7.66	2.61
16-Jun	1908.33	1823.24	80.56	4.54	12.94	0.46	0.72	2.78	0.97
21-Jun	1925.00	1855.91	62.85	6.23	6.36	0.52	0.72	4.13	1.60
29-Jun	1775.00	1698.89	71.43	4.67		0.46	0.69	2.56	1.28
10-Jul	1625.00	1579.38	38.05	7.56	10.61	0.50	0.33	4.07	2.00
24-Jul	1508.33	1450.35	53.39	4.59		0.41	0.37	3.79	1.21
11-Aug	1675.00	1627.99	38.84	8.16	17.38	0.54	1.16		2.37
16-Aug	1633.33	1595.87	25.84	11.61		0.62	1.30		3.22
30-Aug	1675.00	1639.45	16.89	18.64		0.81	0.98	11.89	5.40
14-Sep	1791.67	1738.27	45.78	7.60		0.54	0.89		1.99
21-Sep	1783.33	1745.20	15.33	22.78		0.91	0.96	14.90	5.91
26-Sep	1791.67	1748.72	10.86	32.05		1.12	0.79		8.42
10-Oct	1800.00	1759.94	13.00	27.02		1.00	0.81	17.39	6.86
23-Oct	1883.33	1836.93	10.74	35.62	114.90	1.22	0.58	27.13	8.94

Table 3.3. Stn 81. Seasonal changes in the carbonate chemistry of Lake Ontario at Station 81, 1995 **DIC**; measured concentrations of dissolved inorganic carbon from the epilimnion at Station 81. **cHCO3-**, **cCO3-**, and **$cCO_{2(aq)}$**, all represent calculated concentrations of the different DIC pools. **$mCO_{2(aq)}$** is the measured concentration of **$CO_{2(aq)}$** determined from headspace CO_2 analysis. **$d[CO_2]/dt$**, is the rate at which CO_2 removed from the system could be regenerated given HCO_3^- as the source of CO_2 calculated using the kinetics and equations in Miller and Coleman (1980). **Popt.**, is the maximum observablerate of photosynthesis under conditions of optimal incident irradiance. **ci** is the calculated concentration of CO_2 inside an algal cell to produce a $\delta^{13}C$ identical to that observed in the POM. **pCO_2**, are calculated values for the partial pressure of dissolved CO_2 using Lyman's constants.

Date	DIC µmol C/l	$cHCO_3$ (µmol/L)	cCO_3 (µmol/L)	cCO_{2aq} (µmol/L)	$mCO_{2(aq)}$ (µmol/L)	$d[CO_2]/dt$ (µmol/l*s)	P opt (µmol C/l/h)	ci (µmol)	pCO_2 atm x 10^4
6-Apr	1887.50	1814.37	5.04	68.02		2.79			11.25
11-Apr	1845.83	1766.42	4.44	74.90	52.73	3.28			12.28
20-Apr	1929.17	1889.17	14.97	25.01		1.28			4.01
25-Apr	1900.00	1861.01	18.33	20.63		1.24			4.39
3-May	1908.33	1869.60	18.91	19.80		1.15	0.30		3.67
10-May	1866.67	1827.52	15.53	23.58		1.36	0.71	16.86	4.68
17-May	1679.17	1643.52	12.61	23.01		1.24	0.33		4.37
25-May	1875.00	1835.05	14.94	24.98		1.46	0.28		5.24
1-Jun	1795.83	1757.44	14.18	24.19		1.40	0.29		5.45
6-Jun	1900.00	1860.29	16.98	22.70		1.36	0.52	16.00	5.20
15-Jun	1875.00	1834.80	24.76	15.42	14.66	1.08	0.45	10.55	3.74
20-Jun	1929.17	1889.06	20.62	19.46		1.26	0.48	13.68	4.53
14-Jul	1741.67	1703.35	24.32	13.97	10.91	1.05	0.31	8.35	3.98
27-Jul	1766.67	1690.20	43.81	7.65	9.01	0.74	0.49		2.41
2-Aug	1733.33	1714.31	44.50	7.84	16.98	0.77	0.79	3.99	1.66
15-Aug	1645.83	1607.15	27.61	11.06		0.89	0.43	5.97	3.21
29-Aug	1316.67	1282.84	26.43	7.38		0.64	0.07	4.37	2.06
7-Sep	1325.00	1296.88	14.53	13.58		0.93	0.27		3.69
12-Sep	1158.33	1133.60	11.02	13.69		0.90	0.28	8.18	3.60
20-Sep	1441.67	1411.42	14.02	16.20		1.05	0.30		4.03
27-Sep	1754.17	1717.39	16.79	19.96		1.28	0.02		5.08
4-Oct	1800.00	1761.56	15.12	23.28		1.43	0.70	15.60	5.67
11-Oct	1750.00	1712.40	14.03	23.54		1.42	0.05	19.20	5.67
20-Oct					73.99				

Table 3.4. The $\delta^{13}C$ of particulate organic matter (POM) at Stations 41 and 81. The 0.45 μm to 44 μm to samples were collected with a Westfalia centrifuge, the 20 μm to 44 μm samples were collected on Nitex™ screens, and the 0.2 μm to 1 μm fractions were collected using tangential flow filtration. Duplicate collections for tangential flow filtrate were made August 8 at Station 81.

Date	Stn	$\delta^{13}C$
0.45 - 44 μm		
11-May-95	41	-28.4
19-Jun.-95	41	-29.4
21-Jun.-95	41	-27.8
10-May-95	81	-29.3
20-Jun.-95	81	-29.4
20 - 44 μm		
7-Jun.	41	-23.1
19-Jun.	41	-26.5
21-Jun.	41	-26.9
29-Jun.	41	-22.4
15-Jul.	41	-18.7
1-Aug.	41	-21.9
23-Oct.	41	-25.0
20-Jun.-95	81	-27.4
2-Aug.-95	81	-19.0
20-Oct.-95	81	-25.2
0.2 - 1 μm		
8-Aug.	81	-20.7
8-Aug.	81	-22.8
31-Oct.	81	-25.5
30-Oct.	41	-21.5

climbed from spring minimum values to maximum values during thermal stratification of -22.1 ‰ and -24.3 ‰ respectively (Fig. 3.4, 3.5). In the fall, when thermal stratification starts to break down, the $\delta^{13}C$ of POM drops to below -30.0 ‰ at both sites. Pearson correlation coefficients were calculated with Bonferroni corrected probabilities for: $\delta^{13}C$, chlorophyll *a* (Chl *a*), areal rates of primary productivity (PP), lake surface water temperature, and the calculated $CO_{2(aq)}$ concentrations. No correlation between the $\delta^{13}C$ of POM and Chl *a* or PP could be determined at either site. There was a negative correlation ($r = -0.86$, $p = 0.002$, $\alpha = 0.05$ d.f. = 10) between the $\delta^{13}C$ of POM and the concentration of $CO_{2(aq)}$ at Station 81 and between the $\delta^{13}C$ of POM and temperature ($r = 0.82$, $p = 0.006$) (Fig. 3.5). At Station 41, the $\delta^{13}C$ of POM was not significantly correlated with the concentration of $CO_{2(aq)}$, Chl *a* or PP.

The $\delta^{13}C$ for POM_W (44 to 0.45 μm) and POM_{GFF} collected at the same sites, at the same time, were within 1.5 ‰ of each other. The POM_{20}, had carbon signatures which were slightly enriched in ^{13}C, 1.0 to 2.0 ‰, relative to the POM_{GFF} and POM_W (Table 3.4). During the summer there is departure between the $\delta^{13}C$ of the POM_{GFF} (1 to 20 μm) size fraction and the POM_{20} (44 to 20 μm) (Fig. 3.6, 3.7, Table 3.4). The $\delta^{13}C$ of the POM_{20} fraction was as much as 4.0 ‰ higher than the POM_{GFF} fraction (Fig. 3.6, 3.7, Table 3.4). The POM_{TFF} (0.2 to 1 μm) collected had a $\delta^{13}C$ similar to the POM_{20} fraction at Station 81 (Table 3.4). A POM_{TFF} sample was not collected at Station 41 in August. In October

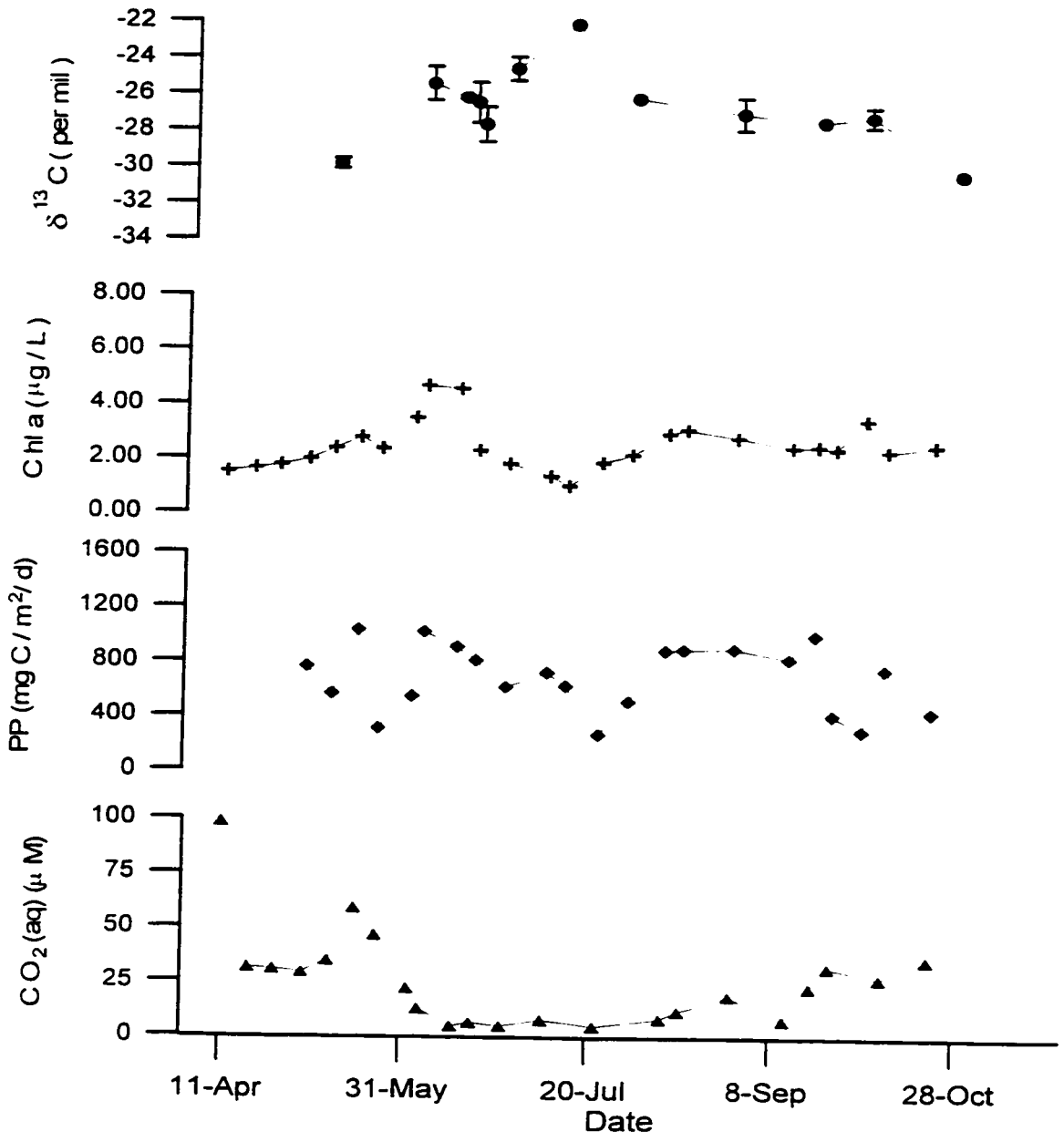


Figure 3.4. A comparison of seasonal trends at Station 41, Lake Ontario, 1995. **A.** The seasonal trend in the $\delta^{13}C$ of POM (20 to 1 µm). **B.** The seasonal change in the concentration of chlorophyll *a* in the epilimnion. **C.** Areal rates of photosynthesis in mg carbon m^{-2} d^{-1}. **D.** The concentration of dissolved CO_2 calculated from measurements of pH, temperature, and an assessment of system alkalinity.

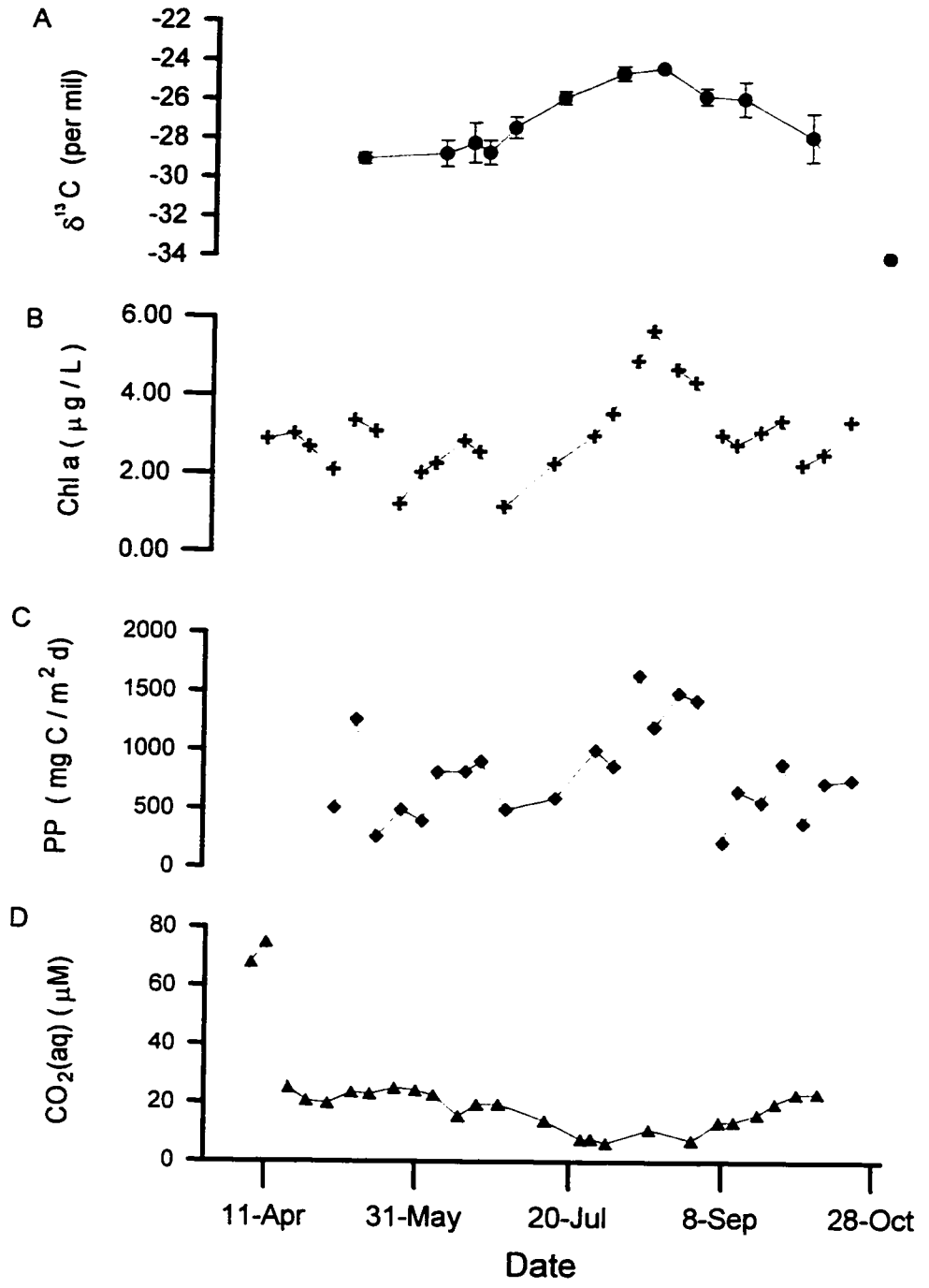


Figure 3.5 A comparison of seasonal trends at Station 81, Lake Ontario, 1995. **A.** (Top) The seasonal trend in the $\delta^{13}C$ of POM (20 to 1 μm). **B.** The seasonal change in the concentration of chlorophyll *a* in the epilimnion. **C.** The areal rates of photosynthesis in mg carbon m^{-2} d^{-1} **D.** The concentration of dissolved CO_2 calculated from measurements of pH, temperature, and an assessment of system alkalinity.

the POM_{TFF} fraction was 4.0 ‰ higher than the POM_{20} fraction and 10.0 ‰ higher than the POM_{GFF} fraction (Fig. 3.6, 3.7, Table 3.4).

The 110 to 210 μm fraction of zooplankton collected was made up of nauplii, smaller copepodids, rotifers, and some bosminids in different proportions, depending on the time of sampling. The 210 to 295 μm fraction contained copepodids, bosminids, and *Diacyclops thomasi*. The > 295 μm fraction contained bosminids, daphnia, calanoid and cyclopoid copepods including *D. thomasi*. All of the size fractions would contain some phytoplankton that could not be removed through filtration.

Zooplankton samples generally demonstrated the same seasonal trends in $\delta^{13}C$ as the POM (Fig. 3.6, 3.7). *Diacyclops thomasi* was available in sufficient quantity to facilitate separation for stable isotope analysis over the entire period studied. The average $\delta^{13}C$ of *D. thomasi* sampled in May was -29.3 ‰ at Station 81 and -28.5 ‰ at Station 41 (Fig. 3.6, 3.7). The carbon signature of *D. thomasi*. peaked at -21.5 ‰ in August at Station 81 before dropping again to -24.5 ‰ by late September (Fig. 3.7). At Station 41 the signature of *D. thomasi* was still as high as -22.8 ‰ September 26 (Fig. 3.6). *Bosmina longirostris* was first available in sufficient numbers to obtain a sample in mid-June at Station 41. The $\delta^{13}C$ of *B. longirostris* in June was approximately -26.0 ‰ at both sites (Fig. 3.6, 3.7). The $\delta^{13}C$ of *B. longirostris* increased to a maximum of -24.5 ‰ by mid-July at both sites and stayed fairly constant until October at Station 41 but dropped to

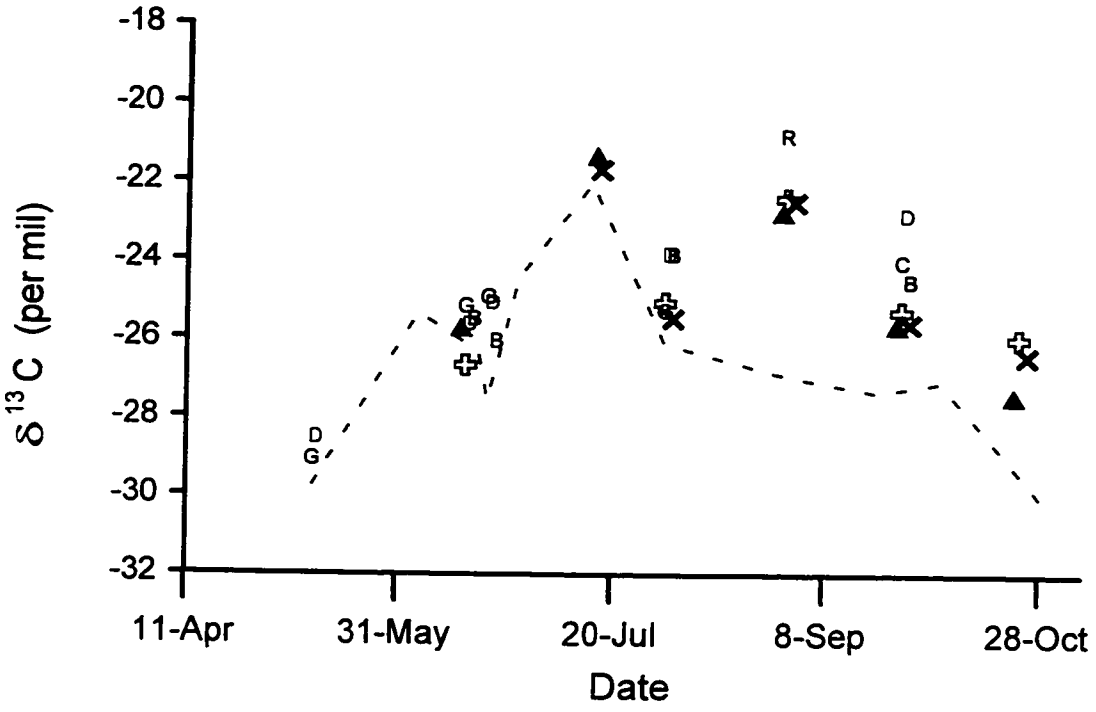


Figure 3.6. The temporal trend in zooplankton $\delta^{13}C$ at Station 41, Lake Ontario, 1995, in relation to the signature of POM collected on GF/F filters (dashed line). Individual species and size fractions are represented as: D *Diacyclops thomasi*; **G** Calanoid copepods; **B** *Bosmina longirostris*; **C** *Daphnia spp;* **R** Rotifers (*Keratella sp.*) ; × Seston 110 - 210μm ; Zooplankton 210 - 295 μm ; ▲ Zooplankton > 295 μm.

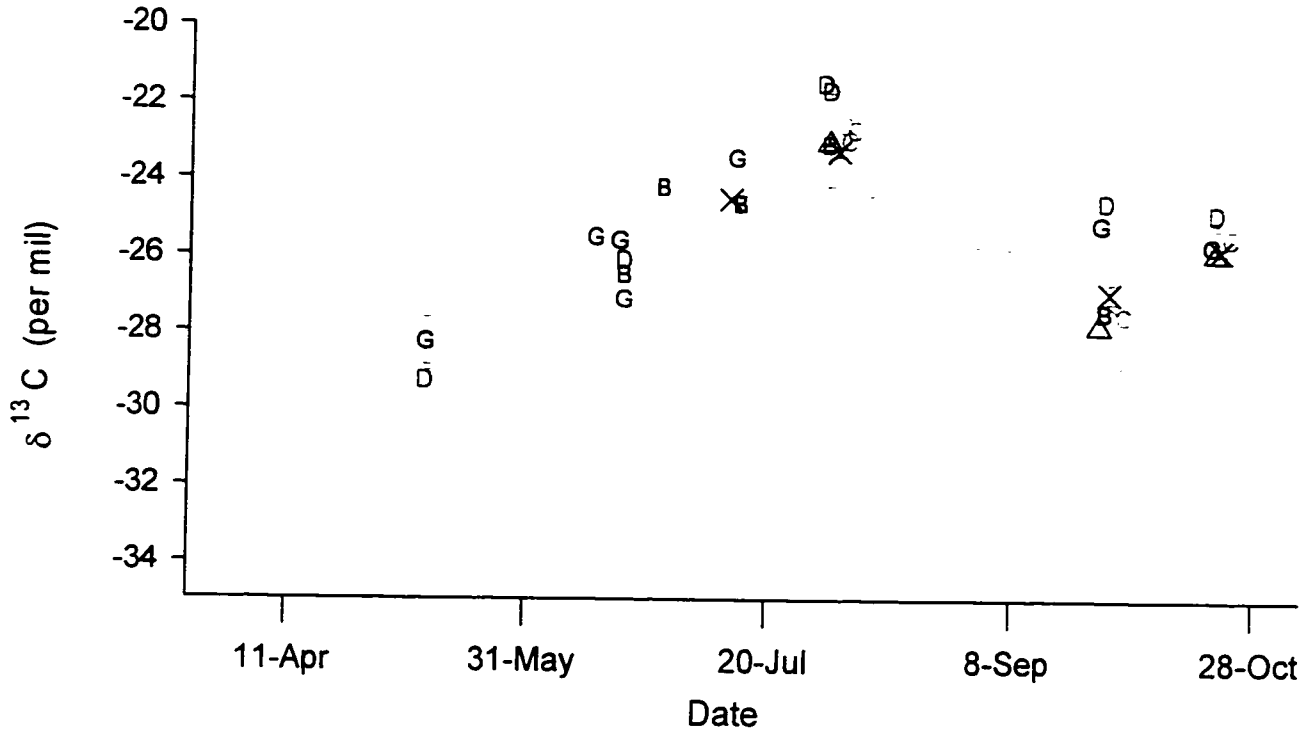


Figure 3.7. The temporal trend in zooplankton $\delta^{13}C$ at Station 81, Lake Ontario, 1995, in relation to the signature of POM collected on GF/F filters (dashed line). **D** *Diacyclops thomasi*; **G** Diaptomid grazers; **B** *Bosmina longirostris*; **C** *Daphnia sp;* × Seston 110 - 210 μm ; - Zooplankton > 295 μm. Where multiple samples were available, the standard deviation is indicated by vertical bars.

-28.2 ‰ at Station 81. *Daphnia spp.* had an isotope composition similar to *B. longirostris* at both sites (Fig. 3.6, 3.7). Species of calanoid copepods were pooled to provide sufficient mass for analysis, samples were made up predominantly of *Leptodiaptomus sicilis* and *Skistodiaptomus organensis*. One sample of *Bythotrephes sp.* was obtained July 15, at Station 41, and was determined to have a $\delta^{13}C$ of -19.4 ‰. A single sample of rotifers, largely *Keratella cochlearis*, was obtained August 30 (Fig. 3.6). The rotifers had a $\delta^{13}C$ which was 2.0 ‰ higher than zooplankton analyzed at the same time (Fig. 3.6). There was no difference in $\delta^{13}C$ between acid-treated and untreated zooplankton samples (Appendix 2). The $\delta^{13}C$ of zooplankton samples which were lipid-extracted or stored in ethanol were 1 to 2 ‰ enriched relative to freeze-dried zooplankton (Appendix 1).

Discussion

The lack of correlation between Chl *a*, the areal PP, and the $\delta^{13}C$ of POM on a seasonal basis suggests that photosynthetic uptake is not the sole determining factor of $\delta^{13}C$ of DIC and POM in Lake Ontario. It's been hypothesized that the observed $\delta^{13}C$ of POM is a function of not only the DIC, but also the mode of uptake and ambient concentration of inorganic carbon available to the cell (Schelske and Hodell 1991; Fogel and Cifuentes 1993). In order to address these hypotheses in the context of our results, an understanding of the processes influencing the $\delta^{13}C$ of DIC and POM on a seasonal basis has to be developed. The trophic dynamics of zooplankton may then be examined using

$\delta^{13}C$ as a tracer against the background of seasonal changes in the $\delta^{13}C$ of the different size fractions of POM.

$\delta^{13}C$ of DIC and POM

The observed 2.0 ‰ change in $\delta^{13}C$ of DIC in Lake Ontario (Fig. 3.3) is hypothesized to be a function of a shrinking pool of available $CO_{2(aq)}$ which is also becoming enriched in ^{13}C. The low level of seasonal change in the $\delta^{13}C$ of DIC, relative to the fluctuation observed in POM_{GFF}, may be attributed to the system's alkalinity and the magnitude of the pool of DIC available in Lake Ontario.

The measured carbon isotope signature of DIC is an integration of all of the carbon molecules making up the total pool of available DIC. Since most of the DIC is HCO_3^-, the $\delta^{13}C$ of DIC is dominated by the $\delta^{13}C$ of HCO_3^-. The $\delta^{13}C$ of $CO_{2(aq)}$ and HCO_3^- are both likely to be dynamic on a temporal basis. However, the equilibrium between $CO_{2(aq)}$ and HCO_3^- is the predominant mechanism for exchange of carbon isotopes in the HCO_3^- pool. Therefore the $\delta^{13}C$ of HCO_3^- should be fairly stable since much more carbon has to be exchanged in order to get a shift in the carbon signature of the entire pool. In contrast, the concentration and the $\delta^{13}C$ of available $CO_{2(aq)}$ in the system is likely to be more dynamic. The calculated seasonal changes in relative proportions of $CO_{2(aq)}$ and HCO_3^- are a function of the physical, chemical, and biological processes influencing the

equilibrium between the two carbonate species. Many of these processes will act directly on the pool of $CO_{2(aq)}$ and will influence the carbon signature.

The rate constants for the dehydration of bicarbonate or hydration of carbon dioxide differ depending on whether the carbon reacting is ^{13}C or ^{12}C (Marlier and O' Leary 1984). This results in a spread in $\delta^{13}C$ between HCO_3^- and $CO_{2(aq)}$ in equilibrium. Isotope fractionation is the term used to describe the change in $\delta^{13}C$ associated with a given process. The fractionation between CO_2 and HCO_3^- is temperature dependent, and has been determined to fall in the range of -12.00 ‰ at 0°C to -8.42 ‰ at 25(C (Mook 1974). The reaction kinetics between HCO_3^- and $CO_{2(aq)}$ are fairly slow in relation to other processes, such as respiration and photosynthesis, which can influence the concentration of $CO_{2(aq)}$ (Miller and Coleman 1980). Since isotope equilibration is a function of discrimination during chemical equilibration, the rate at which isotope equilibrium might be attained kinetically is at least as slow or slower than the rate at which chemical equilibrium can be attained. As a result, under conditions where the rate of $CO_{2(aq)}$ removal or addition is greater than the rate of HCO_3^- dissociation or formation, the chemical equilibrium may be shifted producing a subsequent shift in the isotope composition of the DIC (Goericke *et al.* 1994). The relative impact of these processes with respect to their overall influence on the $\delta^{13}C$ of DIC forms the basis for estimation of the $\delta^{13}C$ of $CO_{2(aq)}$ and HCO_3^-. Under conditions of atmospheric equilibrium, HCO_3^-

will have a characteristic $\delta^{13}C$ signature of 0 ‰ to 1 ‰ (Weiler and Nriagu 1978; Keeling *et al.* 1979).

Assessment of Spring Processes

The temperature of the lake in April or early May is usually between 2 and 4 °C which is near the seasonal minimum (Johannsson *et al.* 1985). Colder winter temperatures enhance $CO_{2(aq)}$ solubility so that $CO_{2(aq)}$ contributes more substantially (5.3 % as opposed to 0.3 % mid-summer) to a slightly larger DIC pool (approximately 1900 μM opposed to 1500 μM) (Tables 3.2, 3.3). The elevated concentration of $CO_{2(aq)}$ results in a DIC pool with a lower $\delta^{13}C$ since $CO_{2(aq)}$ is depleted in ^{13}C relative to HCO_3^-. The -2.2 ‰ signature for DIC measured in the spring represents a seasonal low.

The lake does not freeze over the winter and $CO_{2(aq)}$ is free to exchange with atmospheric CO_2. In the winter and early spring, Lake Ontario is supersaturated with CO_2 and acts as a source of CO_2 to the atmosphere losing between 2 to 3 g C m^{-2} d^{-1} (Weiler 1974). CO_2 exchange across the air-water interface will act to push the $\delta^{13}C$ of $CO_{2(aq)}$ toward -8.0 ‰, the value for the $\delta^{13}C$ of atmospheric CO_2 (Keeling 1979). Given the net flux of CO_2 from the water column, it is unlikely that isotope equilibrium exists between $CO_{2(aq)}$ and atmospheric CO_2. This does not preclude atmospheric gas exchange as a modifying

factor of $\delta^{13}C$ of $CO_{2(aq)}$, just that the level of influence of this process on the overall $\delta^{13}C$ of $CO_{2(aq)}$ in the spring is likely diminished.

The increase in $CO_{2(aq)}$ concentration may be attributed in part to a reduced ratio of photosynthesis/respiration in the lake ecosystem during the winter. Respired CO_2 will closely reflect the source from which it was produced (Jacobsen *et al.* 1970). Therefore, CO_2 from respiration would be expected in the -24.0 to -30.0 ‰ range, characteristic of the $\delta^{13}C$ of lake fauna in Lake Ontario (Kiriluk *et al.* 1995). Oxygen consumption by Lake Ontario biota has previously been estimated (Halfon *et al.* 1996). Respiratory quotients (RQs) for the conversion of oxygen consumption to levels of CO_2 respired for the species inhabiting Lake Ontario generally fall in the range of 0.70 to 1.25. This range in RQs may be used in converting the estimates for oxygen consumption to CO_2 expired calculated as a daily maximum. It may be roughly estimated that respiration adds 0.55 to 0.95 g C m^{-2} d^{-1} (0.53 to 0.92 μM CO_2). This amount is far less than the DIC concentration of 1900 μM C measured in April and May. It does however approach the estimated flux of 2 to 3 g C m^{-2} d^{-1} from Lake Ontario to the atmosphere (Weiler 1974). This suggests that respired CO_2 is a significant influence on the $\delta^{13}C$ of $CO_{2(aq)}$ in the spring.

Photosynthesis will influence the $\delta^{13}C$ of $CO_{2(aq)}$ since ^{12}C is used preferentially in carbon fixation. This leaves the remaining pool of $CO_{2(aq)}$ enriched in ^{13}C. Carbon fixation in

algae is almost exclusively the product of 1,5-bisphosphate carboxylase-oxygenase (RUBISCO) activity (Fogel and Cifuentes 1993; Goericke 1994). Under conditions of CO_2 saturation, this enzyme pathway has been shown to exhibit discrimination against ^{13}C, resulting in isotope fractionation in the order of -29.4 ‰ (Guy *et al.* 1987). The maximum level of fractionation associated with photosynthesis in aquatic systems is normally observed to be -20 to -22 ‰ (Fogel and Cifuentes 1993). The discrepancy between the level of fractionation associated with the enzyme pathway and that observed has been explained as being a function of the amount of carbon actually available to the enzyme pathway (Farquhar *et al.* 1982; Sharkey and Berry 1985). When DIC is scarce, more of the carbon entering the cell will be used in photosynthesis, decreasing the potential for discrimination between isotopes. Thus, under conditions of very low ambient $CO_{2(aq)}$ the isotope discrimination observed in photosynthesis may be virtually eliminated to the point where observed $\delta^{13}C$ is probably associated with discrimination due to diffusive resistance. The fixed carbon produced would have a signature very near that of $CO_{2\ (aq)}$ (Sharkey and Berry 1985). The $\delta^{13}C$ of POM in the spring in Lake Ontario was found to be between -30.0 ‰ and -32.0 ‰. As the amount of available carbon is at a maximum in the spring, it is more probable that there is little or no limitation of carbon at the site of enzyme activity and that the fractionation of the enzyme is fully expressed or close to it. The CO_2 available at the site of enzyme activity would therefore be expected to have a $\delta^{13}C$ of approximately -8.0 ‰ to -12.0 ‰.

The $\delta^{13}C$ of HCO_3^- may then be estimated through isotope mass balance. It was estimated that 5.3 % of the DIC pool in the spring was $CO_{2(aq)}$, the remainder was HCO_3^-. The $\delta^{13}C$ of the DIC was -2.2 ‰ in the spring. If 5.3 % of the DIC pool had a $\delta^{13}C$ of -8.0 to -12.0 ‰ ($CO2_{(aq)}$), the remaining 94.7 % (HCO_3^-) would have a $\delta^{13}C$ of -1.7 to -2.0 ‰ to balance the observed -2.2 ‰ of the DIC. The temperature in the water column in early spring was approximately 5 °C . Laboratory studies have determined that at 5 °C, the carbon isotope fractionation in the equilibrium of HCO_3^- and $CO_{2(aq)}$ was -11.35 ‰ (Mook 1974). The differences between our balance values fall in the range of -6.0 to -10.3 ‰. This suggests that either HCO_3^- and $CO_{2(aq)}$ are not in isotope equilibrium in the spring or that the fractionation associated with photosynthesis larger than -22 ‰.

Assessment of Summer Processes

The $\delta^{13}C$ of DIC increases from -2.2 ‰ in the spring to approximately 0 ‰ in the summer. The $\delta^{13}C$ of POM is observed to increase from approximately -30.0 to -32.0 ‰ in the spring to a mid-summer high of -22.0 to -24.0 ‰ at both sites before dropping below -30.0 ‰ following the breakdown of thermal stratification of the lake (Fig. 3.3, 3.4, 3.5). There are a number of potential explanations for the high $\delta^{13}C$ of POM while the lake is stratified. The 9.0 to 10.0 ‰ change suggests a shift from $CO_{2(aq)}$ as the source of carbon for photosynthesis. The active uptake of DIC, or the facilitated conversion of HCO_3^- to $CO_{2(aq)}$, has been shown to produce elevated carbon signatures in primary producers (Sharkey and Berry 1985; Guy *et al.* 1987; Falkowski 1991).

Alternatively, the change may result from an increase in the $\delta^{13}C$ of $CO_{2(aq)}$ and/or a reduction in the level of isotope fractionation by primary producers due to reduced concentrations of available $CO_{2(aq)}$.

As the temperature increases, the level of photosynthesis increases and the pH of the water in the epilimnion increases to approximately 8.6 by late summer. The concentration of $CO_{2(aq)}$ subsequently drops until it represents less than 0.5 % of the total DIC. The CO_2 system of natural waters is characterized by a number of non-linear relationships which must be taken into consideration when assessing impacts of CO_2 depletion (Talling 1985). When considering the change in the concentration of $CO_{2(aq)}$ in fresh water relative to the change in pH, there is a minimum in the buffering capacity at about pH 8.1 to 8.3 (Talling 1985). Through this pH range, an increase in the pH and subsequent drop in the concentration of CO_2 and pCO_2 due to photosynthesis is easily achieved (Talling 1985). For lakes of high alkalinity (1.5 to 3.0 meq L^{-1}) such as Lake Ontario, the pH range 8.1 to 8.3 also represents an air-equilibrium state, as a result these lakes may be more susceptible to pH elevation and CO_2 depletion than might be expected (Talling 1985). The net flux of CO_2 across the air-water interface is reversed in the summer; Lake Ontario starts to act as a sink for atmospheric CO_2 (Weiler 1974). The influx of atmospheric CO_2 serves to stabilize the concentration and determine the $\delta^{13}C$ of $CO_{2\ (aq)}$.

The concentrations of $CO_{2\,(aq)}$ in Lake Ontario are probably sufficient for diffusive uptake of CO_2 to meet the carbon requirements for a majority of the primary producers. The relationship between the observed $\delta^{13}C$ of POM and the necessary concentrations of CO_2, internal and external to the cell, has been described by Farquhar *et al.* (1982). Applying Farquhar's equation, it is possible to calculate the ratio c_i/c_a: the concentration of CO_2 inside the cell/concentration of CO_2 outside (Fogel *et al.* 1992). The basic premise is that the calculated concentration of CO_2 inside the cell cannot be higher than outside if diffusive uptake is the source of carbon for the cell. Using -9.0 ‰ as the $\delta^{13}C$ for $CO_{2(aq)}$, the CO_2 concentrations inside the cell (c_i) were estimated and found to be less than the external $CO_{2(aq)}$ concentrations throughout the period of investigation (Tables 3.2, 3.3). If the observed rate of photosynthesis is faster than the rate at which $CO_{2(aq)}$ can be regenerated in equilibration with HCO_3^-, the use of bicarbonate may be assumed (Sharkey and Berry 1985). The rate of maximum supply of $CO_{2(aq)}$ from HCO_3^- was calculated using the calculated levels of $CO_{2(aq)}$ and HCO_3^- and by applying the equilibration kinetics. A comparison of these rates to the measured optimal levels of photosynthesis, determined by ^{14}C addition experiments, suggests that the system has an ability to generate $CO_{2(aq)}$ far greater than the maximum rate of photosynthesis is able to remove carbon over the course of the season (Table 3.2, 3.3).

Given that concentrations of $CO_{2(aq)}$ are adequate for diffusion to be the principle mode of uptake, it may be hypothesized that the observed seasonal change in the $\delta^{13}C$ of POM

is a function of more efficient use of carbon diffusing into the cells of primary producers. As mentioned above, fractionation between carbon isotopes in the equilibration of $CO_{2(aq)}$ and HCO_3^- is temperature dependent. The level of fractionation would be approximately -9.0 ‰ at the temperatures normally found in Lake Ontario during the summer months. A derivation of the Farquhar's equation may be used to calculate the difference between cell leakage in the spring and summer (Sharkey and Berry 1985). The level of isotope fractionation observed is a maximum of approximately -22.0 ‰ in the spring. With this level of fractionation and an equilibrium isotope effect of -11.0 ‰ between $CO_{2(aq)}$ and HCO_3^-, cell leakage is estimated to be 37 % in the spring when $CO_{2(aq)}$ is most abundant. Assuming the $\delta^{13}C$ of $CO_{2(aq)}$ is -8.0 ‰, in equilibrium with atmospheric CO_2, and the equilibrium isotope effect between $CO_{2(aq)}$ and HCO_3^- is -9.0 ‰, the calculated level of cell leakage drops to 25 % in the summer.

Neither the direct uptake of inorganic carbon via a DIC concentrating mechanism nor the facilitated conversion of HCO_3^- to $CO_{2(aq)}$ is necessary in order maintain the level of photosynthesis observed. This does not suggest that the diffusion of $CO_{2(aq)}$ into algal cells is the only way that carbon is acquired. Other mechanisms of carbon acquisition, may be utilized by some primary producers. The direct assimilation of HCO_3^- by Lake Ontario picoplankton has previously been observed in ^{14}C-addition experiments (Caron *et al.* 1985). However, the active uptake of HCO_3^- or the facilitated conversion of HCO_3^- to $CO_{2(aq)}$ has an attached energetic cost (Beardall *et al.* 1985; Raven and Lucas 1985).

These mechanisms are therefore more likely to be active under conditions of $CO_{2(aq)}$ limitation. Boundary layer effects, limiting $CO_{2(aq)}$ at the cell surface, or low CO_2/O_2 ratios in the cell may induce some species of primary producers to use compensating mechanisms for carbon uptake (Kaplan 1985; Smith 1985; Prins and Elzenga 1989; Tsuzuki and Miyachi 1989). This may explain some of the variability in the $\delta^{13}C$ of different size fractions of POM collected. The observed differences are possibly a function of species specific differences in uptake and fractionation of carbon isotopes. The 20 to 44 μm fractions of POM collected in the summer are enriched in ^{13}C relative to the 1 to 20 μm fractions collected on the GF/F filters. The approximately 2.0 ‰ enrichment may be attributed to the influence of the boundary layer on the availability of $CO_{2(aq)}$ to the cell. Alternatively, the sample may include algae using HCO_3^- as a carbon source.

Assessment of Fall Processes

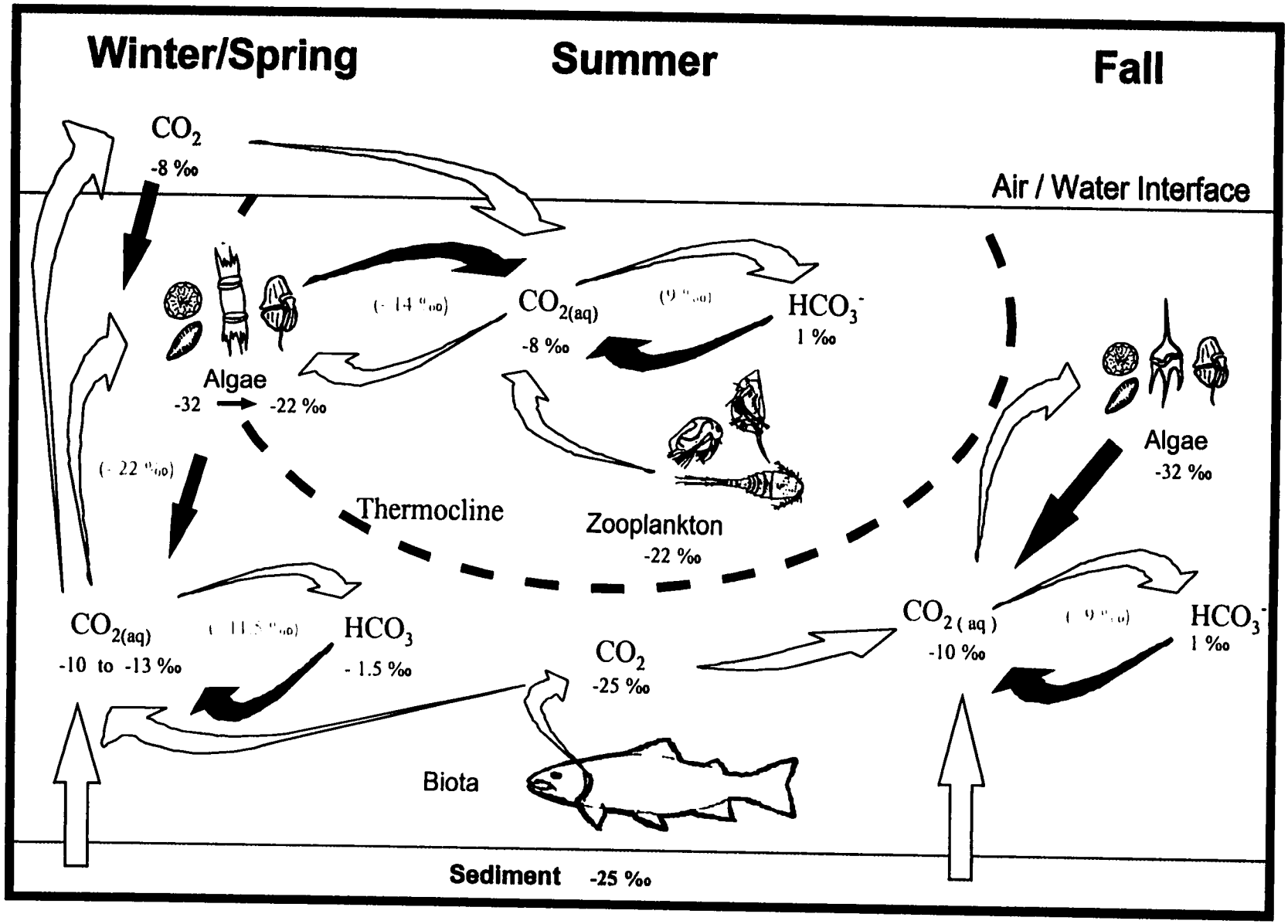
In the fall, there is a breakdown in thermal stratification and a drop in the $\delta^{13}C$ of POM. This drop is due to isotopically depleted $CO_{2(aq)}$ from the hypolimnion being suddenly made available to the epilimnetic algae. Over the course of the summer, there is no mixing of $CO_{2(aq)}$ between the hypolimnion and the upper thermal layers and therefore no CO_2 exchange between the CO_2 produced in the hypolimnion and the atmosphere. Respired CO_2 adds to already elevated $CO_{2(aq)}$ concentrations in the hypolimnion since $CO_{2(aq)}$ is more soluble in the colder waters at greater depths. As stated previously,

isotope equilibration between $CO_{2(aq)}$ and HCO_3^- is temperature dependent and $CO_{2(aq)}$ in the colder waters at greater depths would be expected to be slightly more depleted in ^{13}C. This assertion is supported by the analysis of DIC at increasing depth during the summer (Table 3.1). In addition, respired CO_2 will be approximately the same as its organic source, so $CO_{2(aq)}$ not in equilibrium with HCO_3^- will be further depleted. A similar mass balance to that calculated in the spring indicates that the $\delta^{13}C$ of $CO_{2(aq)}$ in the hypolimnion is approximately -10.0 ‰ to -11.0 ‰. It is the increased concentration of available $CO_{2(aq)}$ causing the drop in $\delta^{13}C$ of POM in the fall.

Model Summary

The results of our analysis may be summarized in a model depicting the dominant processes influencing the $\delta^{13}C$ of $CO_{2(aq)}$ (Fig. 3.8). The onset of spring and subsequent warmer temperatures coincide with elevated levels of primary production. $CO_{2(aq)}$ is used preferentially as a source of carbon for primary producers and concentrations of $CO_{2(aq)}$ start to fall. Isotopically light carbon is drawn out of the $CO_{2(aq)}$ pool as the heavier isotope of carbon is discriminated against by the enzyme systems active in carbon fixation. With increasing temperatures and an increase in primary production, there is a subsequent increase in the pH of the system and a resulting decrease in the solubility of $CO_{2(aq)}$. By the time the lake is thermally stratified in mid-June, the concentration of $CO_{2(aq)}$ has dropped from >70 to <10 μM . Continued removal of $CO_{2(aq)}$ leads to a

Figure 3.8 - A model of the processes influencing the concentration and isotope composition of dissolved CO_2 within Lake Ontario and across the air water interface on a seasonal basis. The left side of the page depicts processes occurring in the spring when the lake is mixed. The centre of the page depicts processes occurring while the lake is stratified. The right side depicts fall processes and signatures when the lake turns over. The flux of CO_2 depleted in ^{13}C relative to the receiving pool is depicted with light arrows. The flux of CO_2 enriched in ^{13}C relative to the receiving pool is depicted with dark arrows. The thermocline is represented by a dashed line separating processes in the epilimnion from processes in the hypolimnion during summer stratification. The $\delta^{13}C$ values for the various pools of carbon are indicated. The level of isotope fractionation associated with cyclic processes are given as ($\delta^{13}C$) values enclosed in arrows.


Winter/Spring
Summer
Fall
CO_2
-8 ‰
Air / Water Interface
$CO_{2(aq)}$
-8 ‰
HCO_3^-
1 ‰
Algae
-32 → -22 ‰
Algae
-32 ‰
Thermocline
Zooplankton
-22 ‰
$CO_{2(aq)}$
-10 to -13 ‰
HCO_3
- 1.5 ‰
CO_2
-25 ‰
$CO_{2(aq)}$
-10 ‰
HCO_3^-
1 ‰
Biota
Sediment -25 ‰

reversal in the exchange of CO_2 with the atmosphere, as the system becomes a sink for atmospheric CO_2.

The combination of these processes are at least partially responsible for the observed increase in the $\delta^{13}C$ of DIC in the epilimnion from -2.2 ‰ to -0.5 ‰, where it remains for much of the summer. As the $CO_{2(aq)}$ pool shrinks and becomes more isotopically enriched, the process of equilibration between $CO_{2(aq)}$ and HCO_3^- becomes faster as there is less carbon in the $CO_{2(aq)}$ pool to turn-over and less total DIC in the system. As the isotope equilibrium is slowly shifted, both species of carbon become more isotopically enriched. At higher temperatures, the carbon isotope fractionation of dissolved CO_2 with respect to dissolved HCO_3^- is less pronounced (-8.97 ‰ at 25°C) (Mook 1974). In addition to the biological and chemical kinetic influences, the influx of atmospheric CO_2 maintains the low levels of $CO_{2(aq)}$ at near equilibrium concentrations. Water in equilibrium with the atmosphere has a free CO_2 concentration of 10 to 20 μM (Boston *et al.* 1989). Atmospheric CO_2 may potentially serve in stabilizing the $\delta^{13}C$ of the $CO_{2(aq)}$ pool at approximately -8.0 ‰, the air-water equilibrium value, since the concentration of CO_2 added is larger than that from epilimnetic respiration and the entire pool is circulated very rapidly. In the hypolimnion, where primary production is not a significant factor influencing the DIC pool, respired CO_2 from inhabiting fauna produces an ambient DIC isotopically depleted relative to epilimnetic DIC. There is little or no mixing of hypolimnetic and epilimnetic $CO_{2(aq)}$ so that atmospheric CO2 is not a mitigating factor.

When the lake turns over in the fall, there is mixing of epilimnetic and hypolimnetic DIC. This addition of isotopically depleted DIC, along with cooler fall temperatures, boosts the concentration of $CO_{2(aq)}$ available to primary producers and results in a decline in the carbon isotope signatures of DIC and POM. The observed decline would be expected to continue into the winter until the lake again reaches a point where the lake attains a balance and a resulting $\delta^{13}C$ signature of approximately -2.2 ‰ for DIC.

$\delta^{13}C$ of Zooplankton

The $\delta^{13}C$ of the zooplankton was usually between the $\delta^{13}C$ of the different size fractions of POM analyzed and was usually consistent with what was known about their feeding ecology. The 110 to 210 μm fraction of zooplankton collected was made up of nauplii, smaller copepodids, rotifers, and some bosminids in different proportions, depending on the time of sampling. The 210 to 295 μm fraction contained copepodids, bosminids, and *D. thomasi*. The > 295 μm fraction contained bosminids, daphnia, calanoid and cyclopoid copepods including *D. thomasi*. All of the size fractions would contain some phytoplankton that could not be removed through filtration.

During the summer there is departure between the $\delta^{13}C$ of the 1 to 20 μm size fraction of POM, collected on GF/F filters, and the 20 to 44 μm fraction collected on 20 μm mesh screen (Fig. 3.6, 3.7, Table 3.4). The 20 to 44 μm fraction had a $\delta^{13}C$ as much as 4.0 ‰ higher than the 1 to 20 μm size fraction (Fig. 3.6, 3.7, Table 3.4). The 0.2 to 1 μm

fraction collected using tangential flow filtration had a $\delta^{13}C$ similar to the 20 to 44 μm fraction at Station 81 (Table 3.4). A 0.2 to 1 μm fraction was not collected at Station 41 in August. However, one sample of rotifers, largely *Keratella cochlearis*, was obtained August 30 (Fig. 3.6). The rotifer sample probably indicates the $\delta^{13}C$ of the 0.2 to 1 μm fraction since that is the size class upon which they would likely be feeding. The rotifers had a $\delta^{13}C$ which was 2.0 ‰ higher than zooplankton analyzed at the same time (Fig. 3.6). In October the <1 μm fraction was 4.0 ‰ higher than the 20 to 44 μm fraction and 10.0 ‰ higher than the 1 to 20 μm size fraction (Fig. 3.6, 3.7, Table 3.4). The discrepancy between different size classes of POM is indicative of varying levels of isotope fractionation in the uptake and assimilation of inorganic carbon by the different algal species collected in each sample. The $\delta^{13}C$ of the 0.2 to 1 μm fraction collected is potentially a function of microbial recycling of available organic carbon. The sources of organic carbon would include zooplankton and the lysed cells of primary producers. It might be expected that the $\delta^{13}C$ of organic carbon available to bacteria would take longer to reflect changes in the carbon fixed by primary producers since it would take a little longer before the organic carbon produced in photosynthesis was available to bacteria in a form they could use.

In late May through mid-June, the $\delta^{13}C$ of all size classes of POM at both sites remained in the -30 to -27 ‰ range, with the exception of the 20 - 44 μm fraction collected June 7, which was -23 ‰ (Fig. 3.6, 3.7, Table 3.4). Colonial diatoms are the dominant primary

producers in the spring and make up most of the mass collected with the Westfalia and on the GF/F at Station 81 (1 - 44 μm). Calanoid copepods, mostly diaptomids, and *D. thomasi* comprise most of the zooplankton biomass in the spring. However, $\delta^{15}N$ analysis suggests selection for food sources other than diatoms by both of these species (Chapter 4). The diaptomids present in Lake Ontario have similar feeding habits, *Leptodiaptomus sicilis* has a broad food range from <10 μm to >53 μm, *Skistodiaptomus oregonensis* prefers large plankton 12 to 24 μm. The $\delta^{13}C$ of the diaptomids analyzed was generally intermediate to the 1 to 20 μm and 20 to 44 μm size fractions over the course of the entire season (Fig. 3.6, 3.7).

The cyclopoid copepod, *D. thomasi*, may be described as a raptorial feeder and is a selective omnivore (Balcer *et al.* 1984). The adults collected and separated from the bulk sample in the spring are likely to feed on dinoflagellates, ciliates, protozoa, cyclopoid copepodids and available diaptomid and cyclopoid nauplii. Diacyclops will expand their feeding to include cladocerans during the summer (Balcer *et al.* 1984). *D. thomasi* is slightly enriched in ^{13}C relative to most other zooplankton analyzed (Fig. 3.6, 3.7). The $\delta^{13}C$ of *D. thomasi* at Station 41 in the summer suggests they are feeding on rotifers or organisms with a similar carbon signature (Fig. 3.6). In previous studies, *D. thomasi* were found not to feed significantly on *Keratella cochlearis* but did feed on rotifer eggs (LeBlanc *et al.*, 1997). The eggs of *Keratella* would be expected to have a $\delta^{13}C$ similar to the rotifer sample collected.

Bosmina longirostris and *Daphnia spp.* appear in abundance in late June about the time the lake starts to stratify. *B. longirostris* is a filter feeder typically consuming algae <10 μm in diameter (DeMott 1982). Bosminids have been observed to feed on organisms as big as 40 to 120 μm in length (Jack and Gilbert 1993). At Station 41, the $\delta^{13}C$ of *B. longirostris* is similar to the 1 to 20 μm size fraction until August (Fig. 3.6). In September, the $\delta^{13}C$ of *B. longirostris* is intermediate between the 0.2 to 1 μm size fraction and the 1 to 20 μm or 20 to 44 μm fraction of POM (Fig. 3.6, Table 3.4). At Station 81, in June, the $\delta^{13}C$ of bosminids are closer to the 20 to 44 μm size fraction (Fig. 3.7, Table 3.4). *B. longirostris* are potentially feeding on larger algae at this point in the season, however, no 0.2 to 1 μm size fraction was collected and although it may have a similar $\delta^{13}C$ to the larger size fraction, it is impossible to make an assessment.

Daphnia spp. are filter feeders able to consume algae over a broad size range. They have been observed to prefer chlorophytes over other algal types (Balcer *et al.* 1984). In Lake Ontario chlorophytes comprise almost 50 % of the available algal biomass immediately following stratification (Chapter 4). The $\delta^{13}C$ of *Daphnia spp.* reflects feeding on algae captured in both the 20 to 44 μm fraction and the 1 to 20 μm size fraction at both sites (Fig. 3.6, 3.7, Table 3.4).

Conclusions

There are a number of processes influencing the $\delta^{13}C$ of DIC and POM at any point in the season. The $\delta^{13}C$ observed depends on which process is dominant at any given time. The lack of correlation between areal primary productivity/ Chl *a* and $\delta^{13}C$ determined in our investigation is likely a function of the time frame chosen for our investigation and a lack of power in our design. A more intensive sampling regimen over a shorter time frame would likely have yielded very different results. The range in $\delta^{13}C$ of different size classes of POM at different times suggests a species specific relationship between the $\delta^{13}C$ availability of $CO_{2(aq)}$ and the $\delta^{13}C$ of the organic carbon produced. Our findings also suggest that $\delta^{13}C$ may not be an effective predictor of productivity in all circumstances. Therefore, using the $\delta^{13}C$ of organic carbon in sediment may not always be a robust means of inferring past productivity in lacustrine systems. The $\delta^{13}C$ of organic carbon in sediment should probably be used as one of many tools in paleolimnological studies. Corroborating evidence should be garnered to support inferences made on the basis of carbon isotopes.

The results of this study, coupled with those obtained in similar investigations, suggest that the level of fluctuation in the $\delta^{13}C$ of POM is largely influenced by the algal response to the ambient concentration of $CO_{2(aq)}$. Any relationship between the $\delta^{13}C$ of POM, levels of productivity, Chl *a*, or the $\delta^{13}C$ of DIC is likely to be system and season specific

depending on the processes controlling $CO_{2\ (aq)}$. Therefore an understanding of system biogeochemistry and potential for change and variability in the $\delta^{13}C$ of primary producers should be an intrinsic part of any study where carbon isotopes are used to make inferences regarding carbon source.

Chapter 4

The Influence of Inorganic Nitrogen Cycling on the $\delta^{15}N$ of Lake Ontario Biota

Introduction

Fractionation between the light and heavy stable isotopes of carbon and nitrogen in the assimilation of food by an organism has been used in ecological and ecotoxicological studies as a means of determining trophic relationships and in tracing the relative importance of potential food sources (Fry and Sherr 1984; Peterson and Fry 1987; Kidd *et al.* 1994; Kiriluk *et al.* 1995). The basis for this is an observed regular pattern of enrichment in naturally occurring ^{15}N relative to ^{14}N between successive links in a food chain (Minigawa and Wada 1984). In contrast, the ratio of ^{13}C to ^{12}C remains relatively constant through trophic transfer and is subsequently used as an indicator of carbon source (Peterson and Fry 1987). Given these relationships, it is presumably possible to map out a food web using a plot of the nitrogen *vs.* the carbon isotope signatures of the dominant organisms in a system (Fry 1991). The relative position of the organisms plotted should be indicative of trophic position (Cabana and Rasmussen 1994).

A key assumption in the use of natural abundance levels of stable isotopes in discerning trophic relationships between organisms is that the isotope signature of the different food sources remains relatively constant. This assumption may not be valid in many ecosystems because the isotope signatures of primary producers vary spatially and temporally (Goering *et al.* 1990; Montoya *et al.*1991; Fogel *et al.* 1992; Yoshioka *et al.*

1994). The $\delta^{15}N$ of primary producers in marine systems depends on the concentration, isotope signature, and form of dissolved inorganic nitrogen (DIN) used as a nitrogen source (Owens 1987; Fogel and Cifuentes 1993; Goericke 1994). A similar situation may occur in freshwater environments. In marine environments, concentrations of DIN are usually thought to be limiting (Hecky and Kilham 1988). In contrast, DIN in freshwater systems is characterized by wider ranges in concentration and greater temporal dynamism (Hecky and Kilham 1988). This has the potential to result in a wide variability in the $\delta^{15}N$ of primary producers, both between and within systems, over time (Yoshioka 1994; Cabana and Rasmussen 1996). Different sources of inorganic nitrogen may be expected to produce a characteristic $\delta^{15}N$ in the organic material synthesized (Owens 1987; Montoya 1990). The observed $\delta^{15}N$ of particulate organic matter (POM) may be compared to the $\delta^{15}N$ of the different pools of DIN. An assessment may then be made of the likelihood that the observed $\delta^{15}N$ of POM could have been produced using a particular source of inorganic nitrogen. Because primary consumers in freshwater ecosystems tend to be relatively small with fast rates of tissue turnover (days), they would be expected to track seasonal changes in $\delta^{15}N$ primary productivity.

To test these hypotheses, a study was conducted to investigate the seasonal variability in the $\delta^{15}N$ of DIN, primary producers, and zooplankton in Lake Ontario of the Laurentian Great Lakes. Lake Ontario is a phosphorus limited system. There are large seasonal changes in concentrations of DIN ranging from 10 to 30 μM. Terrestrial inputs to the

POM are expected to be negligible at both sites. Water, POM, and zooplankton samples were analyzed to determine their nitrogen isotope signatures.

Methods

All of the sampling for this project was done in conjunction with the Lake Ontario Biomonitoring program (Department of Fisheries and Oceans, Canada). Two sampling sites were chosen for comparison. One site was located mid-lake, Station 41, and the other site was in the eastern-basin, Station 81 (Fig. 3.1). Bi-weekly samples were collected from April 20 to September 27 in 1994, and from May 11 to October 31, 1995. Additional samples for $\delta^{15}N$ analysis of nitrate were collected April 23, September 24, and October 23, 1997 from Station 41. Samples for nitrate analysis were collected July 23, 1997 from Station 403.

Water samples

Discrete water samples for nutrient analysis were obtained with Nisken bottles. Samples were taken at 10 m when the lake was thermally homogenous and from the mid-epilimnion once the lake had stratified. Mixing depth was determined from temperature profiles obtained using an electronic bathythermograph. Samples for nutrient chemistry were removed to a shipboard laboratory, filtered, and stored at 4°C. Nutrient analysis of

water samples was conducted by the National Laboratory for Environmental Testing, Burlington, Ontario (Environment Canada 1979).

Ten litre samples of water filtered through GF/F filters were collected in polyethylene containers and stored frozen for $\delta^{15}N$ analysis of NH_4^+ and NO_2^-/NO_3^-. NH_4^+ was extracted from water samples with activated zeolite added to the sample in a 2 L separatory funnel shaken for 5 min. The zeolite was then collected from the sample on a precombusted GF/F filter which was dried in a dessiccator under vacuum. After all of the NH_4^+ in the sample was removed, the NO_2^-/NO_3^- in the sample was reduced to NH_4^+ by passing the sample through a zinc column activated with 2N H_2SO_4. The NH_4^+ obtained from NO_2^-/NO_3^- reduction was then extracted with zeolite as described above. The dried zeolite was scraped from the filters into glass vials and stored at -4 °C prior to analysis.

No $\delta^{15}N$ for NH_4^+ was obtained at Station 81. The analysis of the $\delta^{15}N$ of DIN was made difficult by the low ambient concentrations of ammonium in the lake on many of the occasions when sampling took place. Duplicate standards had $\delta^{15}N$ values for NH_4^+ that were reproducible within 1.2 ‰ when the extraction efficiency was >92 %. For many of the samples the initial concentration of ammonium was low, so that a 92 % extraction resulted in a residual NH_4^+ concentration below the level of detection of 0.005 mg L^{-1} (0.4 μM). The extraction efficiency of the zeolite decreased with lower concentrations. Therefore more zeolite, per milligram ammonium removed, had to be used in samples

with low ammonium concentrations. This created difficulties with the mass of material required for analysis. The concentrations of NO_2^-/NO_3^- were comparatively high, between 10 and 30 μM NO_2^-/NO_3^-. Therefore, after NO_2^-/NO_3^- reduction, greater extraction efficiencies for ammonium were attainable. NO_2^-/NO_3^- reduction efficiencies were at least 95 % and extraction efficiencies were in excess of 92 % for all samples reported.

Particulate Organic Matter

Particulate organic matter (POM) was collected and size-fractionated using a number of different techniques in 1995. This was done to determine the relative efficacy of different sample collection methods. A primary consideration was to obtain a sufficient mass of material for isotope analysis. Since the objective was to characterize the nitrogen isotope signature at the base of the food web, the sampling protocol was also designed to avoid missing a size-fraction which may potentially be providing sustenance to higher trophic levels. A comparison of the $\delta^{15}N$ of the various size classes provided a means of apportioning the $\delta^{15}N$ among different species making up the composite samples of POM.

In 1995, samples of POM_{GFF}, were obtained from 200 L of water pumped from the mid-epilimnion through a 44 μm Nitex™ screen into storage containers. In 1997, samples were prescreened through a 64 μm Nitex™ mesh screen. Most of these samples were

then sequentially filtered through a 20 μm Nitex™ mesh screen and a precombusted (500 °C) 0.7 μm nominal pore size, Whatman GF/F glass fibre filter. The exception was the sample collected on a GF/F filter May 10, 1995 at Station 81 which was not pre-filtered through 20 μm mesh Nitex™. The GF/F filters and samples of particulate collected on 20 μm screens (when available in sufficient mass) were dried at 60 °C prior to analysis. In May and June of 1995, at both sites, additional samples were obtained using a Westfalia continuous-flow centrifuge to separate particulate from 200 L samples collected as described above. The Westfalia effectively removes particles <0.45 μm in size. POM_W obtained from the centrifuge was dried at 60 °C prior to analysis.

In August and October of 1995, tangential flow filtration, as described in Barthel *et al.* (1989), was used to capture particulate approximately 0.2 μm to 1 μm in diameter from 200 L samples filtered through GF/F filters. The sample was continuously passed through a Millipore Pelicon cassette system fitted with a microporous membrane cassette with a rated pore size of 0.2 μm. The filtrate, except for 3 L retained for DIN extraction, was discarded and the remaining sample retentate was recirculated until the sample volume was reduced from 200 L to less than 500 mL. The POM_{TAN} (tangential flow retentate) was subsampled for examination using florescence microscopy in order to make a rough assessment of the composition of the sample and determine the integrity of the glass fibre filtration. No further analysis was done to determine how much of the sample may have been composed of colloidal material or organic material other than

whole cells that were larger than 0.2 μm. The bulk of the retentate was freeze-dried to obtain the POM_{TAN} which was analyzed to determine its isotope signature. The same method was used to collect tangential-flow filtered samples seasonally at Station 41 in 1997. In addition to the 0.2 μm to 1 μm samples, 10 L of the filtrate was kept and refiltered through a 1000 d cassette to obtain a dissolved organic fraction as well as the 0.2 to 1 μm bacterial fraction.

Zooplankton

Zooplankton samples were collected in 1994 and 1995 using 64 μm mesh Nitex™ nets in vertical tows, from 20 m to the surface prior to stratification, and from the thermocline to the surface when the lake was stratified. Samples were sequentially screened through >250 μm, >210 μm, >110 μm and >64 μm mesh Nitex™screens and preserved in ethanol. The >64 μm fraction was not retained in 1995. The POM_{110} or POM_{64} samples consisted of material collected on either 110 μm or 64 μm mesh filters after passing through 295 μm and 210 μm mesh filters. All the POM_{110} or POM_{64} samples were flushed with distilled water to remove as much phytoplankton as possible in order to obtain an assessment of the $\delta^{15}N$ of the rotifers, small cladocerans, nauplii and copepodites retained. Phytoplankton removal was accomplished with varying levels of success. These samples were then viewed under a microscope to roughly determine their content. On separate occasions the material flushed through the 64 μm mesh filters was collected on a 20 μm mesh filter for analysis (POM_{20}). Rotifers did not represent a

substantial portion of the biomass of these samples until June 28. Prior to this, samples contained largely phytoplankton, nauplii and copepod eggs. Selected subsamples were removed from the larger size fractions (>210 μm and >295 μm) and were also viewed under a microscope. These subsamples had all debris removed from the zooplankton prior to analysis. Individual species were obtained through separation under a microscope. Composite samples of many hundreds of individuals were then rinsed with distilled deionized water and dried prior to analysis. The only species available in sufficient quantity over the entire period studied to facilitate separation for stable isotope analysis was *D. thomasi*. Samples of other species were obtained at points during the season when they similarly became available in sufficient numbers to enable enough individuals to be picked for analysis. Calanoid copepods, mostly diaptomids, were pooled to provide sufficient mass for analysis. Samples were made up predominantly of *Leptodiaptomus sicilis* and *Skistodiaptomus organensis*. One sample of *Bythotrephes sp.* was obtained July 15, 1995, mid-lake.

Carbon and nitrogen isotope ratios for all particulate and zooplankton samples were obtained using a VG Optima continuous-flow isotope-ratio mass-spectrometer (CF-IRMS). Instrument accuracy, precision, and range of linearity were monitored using sets of National Institute of Standards and Technology (NIST) -USA standards with a known concentration and isotope composition of nitrogen. Duplicate standards of identical mass

within the linear range of operation consistently had measured $\delta^{15}N$ values within 0.25 ‰ of each other.

All zooplankton samples were stored in ethanol prior to speciation. Ethanol extractable material leached out of the samples during storage is not included in our analysis. No difference in the $\delta^{15}N$ was observed between lipid extracted and/or acid treated zooplankton, zooplankton stored in ethanol, and zooplankton which were fresh-frozen, and analyzed with no other pretreatment (Appendix 2).

Results

Temporal fluctuations in $\delta^{15}N$ were observed in DIN, all size fractions of POM, and in the various species of zooplankton analyzed. Differences were also observed between size fractions of POM collected at the same time and site.

DIN

A distinct difference in the isotope composition of NH_4^+ and NO_2^-/NO_3^- was observed at Station 41 in the spring and fall in 1995. The $\delta^{15}N$ of NO_2^-/NO_3^- for DIN samples collected in October at Station 41 was determined to be 3.1 ‰ ± 1.1 ‰ (S.D.) in 1995 and 3.2 ‰ ± 1.5 ‰ in 1997. The $\delta^{15}N$ of NO_2^-/NO_3^- in the surface water was approximately 1.8 ‰ in the spring of 1995 and 2.6 ‰ in 1997. The mid-summer $\delta^{15}N$

values of NO_2^-/NO_3^- were approximately 3.0 ‰ higher than the spring values (Fig. 4.1). Similar values to those from 1995 were obtained in 1997: on July 23, NO_2^-/NO_3^- was 3.2 ‰; a sample obtained August 15 was 5.4 ‰ (Table 4.1). Spring values for NH_4^- obtained in 1995 were approximately 8 ‰ heavier than the NO_2^-/NO_3^- in the spring, and 6 ‰ heavier after the breakdown of thermal stratification in the fall (Fig. 4.1). No measurements of the $\delta^{15}N$ of NH_4^- were obtained for the stratified period. At Station 81, the $\delta^{15}N$ of NO_2^-/NO_3^- was approximately 3.5 ‰ in the spring and changed only slightly from spring through to the fall (Fig. 4.2).

The $\delta^{15}N$ of NO_2^-/NO_3^- was measured in samples obtained from the hypolimnion in 1997. In the spring, prior to stratification, the $\delta^{15}N$ of NO_2^-/NO_3^- was consistent from the surface to the lake bottom (Table 4.1). After stratification the $\delta^{15}N$ of NO_2^-/NO_3^- in the epilimnion appeared to diverge from the $\delta^{15}N$ of NO_2^-/NO_3^- in the hypolimnion. In October, immediately prior to fall mixing, the $\delta^{15}N$ of NO_2^-/NO_3^- in the hypolimnion was approximately -2.4 ‰ (Table 4.1). The $\delta^{15}N$ of NO_2^-/NO_3^- collected from the epilimnion, at the same time, was approximately 3.2 ‰ (Table 4.1).

Table 4.1. Seasonal changes in concentration and $\delta^{15}N$ of NO_2^-/NO_3^- at various depths from mid-lake sites in Lake Ontario, 1997.

Date	Depth (m)	Concentration (mgL^{-1})	Std dev	n	$\delta^{15}N$ (‰)	Std dev	n
April 23	**3**	0.400	0.006	5	2.60		2
	50	0.388		1	2.38		1
	120	0.420	0.015	3	2.41		2
July 23	**3**	0.299	0.031	4	3.22		2
	75	0.546	0.024	3	1.62		2
August 15	**3**	0.251			5.41		1
September 23	**3**	0.266	0.009	6	4.38	0.950	3
	75	0.450	0.024	3	-1.80	2.010	4
October 23	**3**	0.296	0.015	5	3.155	1.470	4
	75	0.435	0.017	3	-2.35		2

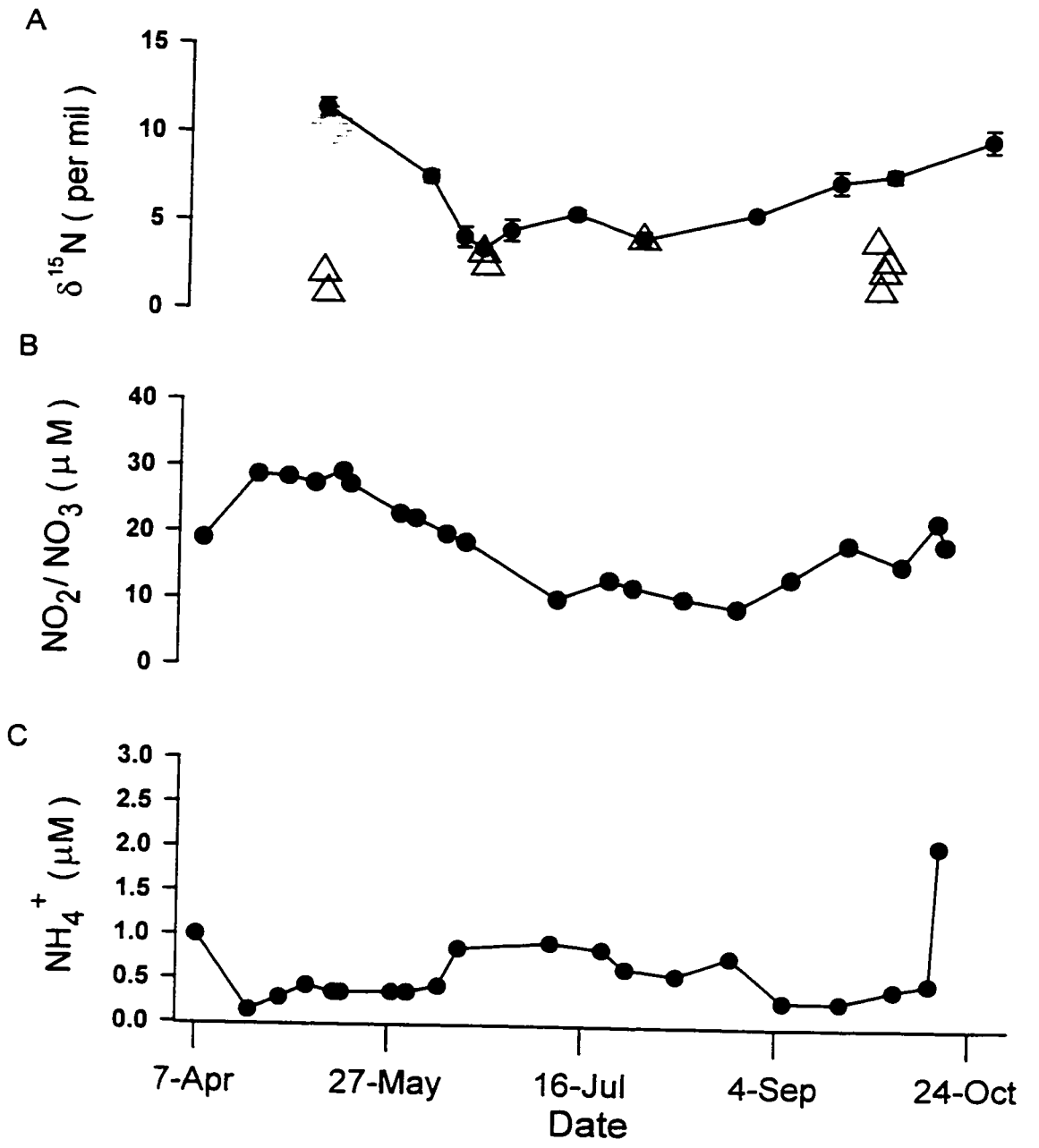


Figure 4.1. **A.** The seasonal change in the $\delta^{15}N$ of POM_{GFF} at Station 41, 1995, in relation to: the $\delta^{15}N$ of NO_2^-/ NO_3^- (—) and the $\delta^{15}N$ of NH_4^+ ( ). **B.** The seasonal change in the concentration of NO_2^-/ NO_3^- in the epilimnion. **C.** The seasonal change in the epilimnetic concentration of NH_4^+.

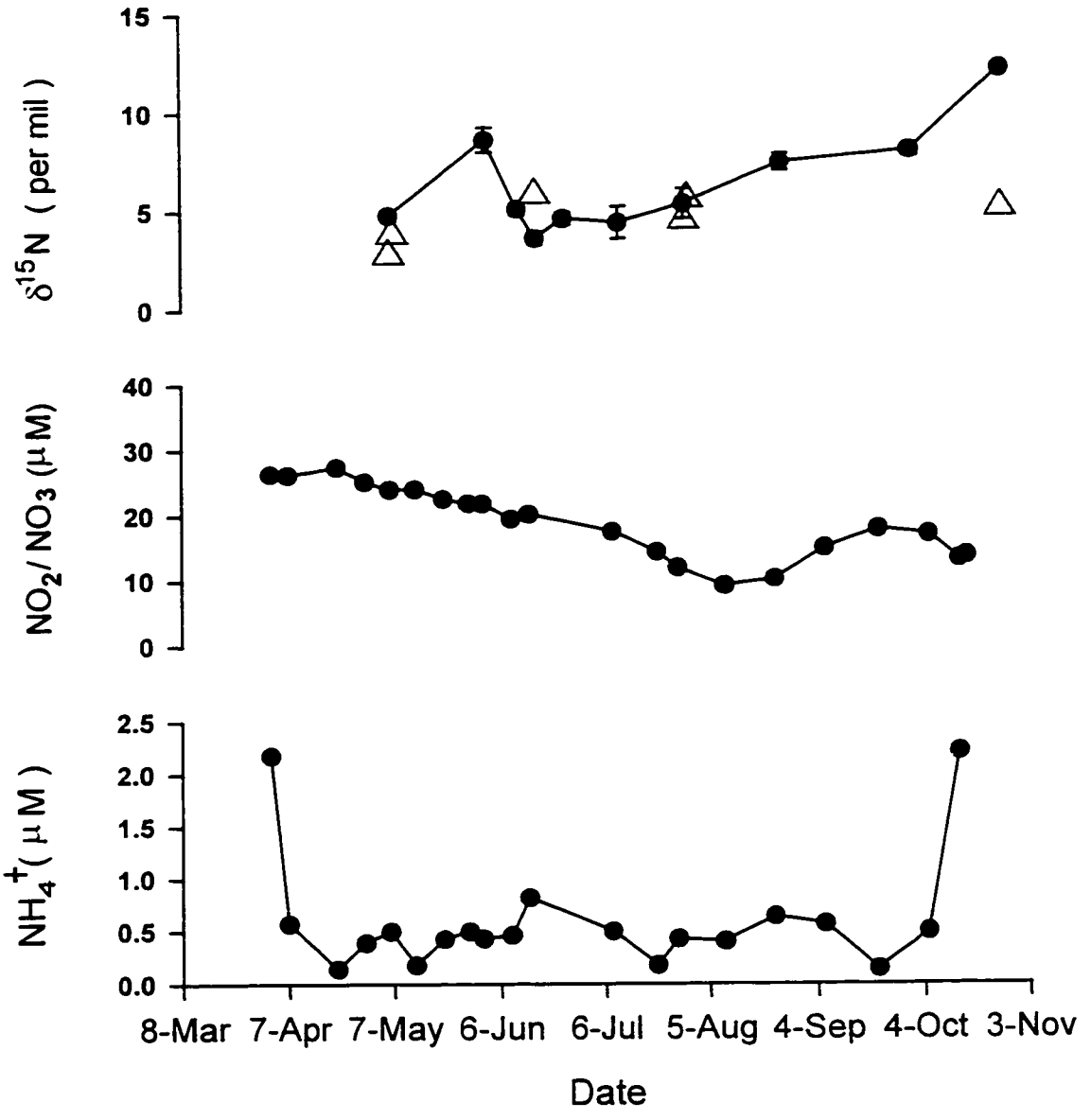


Figure 4.2. **A.** The seasonal change in the $\delta^{15}N$ of POM at Station 81, 1995, in relation to the $\delta^{15}N$ of NO_2^-/ NO_3^- ( ) **B.** The temporal change in the concentration of NO_2^-/ NO_3^- in the epilimnion. **C.** The seasonal change in the epilimnetic concentration of NH_4^+.

Tangential-flow retentate

Microscopic examination of the August and October retentate subsamples showed that the POM_{TFF} fraction contained a few cells estimated to be between 1 and 3 μm in diameter. This indicated that cells larger than the nominal pore size were not excluded by GF/F filtration. However, these cells stood out, as most of the sample was composed of intact cells <1 μm in diameter. The $\delta^{15}N$ of duplicate samples of POM_{TAN}, collected at Station 81 in August 1995 was approximately 6 ‰. This was slightly enriched relative to the $\delta^{15}N$ of NO_2^-/NO_3^- and POM_{GFF} collected at the same time (Table 4.2C, Fig. 4.3). The POM_{TAN}, collected and analyzed at both sites in late October 1995 was not as enriched in ^{15}N on average as the POM_{GFF} (1 - 20 μm) collected, but had a higher $\delta^{15}N$ than any of the NO_2^-/NO_3^- samples obtained in October (Table 4.2C, Fig. 4.2, Fig. 4.3). The $\delta^{15}N$ of the DOM_{TFF} and POM_{TFF} samples collected in April, 1997 were approximately 4 ‰ more enriched than the NO_2^-/NO_3^- (2.9 ‰), and 0.5 ‰ lower than the POM_{GFF} (20 to 1 μm) samples collected at the same time (Table 4.3). The pattern during the summer was the same as what was observed in 1995. POM_{TFF} was enriched in ^{15}N relative to the NO_2^-/NO_3^- or POM_{GFF} (20 to 1 μm) samples analyzed. In contrast to 1995, the smaller tangential-flow filtered samples (0.2 μm to 1 μm), collected in September and October 1997, had the same $\delta^{15}N$ or were slightly more enriched in ^{15}N than the POM_{GFF} sample (Table 4.3).

POM

POM collected on a GF/F filter (POM_{GFF}, 1 to 20 μm) in May of 1995 at station 41, had a $\delta^{15}N$ of 11.4 ± 0.5 ‰. The $\delta^{15}N$ of this material quickly dropped to 3.5 ± 0.2 ‰ immediately after the onset of thermal stratification (Fig. 4.1). Over the course of the summer the isotope signature of POM_{GFF} increased through to fall mixing. The $\delta^{15}N$ of POM_{GFF} was 10.0 ± 0.6‰ by October 31, 1995. The pattern observed in the $\delta^{15}N$ of POM_{GFF} collected at station 81 in the east basin of the lake was very similar, the exception being the May 10, 1995, $\delta^{15}N$ which was 4.7 ± 0.3 ‰, almost 4 ‰ lower than the 8.5 ± 0.6 ‰ measured June 6 (Fig. 4.2). From June through October the pattern of fluctuation is similar to that observed at Station 41 over the period of investigation.

There was a discrepancy between the $\delta^{15}N$ values obtained for POM_W (Westfalia centrifuge retentate, 0.45 to 44 μm) and POM_{GFF}, collected at Station 41 in the spring. The $\delta^{15}N$ of POM_W, collected on May 11 at Station 41, was 7.2 ± 0.8 ‰ as opposed to 11.4 ± 0.4 ‰ for the POM_{GFF}, collected at the same time (Table 4.2A). When the two methods of sampling were employed again on June 21, that discrepancy was diminished: POM_W had a $\delta^{15}N$ 2.5 ± 0.2 ‰ and POM_{GFF} was 3.5 ± 0.4 ‰. There was very little difference between POM_W and POM_{GFF} at Station 81.

A

POM_W 0.45 - 44 μm Date	Stn	$\delta^{15}N$
11-May	41	7.18
19-Jun	41	3.70
21-Jun	41	2.53
10-May	81	4.41
20-Jun	81	4.01

B

POM_{20} 20 - 44 μm Date	Stn	$\delta^{15}N$
11-May	41	10.01
7-Jun	41	3.39
19-Jun	41	1.90
21-Jun	41	0.71
29-Jun	41	7.54
15-Jul	41	7.08
1-Aug	41	8.49
23-Oct	41	9.08
20-Jun	81	4.77
2-Aug	81	6.36
20-Oct	81	8.31

C

POM_{TAN} 0.2 - 1.0 μm Date	Stn	$\delta^{15}N$
8-Aug	81	6.04
8-Aug	81	5.80
30-Oct	41	6.88
31-Oct	81	5.76

Table 4.2. The $\delta^{15}N$ of particulate organic matter at Station 41 and station 81. The 0.45 μm to 44 μm samples (POM_w) were collected with a Westfalia centrifuge, the 20 μm to 44 μm samples (POM_{20}) were collected on Nitex™ screens and the 0.2 to 1.0 μm (POM_{TAN}) fractions were collected using tangential-flow filtration. Duplicate collections were made for tangential flow filtration at station 81, August 8.

Table 4.3. The $\delta^{15}N$ of particulate organic matter (POM) collected at Station 41 in 1997. **DOM_{TFF}**, represents the retentate from tangential-flow filtration that passed through a 0.2 μm membrane and was collected by a 1000 d membrane. **POM_{TFF}**, represents the retentate from tangential-flow filtration that passed through a GF/F filter and was collected by a 0.2 μm membrane. **POM_{GFF}**, is POM that passed through a 20 μm mesh net and was collected on a GF/F filter. **POM 64 - 20 μm,** is POM which passed through a 64 μm mesh net collected on a 20 μm mesh net. POM >110, is POM which was collected on a 110 μm mesh net. The September 24, sample is almost entirely *Ceratium hirundinella*. Zooplankton greater than 210 μm, *Daphnia spp.* and *Diacyclops thomasi* are indicated.

	23-Apr-97	23-Jul-97	15-Aug-97	24-Sep-97	23-Oct-97
DOM_{TFF} (0.2 μm to 1000 dalton)	6.91			7.28	
POM_{TFF} (1 to 0.2 μm)	6.74	7.84	6.37	7.10	7.53
POM_{GFF} (1 to 20 μm)	12.17± 0.15	3.46	3.28	6.17	7.33
POM 64 - 20 μm	6.35	5.65	6.40	6.40	5.20
POM > 110 μm		7.16		7.55*	
Zooplankton > 210 μm		9.08		11.54	
Daphnia sp.		7.12			
Diacyclops thomasi				12.51	

*** Approximately 90 % of the sample is Dinoflagellates**

The $\delta^{15}N$ of the POM_{GFF}, collected on Nitex™ at Station 41 prior to stratification, was relatively low compared to the other POM_{GFF} samples collected: 3.4 ‰ on June 7, 1995 and 1.9 ± 2.4 ‰ on June 19, 1995 (Table 4.2B). However, after stratification this changed. POM_{20} (20 μm Nitex™), collected at Station 41 on June 29 had a $\delta^{15}N$ of 7.5 ‰ and by August 1, the signature of this fraction had increased to 8.5 ± 1.7 ‰ (Table 4.2B).

Spring collections of POM_{64}, from Station 81 in 1994, had a range in $\delta^{15}N$ from 11.3 ± 0.4 ‰ to 1.9 ‰ (Fig. 4.3). Spring samples, determined by microscopic inspection to be 'cleaner'(less phytoplankton), also had higher and less variable $\delta^{15}N$. The $\delta^{15}N$ of POM_{20} was measured at 5.8 ‰ April 19, 1994 and 1.9 ‰ May 25, 1994 (Fig. 4.3).

A large variability in $\delta^{15}N$ of POM_{64} was observed prior to stratification and immediately following thermal mixing in the fall. The $\delta^{15}N$ of POM_{64} appeared to increase slightly while the lake was stratified (Fig. 4.3). POM_{64}, collected June 28, was 5.3 ± 0.5 ‰, and POM_{64}, collected September 13, was 7.7 ± 1.1 ‰. POM_{110} was consistently enriched over POM_{64}, shifting from 7.0 ‰ on July 27, 1994 to 10.2 ‰ September 13, 1994 (Fig. 4.3). The 20 to 44 μm size fraction of POM, collected in 1995, was replaced in 1997 with a 20 to 64 μm size fraction. In comparison to 1995, the $\delta^{15}N$ of the >20 μm fraction from 1997 was consistently lower. The 20 to 64 μm sample, collected in the spring, was

estimated to be comprised of 70 to 80 % diatoms. The POM >110 μm was enriched in ^{15}N relative to other size fractions except POM_{TFF} . The POM >110 μm fraction from 1997 was almost exclusively comprised of *Ceratium sp.*

Zooplankton

Zooplankton samples demonstrated the same seasonal trends in $\delta^{15}N$ as the POM fractions analyzed. Zooplankton are believed to feed predominantly on plankton in the 1 to 20 μm size range (Johannsson and Gorman, 1991). Therefore, the $\delta^{15}N$ of the zooplankton is presented in relation to the POM_{GFF} (1 to 20 μm) (Fig. 4.4, 4.5). The $\delta^{15}N$ of *Bosmina longirostris* and *Daphnia sp.* was consistently between 0 to 2 ‰ higher than the POM_{GFF} collected at Station 41 at the same time (Fig. 4.5). *Bosmina longirostris* was first available in mid June at station 41 and had a $\delta^{15}N$ between 6.3 ‰ and 5.2 ‰ (Fig. 4.3, 4.4, 4.5).

The $\delta^{15}N$ of *Bosmina longirostris* rose steadily to 9.5 ‰ by September 26 at Station 41. At Station 81 in 1995, the $\delta^{15}N$ of *B. longirostris* and *Daphnia spp.* was approximately the same or slightly lower than all the fractions of POM, collected from June through October (Fig. 4.5). At Station 81, *B. longirostris* had a June $\delta^{15}N$ of approximately 5.2 ‰ but showed a more moderate seasonal increase to 7.2 ‰ by September (Fig. 4.5). *Daphnia* had an isotope composition similar to *B. longirostris* at both sites (Fig. 4.3, 4.4, 4.5). The *B. longirostris* samples, as well as the >210 μm and >295 μm samples

collected in 1994 at Station 81, were either slightly enriched in ^{15}N or similar to the POM_{64} samples collected (Fig. 4.3). From June through September, POM_{64} samples, are mainly comprised of rotifers. The POM_{64} samples collected August 1 and August 17, were each rinsed prior to analysis and contained varying levels of phytoplankton, which shows up as a larger range of standard deviation. The lower the percentage of phytoplankton in the sample, the lower the $\delta^{15}N$. The POM_{110} was often enriched relative to cladocerans and the $\delta^{15}N$ of cladocerans was usually lower than the zooplankton size fraction they would normally be found in. The seasonal shift in the $\delta^{15}N$ of *D. thomasi* was very pronounced at both sites in both years, dropping from 18.5 ‰ May 10, 1995 to 8.0 ‰ June 21 before climbing back up to 11.0 ‰ by the end of September (Fig 4.3, 4.4, 4.5). The $\delta^{15}N$ of *D. thomasi* is 3.0 to 5.0 ‰ enriched in ^{15}N relative to *B. longirostris* and *Daphnia spp.* from mid-June through to October.

Discussion

The results of this study suggest that different algal species coexisting in Lake Ontario are using different forms of inorganic or dissolved organic nitrogen resulting in a variation between the $\delta^{15}N$ species and size fractions at different times of the year. The extent to which these species are grazed upon and the relative trophic status of different zooplankton species then determines the isotope signature of the zooplankton and their

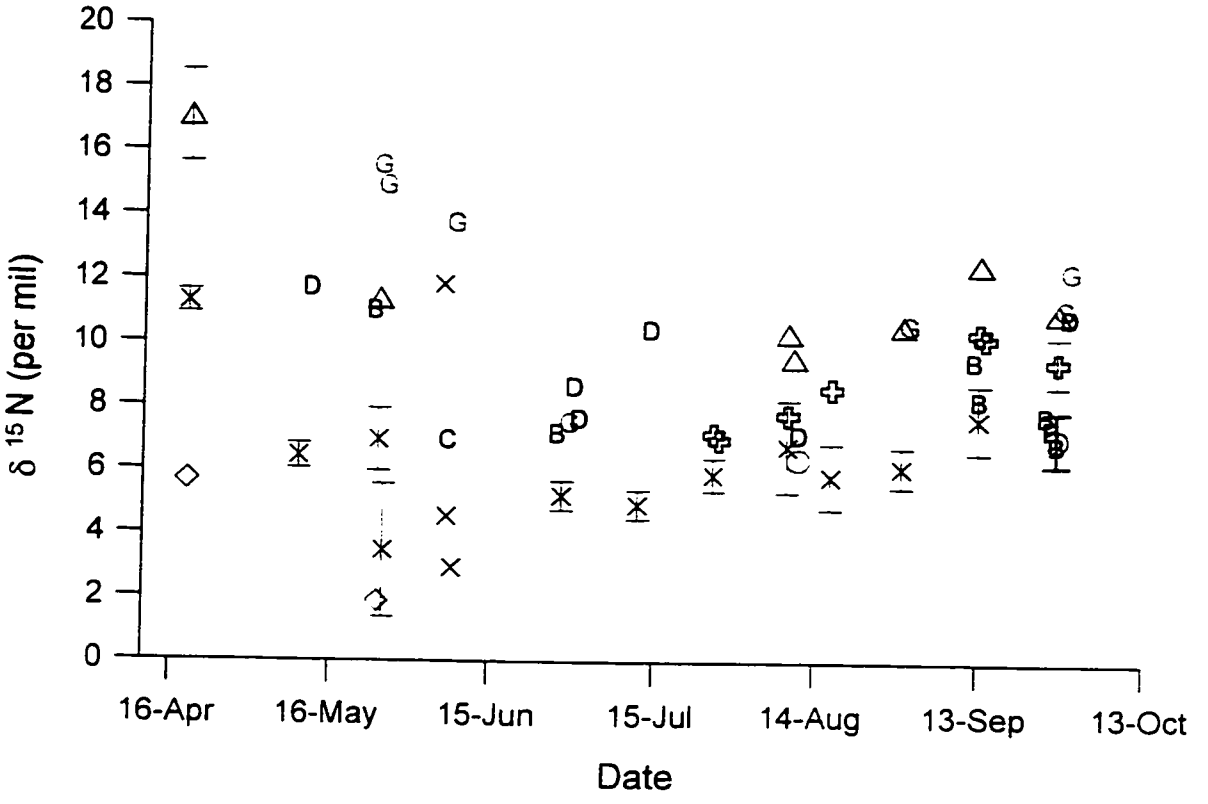


Figure 4.3. Seasonal trends in zooplankton $\delta^{15}N$ at Station 81, Lake Ontario, 1994. Size fractions and species are represented as **D** *Diacyclops thomasi*: Diaptomid grazers: **B** *Bosmina longirostris*: **C** *Daphnia sp.*; ◇ POM, 64 - 20 μm; ×, POM, 64 μm - 110 μm ; POM 110 μm - 210 μm; , Zooplankton, 210 μm to 295 μm; O Zooplankton > 295 μm .

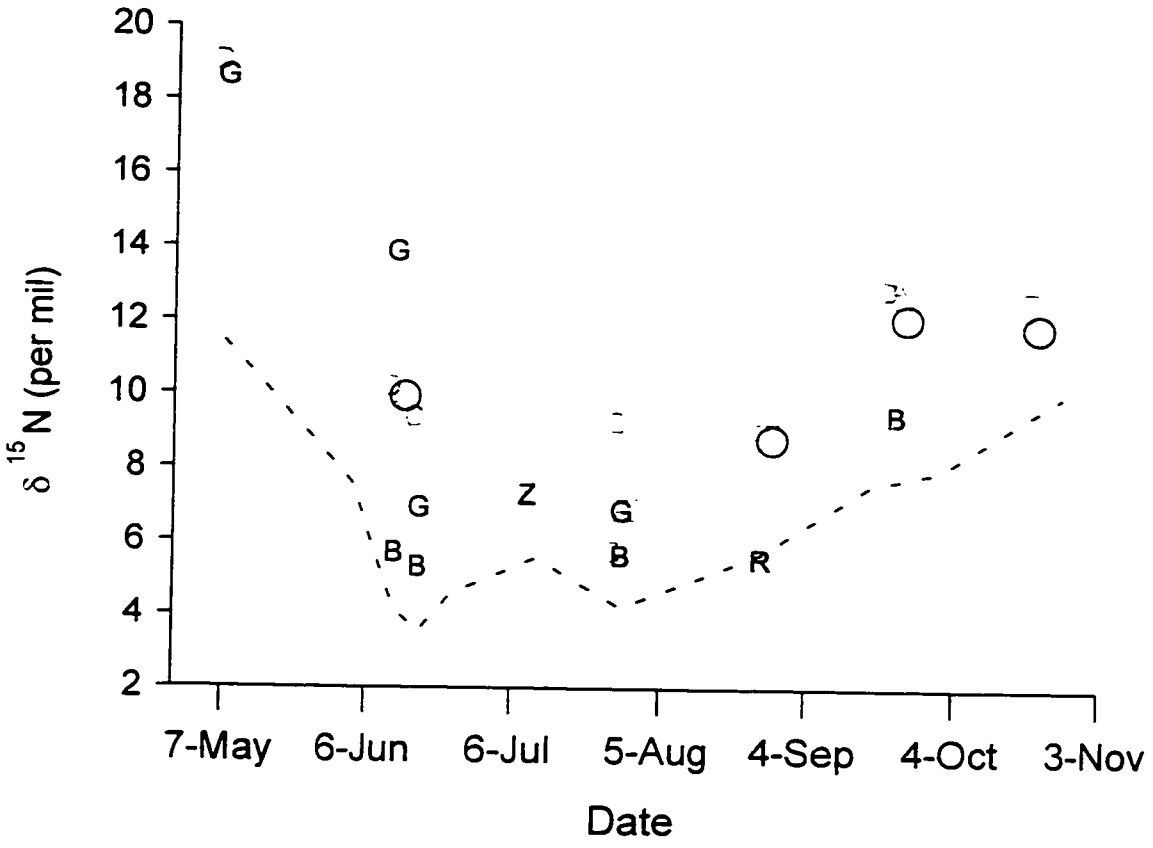


Figure 4.4. Seasonal trends in zooplankton $\delta^{15}N$ at Station 41, Lake Ontario, 1995, in relation to the signature of POM collected on GF/F filters (dashed line) The symbols plotted are: *Diacyclops thomasi*; **G,** Calanoid copepods; **B.** *Bosmina longirostris*; *Daphnia* ; **R,** Rotifers (*Keratella*); **Z,** *Bythotrephes sp.*; POM 110 μm - 210 μm; Zooplankton 210 μm - 295 μm; O, Zooplankton > 295 μm.

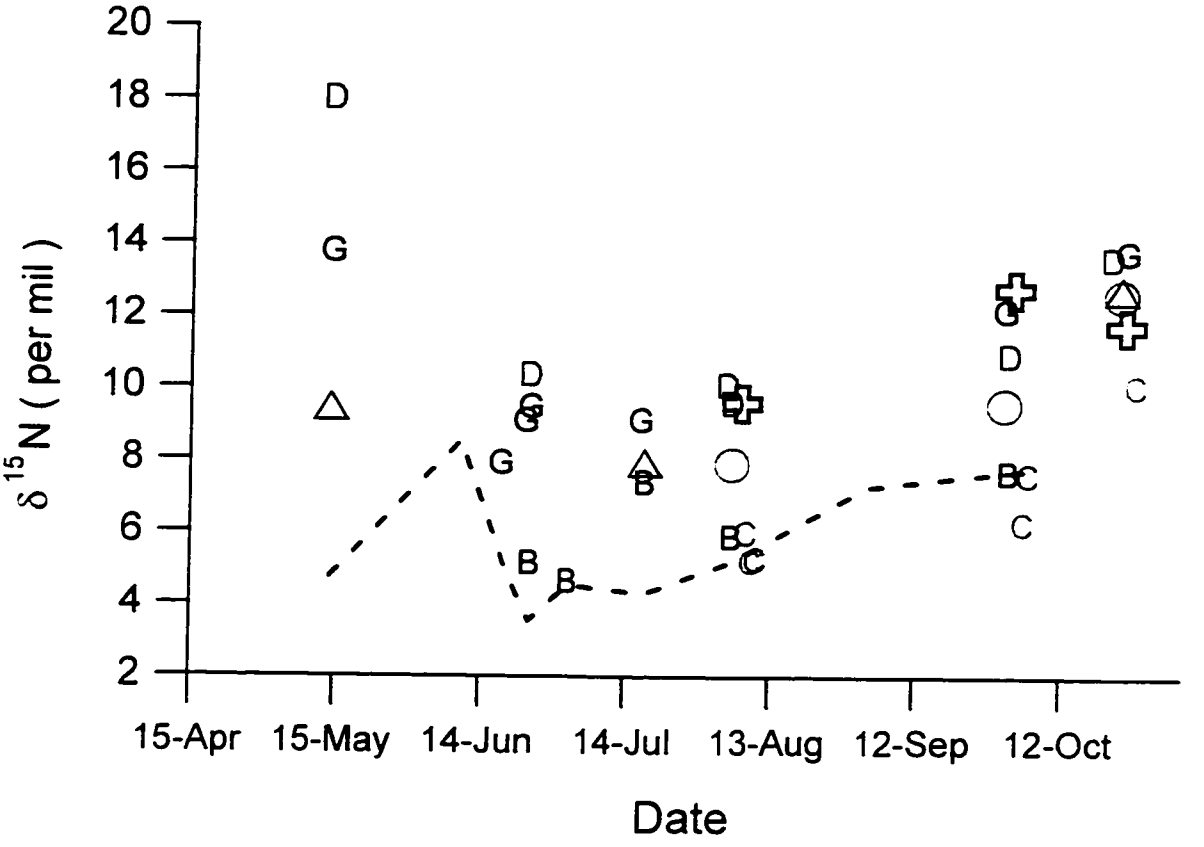


Figure 4.5 Seasonal trends in zooplankton $\delta^{15}N$ at Station 81, Lake Ontario, 1995, in relation to the signature of POM collected on GF/F filters (dashed line) *Diacyclops thomasi*: **G,** Calanoid copepods; **B**, *Bosmina longirostris*: *Daphnia spp.* : POM 110 μm - 210 μm: Zooplankton 210 μm - 295 μm; O, Zooplankton > 295 μm.

predators. Temporal change in the $\delta^{15}N$ of POM and zooplankton is then a function of the dynamics of DIN and seasonal changes in the community structure of the primary producers and mixotrophic algae which comprise the POM. The basis for these hypotheses lies in observations of the relationship between nitrogen uptake and the relative concentrations of NH_4^+ and NO_2^-/NO_3^- .

$\delta^{15}N$ of DIN and POM

The observed $\delta^{15}N$ of any fraction of POM collected will be determined by the species composition of the sample and the signature of inorganic or organic nitrogen those species use as a nitrogen source. The species composition of Lake Ontario phytoplankton has been monitored on an ongoing basis for a number of years (Johannsson *et al.* 1985). The 1994 profile of the succession and the relative abundance of different classes of phytoplankton found offshore in Lake Ontario is presented as a typical seasonal distribution (Fig. 4.6).

There will also be a succession in the dominance of phytoplankton classes and also in individual species within the classes (Fig. 4.6). This POM, collected on filters in May, is likely to be made up of: the Cryptophyte species, including *Rhodomonas minutae*, *Rhodomonas lens and Cryptomonas ovata*, the Dinoflagellates, including *Peridinium aciculiferum* and *Gymnodinium sp.* and the Chrysophytes including *Dinobryon sociale*, and *Chrysochromulina parva*. Most of the Bacillophyceae, hypothesized to use NO_2^-

NO_3^-, are likely to be excluded from the filters by pre-screening. This group becomes much less predominant later in the spring and summer (Fig. 4.6). The dominant cryptophytes immediately following stratification are: *Katablepharis ovalis* and *Rhodomonas minutae* both of which are <20 μm in diameter. Chlorophytes become much more predominant following stratification; by mid-July, *Oocystis parvum* and *Sphaerocystis schroeteri* collectively comprise more than 30 % of the < 20 μm biomass (Fig. 4.6). The dominant dinoflagellates while the lake is stratified are *Gymnodinium sp., Peridinium aciculiferum, and Ceratium hirundinella.* The $\delta^{15}N$ of each species will depend on whether the species uses NO_2^-/NO_3^-, NH_4^+ or dissolved organic nitrogen (DON) as a source of nitrogen for protein synthesis. Patterns of DIN or DON usage will be at least partially determined by the relative concentrations of the different forms of nitrogen available to primary producers and mixotrophs.

When NH_4^+ is available in sufficient concentrations, primary producers will tend to use it as a source of nitrogen preferentially (Pennock 1987). The seasonal levels of NH_4^+ measured at the two sites are typical of much of Lake Ontario, where the ambient concentration historically remains between 0 and 4 μM over much of the year with elevated levels generally recorded in the euphotic zone over the stratified period (Johannsson *et al.* 1985; Lean and Knowles 1987). At these concentrations, NH_4^+ is probably taken up actively, and there may be coincident use of NO_2^-/NO_3^- in species able to utilize NO_2^-/NO_3^- as a source of nitrogen. The active transport of NH_4^+ is stimulated when concentrations of NH_4^+ are below approximately 100 μM (Kleiner 1985).

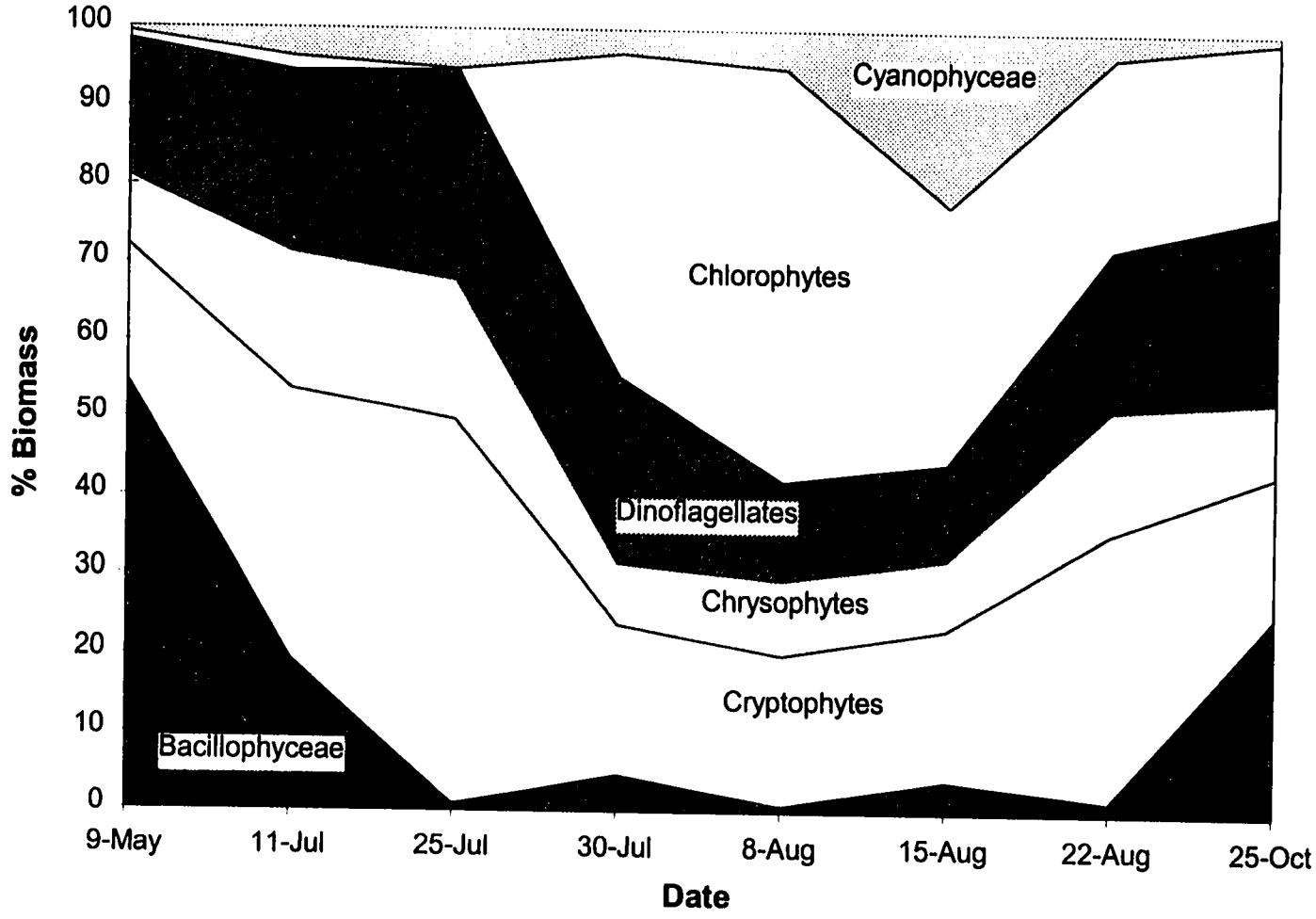


Figure 4.6. The seasonal succession in the relative abundance of different phytoplankton classes expressed as a percentage of the total algal biomass. Samples for species identification and counting were obtained from Station 41 in 1994. The algal classes from bottom to top are; Bacillophyceae , Cryptophyceae, Chrysophyceae, Dinophyceae, Chlorophyceae, Cyanophyceae.

Ammonium concentrations above approximately 2 μM will inhibit NO_3^- uptake by primary producers (McCarthy *et al.* 1977; Paasche and Kristiansen 1982). When available NH_4^+ is depleted to this level, primary producers will effectively switch to utilizing available NO_3^- or NO_2^-. However, coincident utilization of NO_3^- and NH_4^+ at NH_4^+ concentrations above 2 μM has been observed (Pennock 1987). It is uncertain whether preferential uptake of NO_2^- or NO_3^- exists under conditions where both forms of nitrogen are available (McCarthy *et al.* 1975; Pennock 1987; Horrigan *et al.* 1990).

In determining the source of nitrogen for the primary producers on the basis of $\delta^{15}N$, it is important to understand what isotope effects may be associated with the uptake of inorganic nitrogen. There is little available data for the fractionation of nitrogen isotopes in the uptake of NH_4^+. A positive fractionation between 7.0 ‰ and 4.0 ‰ was found for *Chaetoceros sp.* (Wada and Hattori 1978). Estep and Vigg (1985) attributed a 9.0 ‰ difference in the $\delta^{15}N$ of algae and NH_4^+ to positive fractionation in the uptake of NH_4^+ at micromolar concentrations. The fractionation of NO_2^-/NO_3^- has been measured, under field and laboratory conditions, for a number of species and found to vary from -0.9 ‰ to -12.1‰. The level of observed fractionation was dependent on the growth rate of the species and available concentration of NO_2^-/NO_3^- (Montoya, 1990). At the high ambient concentrations of NO_2^-/NO_3^- in Lake Ontario in the spring, fractionation of nitrogen isotopes by primary producers would be expected (Montoya, 1990). Therefore, organisms assimilating NO_2^-/NO_3^- as an inorganic source of nitrogen in Lake Ontario in the spring would be expected to have a $\delta^{15}N$ approximately 2.0 ‰ or less. When the lake

is mixed, the $\delta^{15}N$ of POM_{GFF} is as much as 10.0 ‰ enriched in ^{15}N over the pool of NO_2^-/NO_3^-, and 1.0 to 2.0 ‰ heavier than NH_4^+ (Fig. 4.1). Therefore, it is unlikely that NO_2^-/NO_3^- is the inorganic source of nitrogen for the majority of the material collected on this filter.

Algae, using NH_4^+, would be expected to have a $\delta^{15}N$ of 10 ‰ or more. It follows that NH_4^+ is most likely the source inorganic nitrogen used by primary producers, collected on GF/F filters, at the mid-lake site in the spring. This finding is consistent with the results of isotope addition experiments conducted during a previous assessment of nitrogen cycling in Lake Ontario (Lean and Knowles 1987). ^{15}N tracer studies conducted from April to June, 1982, indicated that smaller cells, < 12 μm, were responsible for most of the NH_4^+ uptake, while larger cells used mostly NO_3^- (Lean and Knowles 1987).

Alternatively, DOM may be the enriched source of nitrogen for the POM_{GFF} in the spring. Algal species coexisting with diatoms in the spring, may be mixotrophic. Mixotrophic algae using the DOM_{TFF} and POM_{TFF} fractions, collected in 1997, may exhibit some positive fractionation in assimilation and therefore might be isotopically enriched relative to coexisting algal species. However, the difference between the $\delta^{15}N$ of the DOM_{TFF} and POM_{TFF} fractions and the POM_{GFF} (1 to 20 μm) fraction is approximately 5 ‰. Nearly a two-fold trophic enrichment would be necessary in order for mixotrophic algae assimilating DOM to be responsible for the observed $\delta^{15}N$ of POM_{GFF}.

The observed range in the $\delta^{15}N$ of POM and seston, collected using different methods suggests multiple sources of nitrogen were being used by species of primary producers. The wide range in $\delta^{15}N$ between different fractions of POM, sampled in the spring, is due to variation in the amount of diatoms included in each sample. The greater the proportion of diatoms in the sample, the lower the $\delta^{15}N$. The algal community of Lake Ontario in the spring is largely comprised of colonial diatoms which are difficult to filter; though their size is less than 5 μm in diameter individually, the long chains formed by the colonies do not allow them to easily pass through a 44 μm mesh filter. Much of this algae was excluded from our sample by the 44 μm nets used in pre-filtering. An even larger fraction was excluded from the POM_{GFF} by pre-filtration through a 20 μm mesh net. POM_W (0.45 - 44 μm), or the POM_{GFF} from Station 81 in May which were not pre-filtered, consequently contained more diatoms than the POM_{GFF} sample, collected at Station 41. POM_{GFF} (1 - 20 μm), collected at the mid-lake sampling site, was enriched in ^{15}N, relative to POM_W (Westfalia centrifuge, 0.45 - 44 μm) or the GF/F filters from Station 81 in May (Fig.4.2). At the mid-lake sampling site, the POM_W had a $\delta^{15}N$ which was 4 ‰ lower than POM_{GFF} in May and 1 ‰ lower in mid-June than both the POM_{GFF} and the extracted NO_2^-/NO_3^-. The POM_{20} fraction from mid-June had an isotope signature similar to the POM_W and 0.5 ‰ lower than the $\delta^{15}N$ of NO_2^-/NO_3^- (Table 4.2B). The 64 to 20 μm fraction, collected at Station 41 in April 1997, was observed to contain approximately 70 % *Melosira. sp.* The relatively low $\delta^{15}N$ of this sample suggests the influence of diatoms on the $\delta^{15}N$ of the POM samples collected (Table 4.3). In

marine environments, diatoms were found to preferentially utilize nitrate over ammonium for growth (Wada and Hattori 1978). The data from this study suggests that the same is also true in Lake Ontario. This is probably the reason for the observed differences between the $\delta^{15}N$ of duplicate POM_{64} samples, collected in the spring of 1994, and the large variance in the $\delta^{15}N$ within those samples, as well as the difference between the $\delta^{15}N$ values obtained in this study and those obtained by Kiriluk *et al.* (1995). Net plankton samples (horizontal tows at 1 m depth with a 153 μm mesh net), collected from Lake Ontario in May of 1992 had a $\delta^{15}N$ of 1.7 ‰ (Kiriluk *et al.* 1995). The collected material would mainly consist of diatoms, resulting in the low value for $\delta^{15}N$. Differences in $\delta^{15}N$ may be attributed to the relative proportion of diatoms and/or any primary producers collected in larger size fractions using NO_2^-/NO_3^- as an inorganic nitrogen source. The low $\delta^{15}N$ of material flushed through the 64 μm mesh filters, and the observation that cleaner samples were isotopically enriched, supports this hypothesis. It also suggests that organisms feeding on phytoplankton in the spring are not feeding on diatoms and are selectively feeding on more ^{15}N enriched sources.

The relative stable isotope signatures of DOM, NH_4^+ and NO_2^-/NO_3^- implicate NH_4^+ as a predominant source of inorganic nitrogen for the isotopically enriched POM_{GFF}, collected prior to the onset of thermal stratification at the mid-lake sampling site. At the onset of stratification, there is a sharp decline in the $\delta^{15}N$ of POM (Fig. 4.1, 4.2). This suggests either a sharp decline in the $\delta^{15}N$ of NH_4^+ or a shift to the use of NO_2^-/NO_3^- by the algal species present. A spring bloom of phytoplankton is a seasonal event at both

sites monitored (Johannsson *et al.* 1985). The marked increase in the biomass of primary producers and subsequent increase in the rate of productivity may be sufficient for inorganic nitrogen demand to outstrip the available supply of NH_4^+, causing organisms to switch to NO_3^- as a source. Alternatively, seasonal succession may replace species using NH_4^+ with species adapted to using NO_2^-/NO_3^- as an inorganic nitrogen source.

Following stratification, the $\delta^{15}N$ of POM_{GFF} increases steadily until fall mixing when it approaches the levels observed in the spring. This suggests that either algal species are using a source of nitrogen which is also becoming enriched, or there are species specific differences in the use of distinct sources of nitrogen, favoring more enriched sources later in the year. In the latter case, the shift in $\delta^{15}N$ would be indicative of which source is predominantly used. The $\delta^{15}N$ of the NO_2^-/NO_3^- pool remains fairly stable throughout the year. Mean values increase slightly at Station 41 from the onset of stratification when concentrations of NO_2^-/NO_3^- are highest, to mid-summer when concentrations of NO_2^-/NO_3^- reach their lowest levels (Fig. 4.2). However, the observed change in the $\delta^{15}N$ of the NO_2^-/NO_3^- is too small relative to the variance in duplicate samples to suggest a trend.

There are several processes simultaneously acting on the epilimnetic pool of DIN which have the potential to alter the $\delta^{15}N$ of NO_2^-/NO_3^- and NH_4^+. Uptake and discrimination against the heavier isotopes of nitrogen by primary producers, or denitrifying bacteria using the available NO_2^-/NO_3^- will push the $\delta^{15}N$ of the remaining NO_2^-/NO_3^- upward.

Denitrification would leave the substrate pool of NO_2^-/NO_3^- enriched in ^{15}N (Mariotti *et al.* 1981). However, Lake Ontario acts as a sink for atmospheric nitrous oxide and has high dissolved oxygen concentrations which suggests that denitrification in Lake Ontario is not likely to be a significant process for NO_3^- loss in the epilimnion (Lean and Knowles 1987).

The $\delta^{15}N$ of the DOM_{TFF} and POM_{TFF} fractions collected in 1997 suggest that the $\delta^{15}N$ of dissolved organic sources of nitrogen remains relatively stable throughout the year. The 0.2 μm to 1 μm fraction contains mostly bacteria and closely reflects the 0.2 μm to 1000 dalton containing organic sources of dissolved nitrogen. The seasonal increase in the $\delta^{15}N$ of POM_{GFF} may reflect an increase in the proportion of mixotrophic algae collected on the filters. The increase in the $\delta^{15}N$ of POM_{GFF} might also be attributed to an increased dependence on NH_4^+ as a nitrogen source by primary producers, as the season progresses. This assumes that the $\delta^{15}N$ of NH_4^+ remains consistently high over the summer months which may not be true. The $\delta^{15}N$ of NH_4^+ has been found to fluctuate temporally in different systems (Cifuentes *et al.* 1989).

The processes influencing the $\delta^{15}N$ of NH_4^+ should be considered in determining whether uptake of NH_4^+ could be responsible for the $\delta^{15}N$ of POM observed. In the euphotic zone, the dominant organic source of regenerated NH_4^+ is zooplankton (Lean and Knowles 1987). The $\delta^{15}N$ of zooplankton was 2 to 8 ‰ heavier than POM due primarily to the trophic enrichment factor described previously. Remineralized NH_4^+ will have a

lower ^{15}N content than the source pool of organic nitrogen (Checkley and Enzeroth 1985). Fractionation, estimated by mass balance between remineralized NH_4^+ from marine copepods and the nitrogen assimilated, was -11.2 ‰ (Checkley and Entzeroth 1985). Nitrification, occurring in the euphotic zone, will tend to produce NO_3^- depleted in ^{15}N while increasing the ^{15}N content of the residual NH_4^+ substrate (Mariotti *et al.* 1981). This may serve to keep down the enrichment in NO_2^-/NO_3^- due to assimilation and enrich the pool of remineralized NH_4^+ from zooplankton in ^{15}N over the course of the summer. The shift in the $\delta^{15}N$ of the POM might then be related to the extent to which the POM collected is made up of primary producers which have assimilated NH_4^+ in the epilimnion.

The POM_{20} fraction, collected in August 1995, was 3 ‰ heavier than that collected on POM_{GFF} (Table 4.2, Fig. 4.2). This suggests that the smaller cells preferred an isotopically lighter source which is the opposite of what was observed in the spring. This may indicate a larger proportion of mixotrophic heterotrophs in the larger fraction later in the year. The 20 to 64 μm fraction of POM, collected in August and September 1997, similarly suggests dissolved organic nitrogen as a source. However, without a measurement of the isotope signature of NH_4^+ during the summer, it is impossible to assess which sources or processes determine the $\delta^{15}N$ of POM while the lake is stratified. It is probable that all of the above sources and processes are influential to some extent.

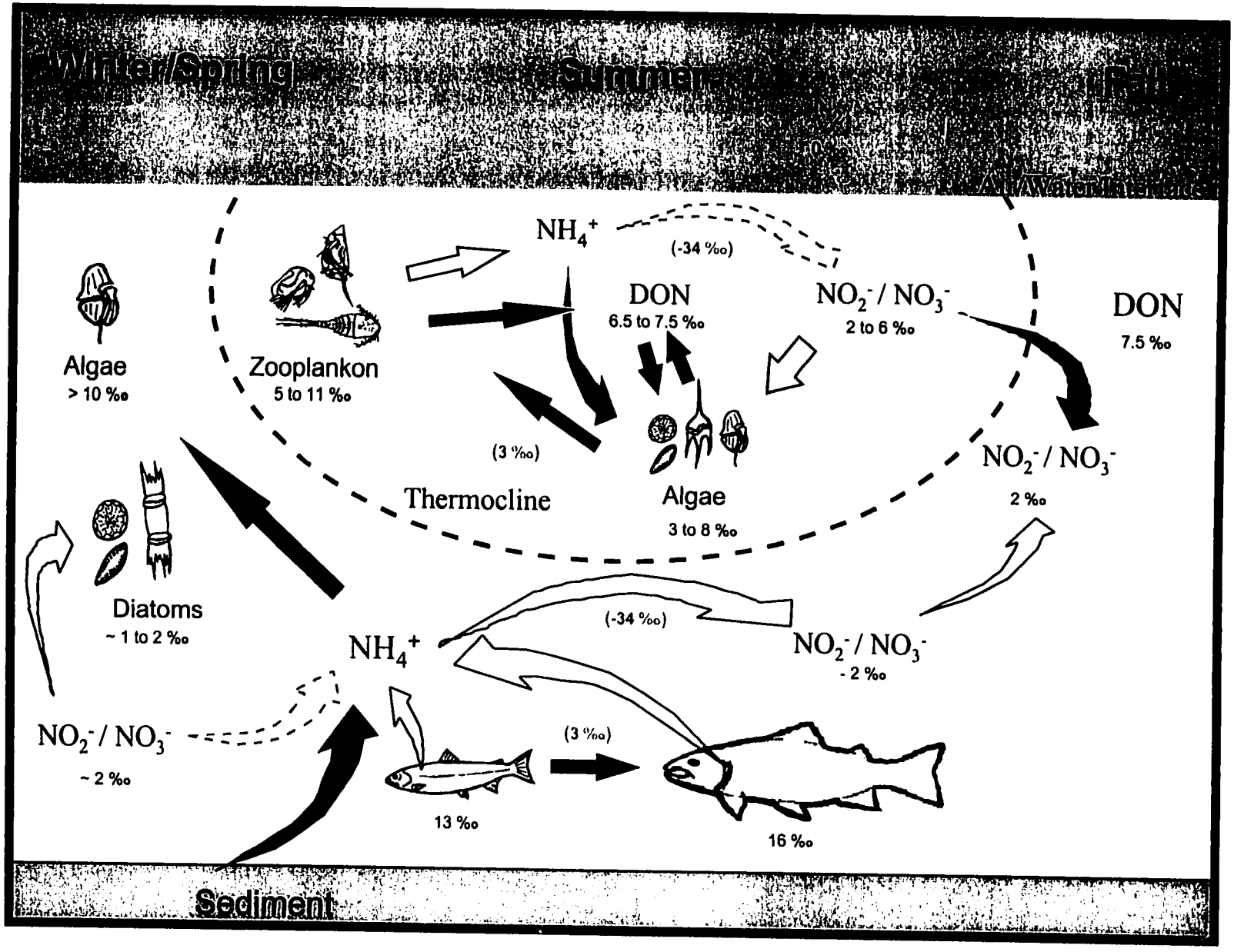
The $\delta^{15}N$ of NO_2^-/NO_3^- from the samples, obtained from the hypolimnion in 1997, suggests that NO_2^-/NO_3^- is the product of nitrification. In previous studies, concentrations of NO_2^-/NO_3^- have been observed to increase from the onset of stratification near the sediments in Lake Ontario (Lean and Knowles 1987). The increase in NO_2^-/NO_3^- has been suggested as evidence that nitrification is responsible for stabilizing NH_4^+ concentrations below the euphotic zone (Lean and Knowles 1987). While a similar increase in the concentration of hypolimnetic NO_2^- / NO_3^- was not observed, the $\delta^{15}N$ of NO_2^-/NO_3^- appeared to undergo a 4 ‰ shift to lower values (Table 4.1). Laboratory experiments with *Nitrosomonas europaea* at 30 °C determined that there is an isotope enrichment factor of -35 ‰ associated with nitrification (Mariotti *et al.* 1981). A reduction in $\delta^{15}N$ of nitrate is probably a function of fractionation during nitrification.

The $\delta^{15}N$ of the remineralized nitrogen from the hypolimnion is likely to be enriched relative to epilimnetic NH_4^+ excreted by zooplankton. In addition to excretion by pelagic biomass, sources of NH_4^+ in deeper waters of the lake include decomposition and release of NH_4^+ from sediment pore waters (Lean and Knowles 1987). Hypolimnetic nitrification would result in the ^{15}N enrichment of an already isotopically enriched NH_4^+ pool. Fall mixing of the water column makes available new sources of NH_4^+ and NO_2^- /NO_3^- to primary producers. The NH_4^+ may be substantially enriched. At the same time, depleted NO_2^-/NO_3^- from the hypolimnion mixed with NO_2^-/NO_3^- in the epilimnion is likely to result in a new equilibrium $\delta^{15}N$ which is lower than that observed in the fall.

To summarize the hypotheses, a model of inorganic/organic nitrogen cycling with respect to the $\delta^{15}N$ of DIN and POM in Lake Ontario is presented (Fig. 4.7). The $\delta^{15}N$ data suggests recycling of nitrogen as an important component of the system. The observed seasonal variability in the measured $\delta^{15}N$ of POM is probably due to the use of remineralized NH_4^+ and a subsequent shift to the use of NO_2^-/NO_3^- as the predominant source of inorganic nitrogen for primary producers after the lake has stratified. It is likely that available ammonium and nitrate are both utilized as a source of nitrogen for primary producers, but over the winter the low ambient levels of NH_4^+ are sufficient to meet the reduced demand for DIN. In the spring, elevated levels of primary productivity may necessitate a change to the use of NO_2^- / NO_3^- as a source of inorganic nitrogen for primary producers. The $\delta^{15}N$ of POM reflects this dependence as the isotope signature of the primary producers drops to a level reflecting the use of NO_2^- / NO_3^-. The observed $\delta^{15}N$ of POM increases and continues to rise into the fall.

As the season progresses algae may be using NH_4^+, NO_2^- / NO_3^-, or DON as a source of nitrogen. The NH_4^+ available in the euphotic zone, would be regenerated by zooplankton and subsequently would not be as enriched in ^{15}N as the NH_4^+ measured in the spring. There may be some enrichment of available NO_2^- / NO_3^- due to fractionation by algal species in uptake, which is reflected in the POM; there may also be fractionation

Figure 4.7. An illustration of the processes influencing the $\delta^{15}N$ of DIN, DON, and POM within Lake Ontario on a seasonal basis. The flux of DIN and DON depleted in ^{15}N relative to the receiving pool is depicted with light arrows. The flux of DIN and DON enriched in ^{15}N relative to the receiving pool is depicted with dark arrows. The dashed line separating processes in the epilimnion from the hypolimnion represents summer stratification. The $\delta^{15}N$ values for the various sinks of nitrogen depicted are displayed beneath the pool described. Literature values for the level of isotope fractionation associated with nitrogen transformation processes are given as ($\delta^{15}N$) values beneath the arrows. The arrows formed with broken lines indicate processes believed not to be influential in determining the $\delta^{15}N$ of DIN in Lake Ontario.


Winter/Spring
Summer
Fall
Water Interface
NH_4^+
(-34 ‰)
DON
6.5 to 7.5 ‰
NO_2^- / NO_3^-
2 to 6 ‰
DON
7.5 ‰
Algae
> 10 ‰
Zooplankton
5 to 11 ‰
(3 ‰)
Thermocline
Algae
3 to 8 ‰
NO_2^- / NO_3^-
2 ‰
Diatoms
~ 1 to 2 ‰
(-34 ‰)
NH_4^+
NO_2^- / NO_3^-
- 2 ‰
NO_2^- / NO_3^-
~ 2 ‰
(3 ‰)
13 ‰
16 ‰
Sediment

associated with epilimnetic nitrification. In the hypolimnion, remineralized NH_4^+ is oxidized creating an isotopically enriched pool of NH_4^+ and a depleted pool of NO_2^- / NO_3^-. When mixing occurs in the fall, this DIN from the hypolimnion mixes with DIN from the epilimnion and re-establishes the concentrations and isotope signatures observed in the spring.

$\delta^{15}N$ of Zooplankton

Almost all of the observed seasonal variability in the $\delta^{15}N$ of the zooplankton species analyzed could be attributed to the seasonal fluctuation in the nitrogen isotope signatures of POM. The variability between species and among different size classes suggests heterogeneity in the $\delta^{15}N$ of source nitrogen at the base of the food web. Trophic enrichment of $\delta^{15}N$ in cladocerans, relative to their food, has been observed in laboratory studies (Graham, pers. com.). Therefore, the lack of observed enrichment in the ^{15}N of cladocerans, relative to POM, suggests they are feeding selectively on a component of the POM which has a lower $\delta^{15}N$ than the rest of the POM in the sample collected (Fig. 4.5).

A proportion of the mass of the >110 μm fraction, obtained during the summer in 1995 and in 1997, would be made up of copepodids. The copepodids may be feeding at a higher trophic level or are feeding on an enriched source of POM, relative to the cladocerans. Ciliates and mixotrophic dinoflagellates may also be a component of this

fraction. The dissolved organic sources of nitrogen used by mixotrophic algae are enriched, relative to *Daphnia spp.* There may be some further trophic enrichment of this fraction by ciliates, feeding on mixotrophic algae or bacteria. The combination of trophic enrichment and mixotrophy is probably responsible for the ^{15}N enrichment observed.

Calanoid copepods are consistently 2 ‰ or more enriched, relative to POM, and often have a $\delta^{15}N$ substantially higher than cladocerans collected at the same time (Fig. 4.3, 4.4, 4.5). The calanoid copepods, collected June 16, 1995, at Station 41, were 7.5 ‰ more enriched than *Bosmina longirostris* (Fig. 4.4). Copepods, collected June 16 1995, at Station 81, were approximately 6 ‰ more enriched than *B. longirostris* (Fig. 4.5). The observed differences may be attributed to a variation in food source between calanoid copepods and *Bosmina longirostris* or to a difference in trophic status. Calanoid copepods may be feeding on mixotrophic algae, which are likely to be more ^{15}N enriched for reasons previously discussed. There may also be a degree of omnivory in the diet of calanoid copepods, feeding partially on ciliates. If this is the case, the calanoids would be feeding at a slightly higher trophic level than coexisting cladocerans.

The $\delta^{15}N$ of spring samples of *Diacyclops thomasi* and calanoid copepods from Lake Ontario were as high as 18 ‰ to 19 ‰ at both sites and in both years of the study (Fig. 4.3, 4.4, 4.5). In order for the copepods to become so enriched in ^{15}N, they must be selectively feeding on material within the system which has a $\delta^{15}N$ approximately 15 to

16 ‰. There are a few possibilities. Primary producers may be using a highly ^{15}N enriched source of nitrogen. It is reasonable to assume that a portion of the biomass collected on the GF/F filter in the spring, is comprised of diatoms, with a $\delta^{15}N$ of approximately 2 ‰. It follows that the rest of the collected material has a $\delta^{15}N$ greater than the measured 11.5 ‰ (Fig. 4.1). If the copepods are feeding on primary producers using ammonium as a source of nitrogen, the primary producers must become nitrogen enriched in assimilation and/or the isotope signature of available ammonium must fluctuate within the system, attaining a $\delta^{15}N$ 4 to 5 ‰ heavier than that measured.

Alternatively, the elevated signatures may be due to a combination of trophic enrichment and the recycling of nitrogen through mixotrophic algae or the microbial loop. The dinoflagellate, *Gymnodinium helveticum*, represents approximately 5 % of the total spring biomass (Johannsson unpublished). It is also known to be mixotrophic, using DON as a nitrogen source. If *G. helveticum* became enriched in ^{15}N relative to DON during assimilation of organic nitrogen, it may have a $\delta^{15}N$ of approximately 10 ‰. If this mixotroph were ingested by ciliates, the ciliates may have a signature of approximately 13 ‰. Ingestion of the ciliates by copepods would result in a $\delta^{15}N$ of approximately 16 ‰ in the copepods. Cannibalism by copepods on earlier life stages may lead to further enrichment. A larger fractionation in the uptake of dissolved organic nitrogen (DON) may also be sufficient to produce the $\delta^{15}N$ signatures observed. There is little evidence to support a stepwise enrichment of copepods. The similarity between the DOM_{TFF} and POM_{TFF} fractions, when both were collected, suggest that enrichment does not occur

during assimilation of organic nitrogen by bacteria (Table 4.3). The samples analyzed do not suggest the existence of a stepwise enrichment pathway. However, the $\delta^{15}N$ of the organisms acting as intermediate steps may be obscured by the presence of diatoms. The 64 to 20 μm fraction, collected in April of 1997, had a $\delta^{15}N$ of 6.35 ‰ (Table 4.3). If this sample was comprised of 70 % diatoms with a $\delta^{15}N$ of approximately 2 ‰, the remaining 30 % would have a $\delta^{15}N$ of approximately 16.5 ‰. Therefore, the hypothesis that copepods may be feeding 3 to 4 trophic levels above primary producers in the spring cannot be ruled out.

The $\delta^{15}N$ of *Diacyclops thomasi* from mid-June through to October, is similarly indicative of *D. thomasi* planktivory or feeding on algae using an ^{15}N enriched food source (Fig. 4.3, 4.4, 4.5). Evidence from feeding experiments of *D. thomasi* in Lake Ontario suggest that the observed signature is a function of both planktivory on ciliates, rotifer eggs and feeding on mixotrophic algae (LeBlanc *et al.* 1997).

Implications for Other Aquatic Systems

The results of our study suggest there may be species specific differences in the $\delta^{15}N$ of algae and differences among zooplankton related to food preference. Depending on the system, naturally occurring stable isotopes of nitrogen may be an effective means of determining food sources at the base of the food chain. In order to maximize the efficacy of this approach, more work needs to be done investigating the fractionation of nitrogen

isotopes in the assimilation of nitrogen by primary producers, bacteria, and mixotrophic algal species.

The observed fluctuation in the $\delta^{15}N$ of POM, seston and zooplankton was consistent in magnitude and timing between sites and between years. This suggests that the observed seasonal pattern is a feature of the system and not a local response to a random event. The $\delta^{15}N$ for *D. thomasi* drops 10 ‰ between May and August and during this period, the trophic status of this copepod likely does not change. This may be presented as a cautionary note. If the fractionation of nitrogen isotopes associated with trophic transfer is used as a means of mapping the food webs in aquatic systems, it is crucial to understand that the results obtained for any organism are system specific and may fluctuate spatially or temporally. Observed fluctuations may occur, not only on the basis of a change in feeding, but also as the result of changes in the nutrient dynamics of the system. At the base of aquatic food webs, the time required to turn-over the tissues of most organisms is quite short (hours to days), and the temporal change in $\delta^{15}N$ and $\delta^{13}C$ may be very large. Researchers run the risk of potential misinterpretation; if different species within a system are collected for stable isotope analysis at one point in time and results are interpreted on the basis of trophic dynamics, without due consideration of other influential processes, or differences in tissue turnover of the organisms examined are ignored. Baseline stable isotope signatures are not constant. The stable isotope

signatures of organisms at the base of the food web are influenced by more than just dietary interactions and interpretation should be done carefully with due consideration of all potential mitigating factors.

Chapter 5

A quantitative determination of the diet of *Mysis relicta* in Lake Ontario

Introduction

The role of *Mysis relicta* in the food webs of freshwater lakes is difficult to characterize. Mysids are omnivorous crustaceans which migrate between the sediment surface and the metalimnion of lakes on a diurnal basis within specific boundaries of light intensity and temperature (Beeton and Bowers 1982; Grossnickle 1982; Rudstam *et al.* 1989; Rudstam 1998). Given their behavioural patterns, mysids may play a key role in the recycling of energy back into the pelagic food web which would otherwise be lost in sedimentation. *M. relicta* will both prey on and compete with zooplankton for food (Johannsson *et al.* 1994). They also compete with and provide a food source for forage fish (Mills *et al.* 1992; Urban and Brandt 1993; Johannsson *et al.* 1994). The presence of *M. relicta* in lakes has been implicated as a contributing factor to increased contaminant loading in top predators due to extension of the food chain (Rasmussen *et al.* 1990).

In order to better understand the role of mysids in aquatic systems, it is important to obtain accurate dietary information. Traditionally, gut content analysis has been relied upon to provide this information. However, gut content analysis of organisms as small as mysids has a number of draw-backs. Besides being arduous, an examination of the gut contents of any given organism only provides a 'snapshot' of what the species is actually feeding on over a longer period of time. To obtain accurate dietary estimates, the analysis must be repeated with many individuals obtained over a broad time scale. In organisms as small as mysids, results are likely to be biased toward material found in the guts which is readily identifiable. It is difficult if not impossible to determine

the source of much of the material found in the guts of small organisms without the use of some form of tracer. The relative ratios of stable isotopes of carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N) in biota have been used in numerous studies as indicators of food source and trophic status and may be used to augment gut content analysis (Fry 1991).

Stable isotope ratios are often expressed as δ values, in units per mil (‰), and their efficacy as tracers is based on an understanding of how their relative ratio is altered in different biological processes (Peterson and Fry 1987). Carbon may be used as an indicator of source, since the complete metabolism of carbon substrates is most often observed. As long as the carbon source utilized is broken down to CO_2 completely in catabolic processes, isotope changes will be minimal (Raven 1990). This will be true throughout the food web, so the $\delta^{13}C$ ratio observed in phytoplankton will be nearly conserved right up to the top predators in a pelagic system, with little change when the whole organism is analyzed. In studies using carbon isotopes, the fractionation of carbon typically results in an enrichment of ^{13}C by a factor of 1 ‰ or less through each trophic transfer (Peterson and Fry 1987). In contrast, nitrogen isotopes are ^{15}N enriched in trophic transfer (DeNiro and Epstein 1981; Peterson and Fry, 1987). In digestion and protein metabolism, cleavage of ^{14}N peptide bonds occurs preferentially (Macko *et al.* 1986; Bada *et al.* 1989). This results in feces which are enriched in ^{15}N relative to the food eaten and the excretion of isotopically light (^{15}N depleted) waste ammonia or urea leaves organisms enriched in ^{15}N relative to their food source (Checkley and Entzeroth 1985; Macko *et al.* 1986; Bada *et al.* 1989; Appendix 3). Therefore, nitrogen isotope ratios may be used as an indicator of trophic status as well as tracers of food source (Cabana and Rasmussen 1994). In order to use stable isotope ratios as a means of

apportionment between the potential food sources, there must be sufficient variation between competing sources to discern between them. Seasonal fluctuations in primary productivity and the availability and source of inorganic carbon in lakes can produce a broad range of carbon signatures for primary producers, over the course of a year (Chapter 2). Similarly, the level of fractionation associated with transformation processes of inorganic nitrogen can be very large (Marriotti *et al.* 1981). Under conditions where inorganic nitrogen is abundant, there is potential for variation in nitrogen signatures at the base of the food web. Seasonal surveys of carbon and nitrogen stable isotopes of primary producers in Lake Ontario have demonstrated that there is a definite seasonal pattern in $\delta^{13}C$ and $\delta^{15}N$ determined by the biogeochemistry of the system (Chapters 3 and 4). These seasonal changes in carbon and nitrogen isotope signatures result in the variation in signatures necessary to discern the level of dependence an organism has on different food sources.

In this study, seasonal fluctuation in $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web and variation in $\delta^{15}N$ due to trophic enrichment, were exploited in determining the diet of mysids in Lake Ontario. In 1995, samples for stable isotope analysis were collected in conjunction with samples collected for analysis of the gut contents of *M. relicta*. The gut content analysis was used as a means of apportioning of the diet of *M. relicta* among potential sources. Stable isotope data was then used to augment the estimates drawn from analysis of gut contents and develop a quantitative assessment of diet, something which was not feasible with either method alone.

Methods

In order to accurately assess the diet of an organism using stable isotopes, sampling should be conducted on a time scale consistent with the tissue turnover time of the organisms under investigation. The sampling in this study was conducted in conjunction with the Bioindex biomonitoring program of the Department of Fisheries and Oceans. In 1995, collections of mysids were made in collaboration with Gideon Gal (Cornell University, Ithica N.Y.). Samples of particulate organic matter and zooplankton, representative of the base of Lake Ontario food web, were collected every two weeks between mid-April and late October of 1994 and 1995, at a mid-lake station on Lake Ontario. Mysids were collected monthly in both years studied. Benthic grab samples were collected in the spring, summer and fall of 1994. In addition to these samples, mysids and zooplankton were collected in October of 1995 from a site near Oswego, New York.

Vertical tows, using 64 μm mesh Nitex™ nets, were used to collect zooplankton from 20 m to the surface or from 1 m above the start of the thermocline to the surface when the lake was stratified. Closing nets were used in 1995 to sample zooplankton from the metalimnion, approximately 20 to 50 m, determined from temperature profiles obtained with an electronic bathythermograph. Hypolimnetic samples were also obtained once the lake had stratified. The $\delta^{13}C$ and $\delta^{15}N$ of zooplankton from these collections was compared with the values of epilimnetic samples. Samples were screened to separate zooplankton into size categories: >64 μm, >110 μm, >210 μm and >250 μm, and were preserved in 99.9% ethanol. The larger size fractions (>210 μm and >250 μm) were sub-sampled, and composite samples of individual species were obtained through separation under

a microscope. These samples were then rinsed with distilled deionized water and dried prior to analysis. When duplicate samples were available, samples were either treated with the addition of 2N HCl to remove residual carbonate and redried prior to analysis, or left untreated to determine the effect of acid addition. Acid addition had no observable effect on either the $\delta^{13}C$ or $\delta^{15}N$ of zooplankton samples, so all samples were included in our analysis ($t_{0.05,23} = 0.896$, $p = 0.38$) (Appendix 2).

Several hundred individual mysids were analyzed for carbon and nitrogen stable isotopes. Mysids were collected in 1994 using an epi-benthic sledge and also in vertical net hauls from the bottom of the lake to the surface, using a 253 μm mesh Nitex™ net. In 1994, all mysids, collected on a particular date, were either stored frozen or preserved in ethanol. Prior to analysis, subsamples of the mysids collected in 1994 were either lipid extracted, treated with 2N HCl addition, were both lipid extracted and treated with acid, or left untreated. In 1995, a comparative analysis of the mysid collections was done. Separate samples of *Mysis relicta* were collected: in the early evening during their ascension up the water column, just before dawn when mysids were descending to the sediment surface, and from the sediment surface using a epi-benthic sledge. Mysids were also classed according to size as either small (<6 mm), medium (7 to 10 mm), or large (>12 mm) for comparative analysis. Mysids were dried at 60 °C prior to isotope analysis. Subsamples of the mysids collected were pooled into composite samples, homogenized with a ball-mill grinder prior to analysis. Most of the results presented are from whole mysids, analyzed as individual samples. Lipid is comparatively depleted in ^{13}C relative to other tissues (Tieszen *et al.* 1983). It was considered that lipid loading over the course of a season had the potential to bias our analysis,

therefore steps were taken to remove the influence of lipid on $\delta^{13}C$ by applying a correction. A regression equation was developed to correct the mysid $\delta^{13}C$ signatures for the influence of lipid. It is the lipid corrected mysid $\delta^{13}C$ signatures which are reported here. Details of the observed relationship between the C/N ratio of *M. relicta* and their $\delta^{13}C$ signature are provided in Appendix 1.

Diporeia hoyi were collected along with mysids using the epi-benthic sledge. Samples of *D. hoyi* were lipid extracted and acid rinsed when sufficient sample was available. Individual *Diporeia hoyi* were analyzed to determine population variability. Composite samples of 40 or more *D. hoyi*, homogenized with a ball-mill grinder, were also analyzed. An Ekman grab sampler was also used to obtain samples of benthic invertebrates. Oligochaetes and *D. hoyi* were removed from sediment samples, rinsed with distilled de-ionized water, and frozen. Frozen samples were thawed and dried at 60°C prior to isotope analysis.

Samples were analyzed on either of two instruments. A Micromass Continuous-flow isotope-ratio mass spectrometer, fitted with a Carlo-Erba elemental analyzer as part of the inlet system (EA/CF-IRMS), was used to determine the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton, *D. hoyi*, and composite samples of *M. relicta*. Where sufficient mass was available, samples were analyzed using a VG-Optima EA-IRMS. Analysis of standards of known isotope composition and duplicate analysis of samples was used to assess the precision and accuracy of analysis between systems and within runs. The difference in $\delta^{13}C$ and $\delta^{15}N$ between duplicates and between standards and a known reference value was consistently 0.3 ‰ or less.

Gut Content Analysis

In order to estimate the contribution of different organisms to the diet of *Mysis relicta*, exhaustive gut content analysis of mysids was conducted at Cornell University (Ithica, N.Y.) under the direction of Ora Johannsson (Fisheries and Oceans, Burlington). Sets of 20 individual mysids, each from ascending, descending and benthic collections in the spring, summer and fall were analyzed. Body parts of zooplankton in the gut contents were identified and from this analysis, the total number of individuals recently ingested was estimated. The remains of rotifers were counted. The total number of individuals of each species of zooplankton, found in the guts of the entire set, was divided by the number of mysids analyzed to get the average number of organisms ingested by each mysid. This value was multiplied by the average mass of the organism to obtain the mass ingested per individual mysid. The mass of all zooplankton was totaled, and the percentage contribution of each species to the total contribution of zooplankton to the diet was determined. Diatoms were identified to species and were classified as either: absent, a few present, many present, or abundant.

Dietary mass balance

The diet of *Mysis relicta* can be assessed by isotope mass balance of either carbon or nitrogen using the following equation, presented for carbon isotopes:

$$\delta^{13}C_M = \delta^{13}C_h \times b_h/b_t + \delta^{13}C_i \times b_i/b_t + \delta^{13}C_j \times b_j/b_t \ldots. \qquad (5.1)$$

where $\delta^{13}C_M$ is the isotope signature of *M. relicta*; $\delta^{13}C_h$ is the isotope signature of *h*, and b_h/b_t is the proportion *h* represents of the total biomass assimilated by *M. relicta*. In using this equation with nitrogen isotopes, adjustments have to be made to account for fractionation, associated with trophic transfer. Ecological studies in which the trophic enrichment of nitrogen isotopes has been examined have been reviewed, and the average level of trophic enrichment was found to be approximately 2.5 ‰ (Owens 1987). Laboratory studies of trophic enrichment of ^{15}N were conducted on *Neomysis intermedia* Czerniawsky, using frozen cladocerans as a food source (Toda and Wada 1990). The $\delta^{15}N$ enrichment of *Neomysis intermedia* in these studies was 3.2 ‰ (Toda and Wada 1990). An intermediate value of 3 ‰ was adopted in this study as an enrichment factor to adjust food source $\delta^{15}N$. A trophic enrichment of approximately 1 ‰ in $\delta^{13}C$ has been reported in numerous studies and used as a foundation for the determination of trophic relationships (DeNiro and Epstein 1979; McConnaughey and McRoy 1979). The observed range in $\delta^{13}C$ of individual mysids and each of their potential food sources including phytoplankton was often greater than 1 ‰ even with the influence of lipid content removed. It was considered that this was a natural level of variability in the population. In relation to the natural variance any trophic enrichment of $\delta^{13}C$ was considered negligible and therefore no adjustment was made.

Results

Gut content analysis

The pooled results of gut content analysis of small mysids from summer and of all collections of adult mysids done in the spring, summer and fall are summarized in Table 5.1.

May Collections

Mysids collected after dusk in the ascending phase of their diel migration were found to contain a large quantity of diatoms in their guts. *Melosira islandica* appeared to be most numerous, *Tabellaria fragella* and *Cyclotella sp.* were less numerous than *M. islandica* but were also present in substantial quantities in most samples analyzed. The remains of 23 rotifers, or 1.2 rotifers per mysid, was found in the guts examined. An estimated 14 *Limnocalanus macrus*, 28 other calanoid copepods, and 14 cyclopoid copepods were ingested by the 20 mysids examined.

Two sets of mysids from epibenthic sledge collections were analyzed. One collection was made at 6:00 p.m. on May 23, 1995, a second collection was made at 5:00 a.m. on the 24th. The average number of *L. macrus* ingested per mysid analyzed was determined to be 1.1 in the evening sample and 1.0 the early morning sample. The average number of calanoid copepods found was 1.0 and 0.2 respectively. The number of cyclopoid copepods in the guts of each mysid was estimated to be 0.7 for both the evening and the morning collections. The evening sample of mysids contained the remains of 13 cladocerans, 28 were found in the guts of the morning collection of mysids. Diatoms were present in the guts of most of the benthic mysids sampled in the evening. Less than half of the mysids sampled at 5:00 a.m. had diatoms in their guts.

Mysids, collected just prior to dawn in the descending phase of their diel migration, had more cyclopoids in their guts, 1.7 per mysid examined. The number of *L. macrus* was 0.6 per mysid, similar to the ascending set of mysids. The remains of only three other calanoid copepods and 13

Table 5.1. A summary of the results of *Mysis relicta* gut content analysis.

	Total	Avg. Mass (µg)	# per ind.	% of total
May 23, 1995				
Adult *Mysis relicta* n=80				
Limnocalanus macrus	66	45.0	0.83	0.79
Calanoid copepods	57	9.30	0.71	0.14
Cyclopoid copepods	73	3.20	0.91	0.06
Cladocerans	57	0.57	0.71	0.01
Rotifers	94	0.03	1.18	0.00
August 15/16 1995				
Adult *Mysis relicta* n=60				
Limnocalanus macrus	7	49.00	0.1	16.1
Calanoid copepods	13	5.76	0.2	3.5
Cyclopoid copepods	112	2.56	1.9	13.4
Daphnia spp.	137	2.03	2.3	13.0
Bosmina spp.	924	0.97	15.4	42.0
Rotifers	1026	0.25	17.1	10.7
Small (< 6 mm) *Mysis relicta* caught in morning nets (descending) n=20				
Limnocalanus macrus	0	49.00	0.00	0.0
Calanoid copepods	0	9.30	0.00	0.0
Cyclopoid copepods	2	2.56	0.20	11.4
Daphnia spp.	0	2.03	0.00	0.0
Bosmina spp.	11	0.97	1.10	23.8
Rotifers	116	0.25	11.60	64.8
September 25/26 1995				
Adult *Mysis relicta* n=60				
Limnocalanus macrus	6	48.00	0.10	13.4
Calanoid copepods	97	6.87	1.62	31.1
Cyclopoid copepods	263	2.35	4.38	28.8
Daphnia spp.	94	1.56	1.57	6.8
Bosmina spp.	391	0.61	6.52	11.1
Rotifers	468	0.40	7.80	8.7

cladocerans were found in the guts examined in addition. 41 rotifers. 2.3 per mysid. were identified. Diatoms were present in relatively few of the mysids in this set.

August Collections

Gut content analysis suggests that cladocerans were the predominant food source in all samples of adult mysids collected in August. Benthic sledge samples were again collected at dusk and dawn. Inspection of the mysids collected from the bottom in the evening and morning placed the average number of bosminids ingested per mysid at 6.6 and 5.3 respectively. The number of *Daphnia spp.* eaten was estimated at 3.8 and 1.8 respectively. The number of cyclopoid copepods per mysid was the same in each set (0.7 and 0.6). The guts of mysids collected with the night sled contained 49 rotifers compared to 80 rotifers found in the guts of mysids from the morning sled. Only 1 *L. macrus* and 2 calanoid copepods were found in the gut contents of the evening set. Similarly, 3 *L. macrus* and 1 calanoid copepod were found in the guts of the morning benthic set.

The gut contents of mysids, collected in net hauls while mysis were descending, had a greater proportion of bosminids in their guts, approximately 22.4 ingested per mysid. It was estimated that each mysid ingested: 29 rotifers, 2.8 cyclopoid copepods and 1.0 *Daphnia spp.* The number of *L. macrus* found in the guts of each mysid was the same as the benthic collection, 0.1. However, more calanoids (0.3) were found in the guts examined. The gut contents of one of the epi-benthic mysids examined contained the remains of an amphipod. Small mysids were analyzed and determined to probably feed predominantly on rotifers. On average, 11.6 rotifers and 1.1

bosmonids were found in the guts of each small mysid. Two cyclopoid copepods were also identified in the guts of the ten small mysids examined.

September Collections

Copepods become a more prominent food source for *M. relicta* in the fall. Ascending mysids were likely to have the remains of 2.3 cyclopoid copepods and 0.7 calanoid copepods in their guts. Descending mysids were estimated to contain, on average, 7.2 cyclopoid copepods, 2.7 calanoid copepods and 0.2 *L. macrus*. The guts of mysids from benthic collections contained an average of 4 cyclopoid copepods, 0.1 *L. macrus* and 1.6 other calanoid copepods. The number of cladocerans found in the guts of mysids collected in the fall was similar in evening, morning and bottom collections. Ascending mysids had an estimated 5.3 bosminids and 0.95 *Daphnia spp.* per mysid. Descending mysids contained 5.8 bosminids and 2.8 *Daphnia spp*. An average of 8.7 bosminids and 1.1 *Daphnia spp* were identified in mysids collected from the lake bottom. *M. relicta* also feeds on rotifers in the water column. Mysids, ascending, or on the bottom, had an average of 3.5 or 4.9 rotifers in their guts respectively. Mysids, descending in the water column, contained 15.9 rotifers on average. The 20 epi-benthic mysids were also found to contain the partial remains of 3 amphipods. Some of the guts examined were green suggesting phytoplankton was ingested.

Isotope Signatures

A comparison was made of zooplankton collected from the epilimnion, metalimnion and hypolimnion to determine if the same species may have a different isotope signature at different depths (Table 5.2). The vertical migration and feeding of *M. relicta* is temperature restricted (Rudstam *et al.* 1998). Therefore, large differences in zooplankton $\delta^{13}C$ and $\delta^{15}N$ between epilimnetic samples and metalimnetic and/or hypolimnetic samples may alter our interpretation. The maximum differences in $\delta^{15}N$ and $\delta^{13}C$ were between samples of *Diacyclops thomasi* obtained at different depths. The observed difference in $\delta^{15}N$ was approximately 1.9 ‰, the maximum observed difference in $\delta^{13}C$ was 1.1 ‰.

Pelagic $\delta^{13}C$

The $\delta^{13}C$ of planktonic organisms from net hauls collected in the spring of 1994 varied between -26 and -32 ‰ (Fig. 5.1). Calanoid copepods sampled were largely made up of diaptomids; *Skistodiaptomus oregonensis, Leptodiaptomus minutus, Leptodiaptomus sicilis*. Spring and fall samples of calanoid copepods may have also contained juvenile and adult *L. macrus* . Bosminid samples were almost exclusively comprised of *B. longirostris*, but also contained *Eubosmina sp.* One sample of rotifers was obtained August 29, 1994 and had a $\delta^{13}C$ of approximately -24.5 ‰. The carbon signatures of calanoid copepods and *D. thomasi* separated from the >295 μm and >210 μm size fractions were enriched in ^{15}N relative to POM <110 μm and >64 μm in size, collected in the same net haul.

Table 5.2. A survey of $\delta^{13}C$ and $\delta^{15}N$ for organisms collected at different depths. All samples are composite samples separated from one to three vertical tows which were pooled. The epilimnetic samples were collected between the surface and 1m above the thermocline. Metalimnetic samples were collected from 50 to 20 m, Hypolimnetic samples were collected from 120 m to 50 m.

	Date	n	$\delta^{13}C$	$\delta^{15}N$
Epilimnion				
Seston 210 - 110 μm	1-Aug.	6	-23.96	6.95
Zooplankton >210 μm	1-Aug.	3	-23.74	7.55
Daphnia spp.	1-Aug.	1	-23.21	4.83
Bosmina spp.	1-Aug.	1	-23.86	4.56
Calanoid copepods	1-Aug.	1	-24.56	6.87
Diacyclops thomasi	1-Aug.	1	-24.37	8.30
Seston 210 - 110 μm	30-Aug.	4	-23.00	9.74
Metalimnion				
Seston 210 - 110 μm	1-Aug.	2	-24.31	6.24
Daphnia spp.	1-Aug.	1	-22.76	4.59
Bosmina spp.	1-Aug.	2	-23.74	5.64
Calanoid copepods	1-Aug.	1	-24.64	8.51
Diacyclops thomasi	1-Aug.	2	-23.27	10.24
Hypolimnion				
Limnocalanus macrus	1-Aug.	3	-29.37	11.91
L. macrus (lipid extracted)	1-Aug.	3	-26.01	11.82
Seston 210 - 110 μm	30-Aug.	1	-24.18	10.48
Diacyclops thomasi	30-Aug.	1	-21.11	11.12
Seston 210 - 110 μm	23-Sept.	1	-30.15	7.62
Seston 210 - 110 μm	23-Sept.	1	-25.87	9.4
Limnocalanus macrus	23-Sept.	5	-29.52	12.61
L. macrus (lipid extracted)	23-Sept.	4	-25.80	12.53

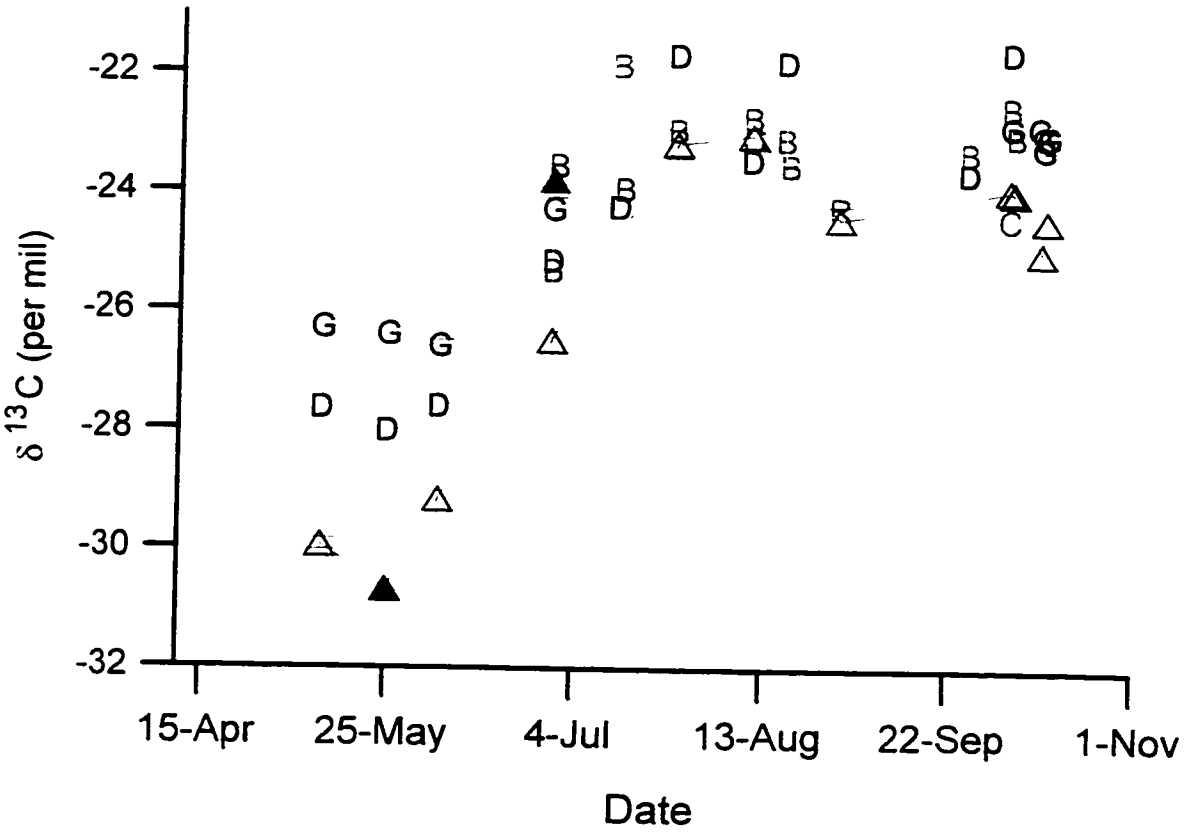


Figure 5.1. The seasonal trend in the $\delta^{13}C$ of epilimnetic zooplankton and particulate organic matter (POM) collected at Station 41 in 1994. The $\delta^{13}C$ of POM <64 μm collected on a 20 μm mesh screen is indicated by ▲;△, is POM <110 μm and >64 μm with standard deviations indicated by vertical bars. **R** represents the $\delta^{13}C$ rotifers; **D** represents *Diacyclops thomasi*; indicates *Bosmina spp.*; chiefly *Bosmina longirostris*; **G,** Calanoid copepods, chiefly diaptomids; , is *Daphnia spp.* chiefly, *Daphnia retrocurva.*

However, the observed level of enrichment was not consistent. The $\delta^{13}C$ of all plankton samples increased to - 22 to -23 ‰ by mid-July and remained within the -22 to -25‰ range through mid-October (Fig. 5.1). Samples of POM collected at the time of stratification had a highly variable carbon signature. This variability was observed to diminish as the season progressed.

Pelagic $\delta^{15}N$

The range in $\delta^{15}N$ of plankton samples collected in 1994 and 1995 was almost 18 ‰ (Fig. 5.2). However the seasonal pattern in $\delta^{15}N$ showed little variation between sites or between different years (Fig. 5.2, 4.5, 6.3). The variability in $\delta^{15}N$ was greatest in the spring. The POM <64 μm was observed to have a signature of 5 ‰. The $\delta^{15}N$ of calanoid copepods (largely diaptomids) and *D. thomasi* collected at the same time was more than 17 ‰. Between May and July there was a 7 ‰ drop in the signatures of calanoids and *D. thomasi* (Fig. 5.2). Insufficient numbers of calanoid copepods were available through the summer months to obtain a sample. After the lake stratified, cladocerans became more abundant. Samples of *B. longirostris* were obtained starting in the latter part of June, one summer sample of *Daphnia spp.* was obtained in mid-August. *D. thomasi* was abundant throughout the entire field season. The $\delta^{15}N$ of *D. thomasi* remained at 9 to 10 ‰ through the summer months, 3 ‰ greater than seston >64 μm collected at the same time. In contrast, the $\delta^{15}N$ of *B. longirostris* remained in the 3 to 5 ‰ range, 1 to 2 ‰ below the $\delta^{15}N$ of seston >64 μm in size collected at the same time (Fig. 5.2). The POM <64 μm in diameter collected on a 20 μm mesh Nitex™ screen June 30, had a $\delta^{15}N$ of 3 ‰.

A sample of rotifers obtained Aug. 29, 1994 had a $\delta^{15}N$ of 2.5 ‰. In the fall, the nitrogen signatures of plankton were observed to increase. The $\delta^{15}N$ of *Diacyclops thomasi* was approximately 12.0 ‰ by mid-October. Calanoid copepods had a signature similar to *D. thomasi* (Fig. 5.2). Calanoid copepods, cladocerans and the seston samples obtained were all in the 7.0 to 10.0 ‰ range.

$\delta^{13}C$ of Benthos

The lipid corrected $\delta^{13}C$ of *Diporeia hoyi* in either 1994 or 1995 was determined to lie in the 27.0 to 29.0 ‰ range (Fig. 5.3). *Diporeia* without any lipid extraction or correction stayed relatively constant at approximately -30.0 to -31.0 ‰. Lipid extracted values were as high as -26.0 ‰ in June. Oligochaetes were enriched in the heavier isotopes of both carbon and nitrogen. Oligochaetes had a $\delta^{13}C$ in the spring of -24.0 ‰, this value increased to approximately -22.0 ‰ in August before dropping to -24.0 ‰ in October.

$\delta^{15}N$ of Benthos

The $\delta^{15}N$ of Oligochaetes stayed fairly consistent at 13.5 to 15.5 ‰ over the course of the entire field season (Fig. 5.4). During the same time period the $\delta^{15}N$ of *D. hoyi* dropped from 13.0 ‰ in the spring to 10.0 ‰ in October (Fig. 5.4).

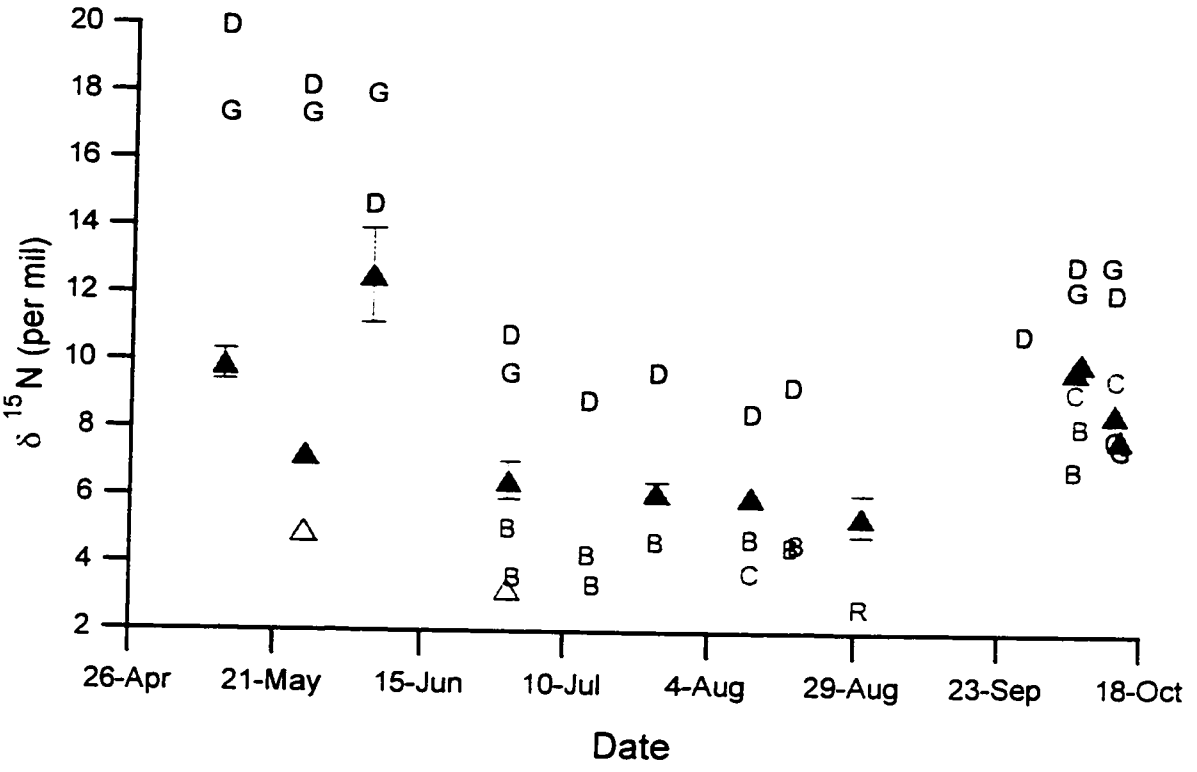


Figure 5.2. The seasonal trend in $\delta^{15}N$ of epilimnetic zooplankton collected at Station 41 in 1994. The symbols plotted are: △, particulate organic matter (POM) <64 μm collected on a 20 μm mesh screen; ▲, POM <110 μm mesh screen collected on a 64 μm mesh screen (vertical bars indicate the standard deviation), **R,** rotifers; **D** represents *Diacyclops thomasi*; **B** indicates bosminids; chiefly *Bosmina longirostris*; **G,** Calanoid copepods, chiefly diaptomids; , is *Daphnia spp.* chiefly, *Daphnia retrocurva* .

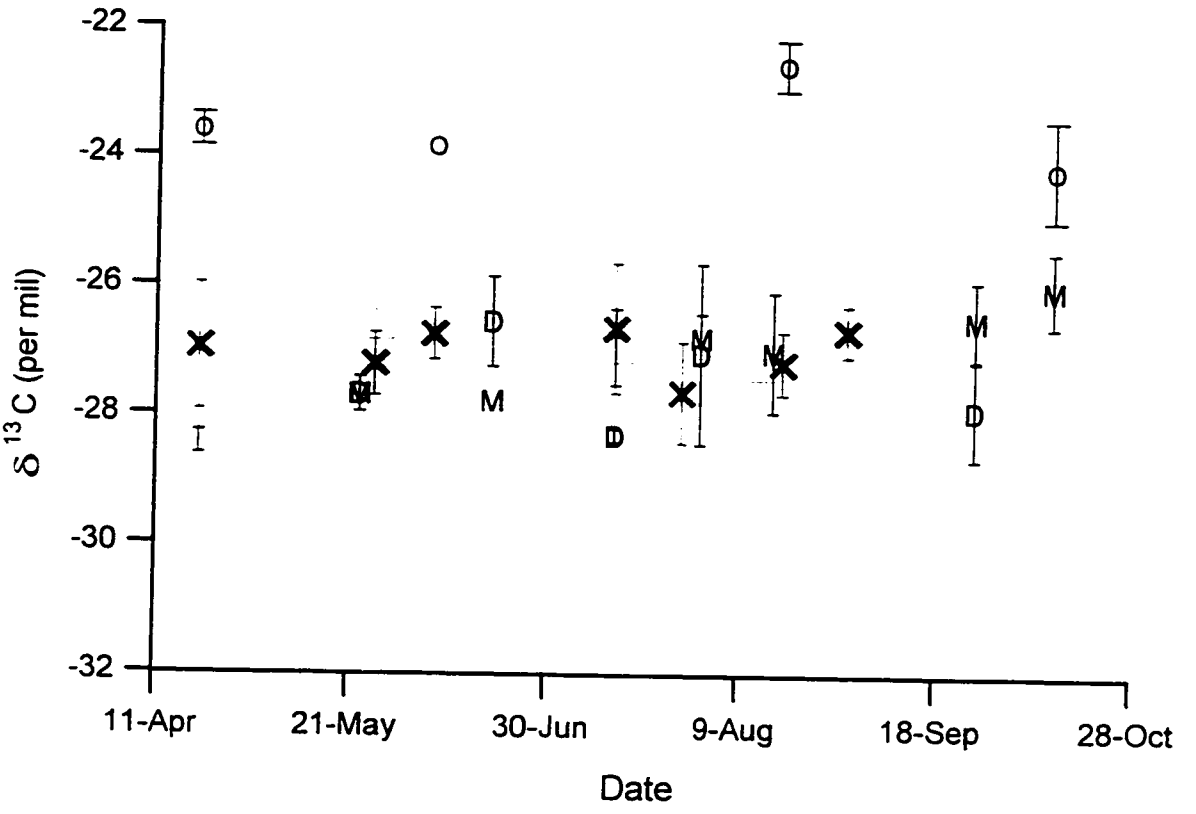


Figure 5.3. The seasonal trend in $\delta^{13}C$ of *Mysis relicta* (lipid corrected) in relation to benthic organisms. **O**; represents composite samples of Oligochaetes collected in 1994. ; *Mysis relicta* collected in 1995, ×; *Mysis relicta* collected in 1994, Δ; *Diporeia hoyi* collected in 1994, **D**; *Diporeia hoyi* collected in 1995. Standard deviations from mean values are indicated as horizontal bars.

$\delta^{13}C$ of Mysis relicta

The $\delta^{13}C$ of untreated mysids remained in the range of -29.0 to -33.0 ‰ from April through October in 1994 and 1995 (Table 5.3). Lipid corrected $\delta^{13}C$ values were consistent with lipid extracted values for mysids, between -26.0 and -28.0 ‰, for the same period (Table 5.3, Fig. 5.3). No seasonal change in the $\delta^{13}C$ was observed in 1994. In 1995 a correlation was observed between the date of collection and the $\delta^{13}C$. There was no significant difference ($\alpha = 0.05$) in $\delta^{13}C$ between ascending, descending or benthic mysids collected from the mid-lake site or further to the east near Oswego, New York (Table 5.4). There was also no significant difference in $\delta^{13}C$ between size classes of mysids (Table 5.4). For both 1994 and 1995, 90% of the variability in $\delta^{13}C$ could be attributed to differences in the C/N ratio.

$\delta^{15}N$ of Mysis relicta

The $\delta^{15}N$ of mysids collected in the spring was 12.0 to 13.0 ‰ in both 1994 and 1995 (Fig. 5.4). The $\delta^{15}N$ of *M. relicta* then dropped to 9.0 to 10.0 ‰ by early August in both years studied. In 1994 the $\delta^{15}N$ of *M. relicta* appeared to increase slightly in late August to approximately 12.0 ‰ before dropping back down to 10 ‰ (Fig. 5.4). No similar increase was observed in 1995. There was a significant difference in $\delta^{15}N$ between small and large mysids at both sites ($F = 19.63$, $P = <0.0001$), larger mysids were more ^{15}N enriched (Table 5.4). No significant difference ($\alpha = 0.05$) in $\delta^{13}C$ or $\delta^{15}N$ between ascending, descending or benthic mysids was observed (Table 5.4).

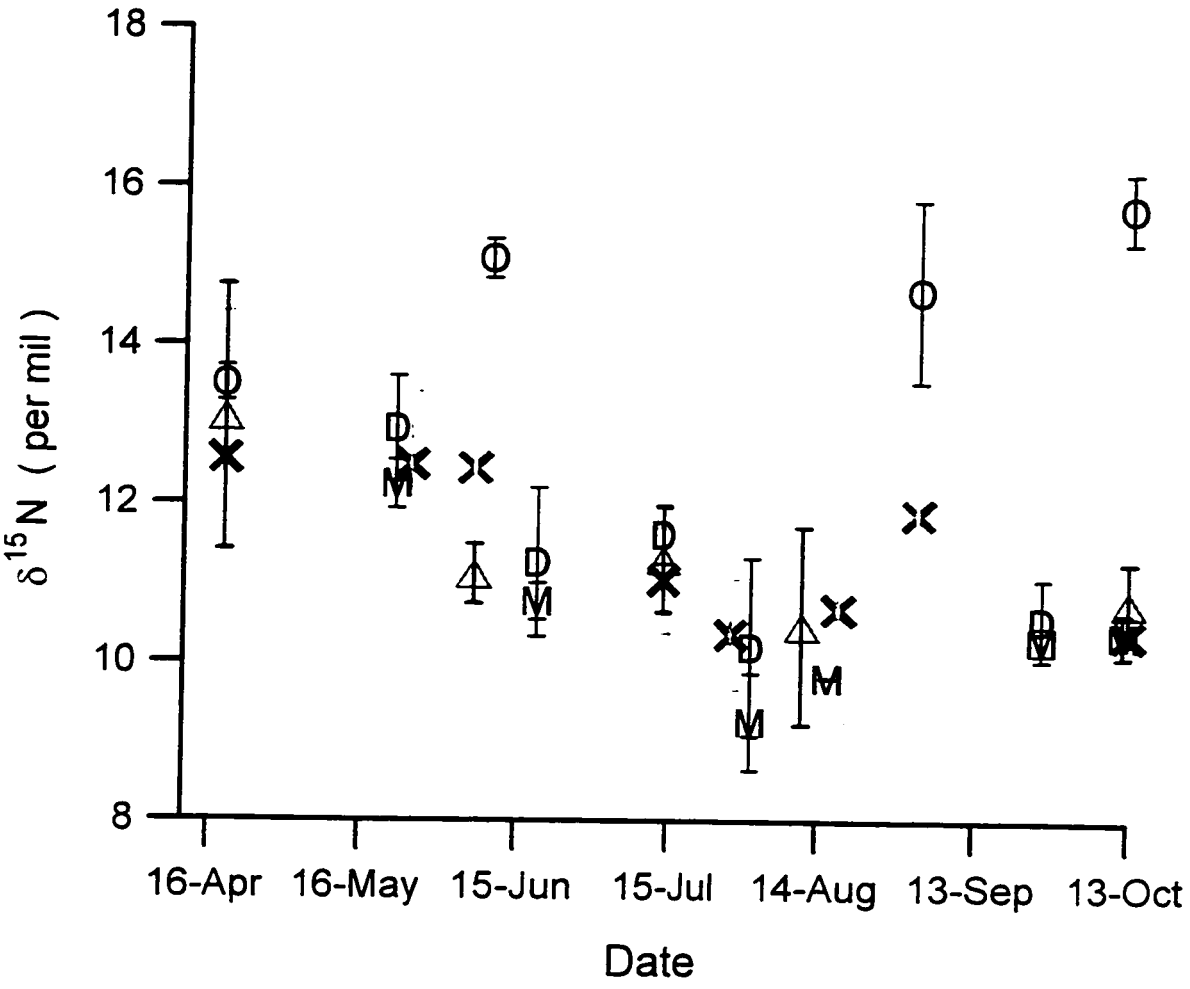


Figure 5.4. The seasonal trend in $\delta^{15}N$ of *Mysis relicta* (lipid corrected) in relation to benthic organisms. **O**; Composite samples of Oligochaetes collected in 1994. The symbols plotted are: ×; *Mysis relicta* collected in 1994, **M;** *Mysis relicta* collected in 1995, △; *Diporeia hoyi* collected in 1994, **D;** *Diporeia hoyi* collected in 1995. Standard deviations from mean values are indicated as horizontal bars.

Table 5.3 A comparison of lipid corrected $\delta^{13}C$ values with untreated and lipid extracted composite samples of *Mysis relicta* collected in 1995.

Date	n*	$\delta^{13}C$ untreated	Std dev	$\delta^{13}C$ lipid extracted	Std dev	$\delta^{13}C$ lipid corrected	Std dev	$\delta^{15}N$	Std dev
23-May-95	4†	-31.68	1.78	-27.52	0.39	-27.03	0.39	12.24	0.31
19-Jun.-95	1	-32.47		-27.87		-27.79		10.77	0.23
1-Aug.-95	3	-31.36	0.86	-27.21	0.53	-26.80	0.53	9.26	0.61
16-Aug.-95	3	-31.67	0.61	-26.50	0.97	-27.02	0.97	9.81	0.01
26-Sept.-95	4	-30.61	0.24	-27.20	0.62	-26.52	0.62	10.30	0.17
12-Oct.-95	1	-31.06		-26.50		-26.03		10.37	0.28

† A single composite sample of 20 mysids is included.
* n represents the number of composite samples of 20 mysids
The standard deviations reported are the standard deviation of the mean values for each of the composite samples analyzed

Table 5.4. A comparison of $\delta^{13}C$ and $\delta^{15}N$ of *Mysis relicta* separated by size, and time and place of collection. Ascending mysids were sampled just after dusk at the beginning of the diel migration of *Mysis relicta* and descending mysids were sampled just prior to dawn.

Mid-lake, Station 41	**n**	**$\delta^{15}N$**	**Std dev**	**$\delta^{13}C$ lipid corrected**	**Std dev**
August 1995					
Small	5	8.61	0.69	-25.64	0.61
Medium	7	9.35	0.70	-26.80	0.37
Large	3*	9.60	0.16	-26.80	1.08
September 1995					
Small	6	8.83	0.69	-26.09	0.42
Medium	4	10.45	0.40	-26.63	0.46
Large	3*	10.27	0.16	-26.84	0.41
Ascending	1*	9.98		-26.81	
Descending	1*	10.33		-26.72	
Benthic	1*	10.11		-27.32	
October 13, 1995, east basin samples, Oswego NY					
Net Plankton	3	12.87	0.20	-25.48	0.20
Ascending	1*	13.05		-25.82	
Descending	1*	13.08		-25.80	
Benthic	1*	13.92		-25.49	
Small	5	11.97	0.67	-24.75	1.02
Medium	5*	12.67	0.49	-25.24	0.30
Large	3*	13.35	0.49	-25.70	0.18

***Composite samples of 20 individuals**

Discussion

Apportionment of potential food sources in the diet of *Mysis relicta* on the basis of stable isotopes requires variation in either the $\delta^{13}C$ or $\delta^{15}N$ of those food sources. The $\delta^{13}C$ and $\delta^{15}N$ of primary producers is a function of the system biogeochemistry, the prevalent environmental conditions, and differences among species in nutrient uptake and fractionation kinetics (Chapter 3, 4). Over the course of a year, primary production in Lake Ontario has a measured range in $\delta^{13}C$ of -32.0 to -22.0 ‰ (Chapter 3). The range of seasonal fluctuation in the $\delta^{15}N$ of POM (1 to 20 μm size fraction) is 2.0 to 12.0 ‰ (Chapter 4). The range of variation in $\delta^{15}N$ within POM samples collected while the lake was stratifying was almost 10.0 ‰. The observed variability in $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web creates some difficulties in interpretation but also provides opportunity in determining feeding relationships among species using isotope signatures.

The variation in $\delta^{15}N$ makes it difficult to assess on the basis of stable isotopes the proportion of the diet of *M. relicta* which consists of phytoplankton in Lake Ontario. Mysids may be exposed to a wide range in isotope signatures of primary producers at any given time during the year. An appreciable amount of particulate matter may be retained in the thermocline due to temperature-related increase in density and viscosity of the water at this depth (Rosa 1985). As mysids migrate, they may be exposed to plankton produced earlier in the season under different environmental conditions than currently exist. To circumvent this uncertainty, the signature of cohabiting species known to feed on algae within a particular zone may be used as an integrative measure of the $\delta^{15}N$ of available plankton. Another difficulty presented by the isotope variability in the system is in

determining a trophic fractionation factor to be used in our analysis. It should be possible to estimate the level of trophic fractionation of nitrogen isotopes by primary consumers from the data presented. However, depending on the size fraction collected, the $\delta^{15}N$ of zooplankton was sometimes lower than the particulate fraction representing their food source. This is possibly a function of zooplankton feeding on selected species of algae with a $\delta^{15}N$ which varies from the average for a particular size class (Chapter 4). Therefore, a fractionation factor based on the literature was adopted to make corrections for trophic enrichment (Owens 1985, Toda and Wada 1990).

In Lake Ontario the seasonal shift in $\delta^{13}C$ creates a difference in $\delta^{13}C$ between benthic and pelagic carbon which can be exploited in dietary analysis of *M. relicta*. Both the magnitude and direction of seasonal change in $\delta^{13}C$ of primary producers depend on what processes dominate the cycling of inorganic carbon at any particular time. Changes in temperature, productivity and availability of inorganic carbon, combine to produce the observed shift in $\delta^{13}C$ of pelagic plankton (Chapter 3). It has been suggested that the carbon isotope signature of the benthos in Lake Ontario is probably established during the spring bloom (Shelske and Hodell 1991). The data presented here seem to support that hypothesis as the signatures of benthic organisms are consistent with the $\delta^{13}C$ of POC produced early in the seasonal cycle, close to -30.0 ‰. In the summer the $\delta^{13}C$ of pelagic sources of energy for mysids increases to between -23.0 and -24.0 ‰, and remains above -25.0 ‰ through the entire season. At the same time, the $\delta^{13}C$ of *D. hoyi*, taken as representative of benthic carbon, remains relatively consistent at -27.0 to -29.0 ‰. Since all the sources of carbon in the spring have the same signature, $\delta^{13}C$ is of little use in discerning the diet of *Mysis relicta*. However the $\delta^{13}C$ of

M. relicta remains at approximately -26.0 to -27.0 ‰ through the summer and fall, which suggests both pelagic and benthic sources of carbon are part of their diet.

A number of studies have used the fractionation of $\delta^{15}N$ between an organism and its food to as a means of establishing trophic relationships (Hobson and Welch 1992; Kling *et al.* 1992; Kiriluk *et al.* 1995). However, as mentioned above, fractionation in trophic transfer is not the only process influencing $\delta^{15}N$ signatures at the base of the food web (Peterson and Fry 1987). In Lake Ontario differences in $\delta^{15}N$ between dissolved organic nitrogen, ammonium and nitrate/nitrite were observed within the system (Chapter 4). These differences are reflected in the $\delta^{15}N$ of primary producers using different forms of nitrogen. In this case, a variability is created between potential food sources of *M. relicta* in the spring that can be used in assessing their relative contribution of each source to its diet. Particularly, the $\delta^{15}N$ of diatoms was determined to be approximately 2.0 to 3.0 ‰, reflecting the use of nitrate as a source of inorganic nitrogen for growth (Chapter 4). This is in contrast to the $\delta^{15}N$ of cyclopoid and calanoid copepods in the spring which were in the 17.0 to 20.0 ‰ range.

Spring dietary analysis

The $\delta^{15}N$ for spring diatoms adjusted for trophic enrichment was 5.0 to 6.0 ‰ in comparison to the spring signature of *M. relicta* of approximately 12.0 ‰. The adjusted signature of copepods was 20.0 to 23.0 ‰. These signatures can be used to establish limits for the contribution of either food source to the somatic growth of *Mysis relicta* in the spring.

The $\delta^{15}N$ of *Mysis* would probably not be as low as the 11 to 14 ‰ range observed if more than 60 % of the diet of mysis was comprised of copepods. Similarly, it is unlikely that the $\delta^{15}N$ of *M. relicta* would be as high as the observed range if diatoms made up more than 60 % of its diet. As indicated, the $\delta^{13}C$ of the mysids collected in the summer and fall indicate that a significant portion of the diet of *M. relicta* is obtained at or near the sediment surface. The $\delta^{15}N$ of *D. hoyi* is approximately 12.0 ‰ which is consistent with the observed signature of *M. relicta* (Fig. 5.4). This suggests a similar trophic status but not necessarily a similar diet. The carbon signatures of *Mysis relicta*, diatoms, copepods and *D. hoyi* are too similar and the range in signatures of individual species is too great for $\delta^{13}C$ to be useful as a tracer of source in the spring (Table 5.6). Therefore, all that can be said for certain is that the $\delta^{15}N$ suggests that the portion of the diet of *M. relicta* obtained in the pelagic zone in the spring consists of roughly a 50:50 mixture of diatoms and copepods.

Summer dietary analysis

Both carbon and nitrogen stable isotopes can be used in the apportionment of the diet of *M. relicta* in the summer (Table 5.6). The $\delta^{13}C$ of *M. relicta* is in the -26.0 to -27.0 ‰ range. This may be compared to the $\delta^{13}C$ of benthic carbon, determined to be approximately -27.0 ‰ to -29.0 ‰, and with pelagic carbon at -22.0 ‰ to -25.0 ‰. The proportion of benthic carbon in the diet of *M. relicta* would have to be 30 % or greater in order for *M. relicta* to have a signature as low as -26.0 to -27.0 ‰.

Table 5.5 Spring dietary analysis of *Mysis relicta*. The contribution to the overall $\delta^{13}C$ and $\delta^{15}N$ of *Mysis relicta* is calculated based on the apportionment of diet from gut content analysis. This theoretical value is compared to the $\delta^{13}C$ and $\delta^{15}N$ of both *Diporeia hoyi* and *Mysis relicta*.

	% of identifiable gut contents	Weighted $\delta^{15}N$ of food item	$\delta^{13}C$ of food item	Contribution to *Mysis relicta* $\delta^{15}N$	Contribution to *Mysis relicta* $\delta^{13}C$
Limnocalanus macrus	39.40	22.0	-28.0	8.7	-11.0
Calanoid copepods	7.03	22.0	-27.0	1.5	-1.9
Cyclopoid copepods	3.10	22.0	-28.0	0.7	-0.9
Cladocerans	0.43	5.5	-28.0	0.0	-0.1
Rotifers	0.00	-	-	-	-
Diatoms	50*	5.5	-29.0	2.8	-14.5
Mysis relicta **(theoretical from gut contents)**				13.7	-28.0
Diporeia hoyi				13.0	-28.5
Mysis relicta **(actual)**				12.2	-27.5

* *approximate apportionment, no quantitative estimate could be drawn from gut contents.*

Table 5.6 Summer dietary analysis of *Mysis relicta*. The contribution to the overall $\delta^{13}C$ and $\delta^{15}N$ of adult and small *Mysis relicta* is calculated based on the apportionment of diet from gut content analysis. This theoretical value is compared to the $\delta^{13}C$ and $\delta^{15}N$ of both *Diporeia hoyi* and *Mysis relicta*.

Adult *Mysis relicta*

	% of identifiable gut contents	Weighted $\delta^{15}N$ of food item	$\delta^{13}C$ of food item	Contribution to *Mysis relicta* $\delta^{15}N$	Contribution to *Mysis relicta* $\delta^{13}C$
Limnocalanus macrus	16.06	14.5	-29.4	2.3	-4.7
Calanoid copepods	3.51	9.0	-24.6	0.3	-0.9
Cyclopoid copepods	13.41	12.0	-23.3	1.6	-3.1
Daphnia	13.05	9.0	-22.8	1.2	-3.0
Bosmina	41.96	9.0	-23.7	3.8	-10.0
Rotifers	10.67	6.5	-23.0	0.7	-2.5
Mysis relicta **(theoretical from gut contents)**				9.9	-24.1
Diporeia hoyi				11.0	-28.0
Mysis relicta **(actual)**				10.0	-26.5

Summer dieatry analysis, small (< 6 mm) *Mysis relicta*

	% of identifiable gut contents	Weighted $\delta^{15}N$ of food item	$\delta^{13}C$ of food item	Contribution to *Mysis relicta* $\delta^{15}N$	Contribution to *Mysis relicta* $\delta^{13}C$
Limnocalanus macrus	0.0	14.5	-29.4	0.0	0.0
Calanoid copepods	0.0	9.0	-24.6	0.0	0.0
Cyclopoid copepods	11.4	12.0	-23.3	1.4	-2.7
Daphnia	0.0	9.0	-22.8	0.0	0.0
Bosmina	23.8	9.0	-23.7	2.1	-5.7
Rotifers	64.8	6.5	-23.0	4.2	-14.9
Mysis relicta **(theoretical from gut contents)**				7.7	-23.2
Diporeia hoyi				11.0	-28.0
Mysis relicta **(actual)**				8.60	-25.60

The $\delta^{15}N$ of *M. relicta* is 9.0 to 11.0 ‰ in August. Since approximately 30 % of assimilated carbon in *M. relicta* also has a benthic origin it may be assumed that 30% of the $\delta^{15}N$ is from the same source. The $\delta^{15}N$ of *D. hoyi* is approximately 11.0 ‰ and used as the benthic signature. The remaining 70 % is from zooplankton ingested higher in the water column. The percentage contribution to the diet of *M. relicta* attributed to each zooplankton species can be derived from the results of the gut content analysis (Table 5.6). Given this apportionment, a theoretical $\delta^{15}N$ signature can be calculated for *M. relicta* by determining the contribution of each species to the overall $\delta^{15}N$ signature. This theoretical value may be compared to the average value actually observed. To obtain the average values for gut contents and the $\delta^{15}N$ signatures of *Mysis relicta*, the gut content apportionment and isotope signatures of mysids from morning and evening epibenthic sledge collections and from pre-dawn collections of *M. relicta* in vertical hauls were pooled. The gut content data was adjusted using the average biomass of the organisms to reflect that the proportions listed are the percentage of the total mass of ingested zooplankton. On the basis of the $\delta^{13}C$ analysis, this portion represents about 70 % of the total diet. Adjusting for the contribution to the diet from benthic sources the following proportions were obtained: *L. macrus* represents 1 to 7 % of the diet of *Mysis relicta*, cladocerans accounted for 40 to 60 % of the zooplankton ingested, rotifers represented approximately 10 % and cyclopoid copepods were 7 to 14 % of the diet of adult mysids during the summer months. Whether or not these proportions reflect reality can be tested by setting up an isotope mass balance of $\delta^{15}N$ as described using equation 5.1. The average $\delta^{15}N$ of cladocerans in August was ~ 6.0 ‰, the value used in the equation is 9.0 ‰, which is the observed value adjusted for trophic enrichment. Similarly a $\delta^{15}N$ of 14.5 ‰ is used for *L. macrus*, 6.5 ‰ is used for rotifers and 12.0 ‰ is used for cyclopoid

copepods. Using the given range for each species with the appropriate $\delta^{15}N$ in different combinations of apportionment in equation 5.1 consistently results in a calculated $\delta^{15}N$ for mysids in the 9.0 to 11.0 ‰ range. This suggests that the combined stable isotope/gut content apportionment accurately reflects the diet of mysis during the summer months.

Fall dietary analysis

A balance of $\delta^{13}C$ for *M. relicta* between benthic and pelagic carbon in late September suggests a diet similar to what was derived in August. The $\delta^{13}C$ of lipid corrected/extracted *M. relicta* remains relatively unchanged at -26.0 to -27.0 ‰. This range in signatures is similar to *L. macrus* (-25.0 to -26.0 ‰), and intermediate to the $\delta^{13}C$ of *D. hoyi* (-28.0 to -29.0 ‰) and the $\delta^{13}C$ of pelagic zooplankton. The zooplankton $\delta^{13}C$ is slightly lower than it is in the summer with an upper limit of -23.5 ‰. However, none of the samples obtained higher in the water column had a $\delta^{13}C$ as low as the range observed for *M. relicta*. A balance of $\delta^{13}C$ values using equation 5.1 suggests 20 % of the diet of *M. relicta* is obtained from the benthos.

The $\delta^{15}N$ of *D. hoyi* in late September was approximately 11.0 ‰. In contrast to the situation in August, the apportionment of the pelagic fraction of the diet of *M. relicta* on the basis of the gut content analysis does not balance with respect to $\delta^{15}N$ (Table 5.7). The $\delta^{15}N$ of *M. relicta* is approximately 10.0 to 11.0 ‰ (Fig. 5.4). If 20 % of the $\delta^{15}N$ of *M. relicta* is derived from the benthos with a trophic adjusted $\delta^{15}N$ of 11.0 ‰, then the remaining 60 to 80 % of the diet has to have an average $\delta^{15}N$ of 9.0 ‰ to 11.0 ‰. The apportionment of *M. relicta's* diet derived from the gut contents is weighted towards carnivores or organisms with an elevated $\delta^{15}N$. The average $\delta^{15}N$

Table 5.7 Fall dietary analysis of *Mysis relicta*. The contribution to the overall $\delta^{13}C$ and $\delta^{15}N$ of *Mysis relicta* is calculated based on the apportionment of diet from gut content analysis. This theoretical value is compared to the $\delta^{13}C$ and $\delta^{15}N$ of both *Diporeia hoyi* and *Mysis relicta*.

	% of identifiable gut contents	Weighted $\delta^{15}N$ of food item	$\delta^{13}C$ of food item	Contribution to *Mysis relicta* $\delta^{15}N$	Contribution to *Mysis relicta* $\delta^{13}C$
Limnocalanus macrus	13.4	16.0	-23.2	2.1	-3.1
Calanoid copepods	31.1	14.2	-22.5	4.4	-7.0
Cyclopoid copepods	28.8	15.0	-24.6	4.3	-7.1
Daphnia	6.8	12.2	-24.4	0.8	-1.7
Bosmina	11.1	10.5	-23.3	1.2	-2.6
Rotifers	8.7	10.0	-25.0	0.9	-2.2
Mysis relicta **(theoretical from gut contents)**				13.8	-23.6
Diporeia hoyi				11.0	-28.0
Mysis relicta **(actual)**				10.3	-26.6

of *L. macrus* is 13.0 ‰, and the $\delta^{15}N$ of *D. thomasi*, representative of cyclopoid copepods, is 12.0 ‰. These values are adjusted to 16.0 ‰ and 15.0 ‰ respectively to compensate for trophic enrichment. On average, the remains of 0.1 to 0.2 *L. macrus* were found in the gut contents of *M. relicta*. Adjusted for the average mass of individual *L. macrus*, this number represented 13.0 to 14.0 % of the total ingested biomass of zooplankton. In the average mysid the remains of 46 cyclopoid copepods could be also be found. This number of cyclopoids represented 30 % of ingested biomass of zooplankton. If 30 % of the assimilated nitrogen in *M. relicta* has the $\delta^{15}N$ of *L. macrus* and cyclopoids, then the remaining plankton in the diet must have an average $\delta^{15}N$ not greater than 5.0 ‰ which translates to 8.0 ‰ after adjusting for trophic enrichment.

Neither the POM analyzed or any of the zooplankton measured in the fall had a $\delta^{15}N$ which was that low. The analysis of gut contents suggests that cladocerans make up roughly 20 % of the ingested zooplankton, calanoid copepods 31 %, and rotifers 7 %. The lowest measured $\delta^{15}N$ in late September of 1994 or 1995 was 7.5 ‰. If 20 % of the diet of mysids is benthic, and the remaining 80 % is apportioned on the basis of the gut contents, the average $\delta^{15}N$ of *M. relicta* would be approximately 13.0 to 15.0 ‰. The discrepancy may be a reflection of the pelagic component of the diet of mysids being made up partially of phytoplankton. However, in order to shift the signature of *M. relicta* 1 ‰ the phytoplankton would have to comprise more than 15 % of the diet of *M. relicta* in the fall. If the proportion of rotifers in the diet is 10 %, cyclopoid and calanoid copepods are each 15 %, *L. macrus* is 5 % and cladocerans and benthos are both 20 % the mass balance of $\delta^{15}N$ still works out to ~ 13 ‰. This calculated value for *M. relicta* suggests a bias in our apportionment toward organisms enriched in $\delta^{15}N$.

There may be some error in our analysis associated with dietary apportionment made directly from gut content estimates of numbers ingested and conversion to biomass. Studies of mysid feeding suggest that mysids are often inefficient consumers, only ingesting a portion of their prey in some instances (Smolkarowski pers. com.). Therefore the body parts found in the guts may be disproportionate to the overall contribution of any food source to the diet of *M. relicta*. This is especially true for organisms such as *L. macrus*, where the individual mass used to estimate the percentage contribution to the zooplankton biomass ingested is relatively large. The potential to over-estimate the dietary importance of large organisms to *M. relicta* increases. Therefore, *Diporeia hoyi* were not included in our analysis as a separate food source for *M. relicta* even

though the remains of amphipods were identified in two of the mysids collected during the summer. Also, by using *D. hoyi* as a surrogate for benthos the assumption is made that these organisms are obligate detrivores. Omnivory in the diet of *D. hoyi* may increase their $\delta^{15}N$ and bias the analysis of the diet of *M. relicta*.

The importance of rotifers to the diet of *M. relicta* may similarly be under-estimated. Even though large numbers of rotifers were found in the guts of mysids, their contribution to the total ingested biomass of zooplankton is small due to their small average size. The assumption is also made that the assimilation efficiency for each prey item is the same, i.e., there is a consistent percentage of the prey item consumed assimilated. Hard parts of different prey may pass out of the gut at different rates, soft bodied organisms may not be visible in the guts after a short period of time. This may explain some of the discrepancy between the gut content analysis and the stable isotope signature of *M. relicta* observed in the fall.

This study has demonstrated the effectiveness of coupling multiple stable-isotope analysis with gut content analysis. The $\delta^{13}C$ and $\delta^{15}N$ mass balance provided a mechanism for quantitative estimation of components of the diet of *M. relicta* which could only be alluded to by using gut content analysis alone. However, the ability to effectively utilize multiple stable isotopes as a tool in determining the energy provenance of zooplankton may not be feasible in all circumstances. In Lake Ontario, a seasonal shift in the $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web coupled with the trophic enrichment of $\delta^{15}N$ provided sufficient variation in the dietary sources of *Mysis relicta* to make analysis of diet using isotopes possible. In other systems, the range in seasonal fluctuation in

$\delta^{13}C$ and $\delta^{15}N$ may not be sufficient to make the kind of analysis applied here feasible. However isotopes are only one form of tracer which may be employed. The ability to quantitatively determine the relative contribution of different sources of energy to the diet of zooplankton may be further enhanced by the addition of other tracer technologies such as phaeopigment analysis of the gut-contents in future endeavors (Quiblier-Llobéras *et al.* 1996).

Chapter 6

A Stable Isotope Analysis of Food Sources of Lake Ontario Forage Fish

Introduction

The relative ratios of the stable isotopes of carbon and nitrogen in an organism can be used as a means of determining dietary sources (Peterson and Fry 1987). The ratio of stable carbon isotopes, ^{13}C and ^{12}C are expressed as carbon signatures or δ values, $\delta^{13}C$, in units per mil (‰). The ratio of the two isotopes is approximately conserved in the assimilation of carbon by an organism from its source of food (DeNiro and Epstein 1978). In contrast, ^{15}N is enriched relative to ^{14}N in trophic transfer (DeNiro and Epstein 1981). Therefore, stable isotopes may be used as naturally occurring tracers of energy in biological systems (Fry 1991). Using carbon, as an indicator of source, and nitrogen, as an indicator of trophic position, it should be possible to infer the relative trophic status of individual organisms within the food web (Fry 1991; Cabana and Rasmussen 1994).

This basic theory was applied in a study of organic contaminant biomagnification in the Lake Ontario pelagic food web (Kiriluk *et al.* 1995). On average, an increase in $\delta^{15}N$ was observed in successive trophic levels. However, there were some discrepancies between the applied theory and the results obtained (Kiriluk *et al.* 1995). The $\delta^{15}N$ and $\delta^{13}C$ of lake trout was observed to occur over a wide range, 13.0 to 20.0 ‰, and -29.0 to -22.0 ‰ respectively. The range in $\delta^{15}N$ is theoretically equivalent to 2 to 3 trophic levels. An even wider range was observed between net plankton samples collected at different times

of the year (Kiriluk *et al.* 1995). The underlying assumption in the use of nitrogen isotopes to assess relative trophic status is that baseline levels of nitrogen isotopes stay relatively constant or fluctuations are accounted for in some manner (Rasmussen and Cabana 1996). Further study of Lake Ontario indicated that $\delta^{15}N$ of primary producers fluctuated seasonally as a function of inorganic nitrogen cycling producing the observed change in the baseline plankton samples in the earlier study (Chapter 4). Seasonal fluctuation in $\delta^{13}C$ of primary producers was also determined to produce the range in $\delta^{13}C$ signatures observed in the pelagic food web (Chapter 3). In order to determine if the seasonal fluctuation in primary production was responsible for producing the observed range in $\delta^{15}N$ of lake trout it was necessary to establish a range in $\delta^{15}N$ within the lake for forage fish and determine the dominant factors influencing the isotope signatures of forage fish.

Methods

Forage fish were obtained over a wide spatial distribution to facilitate comparison with existing data and obtain an assessment of the range of variability in forage fish across the lake. Seasonal sampling of forage fish, and their potential food sources, were conducted to obtain an assessment of temporal variability in forage fish and the temporal and spatial variability in their food sources.

Zooplankton and particulate organic matter (POM) samples were collected at Station 81, near Main Duck Island in the east basin of Lake Ontario (Fig. 3.1). Samples were collected every two weeks from late April through to late September of 1994. Prior to stratification, samples were collected from 20 m to the surface in vertical tows using 64 μm mesh Nitex™ nets. After the lake had stratified in June, samples were collected from 1 m above the start of the thermocline to the surface. Samples were sequentially screened, as a cleaning process, to remove as much phytoplankton and detritus as possible and also to separate zooplankton into size categories: >295 μm, >210 μm, >110 μm, and >64 μm. The size fractionated plankton samples were preserved in ethanol. Each sample was examined under a microscope, an estimate of species composition was recorded and any detritus or phytoplankton was removed from each sample. Selected subsamples were removed and samples of individual species were obtained through separation under a microscope. Composite samples of many hundreds of individuals were then rinsed with distilled deionized water and dried prior to analysis. As part of a separate experiment to determine the effects of acid addition to $\delta^{15}N$ and $\delta^{13}C$ signatures samples were either treated with the addition of 2N HCl to remove residual carbonate and redried prior to analysis or left untreated. Acid addition was determined to have no influence on the $\delta^{13}C$ or $\delta^{15}N$ of zooplankton samples so all samples were included in the analysis (Appendix 2).

Samples of benthic invertebrates were collected from Station 81, in late April and early August of 1994. Box-core samples were taken from the sediment surface and samples of

D. hoyi and oligochaetes were removed. These samples were rinsed in distilled deionized water, subsamples had 2N HCl added to them, all samples were then oven dried at 60 °C. Slimy sculpin (*Cottus cognatus*) were obtained in July 1994, as by-catch during epi-benthic sled collections of benthic invertebrates at Station 81 as part of the Department of Fisheries and Oceans, Bioindex biomonitoring program. The samples were fresh frozen aboard ship, and stored frozen prior to analysis.

Alewife (*Alosa pseudoharengus*), and smelt (*Osmerus mordax*) samples from the vicinity of Main Duck island, near Station 81, in the east basin of Lake Ontario were collected in trawls by the Ontario Ministry of Natural Resources as part of their routine monitoring of Lake Ontario fish stocks in June, July and September of 1994 (Fig. 3.1). Individual fish were weighed and measured, had their digestive tract removed and were then frozen prior to analysis.

Samples of slimy sculpin (*Cottus cognatus*), alewife (*Alosa pseudoharengus*), and smelt (*Osmerus mordax*) were collected in trawls on east-west transects along the south shoreline in June of 1994. Fish were collected with a 12 m bottom trawl with a 9 mm mesh in the cod end at three locations offshore of Oswego, Rochester and Olcott, New York (Fig. 3.1). Smelt, sculpin and alewife were collected at depths of 55m, 75m, 95m, and 130m. The fish collected were packed in dry ice aboard ship and frozen prior to processing for analysis.

Fish samples analyzed for isotopes were thawed, and measurements of fork length and weight, were recorded. The samples were then homogenized in a commercial meat grinder and freeze-dried. Prior to stable isotope analysis samples were further homogenized using a ball-mill grinder, visible fragments of scale and bone were removed from the sample.

Stable isotope analysis of zooplankton and benthic samples were done using a Micromass Continuous-flow isotope-ratio mass spectrometer fitted with a Carlo-Erba elemental analyzer as part of the inlet system (EA/CF-IRMS). The accuracy, precision and range of linearity were continuously monitored with sets of National Institute of Standards and Technology (NIST) -USA standards of known concentration and isotope composition. Duplicate standards within the linear range of operation consistently had measured values within 0.3 ‰ of each other and the known value.

The forage fish samples as well as selected zooplankton samples with a mass greater than 2 mg were analyzed using a VG-Optima continuous-flow isotope-ratio mass-spectrometer (CF-IRMS). Analysis of standards of known isotope composition and duplicate analysis of samples was used to assess the precision and accuracy of analysis between systems and within runs. The difference in $\delta^{13}C$ and $\delta^{15}N$ between duplicates and between standards and a known reference value was consistently within 0.3 ‰ .

An allometric model for the tissue turnover of $\delta^{13}C$ in fish developed from laboratory studies of growing whitefish was applied to analysis of Lake Ontario alewife (Hesslein 1993, Table 6.1A). Input parameters for dietary apportionment were obtained from the gut content analysis of Hewitt and Stewart (1989) (Table 6.1B). The equation for the model is;

$$C = C_n + (C_o - C_n)\, e^{-(k+m)t} \qquad (6.1)$$

Where; C = δ value of fish, C_o = δ value of fish in equilibrium with old food, C_n = δ value in equilibrium with new food, k = growth rate (per day), t = time (days), m = metabolic turnover constant. For the analysis in this chapter the metabolic turnover constant was set as 0, any change in signature would therefore be due to carbon or nitrogen assimilated in growth. In over-wintering, when there was an average weight loss it was assumed that all of the food ingested was used to produce energy. No adjustment was made to the carbon signatures for trophic enrichment. A fractionation factor of 2.5 ‰ was added to each of the $\delta^{15}N$ values to account for trophic enrichment (Owens 1987). Average seasonal isotope signatures for *Diacyclops thomasi* were used to represent copepods (Table 6.1A). Isotope signatures for *Bosmina longirostris* and *Daphnia spp.* from 1994 were pooled when both were available and used as seasonal values for cladocerans (Table 6.1A).

Table 6.1A. The allometric model for the estimation of the stable isotope composition of alewife. Stable isotope input data are from collections made in the east basin of Lake Ontario in 1994. The exception is the data for *Mysis relicta* which was collected near Oswego.

Allometric Model

$C = C_n + (C_o - C_n) * e^{-(k+m)t}$

C	=	del value of fish
C_o	=	del value of fish in equilibrium with old food
C_n	=	del value in equilibrium with new food
k	=	growth rate (per day)
t	=	time (days)
m	=	metabolic turnover constant

$dC/dt = -k\,(W_o / W)\,(C_o - C_n)$

W_o = fish weight when food was switched
W = fish weight in grams at time of measurement

Stable Isotope Values

	$\delta^{13}C$	Std.Dev.	$\delta^{15}N$	Std.Dev.
Alewife	-24.5	0.75	12.6	0.74
Mysis	-26.38	0.52	12.67	0.49
Diporiea	-28.03	0.28	11	
Cladocerans (spring)	-26.26		5.60	
Cladocerans (summer)	-23.60	0.98	6.37	1.08
Cladocerans (fall)	-27.15	1.24	7.54	1.19
Copepods (spring/winter)	-28.97	0.22	17.71	0.19
Copepods summer	-21.58		9.86	
Copepods (fall)	-24.70		12.29	

Table 6.1B. The dietary input parameters and results of the stable isotope/allometric model estimation of the stable isotope composition of alewife. The alewife age and weight data are obtained from Hewitt and Stewart (1989).

Alewife diet (Hewitt and Stewart 1989)
Growth, Dietary Apportionment and Projected Stable Isotope Composition

Date	Age	Weight	Days	Gain	Cladocerans	Copepods	Diporiea	Mysis	C ($\delta^{13}C$)	C ($\delta^{15}N$)
11-Jul	L	0.0001							-24.5	
19-Aug	1	0.161	38	0.1609	0.8	0.2			-23.20	10.07
1-Oct	1	2	42	1.839	0.8	0.2			-26.11	9.65
1-Jul	2	7.66	274	5.66	0.2	0.8			-28.42	17.75
1-Oct	2	17.07	92	9.41	0.68	0.28	0.04		-23.21	10.03
1-Jul	3	23.92	274	6.85	0.15	0.75	0.1		-28.46	17.71
1-Oct	3	29.53	92	5.61	0.4	0.3	0.2	0.1	-21.55	11.49
1-Jul	4	33.64	274	4.11	0.1	0.35	0.4	0.15	-26.53	15.49
1-Oct	4	39.87	92	6.23	0.4	0.3	0.2	0.1	-25.48	11.48
1-Jul	5	40.56	274	0.69	0.1	0.35	0.4	0.15	-26.04	13.51
1-Oct	5	47.31	92	6.75	0.4	0.3	0.2	0.1	-25.48	11.47
1-Jul	6	46.4	274	-0.91	0.1	0.35	0.4	0.15	-25.47	11.45
1-Oct	6	54.04	92	7.64	0.4	0.3	0.2	0.1	-25.48	11.47
1-Jul	7	52.82	274	-1.22	0.1	0.35	0.4	0.15	-25.47	11.45
1-Oct	7	60.87	92	8.05	0.4	0.3	0.2	0.1	-25.48	11.47
1-Jul	8	58.03	274	-2.84	0.1	0.35	0.4	0.15	-25.47	11.45
1-Oct	8	66.8	92	8.77	0.4	0.3	0.2	0.1	-25.48	11.47

Results

Carbon and nitrogen isotopes were determined to be effective tracers of dietary sources but were poor predictors of relative trophic status in the forage fish analyzed. The $\delta^{13}C$ of any individual fish was determined to be a function of whether its energy was obtained from benthic or pelagic sources and also the timing of food ingestion for growth. The observed $\delta^{15}N$ was found to be site dependent in benthic feeding sculpin and varied among species as a function of the benthic component in the diet.

The >110 μm, and >64 μm samples of POM often contained substantial quantities of phytoplankton, in addition to rotifers, nauplii copepodids. The >295 μm, and >210 μm size classes were comprised almost exclusively of zooplankton. The species composition of each fraction varied with the time of year.

The $\delta^{13}C$ of the POM and zooplankton samples, collected seasonally, increased from approximately -32.0 ‰ to -30.0 ‰ in the spring to between -24.0 ‰ and -22.0 ‰ in late summer and fall (Fig. 6.1, Fig. 6.2). A comparison was made between the $\delta^{13}C$ of *Diacyclops thomasi* and *Bosmina longirostris* at Station 81 and the seasonal analysis from Station 41 a mid-lake site described in previous chapters. The seasonal pattern in $\delta^{13}C$ of *D. thomasi* and *B. longirostris* was found to be consistent between sites and also interannually (Fig. 6.3).

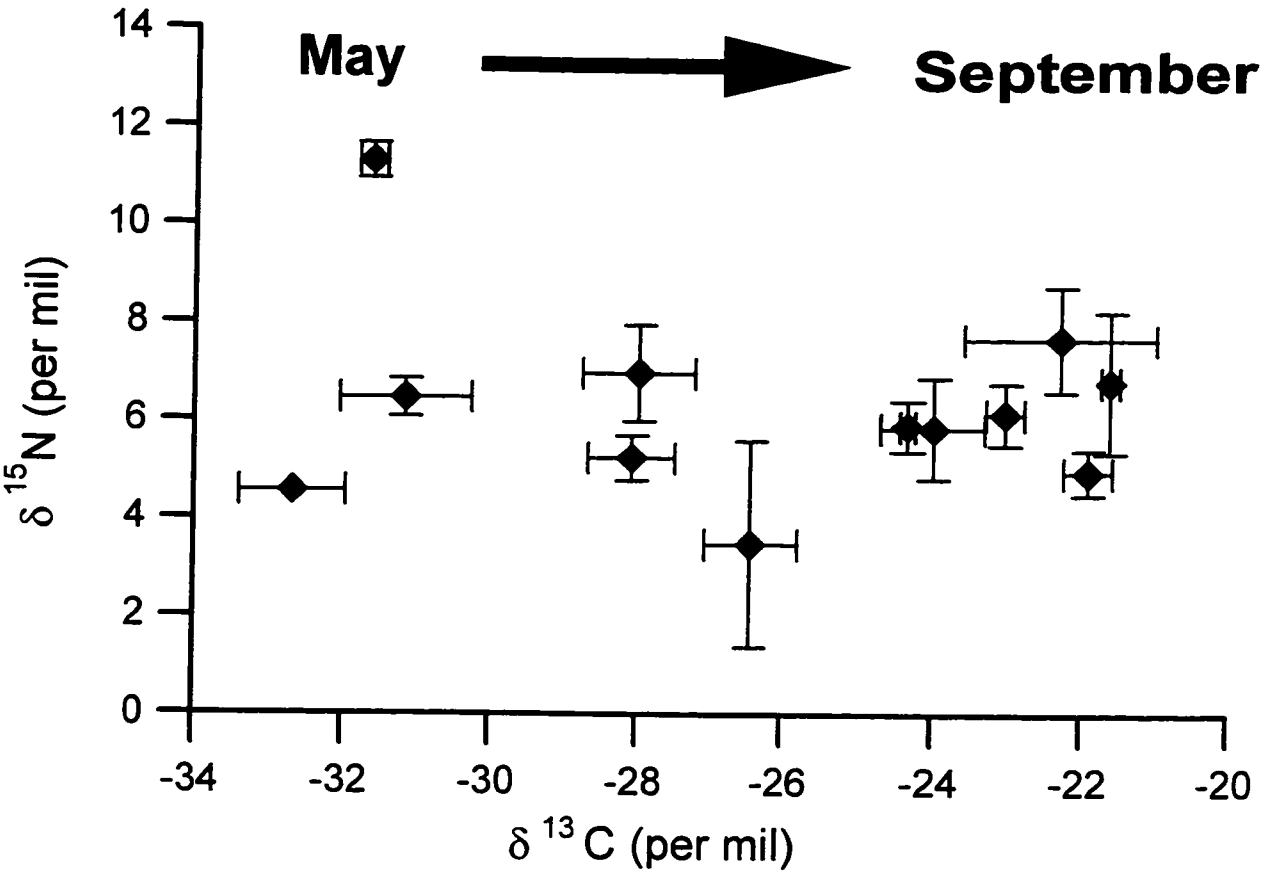


Figure 6.1. A seasonal trend in $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter (POM) passing through a 110 μm mesh screen, collected on a 64 μm mesh screen at station 81, 1994. Standard deviations from the mean values are indicated by vertical and horizontal bars.

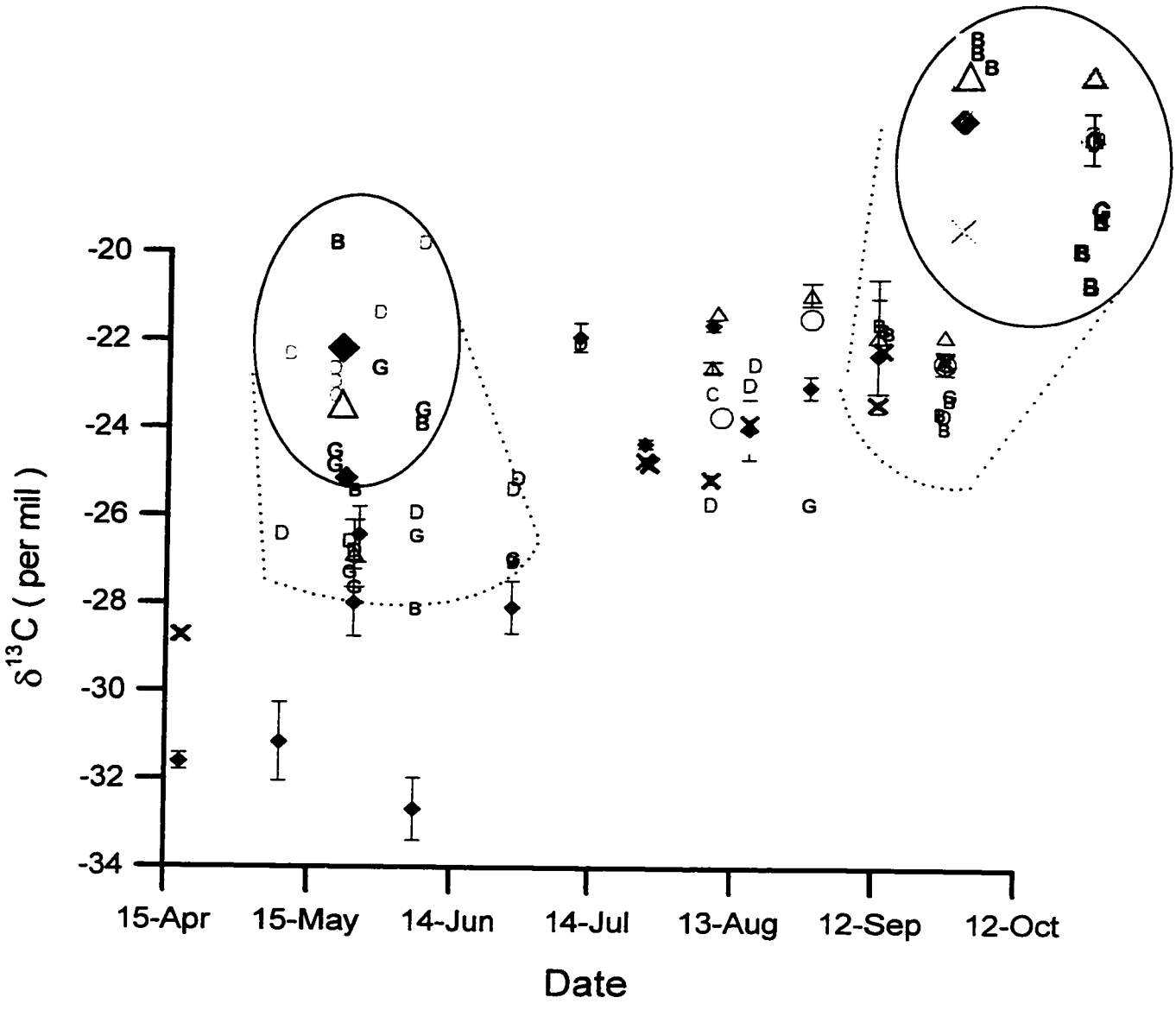


Figure 6.2. The seasonal trend in $\delta^{13}C$ of epilimnetic zooplankton collected in 1994 from station 81 in the east basin of Lake Ontario. The plotted symbols are: ◆, particulate organic matter(POM) <110 μm mesh collected on a 64 μm mesh screen; ✓. POM <210 μm collected on a 110 μm mesh screen; Δ, zooplankton > 210; O zooplankton > 295; D. *Diacyclops thomasi*; **B,** bosminids, chiefly *Bosmina longirostris*; **G,** Calanoid copepods, chiefly diaptomids; C, *Daphnia spp.* chiefly *Daphnia retrocurva* . All of the samples plotted are composites, where three or more replicates have been analyzed the standard deviation from the mean value is indicated as a vertical bar.

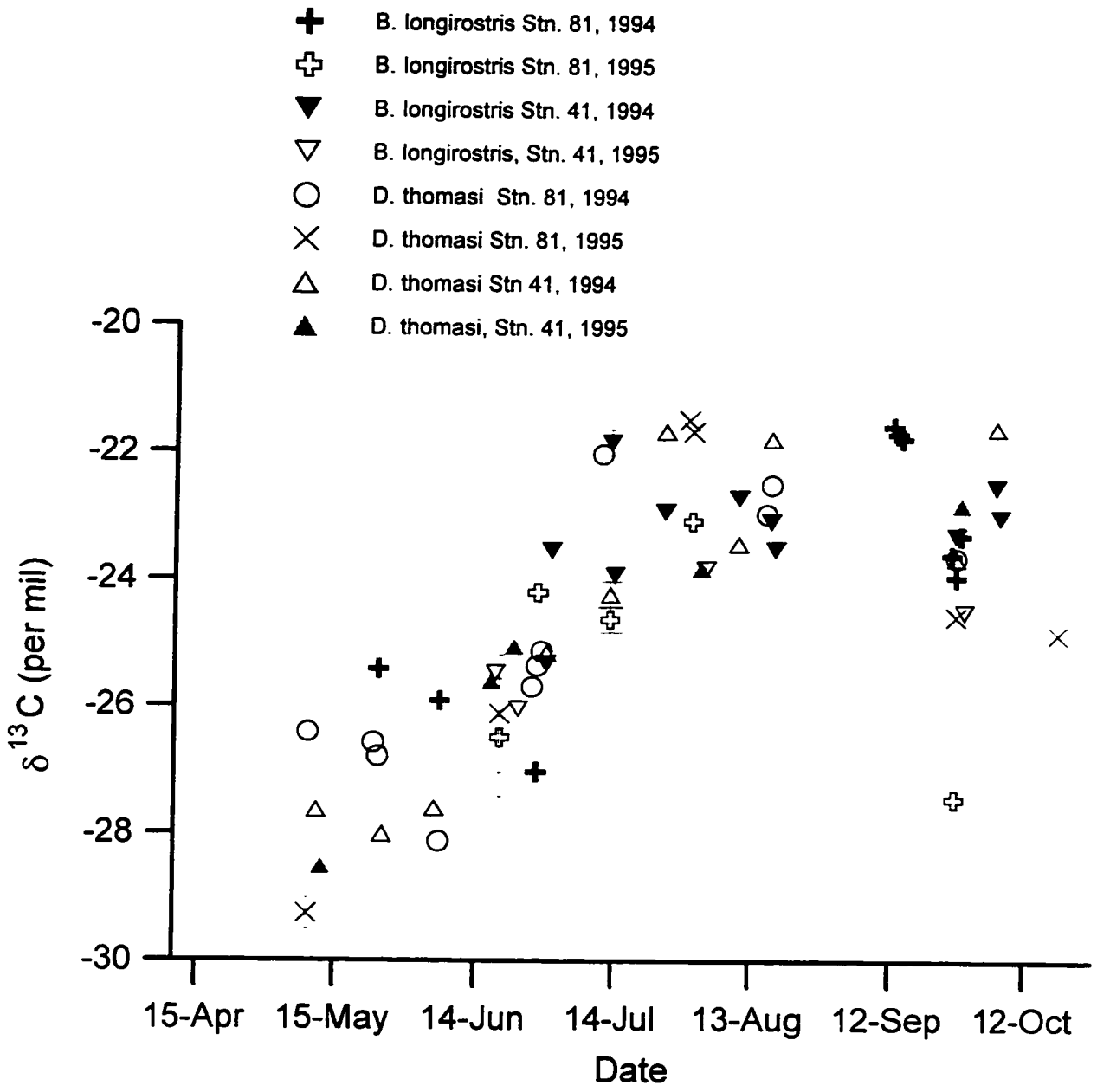


Figure 6.3. A between year comparison of the seasonal pattern in $\delta^{13}C$ of *Diacyclops thomasi* and *Bosmina longirostris* collected at a mid-lake site (Station 41) and a site in the east basin (Station 81) of Lake Ontario.

The $\delta^{15}N$ of POM and zooplankton has a broad range (18.0 ‰ to 5.0 ‰) in the spring prior to thermal stratification in the lake (Fig. 6.1). After this time the observed variability in the $\delta^{15}N$ of algae and consumers gets smaller, and all samples eventually fall in the range of 6.0 to 10.0 ‰ (Fig. 4.3).

The isotope signatures of benthic invertebrates collected at Station 81 were consistent with the signatures of similar samples collected at the mid-lake site (Chapter 5). The $\delta^{13}C$ of oligochaetes was approximately -24.0 ‰ and the $\delta^{15}N$ was 15.0 ‰. Both the $\delta^{13}C$ and $\delta^{15}N$ of oligochaetes are enriched in the heavier isotope relative to *Diporeia hoyi* . The average $\delta^{13}C$ and $\delta^{15}N$ of *D. hoyi,* in May, was -28.2 ‰ and 13.6 ‰ respectively. In July, the average $\delta^{13}C$ and $\delta^{15}N$ of *D. hoyi* was -27.4 ‰ and 10.6 ‰.

The stable isotope signatures of alewife, collected in different parts of the lake, were similar (Fig. 6.4). The same was also true for smelt (Fig. 6.4). However, the isotope composition of sculpin was observed to be site dependent (Fig. 6.4). Significant differences in $\delta^{13}C$ were observed between species of forage fish ($F_{0.05, 119} = 97.19$, $p = < 0.0001$) (Fig. 6.5). The $\delta^{13}C$ of sculpin was determined to be -27.0 ‰ or less at all sites where samples were collected. Smelt were found in the range of -28.0 ‰ to -26.0 ‰. The $\delta^{13}C$ of alewife was between -23.0 and -25.0 ‰ at all sites analyzed, distinctly different from the other two species of fish (Fig. 6.5).

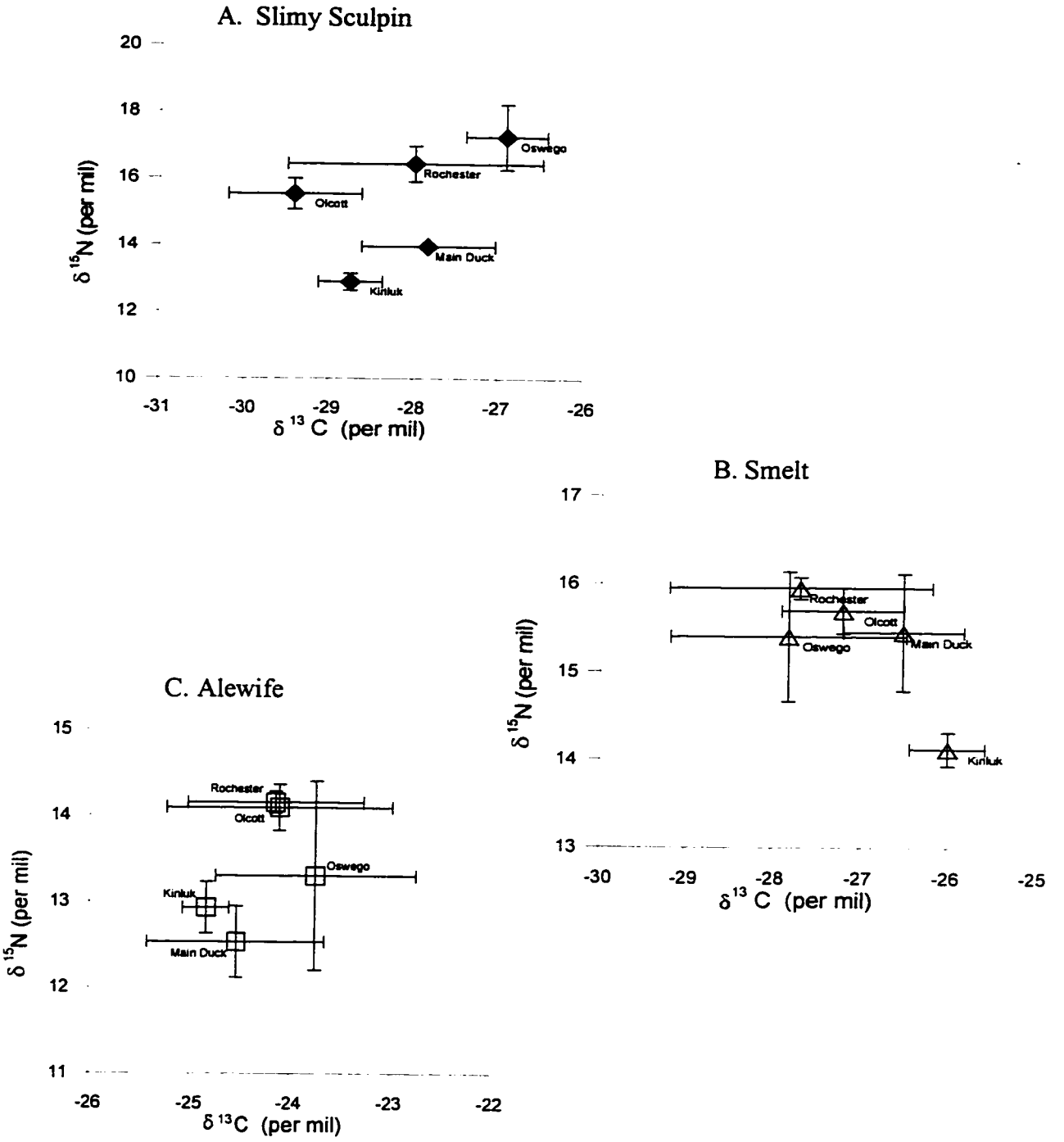


Figure 6.4. Forage fish $\delta^{13}C$ vs $\delta^{15}N$ grouped by species. Collection sites are indicated on the graphs. Collections made by Kiriluk *et al.* in 1992, at locations along the north shore and western end of Lake Ontario, were pooled and are reported as one sample. The plots are; A. ◆, Slimy sculpin (*Cottus cognatus*); B. Δ ,Smelt (*Osmerus mordax*); C. □, Alewife (*Alosa pseudoharengus*).

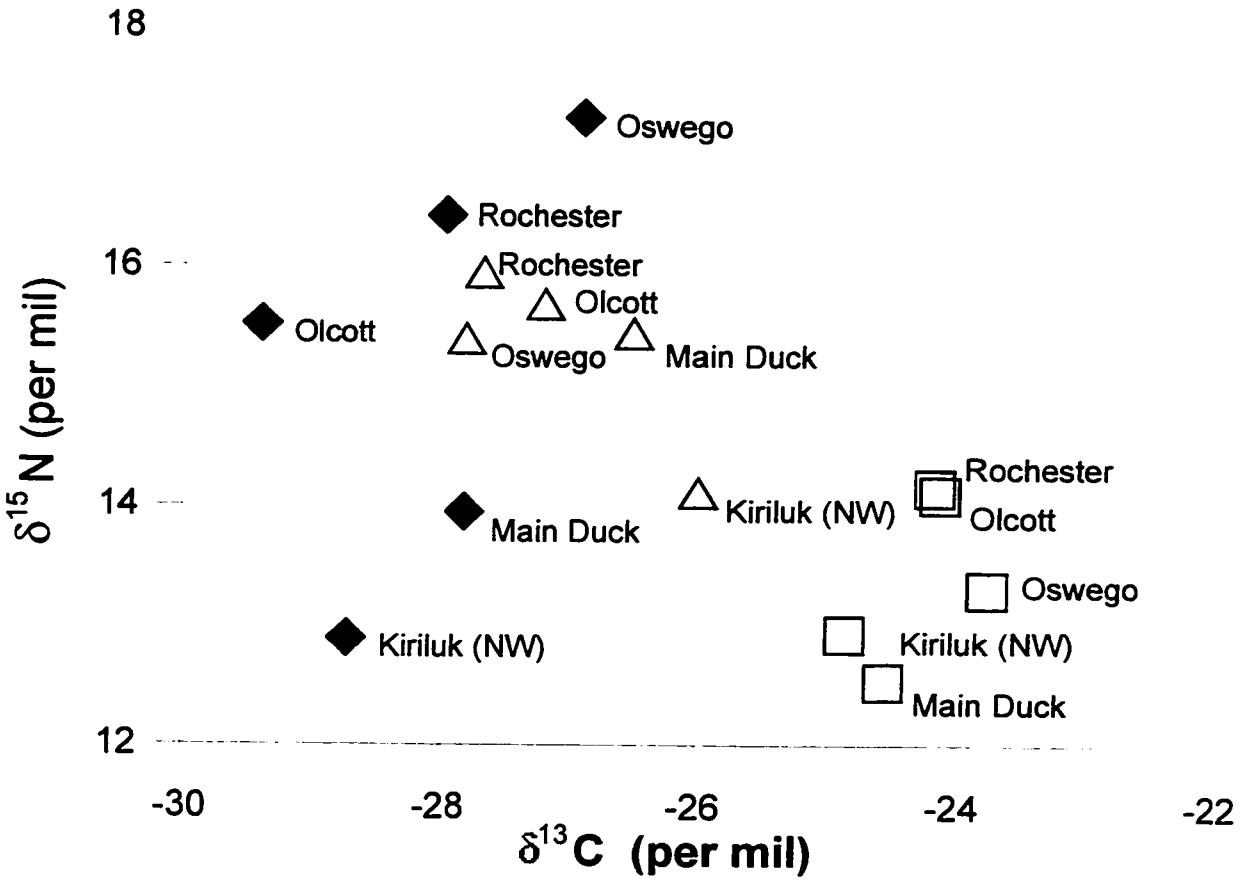


Figure. 6.5. Forage fish $\delta^{13}C$ vs $\delta^{15}N$ grouped by site of collection. Collections made by Kiriluk *et al.* in 1992 at locations along the north shore and western end of Lake Ontario were pooled and reported as one sample. □ Alewife (*Alosa pseudoharengus*), ◆ Slimy sculpin (*Cottus cognatus*), △, Smelt (*Osmerus mordax*).

Significant differences in $\delta^{15}N$ were determined to exist between individual species of forage fish ($F_{0.05,119} = 26.95$, $p = <0.0001$). There were no significant differences in $\delta^{15}N$ between sites in alewife and smelt (Fig. 6.4, 6.5). Significant differences were observed between sites of collection of sculpins ($F_{0.05, 27} = 11.45$, $p = <0.0001$) (Figs. 6.4, 6.5).

There was no significant difference in $\delta^{13}C$ or $\delta^{15}N$ between fish of the same species caught on transects along the shoreline corresponding to different depths (Table 6.2). There was also no difference observed in the isotope signatures of alewife and smelt collected near Main Duck Island at different times during the season (Table 6.2). The results obtained by Kiriluk *et al.* (1995) for 1992 collections of forage fish on the north shore and western basin of Lake Ontario are presented for comparison (Kiriluk *et al.* 1995). (Table 6.2, Fig. 6.4, 6.5).

Using the alewife dietary apportionment of Hewitt and Stewart (1989), the stable isotope/allometric model predicted the $\delta^{13}C$ of alewife to be approximately -25.5 ‰ (Table 6.1). The predicted $\delta^{15}N$ was 11.5 ‰. Both of these values are 1.0 ‰ lower than the average signature observed (Table 6.1).

Table 6.2. Average values for the $\delta^{13}C$ and $\delta^{15}N$ of alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), and slimy sculpin (*Cottus cognatus*) collected in the east basin of Lake Ontario (Station 81) and along the south shore. The results obtained from Kiriluk *et al.* (1995) for the same fish species collected in the north west portion of the lake are included for comparison.

	Date	Site	Depth (m)	n	$\delta^{13}C$	Std Dev	n	$\delta^{15}N$	Std Dev
Sculpin									
	15-Jun-94	Olcott		5	-29.41	0.79	5	15.52	0.46
	15-Jun-94	Rochester		3	-27.99	1.50	3	16.43	0.53
	15-Jun-94	Oswego	75m	8	-26.76	0.83	5	17.42	1.25
	15-Jun-94	Oswego	95m	4	-26.02	0.13	4	16.95	0.56
	15-Jun-94	Oswego	130m	8	-27.95	0.47	8	17.38	1.13
	18-Jul-94	Main Duck Isl.		3	-27.82	0.79	2	13.95	
	Apr.-May, '92	*Kiriluk (NW basin)*		*9*	*-28.74*	*0.38*	*9*	*12.89*	*0.25*
Alewife									
	15-Jun-94	Olcott		6	-24.12	1.13	5	14.10	0.27
	15-Jun-94	Rochester		4	-24.16	0.88	4	14.16	0.13
	15-Jun-94	Oswego	55m	5	-25.19	0.77	5	14.90	2.38
	15-Jun-94	Oswego	75m	6	-24.02	0.83	3	13.17	0.29
	15-Jun-94	Oswego	95m	10	-23.80	0.66	5	13.94	0.99
	15-Jun-94	Oswego	130m	4	-23.45	1.52	4	12.80	2.02
	15-Jun-94	Main Duck Isl.		4	-25.30	1.15	4	12.70	0.05
	18-Jul-94	Main Duck Isl.		4	-24.01	0.74	4	12.60	0.74
	21-Sep-94	Main Duck Isl.		5	-24.33	0.76	5	12.30	0.45
	Apr.-May, '92	*Kiriluk (NW basin)*		*8*	*-24.85*	*0.23*	*8*	*12.93*	*0.30*
Smelt									
	15-Jun-94	Olcott		4	-27.20	0.70	4	15.69	0.26
	15-Jun-94	Rochester		5	-27.69	1.51	5	15.95	0.12
	15-Jun-94	Oswego	55m	8	-27.89	1.57	8	14.80	0.99
	15-Jun-94	Oswego	75m	7	-27.88	0.99	4	15.45	1.10
	15-Jun-94	Main Duck Isl.		5	-26.00	0.60	5	15.15	0.24
	18-Jul-94	Main Duck Isl.		2	-26.81		4	16.00	0.84
	21-Sep-94	Main Duck Isl.		4	-26.70	0.78	3	15.20	0.91
	Apr.-May, '92	*Kiriluk (NW basin)*		*8*	*-25.99*	*0.43*	*8*	*14.12*	*0.19*

Discussion

The strong seasonal trend in $\delta^{13}C$ of zooplankton and POM is a function of seasonal differences in the concentration and isotope composition of dissolved inorganic carbon (DIC) available to primary producers (Chapter 3). Seasonal changes in the isotope composition of primary producers are reflected in the $\delta^{13}C$ of forage fish (Fig. 6.4, 6.5). The $\delta^{13}C$ of forage fish can therefore be used to determine where, and in the case of Alewife, also *when*, they are obtaining the majority of the carbon assimilated as new tissue. The $\delta^{15}N$ of available sources can be used to roughly apportion the diet of forage fish where there is sufficient difference between competing sources to tell them apart.

The distinction between forage fish on the basis of $\delta^{13}C$ is a function of seasonal change in the $\delta^{13}C$ of primary productivity and the mass flux of particulate organic carbon to the sediment (Chapter 5). The $\delta^{13}C$ of benthic amphipods was determined to be seasonally stable and consistent with particulate organic carbon from the spring bloom (Chapter 5). This result lends support to the hypothesis that organic carbon produced over the winter and during the spring bloom, which isn't grazed as effectively as summer primary productivity, dominates the organic carbon found on the sediment surface (Schelske and Hodell 1991). It was hypothesized that the reason for the difference in signature between *Diporeia hoyi* and zooplankton was that *D. hoyi* was using this carbon as a source of nutrition and therefore tends to have a $\delta^{13}C$ which reflects that usage. In the epilimnion, $CO_{2(aq)}$ concentrations drop as the lake gets warmer and productivity increases, resulting in an increase in the $\delta^{13}C$ of primary producers and organisms feeding on primary

producers (Chapter 3). This results in a separation between organisms inhabiting the epilimnion and metalimnion and those found primarily in the hypolimnion and on the sediment surface, on the basis of $\delta^{13}C$. In the case of *Mysis relicta*, which migrates between the metalimnion and the sediment surface, the observed $\delta^{13}C$ is intermediate between the two extremes (Chapter 5). Alewife feed in the epilimnion and so have a higher $\delta^{13}C$ (Brandt 1986; Kiriluk *et al.* 1995; Urban and Brandt 1993). Sculpin feed on benthos and so have a more negative carbon signature. Smelt are believed to feed predominantly on *M. relicta* and so similarly have a signature intermediate between the benthic and pelagic sources of carbon.

The great exceptions within this scenario of benthic/pelagic separation on the basis of carbon signatures are the oligochaetes which were obtained in the box core samples. The enrichment of the oligochaetes is probably a function of their diet in relation to the diet of other benthic invertebrates. Oligochaetes feed immediately beneath the sediment surface. They ingest sediment particles and remove bacteria feeding on the organic material in the sediment as the ingested sediment passes through their alimentary canal (Dermott, D.F.O. Canada, pers. com.). The observed enrichment in oligochaetes is possibly a function of both trophic enrichment as well as biogenic degradation of organic matter (Kikuchi and Wada 1996). A number of studies have observed an enrichment in $\delta^{15}N$ associated with sediment diagenesis (Wada 1980, Macko *et al.* 1993). Peptide bond hydrolysis is one of the primary processes in protein degradation within sediments, preferential cleavage of ^{14}N, C-N bonds will result in enrichment in residual peptides and amino acids (Macko *et*

al. 1993, Appendix 3). Oligochaetes feeding on bacteria which has assimilated these enriched sources of organic nitrogen may have a greater level of enrichment than might be expected from trophic fractionation. The observed $\delta^{13}C$ of oligochaetes is more enriched than the $\delta^{13}C$ of *Diporeia hoyi* with the lipid removed. The enrichment may also be the result of sediment diagenesis. Generally, diagenetic breakdown of organic matter has been associated with preferential removal of relatively light lipid carbon (Macko 1981, Macko *et al.* 1993). However, the oligochaetes signatures suggest the uptake of enriched carbon sources. Carboxyl groups of amino acids tend to be enriched and their removal may enrich the organic carbon in the sediment (Macko *et al.* 1993). There may also be some degree of trophic enrichment between the oligochaetes and their food source (DeNiro and Epstein 1978). For these reasons the isotope signature of oligochaetes was not considered to be representative of the benthic organic material available to the forage fish and invertebrates feeding at the sediment surface.

Sculpin

The $\delta^{13}C$ of sculpins at all sites suggests a diet highly dependent on benthic sources of carbon. Studies of sculpin gut contents indicate that *D. hoyi* is their main food source and that *M. relicta* is of secondary importance (Christie *et al.* 1987; Kiriluk *et al.* 1995). The $\delta^{13}C$ of sculpin is consistent with this scenario. The $\delta^{13}C$ of sculpin collected at Olcott was significantly lower than the fish from Oswego. The observed difference was not related to differences in lipid content between samples. The observed difference may

be a function of increased recycling of particulate organic material in the surface sediments along the south shore.

The variation in the $\delta^{15}N$ of sculpin is interesting, not only in the observed range and differences between sites, but also in where the most enriched and depleted signatures were found to occur. Enrichment in the $\delta^{15}N$ of fish within different lakes has been correlated with the population density surrounding a lake basin (Cabana and Rasmussen 1996). Particulate organic sewage inputs are likely to result in ^{15}N enrichment of sediments. Isotopically enriched dissolved inorganic nitrogen from sewage outfall is similarly likely to result in spatial patterns in the $\delta^{15}N$ of primary producers incorporating that inorganic nitrogen. It might be expected that spatial differences in $\delta^{15}N$ of benthos would exist related to the sewage outfalls of the major metropolitan centres situated around Lake Ontario. This could ostensibly be used as a reason for the observed broad range in $\delta^{15}N$ of sculpins. What is surprising is that the lowest $\delta^{15}N$ observed in sculpin was for the samples collected in 1992 by Kiriluk *et al.* (1995). All of the samples from this earlier study were collected from the north west basin of the lake, on either side of the Toronto sewage outflow nearest the largest population density in the basin (Kiriluk *et al.* 1995). That fact that the $\delta^{15}N$ of sculpin at all sites along the south shore were elevated in relation to the samples collected on the north shore and western basin could be indicative of the extent to which benthic production along the south shore is driven by inputs from the Niagara River. Studies of the distribution of mirex and hydrophobic organic contaminants into Lake Ontario from the Niagara River demonstrated a pattern of

dispersal from the river along the south shore (Whittle and Fitzsimmons 1983). The high $\delta^{15}N$ of sculpin collected near Oswego may be a function of the extent to which this portion of the lake acts as a recipient for particulate inputs from Rochester and the Niagara River. Studies of sediment accumulation suggest particulate from the river settles predominantly in the deeper basin of the lake near Oswego. If sediment organic matter is resuspended and recycled as it moves along the south shore the isotope signatures of carbon and nitrogen would be expected to increase. Organisms feeding on recycled organic material would subsequently have elevated isotope signatures. Recycling of sediment POM may be responsible for the range of signatures observed in sculpin along the south shore of Lake Ontario.

Alternatively, elevated $\delta^{15}N$ of particulate organic matter could be a function of elevated levels of production and/or nitrification producing enrichment in source DIN (Chapter 4). However, the range in $\delta^{15}N$ of alewife, which feed predominantly on primary consumers in the epilimnion, is approximately 2.0 ‰, less than half the range observed in sculpin. If within lake processes were responsible for the wide range in $\delta^{15}N$ of sculpin a similar range would be expected in all of the forage fish. Incorporation of oligochaetes into the diet of sculpins would also increase the $\delta^{15}N$ and $\delta^{13}C$ of sculpins simultaneously. However, it is unclear why sculpins at Oswego would feed more heavily on oligochaetes than sculpin at other sites. It seems most likely that the source of enrichment is recycling of particulate from the Niagara River.

Smelt

Gut content analysis and studies of the diet of smelt indicate that while they are more likely to be piscivorous than other forage fish they rely primarily on *M. relicta* and secondarily on *D. hoyi* as a source of food (Urban and Brandt 1993; Kiriluk *et al.* 1995). The $\delta^{13}C$ of smelt was consistent with this analysis. The $\delta^{13}C$ of *M. relicta* was between the benthic and pelagic sources of carbon (Chapter 5). At all sites except Oswego, the $\delta^{13}C$ of smelt was also intermediate the benthic and pelagic sources. However the $\delta^{13}C$ of smelt from all the sites along the south shore was more negative than the lipid adjusted $\delta^{13}C$ of mysid from mid-lake or from Oswego, approximately -26.0 ‰, which suggests that these fish have additional benthic dietary sources. The opposite is true of samples collected on the north shore and western basin. The average $\delta^{13}C$ of smelt at the northwest end of the lake is closer to the signature of alewife than any of the other samples collected, potentially indicative of a greater dependence on pelagic sources of carbon than fish collected on the south shore.

The uniformity and magnitude of the $\delta^{15}N$ of smelt suggests a diet dominated by *M. relicta*. The similarity in observed $\delta^{15}N$ of smelt reflects a dependence on pelagic sources of energy which appear to be fairly consistent throughout the lake. If most of the smelt diet had been obtained from benthic sources a range in signatures similar to sculpin might be expected. The $\delta^{15}N$ of *M. relicta* collected in the spring and fall of 1995, at the mid-lake site was 12.5 ‰ and 10.0 ‰ respectively (Chapter 5). In contrast, the spring and fall nitrogen signatures for *M. relicta* collected in the northwestern part of the lake were 11.0

and 9.0 ‰ respectively in 1992 (Kiriluk *et al.* 1995). The $\delta^{15}N$ of *M. relicta* collected at Oswego in October had a $\delta^{15}N$ of 12.0 to 13.0 ‰ (Chapter 5). The approximately 1.0 to 2.0 ‰ difference in the $\delta^{15}N$ of *M. relicta*, between the north and south shores, is reflected in the smelt collected at either location.

The observed difference in $\delta^{15}N$ between smelt and autumn *M. relicta/D. hoyi* at Oswego was 3 ‰, consistent with a difference of one trophic level. The difference in $\delta^{15}N$ between *M. relicta/D. hoyi* and smelt was 4 ‰ in 1992 (Kiriluk *et al.* 1995) and 4.5 ‰ in 1994 (Main Duck Island). The jump is greater than that expected for a single trophic level and is potentially indicative of some level of piscivory in smelt at these sites and times (Kiriluk *et al.* 1995). If *M. relicta* comprised 65 to 70 % of the diet of smelt, and trophic enrichment was assumed to be 2.5 to 3.0 ‰, then the remainder of the diet would be expected to have a $\delta^{15}N$ of approximately 16.5 ‰ in 1994 and 14.0 ‰ in 1992. These $\delta^{15}N$ values are similar to those of smelt present in each year (14.0 ‰ in 1992, and 14.0 to 16.0 ‰ in 1994), and may indicate some cannibalism of juveniles (Brandt and Mason 1986). Sculpin have a lower $\delta^{15}N$ and $\delta^{13}C$ at these two times and locations and are therefore unlikely to be the 'missing dietary item'. Alternatively, if the $\delta^{15}N$ of *M. relicta* in the spring is representative of the signature of mysids all winter, the $\delta^{15}N$ of smelt may be indicative of reliance on *M. relicta* and *D. hoyi* as a steady and available source of food year round.

Alewife

The isotope model analysis/isotope mass balance supports the conclusion made by Hewitt and Stewart (1989) that the grazing by alewife is highly seasonal and also suggests that summer grazing on zooplankton supplies most of the carbon used by alewife in growth. At all the sites where alewife was collected, the $\delta^{13}C$ of alewife was found to be between - 23.0 and - 25.0 ‰. This signature is consistent with the seasonal maximum values of particulate organic matter (POM) from primary production in the water column during the summer, and the zooplankton feeding on that POM (Fig. 6.1, 6.2, 6.3). This suggests that the majority of the carbon assimilated by alewife consists of zooplankton, and also that the majority of the carbon assimilated was ingested between July and October. A detailed seasonal analysis of alewife diet in Lake Michigan determined that predation by alewife was strongly seasonal (Hewitt and Stewart 1989). Approximately 45 % of the total annual consumption occurred in August and September and 73 % occurred during July through October (Hewitt and Stewart 1989). These assertions were tested by isotope mass balance using the $\delta^{13}C$ of alewife and zooplankton collected near Main Duck Island as an example (Table 6.1, 6.2, Fig. 4.3). The observed seasonal range in $\delta^{13}C$ of zooplankton was -29.0 ‰ to -22.0 ‰ (Fig. 6.2, 4.3). *D. hoyi* was -28.0 ‰ and *M. relicta* are -26.0 ‰ to -28.0 ‰. A factor of 1 ‰ may be added to all of the dietary sources as an allowance for any trophic enrichment that may occur. If 25 % of the diet of alewife consisted of organisms with a $\delta^{13}C$ of -27.0 ‰ or less, then at least 75 % of its

assimilated carbon would have to have a signature -24.0 ‰ or greater in order for alewife to have a $\delta^{13}C$ of -24.0 ‰. The only pelagic source of carbon that enriched in ^{13}C is zooplankton from late July through early October.

The model, used in analyzing the dietary apportionment of alewife from Hewitt and Stewart (1989), suggests that the diet as described is a fairly accurate approximation. However, the difference between the observed and predicted $\delta^{13}C$ and $\delta^{15}N$ of alewife may indicate that the dietary apportionment is skewed toward isotopically lighter sources of carbon and nitrogen. The amount of *D. hoyi* in the diet from July through October may be less than what is indicated and the percentage of copepods greater. This would result in an increase in $\delta^{13}C$, the $\delta^{15}N$ would remain roughly the same. Alternatively, the difference between the predicted and observed signatures may be ascribed to trophic enrichment. A 1.0 ‰ enrichment in carbon and a 3.0 ‰ instead of 2.5 ‰ enrichment in $\delta^{15}N$ would erode the difference between real and predicted values observed.

The model suggests that seasonal stability in the $\delta^{13}C$ and $\delta^{15}N$ would not likely be observed in this fish population until the fish attain a weight of approximately 30 g after 3 years. Prior to this, the seasonal fluctuations in isotope signatures of the alewife diet in Lake Ontario are likely to be reflected in the $\delta^{13}C$ and $\delta^{15}N$ of alewife, given the pattern of growth described by Hewitt and Stewart (1989).

The results of the analysis of alewife diet using the stable isotope/allometric model are based on several assumptions that should be addressed. It was assumed that there was no change in isotope signature associated with weight loss and that metabolic turnover was zero. In laboratory studies of growing broad whitefish most of the change in isotope signature was associated with growth dilution (Hesslein *et al.* 1987). In relation to growth, metabolic fractionation was negligible (Hesslein *et al.* 1987). Since there was no somatic increase when alewife lost weight there could be no change in isotope signature related to growth dilution. However, the laboratory studies did not test fractionation under conditions of starvation. With respect to carbon isotopes, changes in $\delta^{13}C$ during starvation are likely to be related to changes in the relative proportion of tissues making up the organism. The loss of more depleted lipids and carbohydrates may leave the fish more enriched. Metabolic fractionation of nitrogen are a possibility. In the catabolism of proteins cleavage of isotopically lighter peptide bonds and preferential excretion of ^{14}N ammonium should produce an increase in the proportion of nitrogen which is ^{15}N. However, a mass balance suggests that the weight loss would have to be severe to observe a substantial change in $\delta^{15}N$. Overall, the model should provide an effective means of testing dietary apportionment.

Conclusions

The wide range and fluctuation in $\delta^{15}N$ within this system makes the accurate assessment of the relative trophic status of individual fish on the basis of stable isotopes very difficult

in this lake. The $\delta^{15}N$ of lake trout will likely depend as much on where it was eating as on what it was eating. The wide range in $\delta^{15}N$ of sculpin is probably the reason for the wide range in $\delta^{15}N$ of lake trout observed by Kiriluk *et al.* (1995) since slimy sculpin constitutes a large portion of the diet of lake trout, especially in juveniles (Elrod 1983; Brandt 1986). The range of lake trout migration may span the entire lake. A lake trout caught in the east basin may have been feeding along either the south or north shore before being caught and subsequently may have a high or low signature related to where it obtained most of its food.

The use of stable isotope analysis has been shown in this study to be an effective tracer of nutrient sources in forage fish which do not have the range of the major predatory species in the lake. Isotopes were demonstrated in this study to be useful in discerning the diet of fish and where and when they were feeding. In studies of diet on the basis of relative stable isotope compositions, consideration must be given to the potential sources of nutrition and the allometry of those sources. Organisms should be sampled on a time scale consistent with their rates of tissue turnover so that fluctuations in the baseline may be accounted for and used in analysis.

Chapter 7

General Conclusions and Research Implications with Respect to Organic Contaminant Concentrations in Lake Ontario Biota

The research described in the preceding chapters has addressed several questions and established a number of avenues for future research directions. The primary objective of this study was to attain a better understanding of the dynamics of the Lake Ontario pelagic food web using carbon and nitrogen stable isotopes. The information obtained from stable isotope analysis can be applied in making inferences on how HOCs are cycled within the system. Another goal was to develop a better understanding of what determines the carbon and nitrogen isotope signatures of each organism, from the assimilation of nutrients at the baseline to top predators. This chapter reviews and draws upon some of the results in previous chapters to provide a broader synthesis of earlier conclusions and to address the objectives described. Recommendations are made as to how future research endevours might benenfit from consideration of the results of the studies described in this thesis.

The $\delta^{13}C$ of particulate organic matter (POM) in Lake Ontario may be altered by any process changing the relative magnitudes of fluxes or pools of inorganic carbon within the carbon cycle of the lake. A comparison of the seasonal fluctuation in $\delta^{13}C$ of filtered POM with estimations of the rate of primary productivity using ^{14}C additions demonstrated that the $\delta^{13}C$ of POM was not solely a function of increased primary

production. The balance of carbon cycling with respect to carbon isotopes suggested that many sources contribute to the $\delta^{13}C$ of POM.

The $\delta^{13}C$ of primary consumers fluctuated by as much as 10.0 ‰ seasonally. In contrast, the $\delta^{13}C$ of benthic primary consumers remained relatively constant on a seasonal basis. The $\delta^{13}C$ of benthic invertebrates was consistent with the $\delta^{13}C$ of POM produced in the spring and late fall which is consistent with the hypothesis that benthic primary consumers rely on carbon produced early in the seasonal cycle (Shelske and Hodell 1991). The $\delta^{13}C$ of pelagic primary consumers shifts with the seasonal change in $\delta^{13}C$ of POM, which is a function of the carbonate chemistry of the lake. As a result, the $\delta^{13}C$ of pelagic primary consumers is distinct from the benthic primary consumers for much of the year. This feature of the system can be exploited in dietary apportionment of species with both benthic and pelagic food sources.

There may be species specific differences in the $\delta^{15}N$ of algae and differences among zooplankton related to food preference. The $\delta^{15}N$ of primary consumers was expected to be consistently 2.0 to 4.0 ‰ higher than the $\delta^{15}N$ of POM (Owens 1987; Peterson and Fry 1987). Whether or not primary consumers were enriched in ^{15}N relative to POM depended on: the size fraction of POM, the species composition of the POM collected, and the time of year. More work needs to be done investigating the fractionation of nitrogen isotopes in the assimilation of nitrogen by primary producers. The work that has been done suggests a concentration dependence with fractionation and inorganic nitrogen

uptake which may partially explain why nitrogen isotope signatures appeared to be higher when nitrate concentrations were more depleted (Pennock 1987; Montoya 1990; Pennock *et al.* 1996). A continued fractionation in uptake favoring more depleted nitrate would leave the remaining pool of nitrate enriched in $\delta^{15}N$. If most of the species of primary producers making up the algal community were dependent on nitrate as a source of DIN the enrichment in ^{15}N in nitrate would be reflected in the POM.

The observed seasonal pattern in $\delta^{15}N$ of POM and zooplankton is a function of the cycling of inorganic nitrogen and the level of dependence of primary producers on nitrate as a source of inorganic nitrogen in Lake Ontario. There are numerous inputs of inorganic nitrogen into Lake Ontario which produce the high concentrations of DIN observed. While the concentration of DIN is high, the concentrations of ammonium remains low enough for nitrate to be the predominant but not the sole source of inorganic nitrogen for primary producers (Pennock 1987). The baseline $\delta^{15}N$ of POM is determined not only by the inputs of DIN, but also by: the seasonal succession of algal species and the form of inorganic or organic nitrogen they use; fractionation in the uptake of inorganic nitrogen by primary producers; bacterial reduction and oxidation of inorganic nitrogen through processes of denitrification and/or nitrification.

In Lake Ontario, stratification leads to a separation of available inorganic nitrogen into two layers influenced by different biogeochemical processes as the season progresses. By

the end of the stratified period there is a difference between the $\delta^{15}N$ of nitrate from the epilimnion and the $\delta^{15}N$ of nitrate from the hypolimnion. Lake mixing in the fall serves to reset the $\delta^{15}N$ of nitrate to lower levels as the more ^{15}N depleted nitrate from the hypolimnion is mixed with more ^{15}N enriched nitrogen from the epilimnion. This is what determines the seasonal pattern of change in the $\delta^{15}N$ of POM and partially determines the baseline $\delta^{15}N$ signature for isotope studies of the pelagic food web of Lake Ontario.

In addition to the evidence supporting this conclusion presented in Chapter 4 the following may be considered. The consistency of the $\delta^{15}N$ of POM and zooplankton between sites, and the consistency in the observed level and timing of the seasonal pattern of fluctuation in $\delta^{15}N$ from year to year, indicates that the shift is due to a system wide process and not a sewage plume or storm event. The consistency in the $\delta^{15}N$ of alewife from sites around the lake further suggests that the $\delta^{15}N$ of their primary food sources, POM and zooplankton, are similar throughout the lake.

Relative to many other northern temperate lake systems Lake Ontario would be considered rich in DIN. The level of fractionation expressed as a result of within lake processes may be maximized since the pools of available inorganic nitrogen are relatively abundant. In more pristine systems, without the inputs of DIN that Lake Ontario receives, the available nitrogen will be more tightly cycled, potentially limiting the opportunity for isotope fractionation within the system. However, in order for there

to be no fractionation it must be assumed that available DIN is depleted largely through a single unidirectional process, i.e., that a particular pool is converted entirely into another form of nitrogen by one process, with no additions, resulting in no fractionation (Peterson and Fry 1987). More realistically, a number of different competing processes will be acting on any particular pool of available DIN, each with its own reaction kinetics and process specific level of fractionation. A tighter nitrogen cycle might dampen these isotope effects but it would seem unlikely that they would disappear entirely and so it might be expected that the $\delta^{15}N$ of POM would fluctuate seasonally in many systems.

Some of the observations made in this study may be applied in developing a hypothesis of the routes of contaminant transport within the Lake Ontario pelagic food web. The magnitude and direction of contaminant mass flux within the pelagic system will be influenced by a number of different factors acting in unison to establish the concentrations of contaminant available at the base of the food web. The body burden of HOCs within epilimnetic primary consumers will be determined by the concentrations of HOCs in their diet. Therefore, dietary preferences, the seasonal succession of algal species, biomagnification, the kinetics of HOC uptake by phytoplankton and the equilibrium partitioning of HOCs, will all be determining factors of primary consumer HOC body burden.

The observation that diatoms are not grazed extensively by calanoid and cyclopoid copepods in the epilimnion in the spring, and that the $\delta^{13}C$ signature of *Diporeia hoyi* is consistent with carbon produced during the spring bloom, supports the contention that sedimentation of diatoms may play a central role in contaminant fate and transport (Baker *et al.* 1986). There are several factors influencing the uptake of HOCs by primary producers such as: the cell surface area, the lipid content, the composition of the lipid, and the cell growth rate (Stange and Swackhamer 1994). The kinetics of phytoplankton HOC uptake are biphasic, with a fast adsorption phase followed by a slower equilibrium-partitioning phase (Richer and Peters 1993; Stange and Swackhamer 1994). Laboratory studies of the kinetics of PCB bioaccumulation by phytoplankton demonstrated that after the initial adsorption of PCBs to the cell surface, the rate of equilibration between primary producers and the surrounding media was slow in relation to the kinetics of phytoplankton growth under bloom conditions (Swackhamer and Skoglund 1993). As a result, faster growing algal cells are less likely to reach an equilibrium steady-state with the surrounding media (Skogland *et al.* 1996). Diatoms have a higher lipid content than many algal species, a slower growth rate and longer residence time than primary producers growing in the summer months (Shifrin and Chisholm 1981). Therefore diatoms have a greater opportunity to reach an equilibrium with the water column with respect to HOC concentration. If diatoms are not grazed extensively in water column, they would effectively act as a transport vector for HOCs to the sediment. In contrast, primary producers growing later in the season may be less able to reach a equilibrium steady-state with the HOCs in the surrounding water. As a result, food sources for

primary consumers during the summer months may have a lower contaminant concentration than the food sources of benthic consumers, even if concentrations of HOCs in the water column stay relatively consistent.

Seasonal changes in the physical conditions within the lake may also serve in reducing contaminant bioavailability. Seasonal 'whiting', the precipitation of calcite from the water column, is a feature of the system (Lean *et al.* 1987). The surface adsorption of HOCs to particulate may limit the bioavailability of HOCs to coexisting algae (Voice 1983, Baker *et al.* 1996, Taylor *et al.* 1996). Surface adsorption of HOCs to precipitating calcite would further serve in shunting contaminant from the water column to the sediments.

The discrepancy between $\delta^{15}N$ and contaminant concentrations of forage fish reported by Kiriluk *et al.* (1995) may be partially explained by a seasonal variation in HOC loading by primary producers. The sedimenting algae from the spring bloom, comprised largely of diatoms, would contain a higher concentration of HOCs per unit mass than primary producers growing in the summer. Benthic invertebrates feeding at the sediment surface would have an elevated body burden of HOCs relative to pelagic primary consumers feeding on primary producers in the epilimnion (assuming their rate of ingestion relative to their body mass is nearly similar). Sculpin rely primarily on benthic sources of energy (Brandt and Madon 1986). Therefore sculpin would be expected to have higher body

burdens of HOCs than fish feeding in the water column. Alewife, feeding at approximately the same trophic level as sculpin, would have a lower contaminant body burden, since their diet would be comparatively less contaminated. The apportionment of the diet of *Mysis relicta* is reflected in the contaminant loading of smelt. The benthic component of the diet of *M. relicta* would have an increased contaminant load, the diel migration of *M. relicta* recycles benthic carbon and contaminants to smelt and alewife. Since the diet of smelt is dominated by *M. relicta* it has a higher $\delta^{15}N$ than the other forage fish collected on the north shore reflecting a slightly elevated trophic status. However, the observed body burdens of HOCs in smelt are no greater than sculpin since 60 to 70 % of the contaminant loading of smelt has a pelagic origin (Metcalfe and Metcalfe 1997).

It is difficult to characterize the influence of POM recycling on the HOC loading of forage fish using stable isotopes because the routes of isotope enrichment are likely to diverge from routes of enrichment of contaminant concentrations. The organic contaminant concentrations of recycled organic material will move toward equilibrium with the surrounding media or drop as contaminant is removed through bioconcentration in benthic organisms (Morrison *et al.* 1997). In contrast, as the POM is recycled it will become more enriched in ^{13}C and ^{15}N. Sculpin collected at the east end of the south shore should therefore have similar or slightly lower contaminant levels to fish caught closer to the Niagara River even though the $\delta^{15}N$ signature increases.

The large range in the $\delta^{15}N$ of lake trout observed by Kiriluk *et al.* (1995) is likely related to the spatial variability in the $\delta^{15}N$ signatures in sculpin. The $\delta^{15}N$ signatures in adult lake trout will be strongly influenced by their diet during the early, rapidly growing juvenile phase when they feed primarily on sculpin (Elrod 1983). The contribution of sculpin to the diet of adult lake trout is reduced significantly, as they shift their feeding to more pelagic species (Brandt 1986). However, the nitrogen signature of lake trout muscle is likely to turn-over slowly, since the shift in diet occurs over two to three years and much of the muscle mass of a 4 year old lake trout is accrued during its juvenile stages of development (Elrod 1983). With limited growth dilution, the previous dependence on sculpin in the diet of lake trout will be reflected in their $\delta^{15}N$ signatures. The high variability in the $\delta^{15}N$ signatures of lake trout is therefore a result of current and past feeding of sculpin which are spatially variable in their $\delta^{15}N$ signatures. This limits the use of $\delta^{15}N$ signatures of lake trout tissue to infer their trophic status and therefore to predict bioaccumulation of HOCs for individual trout.

The research described in previous chapters of this thesis has demonstrated the utility of stable isotopes as a tool in understanding the dynamics of the Lake Ontario pelagic food web. This work can be further advanced by incorporating biomass estimates and rates of ingestion with these findings to quantitatively estimate the flow of carbon within the system and move closer toward a mass balance of carbon. Additionally, this research has demonstrated that stable carbon and nitrogen isotopes used as tracers should not be

considered a replacement for other means of determining trophic relationships. There are many variables in addition to trophic effects that can influence isotope signatures. What was shown was that stable isotopes can be a valuable tool when used to augment other forms of analysis in elucidating the trophic dynamics of a system.

Recommendations

- If stable isotopes of carbon and nitrogen are used to make inferences on the relative trophic position of organisms within a system, the organisms and their food sources should be sampled on a time scale consistent with their rate of tissue turnover. This is necessary in order to effectively characterize the relative isotope signatures of organisms and their variability within species as well as the isotope signatures of their forage base.

- The potential forage base for any organism should be characterized as fully as possible - generalizations should be avoided unless the biogeochemistry of a system is understood sufficiently to enable prediction.

- Attention should be paid to system inputs and the influence of inputs, such as the Niagara River in this study, on the signatures of organisms within the system.

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Appendix 1

A process and rationale for lipid correction of $\delta^{13}C$ data used in food web analysis

The ratio of ^{13}C to ^{12}C in any given organism is a reflection of it's food source (Peterson and Fry 1987; Fry and Sherr 1984). Since oxidative respiration of source carbon is a nearly complete process, there is little opportunity for isotope fractionation. (Jacobsen 1970). Therefore, the ratio of heavy to light isotopes of carbon (expressed as δ values in units per mil (‰)) will be maintained in the whole organism relative to its food (Fry and Sherr 1984). There are factors besides differences in the isotope composition of source carbon that can produce variability in the $\delta^{13}C$ (carbon signatures) of organisms in aquatic systems, such as the influence of residual carbonate and lipid loading.

There will be tissue specific variation due to differences in the kinetics of metabolism between carbon isotopes (DeNiro and Epstein 1977; Tieszen *et al.* 1983). In particular, fractionation of carbon isotopes occurs in the formation of lipids resulting in lower δ values in this tissue (DeNiro and Epstein 1977). If an organism has a high lipid content relative to its body mass, it may have an artificially low $\delta^{13}C$ (Tieszen *et al.* 1983). Temporal differences in lipid loading may cause fluctuation in the $\delta^{13}C$ of an organism that are not related to changes in diet. In order to interpret differences in the carbon isotope signatures on the basis of energy sources, it is necessary to account for sources of variability, other than changes in the isotope composition of the diet carbon. In this section, variation in the $\delta^{13}C$ of *Mysis relicta* due to variable lipid content is examined.

In dealing with variation due to lipid content, there are three routes investigators may follow. Researchers may choose to remove this source of variability by extracting the lipid from an organism before isotope analysis (Sholto-Douglas *et al.* 1991; Hobson and Welch 1992; Malej, Faganeli & Pezdic 1993). They may use some form of normalization of data to account for lipid loading (McConnaughey and McRoy 1979; Rau *et al.*, 1991; Rau *et al.* 1992). Or, they may ignore lipid content as a potential mitigating factor (Yoshioka and Wada 1994; Kiriluk *et al.* 1995; France 1996; Keough *et al.* 1996).

Whether or not the influence of lipid can be ignored will depend on the observed differences between competing sources of carbon and the study's objectives. If there is a large spread in $\delta^{13}C$ of source carbon (in excess of 3.0 ‰), the influence of lipid on the $\delta^{13}C$ of an organism may not confound the determination of which source of carbon has been predominantly assimilated. Ignoring lipid content implies that seasonal variability in lipid levels does not influence the $\delta^{13}C$ of organisms enough to confound interpretation of relative signatures on the basis of food source. It may, however, undermine efforts to obtain any quantitative estimation of the extent of an organism's reliance on different carbon sources. In the study of energy provenance of *Mysis relicta* in Lake Ontario using carbon isotopes, virtually all of the carbon is of autochthonous origin, and any variability is related to seasonal biogeochemical influences (Rosa 1985; Chapter 5). Under these circumstances, differences in $\delta^{13}C$ are subtle. The $\delta^{13}C$ of zooplankton shifts 4.0 to 5.0 ‰ seasonally in response to the change in primary producers (Chapter 3). If the

proportion of zooplankton in the diet is large enough, and the tissue turnover rate of *M. relicta* is fast enough, a seasonal shift of similar magnitude might be expected for *M. relicta*. Lipid content has been observed to vary by as much as 20 % seasonally in studies of *M. relicta* in northern Ontario lakes (Adare and Lasenby 1994). Changes in the lipid content of this magnitude could easily offset any change in the $\delta^{13}C$ of mysids due to changes in food source and so might lead to error in interpretation. For the purposes of this investigation, it was considered advantageous to be able to deal with the influence of lipid on $\delta^{13}C$ in some manner.

Lipid Extraction

In addition to added time and energy associated with sample preparation, lipid extraction alters the sample composition. On the surface, lipid removal seems to be at odds with the purpose of most stable isotope surveys of food webs. Lipid is the major reserve of food energy in organisms. If the objective in stable isotope analysis is to determine an organism's source of energy, why would an investigator remove the major reserve of food energy? While fluctuating lipid levels may confound interpretation wouldn't lipid removal be just as detrimental to interpretation? The answer lies in how the body processes food. Lipids in food which are not burned right away to meet an organism's energy requirements will be stored in the form in which they are ingested, as lipid (Lehninger 1981).

This conservation has been exploited through the analysis of fatty acid structure undertaken as means of obtaining similar information to stable isotope analysis (Iverson *et al.* 1996). Alternatively, proteins and carbohydrates can be taken up, broken down, assimilated in the formation of new proteins and carbohydrates and either used as food energy or stored as lipid (Lehninger 1981). Since lipid, which is ingested generally, isn't assimilated into other bodily tissues, the carbon isotope signature of the ingested lipid won't be reflected in the tissues of an organism with the lipid extracted. Given this, if the carbon isotope signatures of all of the potential sources of carbohydrate and protein potentially ingested are known, then all of the tools are available to trace the path of energy transfer. There is a consequence in that lipids formed by the organisms are isotopically light (contain more ^{12}C), and therefore the remaining tissues will be isotopically enriched and heavier than their food source. This is probably why trophic enrichment of carbon has been observed in so many studies (McConnaughey and McRoy 1979; Rau *et al.* 1983; Fry and Sherr 1984; Sholto-Douglas *et al.* 1991). Ingested carbohydrates and proteins not assimilated are stored as lipid, mass balance would suggest that this lipid will be enriched relative to the ingested lipid. As the pool of lipid reserves is turned-over, the lipid pool and the remaining tissue will become more enriched. The depleted lipids are 'burned' and a slightly depleted CO_2 is respired leaving the enriched tissues behind. The process of lipid formation and respiration should, therefore, ensure that there is trophic enrichment of carbon isotopes with successive jumps up the food-chain. It also suggests that the level of trophic enrichment of carbon

isotopes may be dependent on the adequacy of the food supply to meet the energetic needs of an organism.

There are other considerations with lipid extraction. As in this study, much of the isotope analysis currently being published was performed on continuous-flow isotope-ratio mass spectrometers (CF-IRMS) that produce both $\delta^{13}C$ and $\delta^{15}N$ numbers in a single analysis. There has been much written about the potential for alteration of $\delta^{15}N$ numbers by acid addition to samples in order to remove residual carbonates. The same potential dangers may exist with lipid extraction.

As a component of the seasonal study of the diet of *M. relicta* in Lake Ontario, using carbon and nitrogen isotopes as tracers of food source, the effects of lipid extraction on carbon and nitrogen isotope signatures were investigated, Mysids, collected monthly from Station 41 in Lake Ontario, were used. The influence of lipid extraction on composite samples of zooplankton was tested, as was the difference between lipid extraction and storage in 99.9% ethanol. A method of preservation was critical in order to facilitate cleaning and separation of zooplankton. Pure ethyl alcohol was chosen since it would not 'fix' itself in the tissues as would formalin. Therefore, it could be rinsed away completely, so that the signature of the tissue analyzed did not include the signature of ethyl alcohol. However, ethanol is likely to alter the sample composition by leaching soluble lipids out of the organisms into the solvent. This could be considered as a

benefit, if the lipid removal associated with sample storage in ethanol is extensive, further extraction may not be necessary to produce results free of any lipid effects.

Samples collected July 27, 1994 were used as a test. Samples were separated into three groups: untreated samples, samples fully lipid extracted, and samples stored in ethanol. Extraction was carried out by first leaving the sample submerged in methanol for 16 hrs. The methanol was then decanted, and the sample was emersed in DCM and vortex mixed for 10 min., before again decanting the solvent. The DCM addition and vortex mixing procedure was repeated three times. Acetone was then added, and the sample was vortex mixed for 10 min. Methanol was added, and the sample was again vortex mixed for 10 min. Distilled deionized water was used to rinse the sample which was then oven dried at 60 °C. Using the C/N ratio as an indicator of extraction efficiency, there was no significant difference between this method and 24 hr sohxlet extraction of mysids with 50:50 DCM:Hexane. There was no correlation between length or weight of the lipid extracted mysids analyzed and the C/N ratio as a measurement of the efficiency of extraction. Similarly, no significant difference was found between samples stored in ethanol and lipid extracted samples. There was a significant difference in $\delta^{13}C$ between lipid extracted and untreated samples ($F = 15.82$, $p = < 0.0001$) (Fig. A1.1). The measured $\delta^{13}C$ was highly correlated with the C/N ratio in all samples analyzed ($r = -.92$, $p = < 0.0001$); $\delta^{13}C$ was also correlated with the C/N ratio in the lipid extracted

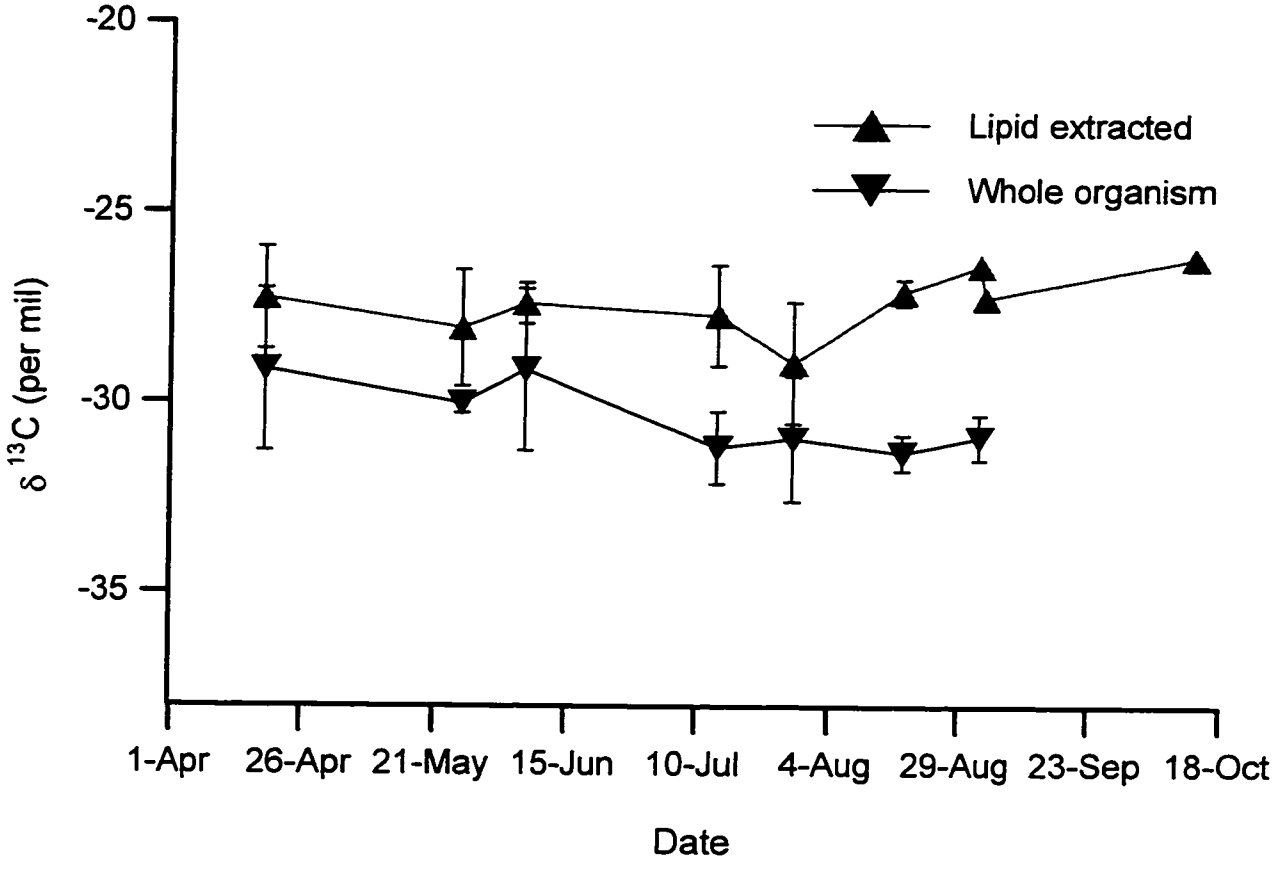

Figure A1.1. Temporal trend in $\delta^{13}C$ of lipid-extracted and whole (untreated) *Mysis relicta* collected in 1994.

subgroup ($r = -0.5372$, $p = 0.003$). This was taken to indicate that less than perfect extraction efficiency would increase the variability observed in the measured $\delta^{13}C$.

Table A1.1 The $\delta^{13}C$ and C/N for ethanol stored, lipid extracted and untreated *Mysis relicta* collected from Station 41, Lake Ontario 1994..

	No Treatment		**Ethanol Storage**		**Lipid Extraction**	
	$\delta^{13}C$	C/N	$\delta^{13}C$	C/N	$\delta^{13}C$	C/N
Mean	**-32.22**	**8.17**	**-28.92**	**4.23**	**-28.58**	**4.28**
Standard Deviation	**0.67**	**1.21**	**0.58**	**0.31**	**1.81**	**0.83**

The hypothesis that lipid extraction altered the $\delta^{15}N$ of the organism was tested ($Ho: \delta^{15}N_{extracted} = \delta^{15}N_{untreated}$). Mysid samples collected the same date were paired with mysids of similar size. One set was placed on tin foil and oven dried at 60 °C. Lipid extraction was conducted using the vortex method described previously. All of the samples were then packed in tin foil cups and analyzed on a VG Optima IRMS. The analysis of standards of known isotope composition and duplicate samples were within 0.3 ‰ of each other or the known value. The mean $\delta^{15}N$ of the untreated samples was 11.6 ‰ with a standard deviation of 1.4 ‰. The mean $\delta^{15}N$ of the extracted samples was 11.4 ‰ with a standard deviation of 1.07 ‰. A two-sample t-test of paired samples of

independent means yielded a pooled-variance $t_{46} = -0.6969$, $p = .49$. This indicates that there is insufficient evidence to reject the null hypothesis that the difference between the two means is zero. The power in this analysis is approximately 0.1 with $\alpha = .05$, not resounding evidence, that the means are indeed the same. However, over 750 mysids would be required (a prohibitive level of preparation and analysis) to be 80 % certain that the means were identical. This study has therefore proceeded under the assumption that lipid extraction may have an effect on $\delta^{15}N$, but the level of influence is minimal. Lipid extraction is unlikely confound interpretation on the relative trophic position of organisms based on the evidence given here.

Lipid Normalization

Lipid normalization of isotope data is clearly the easiest method of dealing with the influence of lipid. However, any normalization must consider that prior to analysis all tissues are combusted to CO_2 which is the substance actually analyzed. Since there is more carbon per unit mass of lipid, the percentage lipid in the sample is likely to underrepresent the contribution of lipid to the overall $\delta^{13}C$ of sample analyzed. Any method of normalization must take this into consideration. Simultaneous analysis of carbon and nitrogen content with IRMS provides a means of circumventing this problem. By using the carbon to nitrogen (C/N) ratio as a surrogate for lipid content a relationship can be derived to 'correct' the $\delta^{13}C$ values measured to a standard C/N. This method was employed by McConnaughey and McRoy (1979), to develop a lipid factor;

$$L = (93/(1 + 1/(0.246(C/N) - 0.775)) \quad \text{(A1.1)}$$

which was then used to calculate a lipid corrected $\delta^{13}C'$;

$$\delta^{13}C' = \delta^{13}C + 6((3.9/(1 + 287/L))-1). \quad \text{(A1.2)}$$

As mentioned above, there was a high degree of correlation between C/N and $\delta^{13}C$ even within lipid extracted samples. In order to characterize the relationship between $\delta^{13}C$ and C/N, the lipid extracted, ethanol stored, untreated mysid samples and the extracted lipid were pooled for larger sample sizes and wider range of variation in lipid contents. All of these samples were plotted and least-squares regression analysis was used to fit the curve(Fig. A1.2, Fig. A1.3). The minimum $\delta^{13}C$ was -35.9 ‰. The minimum C/N ratio was approximately 3.2. These were taken as limiting values of 100 % lipid and 0 % lipid respectively. The -36.0 ‰ value was subtracted from the measured $\delta^{13}C$, the difference being a measure of the level of lipid extraction, which is a lipid analysis factor. The natural logarithm of this value was plotted against the natural logarithm of the C/N ratio to produce a linear relationship (Fig. A1.4).

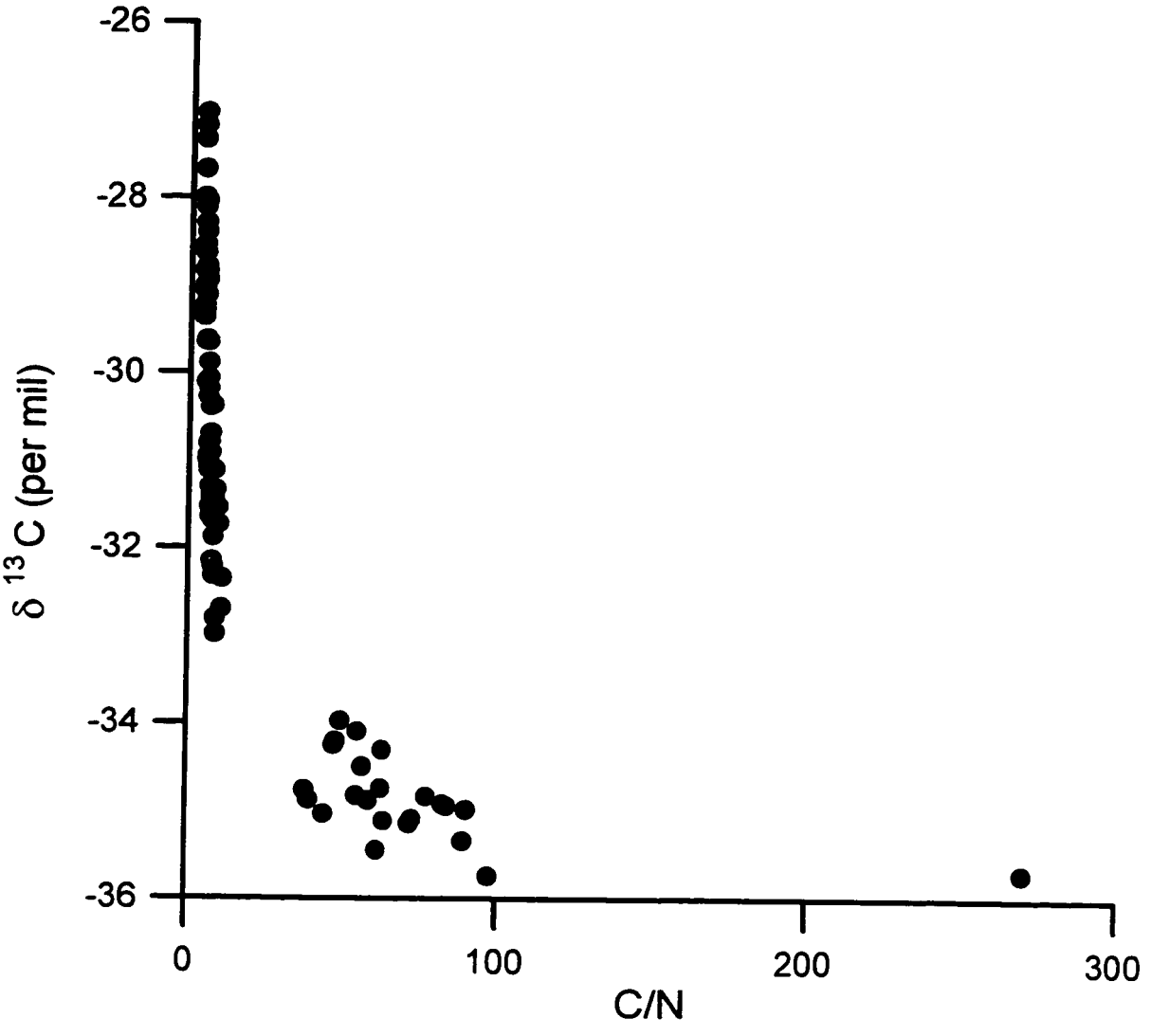

Figure A1.2. Pooled $\delta^{13}C$ vs C/N ratio of *Mysis relicta* collected in 1994 including lipid extracted *M. relicta* and lipid samples.

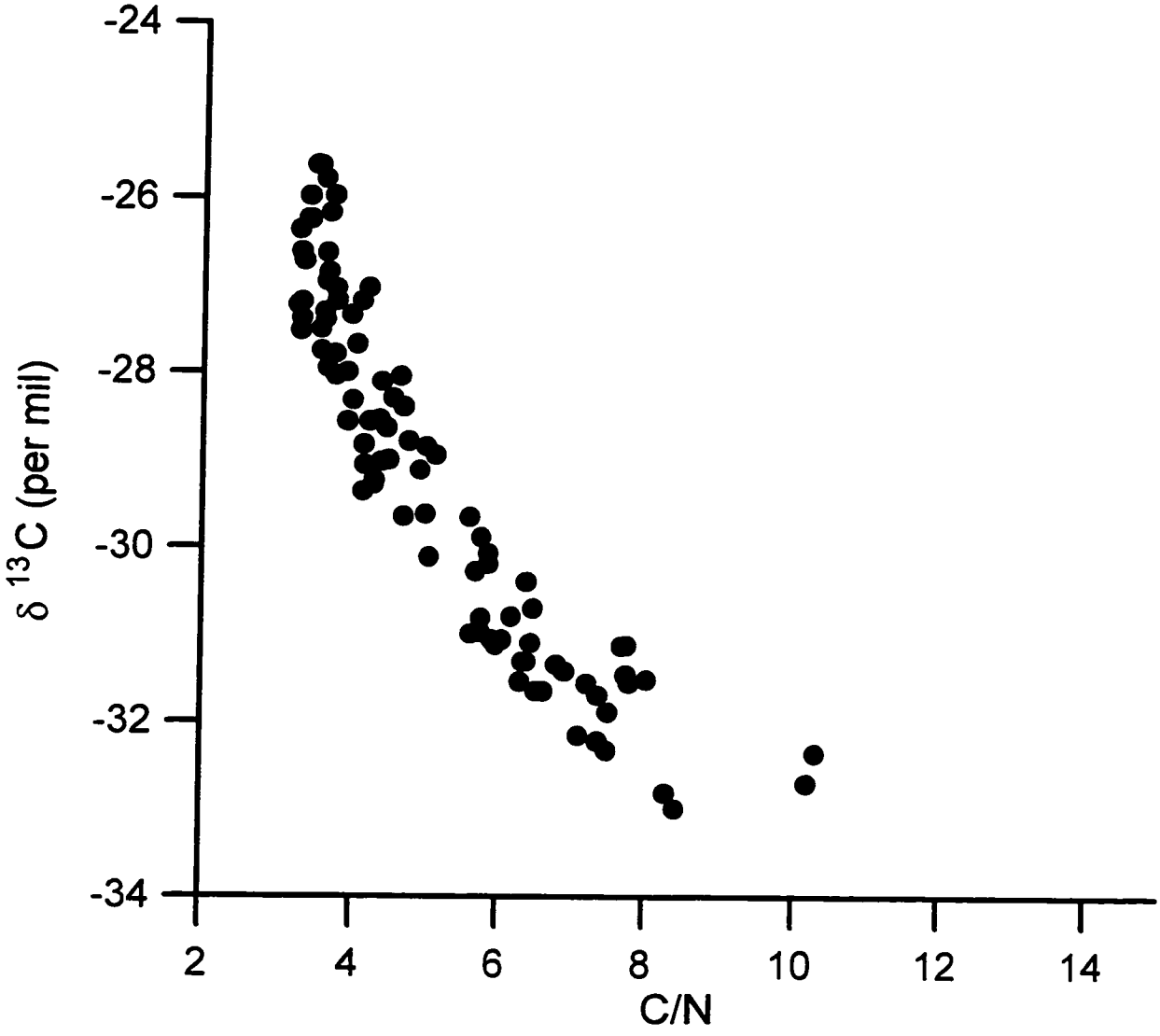

Figure A1.3. Pooled $\delta^{13}C$ vs C/N ratio of *Mysis relicta* collected in 1994 excluding lipid samples.

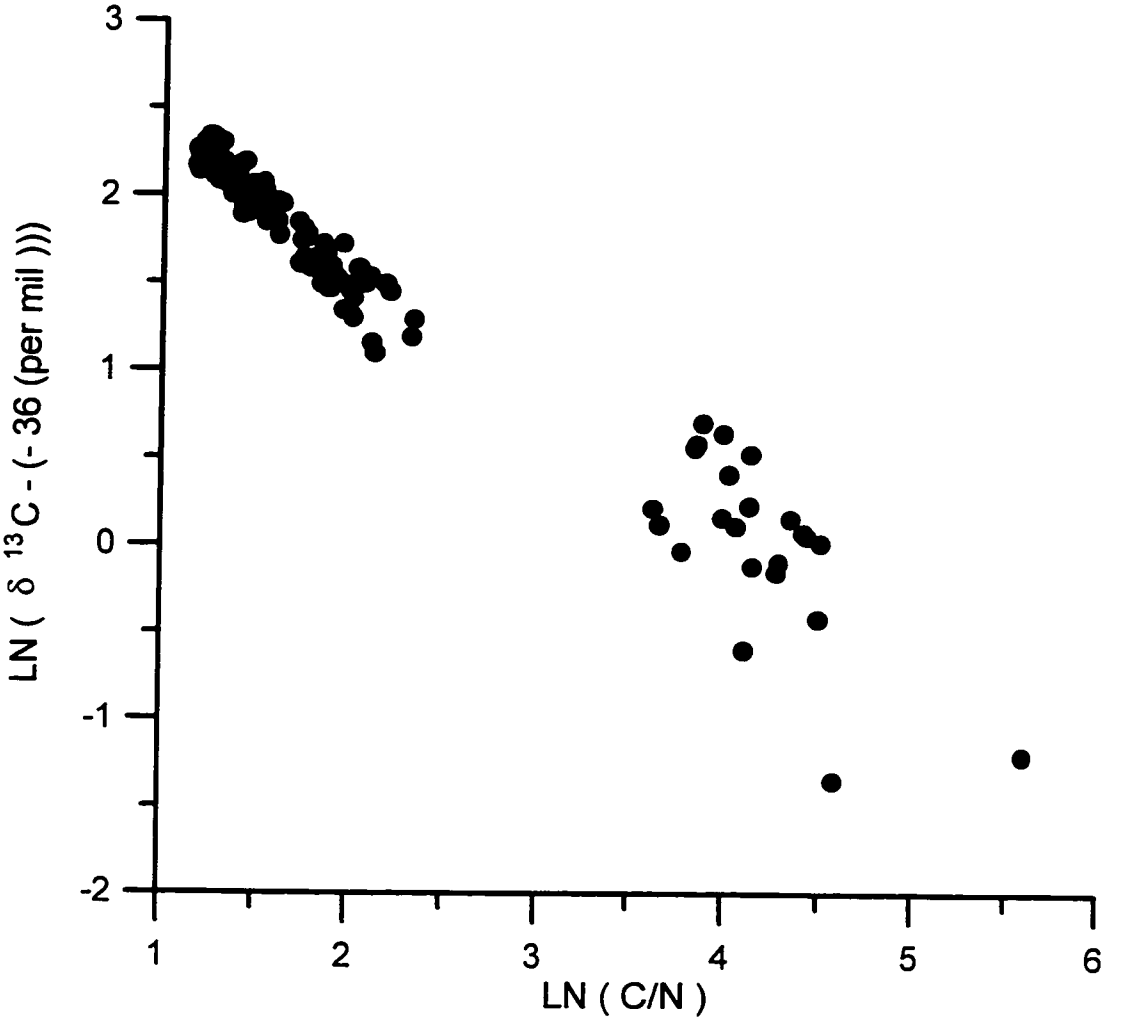

Figure A1.4. The natural logarithm of the *Mysis relicta* lipid analysis factor vs the logarithm of the C/N ratio.

The equation describing the relationship from regression analysis is:

$$\delta^{13}C = e^{3.1509 - 1.2045 \, Ln(C:N)} - 36‰ \qquad (A1.3)$$

As might be expected with the inclusion of the lipid data, the relationship between C:N and the observed $\delta^{13}C$ is very strong. Most of the variation in $\delta^{13}C$ of samples analyzed could be explained by variation in the C:N ratio ($r^2 = 0.94$, $p = <0.0001$), the regression is highly significant ($F_{1, 0.05, 1, 121} = 1895.25$, $p = <0.0001$).

In order to 'normalize' our existing $\delta^{13}C$ data, this predictive ability has to be translated into a corrective factor. The relationship between $\delta^{13}C$ and C/N is nearly linear in the C/N = 3 to C/N = 10 range. This range also encloses the C/N ratios of all the *M. relicta* collected and analyzed intact. The regression constant 3.15 was subtracted from the measured C/N and this value was multiplied by the slope (-1.2045) to obtain a factor to be subtracted from the measured $\delta^{13}C$:

$$\delta^{13}C_{corrected} = \delta^{13}C_{measured} - (-1.2045)\,(C:N_{measured} - 3.15) \qquad (A1.4)$$

The $\delta^{13}C_{corrected}$ approximates the $\delta^{13}C$ of the organism with the lipid removed. This correction was contrasted with the equation developed by McConnaughey and McRoy (1979). Untreated samples collected in 1994 were corrected using both the factor derived

here and the McConnaughey and McRoy equation. These corrected results were plotted with the original data as well as the lipid extracted samples (Fig. A1.5). The McConnaughey and McRoy equation normalizes the data at very depleted levels, approaching the values obtained for the extracted mysid lipid. In contrast, our corrected values were comparable to the $\delta^{13}C$ of the lipid extracted mysids with a tendency toward overcorrecting at higher C/N ratios (Fig. A1.5).

Analysis of variance (ANOVA) was conducted using the 1995 data for *M. relicta* to determine if there was a relationship between the date of collection of the mysids and $\delta^{13}C$ of the mysids collected. It was determined that there was a relationship between $\delta^{13}C$ and the date of collection ($n = 67$, $r^2 = 0.21$, $F = 3.1985$, $p = 0.0125$). In order to determine when the date was most influential on the $\delta^{13}C$ of *M. relicta*, the regression developed above was applied using the C/N ratios obtained for the 1995 mysid data. The $\delta^{13}C$ values from the regression were subtracted from the measured $\delta^{13}C$ values to determine if there was a seasonal change in the predictive ability of the regression (Fig. A1.6). A significant difference from 0 would indicate that the regression, i.e. the C/N ratio, was not the most influential determinant of $\delta^{13}C$ of *M. relicta*. In the spring, the $\delta^{13}C_{measured}$ was approximately 1 per mil lower on average than predicted by the regression with C/N. This is indicative of mysid feeding on more isotopically depleted sources of carbon during the spring. The residuals in the early October were ^{13}C enriched relative to those predicted by the regression, which suggests feeding on a more

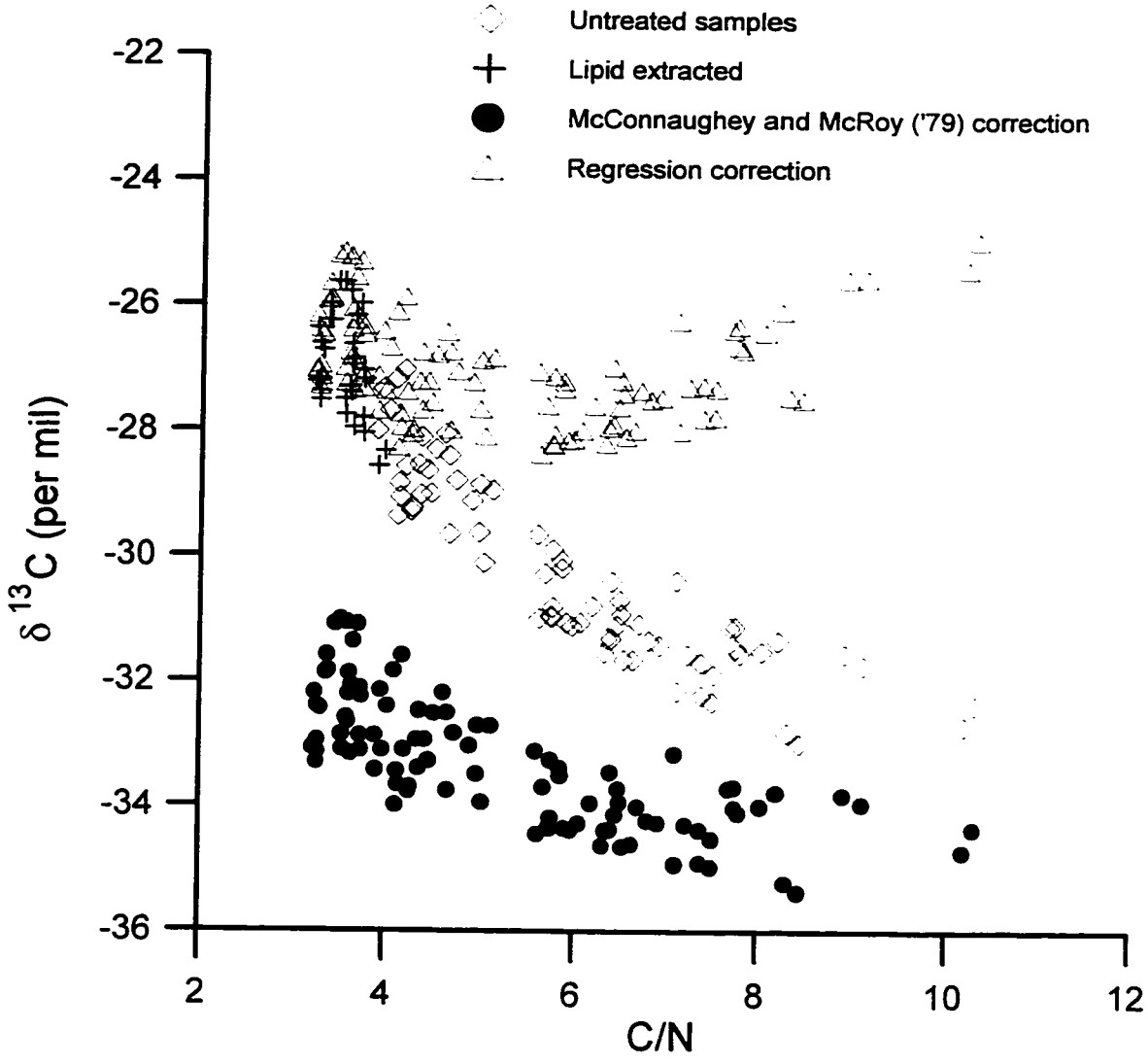

Figure A1.5. A plot of the $\delta^{13}C$ vs the C/N for; lipid extracted *Mysis relicta*, whole untreated *M. relicta*, *M. relicta* with the correction factor derived from regression applied, *M. relicta* with the McConnaughey and McRoy (1979) correction factor applied.

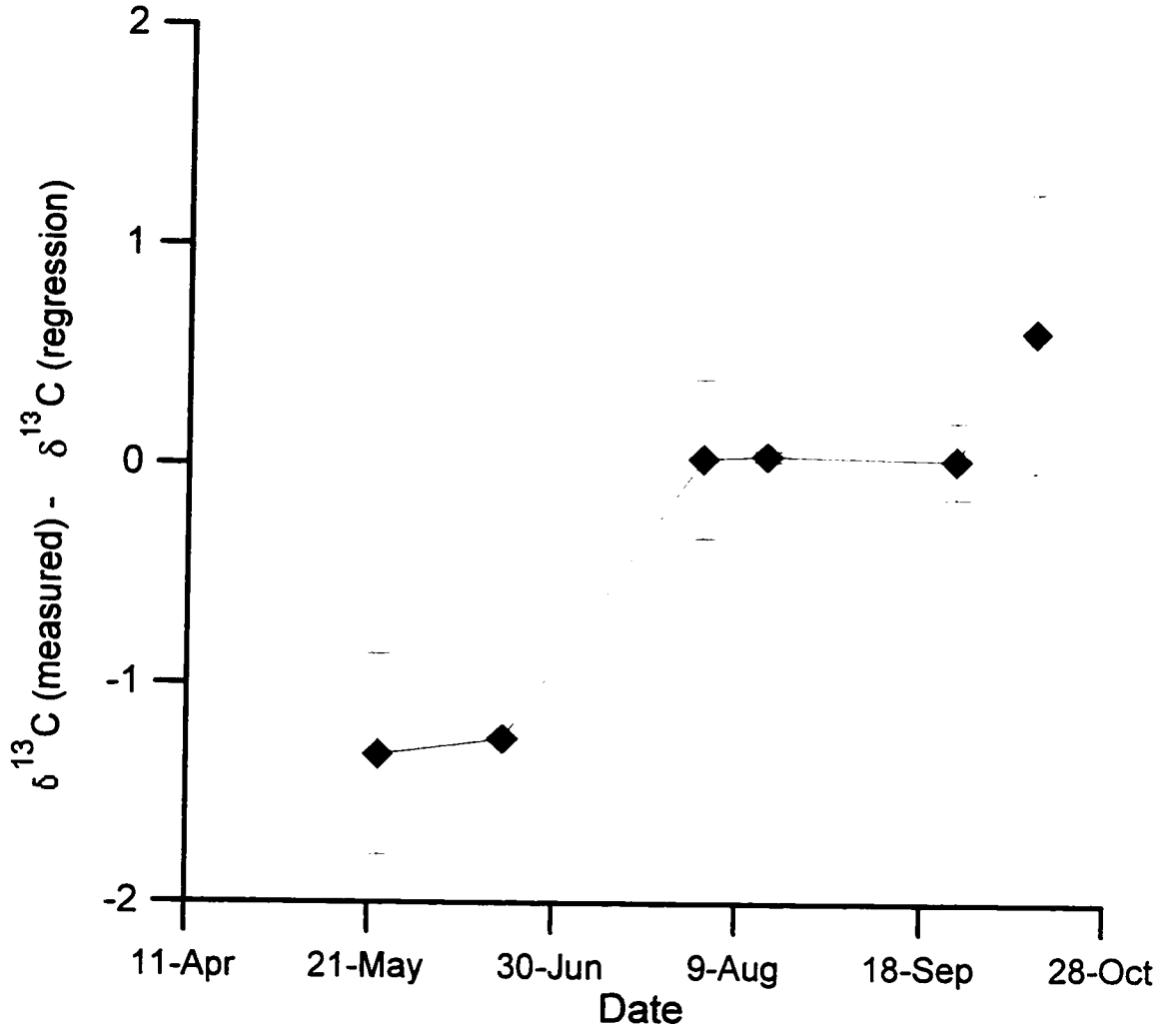

Figure A1.6. Temporal differences in the residuals of the difference between measured $\delta^{13}C$ of *Mysis relicta* (1994) and the predicted $\delta^{13}C$ from the regression of $\delta^{13}C$ of *M. relicta* and the C/N ratio.

enriched source of carbon in the fall. A discrepancy was observed between the values obtained in 1994 and 1995 when the predicted value based on the C/N ratio, was subtracted from the measured value. In 1994, the residuals were not significantly different from zero for any time of the year. In 1995, the spring measured values were significantly lower than the values predicted by the regression on C/N. This indicates that the $\delta^{13}C$ of *M. relicta* in the spring was lower for reasons other than an elevated lipid content.

It is important to note that this type of lipid correction or any lipid correction factor for $\delta^{13}C$ using C/N ratios is going to be somewhat species specific. Application of this particular regression equation to other species or even mysids outside the system may be inappropriate for two reasons. First, lipid extracted C/N ratios will vary for different organisms. The size and composition of organisms will be different, there may be more or less chitin on average etc., which will alter the correction factor since the correction is derived from the difference between the average lipid extracted C/N and the C/N ratio measured. Second, the slope of the equation used as a correction will be a function of how much the lipid added alters the overall signature of the organism in question. This will depend on the mass of lipid, which is normalized, and also the signature of the lipid which will be somewhat system and origin specific. It would be inappropriate to apply a correction derived from a regression where the lipid has a signature of -35 ‰ to an organism with a lipid signature of -10 ‰. The change in $\delta^{13}C$ for the same level of incremental change in C/N would be different. The assumption of consistency in lipid

$\delta^{13}C$ is implicit in the use of any correction factor pulled from the literature. Therefore the analysis presented here should be taken as a framework for analysis, the results should not be used as a surrogate for similar analysis, with the exception of application to future studies of *M. relicta* in Lake Ontario.

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Appendix 2

The Influence of Acid Addition in Sample Pretreatment on the $\delta^{15}N$ of Biological Samples.

Continuous-flow, isotope-ratio, mass-spectrometry (CF-IRMS) provides both carbon and nitrogen isotope analysis in a single run. In the analysis of biological samples from aquatic systems with high alkalinity, residual carbonate on the organism's surface can, and will, interfere with the carbon isotope analysis. The addition of dilute (< 2N) HCl removes residual carbonate and is commonly utilized as a pretreatment strategy for samples being prepared for carbon isotope analysis. However, it has been suggested that pretreatment of samples with acid may alter the nitrogen isotope signatures of the material being analyzed (Bunn *et al.* 1995). Acid washing was found to increase the mean $\delta^{15}N$ of penaeid shrimp (*Metapenaeus* spp.) approximately 3 ‰, and decreased the $\delta^{15}N$ of seagrass (*Enhalus acoroides*) by approximately 1.8 ‰ (Bunn *et al.* 1995). Acid washing was also determined to increase the variability of $\delta^{15}N$ signatures (Bunn *et al.* 1995).

Residual carbonate was a problem that had to be addressed in many of the samples analyzed (Fig. 3.2). The effect of acid treatment on the $\delta^{13}C$ and $\delta^{15}N$ of samples was tested by comparing acid treated and non-acid treated samples. Acid addition to fish

tissue was deemed to have no effect on $\delta^{13}C$ (Kiriluk *et al.* 1995). The same may be true for zooplankton samples.

The problems with acid rinsing, encountered in previous studies, may be avoided by altering the methods used. The losses of isotopically light nitrogen from shrimp in acid washing, reported by Bunn *et al.* (1995), may be due to the preferential hydrolysis of ^{14}N peptide bonds in the muscle tissue of the shrimp analyzed. In the study by Bunn *et al.* (1995), half the tissue from the tail of each shrimp was ground and analyzed without treatment, the other half was bathed in 0.1 N HCl and then rinsed with distilled water, dried, and ground prior to analysis. If the dilute acid hydrolyzed the muscle proteins, comparatively light peptide chains and amino acids would probably be suspended in solution and subsequently rinsed away. The same basic principle may be used to explain the observed decrease in the $\delta^{15}N$ of seagrass. If the dilute acid dissolves a $\delta^{15}N$ enriched component of the plant tissue preferentially, rinsing would leave the remaining tissue relatively depleted. If this is the case, then it is not the addition of acid that is compromising the sample integrity as much as it is the act of rinsing. The objective of acid addition is the removal of residual carbonate. The carbonate is lost as CO_2 on contact with the acid. Soaking in acid is probably not required and dilute HCl is unlikely to contribute a significant isotope signature. Therefore, rinsing should be unnecessary.

Considering the above observations, the influence of acid addition on $\delta^{15}N$ of particulate organic material on filters (POM), zooplankton, and mysids was determined. A total of

48 mysid samples, paired by date of collection, were analyzed, 24 with no pretreatment and 24 with dilute acid (2N) added to the sample dropwise. All samples were dried at 60 °C prior to stable isotope analysis and were analyzed individually. The $\delta^{15}N$ values obtained were compared using a t-test for independent means.

Composite samples of zooplankton and seston (>110 μm) were oven dried at 60 °C and then split into two separate sub-samples. Half of the samples had dilute acid added and were redried. The samples were analyzed and the $\delta^{15}N$ was compared using a paired samples t-test. The results of these tests suggested that acid-rinsing had little effect on $\delta^{15}N$, $t_{0.05,23} = 0.8955$, $p = 0.3798$, and $t_{0.05,54} = 0.2535$, $p = 0.8008$, respectively (Table A2.1).

Table A2.1. A comparison of the $\delta^{15}N$ values obtained from acid treated and untreated samples of *Mysis relicta* and zooplankton/seston.

	Mysis relicta	Zooplankton/Seston
n	48	55
Mean, untreated samples	11.79	8.99
Mean, acid-rinsed samples	11.39	8.95
95 % confidence interval	-0.51 to 1.30	-0.23 to 0.29
Std deviation difference	2.15	0.97

The filters which were used to collect POM for isotope analysis were subsampled. The subsample of the filter had dilute acid added to it, the remaining filter was left untreated. Filters were dried at 60 °C and analyzed. Filters were categorized by date and the results compared by ANOVA. Like the mysid and the zooplankton samples analyzed, there was no significant difference in $\delta^{15}N$ between acid treated and untreated filters.

It was concluded from this analysis that acid addition in the manner described would not influence the $\delta^{15}N$ of the treated sample sufficiently to influence the interpretation of the data. Therefore, the $\delta^{15}N$ results of acid treated and untreated samples were pooled in Chapters 3, 4 and 5.

References:

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Appendix 3

'Nitrofish' A Model Based on the 'Fish' Fugacity Model to Trace the Fractionation of Nitrogen Isotopes Between a Fish and its Food

Introduction

Knowing the routes of exposure of a chemical to an organism is important in determining what potential impacts the chemical may have. The potential impact is dependent on the dose of chemical the organism would receive. This can be elucidated from the real or projected concentrations in exposure media and the physical properties of the chemical of interest. For aquatic systems, organisms can be exposed through food and water. There are several chemicals found in the environment in which food is the predominant route of contaminant exposure for an organism (Oliver and Niimi 1988). These chemicals collectively share the properties of being persistent, resistant to breakdown under environmental conditions and not readily metabolized, as well as being hydrophobic, an example being PCBs (Mackay 1989). They are said to 'biomagnify' and are found in higher concentrations in predators than their prey, at least more so than can be explained by differences in size and lipid levels (Borgmann and Whittle 1992). Therefore, understanding the trophic dynamics of an ecosystem (what eats what) can be very critical in ecotoxicological assessment.

It is often difficult to assess the level of dependence of one species on another as a food source relative to other potential food sources, especially over an extended time period. For some organisms, the trophic relationship is readily apparent and can be discerned through analysis of gut contents. At lower trophic levels, gut content analysis may reveal little or nothing about where an organism obtains its energy requirements, as gut contents may not be recognizable. The use of naturally occurring tracers is an alternative or complimentary means of investigating the bioenergetics in an ecosystem. A tracer that may be used is the relative ratio of stable isotopes (Peterson and Fry 1987). This analysis uses the fractionation of stable isotopes of the same element in biological systems as a means of tracing the flow of elements, and by extension, the flow of energy (Peterson and Fry 1987).

In nature, the majority of atoms of any element are normally configured so that the number of protons in the nucleus of an atom is matched by an equal number of neutrons. A small fraction of atoms of an element have more neutrons than protons and may be described either as radioactive or stable isotopes of the element. Radioactive isotopes may emit positive or negative β-particles, an α-particle, a γ-ray, or capture an orbital electron in order to transform to achieve a more energetically favorable state, usually characterized by an even number of protons and neutrons (Mahan 1975). Stable isotopes undergo no such transformation and react chemically in the same manner as other isotopes of that particular element with some subtle differences due the difference in mass. It is these differences in mass, between isotopes, that can and have been exploited

as tracers in biological reactions and as indicators of reaction conditions (Peterson and Fry 1987). There are equilibrium and kinetic effects in reactions that result in isotope fractionation (Peterson and Fry 1987).

Equilibrium isotope effects can occur under conditions where there is free exchange between two molecules. Heavy isotopes tend to accumulate in the molecule in which the bond strength is greatest (Urey 1947; Stacey *et al.* 1952). An example of this is the exchange of carbon between CO_2 in air and bicarbonate in ocean waters. Heavy isotopes of carbon accumulate in bicarbonate (Mook 1974). The level of fractionation that will occur is predictable from bond strength measurements (Stacey *et al.* 1952).

Kinetic isotope effects occur in processes where the isotope of heavier mass is discriminated against in the reaction process usually by faster reaction of substrates made up of the lighter isotope. An example of this is diffusion processes where the lighter isotopes tend to move faster and through membranes more readily, thereby creating an isotope gradient (Peterson and Fry 1987).

In a biological reaction, the fractionation in the final end product is the result of fractionation in the reaction processes leading to that product. Many processes occur by means of a series of linked enzymatic reactions. If the entire pool of a substrate is reacted, there can be no fractionation. This is true of enzymatically mediated reactions, where all of the intermediary substrate is carried forward in the reaction process (Macko 1986).

Therefore, any isotope fractionation observed has to be the result of the initial reaction in the sequence (Peterson and Fry 1987). Where a reversible reaction occurs before an irreversible reaction, the level of fractionation depends on the direction favored in the process, i.e., which process dominates. The uptake of CO_2 into the leaves of plants as an initial step of the photosynthetic pathway is an example of this type of reaction sequence (Wada and Hattori 1978; O'Leary 1981; Peterson and Fry 1987). The CO_2 is able to back-diffuse, if it is not all utilized in carboxylation, the leaf and atmospheric CO_2 will attain an equilibrium and the fractionation effect of the carboxylation process dominates. If back-diffusion is negligible, then most of the CO_2 diffusing in is used and the fractionation due to diffusion dominates (Roeske and O' Leary 1984). In branched reactions, the level of fractionation depends on the fractionation in each step as well as partitioning of the mass flow within the system. Most metabolic pathways are branched reaction pathways and this results in differential isotope ratios in many tissues within organisms, for example lipid and muscle tissue (Tieszen *et al.* 1983).

A useful phenomenon occurs with respect to the ratios of naturally occurring stable isotopes of Nitrogen, ^{15}N and ^{14}N. Feeding studies and studies in ecosystems where the trophic dynamics were well known, have illustrated that there is an increase in the whole body ratio of ^{15}N to ^{14}N in an organism over the ^{15}N to ^{14}N in the organism's food (DeNiro and Epstein 1981; Minagawa and Wada 1984). This enrichment is measured as the parts per mil difference from a standard expressed in δ units and calculated by the following equation:

$$\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1] \times 10^{3}$$

The enrichment in ^{15}N through an aquatic food chain has been estimated to be approximately 3 ‰ (Minagawa and Wada 1984). DeNiro and Epstein (1981) did feeding studies and did not observe the same consistency in the level of ^{15}N enrichment between organisms and their source of food. They also found that different individuals of a species may vary with respect to their level of enrichment relative to a common food source, and that two species fed the same diet may differ in their $\delta^{15}N$ by as much as 3 ‰ (DeNiro and Epstein 1981). The observed differences may be the result of the form of nitrogenous waste predominantly utilized in excretion by the organism. DeNiro and Epstein (1981) used blowflies and houseflies which excrete nitrogenous waste as uric acid, relative to fish species which excrete mostly NH_3. It should be noted that while enrichment in ^{15}N does occur through the food chain and can be utilized as an indicator of trophic status, the level of understanding of the processes resulting in isotope fractionation needs further research.

Modeling Isotope Enrichment

The "fish" fugacity model lends itself to application in tracing the enrichment of nitrogen isotopes through the fish and through a food web. The original model and definition of the terms in the model are described in Mackay (1981). The operational definitions of the D values associated with uptake and elimination processes must be revised and the mass

balance equation altered. This is done to account for differences in the way in which nitrogen is taken up by an organism relative to most chemical contaminants. The result is a new slightly altered version of 'Fish' entitled here as 'Nitrofish'.

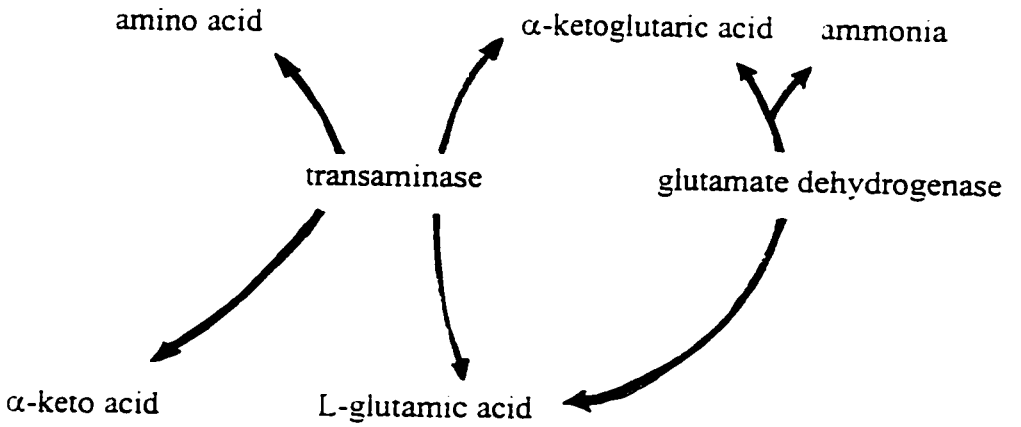
There are numerous investigations dealing with the level of isotope fractionation occurring in different chemical processes. Equations have been developed to describe fractionation in terms of relative rate constants for different isotopes. We will make use of the 'Rayleigh' equation, derived by Lord Rayleigh for the fractional distillation of a mixed liquid (Mariotti *et al.* 1981):

$$R_S/R_{S,o} = m^{(\alpha p/s\ -1)}. \qquad \text{(A3.1)}$$

where R_S is the ratio of heavy to light isotopes in the substrate remaining unreacted; $R_{S,o}$ is the initial isotope ratio of substrate before the reaction process; $\alpha_{p/s}$ is the ratio of the isotope ratios of the product and the substrate; and m is the ratio of the concentration of ^{14}N in the substrate to the concentration in ^{14}N that was in the substrate prior to the reaction.

In the system of interest, we hypothesize a linked reaction chain with two dominant first order reactions. Fractionation, of isotopes occurs in the gut as a result of discrimination in peptide bond cleavage. The zero-point energy for the ^{12}C-^{14}N bond greater than that of the ^{12}C-^{15}N, so peptide bonds of ^{12}C-^{14}N should rupture preferentially (Bada *et al.* 1989).

Fractionation occurs in transamination and deamination processes (Macko *et al.* 1986). Isotopically lighter nitrogen is preferentially excreted. Ostensibly, this occurs for the same reason that isotopically lighter amino acids are absorbed preferentially: the ^{14}N-C bond is cleaved more readily than ^{15}N-C by the action of peptidase in the gut and so is excreted preferentially. Glutamic acid is utilized in the fish as an intermediate in the transamination process. Ammonia is either absorbed and utilized in the formation of new amino acids, or it is liberated for excretion, depending on the requirements of the fish at any particular time (Vellas and Serfaty 1974). The reaction pathway is schematically shown here:



Not all amino acids undergo transamination: essential amino acids are not altered: whereas other amino acids such as serine are the product of a series of transamination reactions (Macko *et al.* 1986). In our model, we take the net fractionation due to metabolic processes to be one reaction. The fractionation associated with the hydrolysis

of peptide bonds by protease in the gut is said to be another reaction. The two reactions are treated as a single process, the fractionation associated with the former being offset by a factor describing the fractionation associated with the latter process.

Comparison of the Fish and Nitrofish Fugacity Models

The mass balance equation for nitrogen in Nitrofish may be derived from the mass balance in the 'Fish' model of Mackay (1981) and the various components can be redefined for nitrogen . Then we can look at the model inputs and potential model modifications as follows:

$$D_v f_w \times D_A f_A = D_V f_f + D_m f_f + D_E f_f + D_G f_f \qquad \text{(A3.2)}$$

This is the mass balance equation for the fish model: D values are transport parameters in units mol/Pa.h. D = GZ; f is the fugacity, a measure propensity to disperse, or a 'fleeing factor'; D=GZ an d f = C/Z where C is the concentration of a substance in a given medium; Z is a proportionality constant, the 'fugacity capacity', in units mol/m^3Pa; and G is the flow rate in m^3/h.

Therefore, the different Df terms represent fluxes for gill uptake/loss, food uptake, metabolism, elimination, and growth.

Nitrogen is naturally found in water in the form of nitrates, nitrites, ammonia, and other nitrogen containing compounds. Nitrogen is passively and actively removed from the

fish through the gill by diffusive and active transport processes. The active transport of sodium ions into the fish through the gill surface is linked to the removal of ammonium ions (Love 1980). The diffusion of nitrogen into the fish via the gills is negligible: f_w is very small so that the $D_v f_w$ term can be eliminated. Nitrogen from food is the only source of concern so the mass balance becomes:

$$D_A f_A = D_V f_f + D_m f_f + D_E f_f + D_G f_f \qquad \text{(A3.3)}$$

D_v, D_E, D_G and D_m are not independent loss mechanisms in Nitrofish as they are in 'Fish'. The details of this are described later when these terms are defined. The implications for writing the mass balance equation are that the metabolism term cannot be used, as the other D values describe components of the same process. So the mass balance in Nitrofish is further reduced to become:

$$D_A f_A = D_V f_f + D_E f_f + D_G f_f \qquad \text{(A3.4)}$$

$D_A = G_A Z_A E_O$ in the fish model. For Nitrogen, this will remain, except Z_A will be redefined. $Z_A = L_A Z_O$ in the contaminants model, where L_A is the lipid fraction of G_A, the food ingestion rate as a percent volume. In the contaminants model, the fish is essentially represented as a bag of lipid. This works because hydrophobic organic contaminant loading is strongly correlated with lipid levels: the fattier the fish the higher it's Z value

with respect to contaminants. In contrast the main source of nitrogen is the amino acid of proteins and lipid levels are of little consequence. Thus, for the purposes of modeling nitrogen we introduce a new factor P_A, which is the percentage of nitrogen found in the ingested food G_A . Fish are limited in the amount of protein they are able to convert into body material. Overfeeding a fish does not result in excess material passing through the gut unchanged and being voided in the feces (Gerking 1955). Increasing the rate of feeding does not effect the efficiency of protein absorption. The efficiency remains constant and has been measured in a range of 80 to 90 % . For the purpose of demonstration, E_O is set at 86 %. Another factor must be introduced into the model to account for metabolic effects on the relative rates of uptake of ^{15}N to ^{14}N. The rate of absorption is multiplied by a protease factor to reflect differential rates of peptide bond cleavage in the gut. As previously mentioned, zero point energy of peptide bonds between ^{15}N and ^{12}C is greater than that between ^{14}N and ^{12}C and as a result, cleavage of these bonds occurs at a slower rate. The peptides and amino acids containing ^{14}N are liberated faster and therefore are absorbed to a greater extent which results in the enrichment of ^{15}N in feces versus food.

Therefore D_A is given by:

$$D_A = (G_A P_A) Z_A E_O k_P \qquad \text{(A3.5)}$$

$Z_O = K_{OW} Z_W$ and again, this reflects the relation between contaminant flow and lipid. For nitrogen, Z_O must be = 1, since the mass of material is given by G_A. We know that since

Z_W is very high, the lake is capable of assimilating a great deal of nitrogen. Therefore, the K_{OW} must be the very small reciprocal of the Z_W value.

The amount of nitrogen diffusing back into the gut is negligibly small . For the purposes of these calculations the nitrogen in the feces is the nitrogen not absorbed in digestion and is calculated as:

$$D_A(1 - E_O)\,(1/k_P) \qquad \text{(A3.6)}$$

$1/k_P$ is used to reflect that the nitrogen isotope composition of the remaining nitrogen in the feces are enriched at a reciprocal rate to the rate of uptake of the light isotopes. To the gut elimination portion is added the amount of nitrogen excreted as urea, which is 10 % of the total amount of nitrogen metabolized and excreted by the fish. Therefore:

$$D_E = (1 - E_O)D_A\,(1/k_P) + 0.1D_M \qquad \text{(A3.7)}$$

D_v, reflects the movement of contaminant across the gill surfaces in the contaminants model. Movement of contaminant across the gill surface is diffusive and dependent on the relative resistance the contaminant faces in moving from the water into the gill and vice versa. The transport of nitrogen across the gill surface is more complex. The gills play an extremely important roll in the transport of nitrogenous waste out of the fish. Some of the ammonia in the bloodstream diffuses across the gill surface. The majority of

nitrogenous waste created by the fish is actively removed from the bloodstream of the fish at the gill (Love 1980). Ammonia is protonated and is excreted as ammonium ions in an exchange process in which sodium ions are actively removed from the water passing over the gill surfaces, thus maintaining an osmotic balance within the fish. The excretion of nitrogenous waste as ammonia is energetically favorable, and possible, since the fish is able to rid itself of toxic ammonia on a continuous basis. Organisms unable to do this must cope by storing nitrogenous waste as urea or uric acid. Fish do, however, have kidneys and a bladder. For a small percentage of the amino acids metabolized, fish produce urea in the kidneys (which serves in sodium conservation) and store it in the bladder. Thus, approximately 10% of the nitrogenous waste in fish is excreted as urea (Love 1980). The amount of ammonia excreted via the gills has been measured in several studies and expressed as a mol/kg/hr value. D_v may be calculated as the parallel sum of the resistance for the diffusive transport of ammonia across the gill surface, D_D, and the active transport of ammonium ions across the gill surface, D_T:

$$D_v = 1/(D_D + D_T) \qquad \text{(A3.8)}$$

Both D_D and D_T are a function of: the concentrations of free amino acids, nitrogen containing byproducts and ammonia in the blood stream of fish, and the rate of blood circulation. $D_v f_f$ can be measured and D_T might be estimated from the level of sodium uptake.

In the model for contaminants, the metabolism D value, D_M, refers to a removal process. This is not the case for nitrogen. Metabolism of proteins is what leads to isotope fractionation, but metabolism, in itself, does not remove nitrogen from the organism . The metabolism of proteins results in the nitrogenous waste being produced. Therefore, the D value for metabolism must be linked to the D value for transport of ammonia from the fish via the gills, D_v as well as to excretion as urea. The reason for this is that 90% of the nitrogenous waste produced in fish, regardless of where it is produced, is excreted via the gills, $D_v = .9D_m$ (Love 1980).

Nose (1971) found that the efficiency of protein utilization for growth began to decrease and the level of excretion of nitrogen increased with increased protein feeding. The rate of metabolism, k_m, is defined as the level of protein deamination, or transamination, which correlates directly with the rate of respiration. Approximately 90% of the nitrogen absorbed undergoes a transamination process. The D value for metabolism in Nitrofish is the sum of the nitrogen absorbed and the fish tissue broken down :

$$D_M = ((G_A P_A) Z_A E_O k_P + V_F (-k_G) Z_F) k_M . \qquad (A3.9)$$

V_F is the total nitrogen pool of the fish, estimated as the amount of protein in the fish. This factor is multiplied by another factor, k_G, which is the percentage change in fish volume over a given time period. If G_A is zero, metabolism still occurs and the D value for metabolism is positive when k_G is negative. In defining D_M in this manner, losses in

protein nitrogen during periods of starvation can be accounted for. When k_G is positive, D_M is reduced, and even though the rate of metabolism, k_M, is constant, the amount of nitrogen being excreted by the gills, D_v, is reduced.

The D_G term does not represent a 'dilution' factor, as it does in the Fish model. Since nitrogen is incorporated into the tissues which are growing, the mass of nitrogen within Nitrofish will increase at a rate directly proportional to the growth in overall fish mass. The growth in fish as a percentage of volume, is therefore equal to the increase in nitrogen and $D_G = dV_F/dt\ Z_F$.

Calculating Fugacities in Nitrofish

Input values	Z values and physical constants
$D_v f = .00026$ mol/h*Pa for a 1 kg fish (10 cm^3	$Z_w = 10^{11}$
$E_o = .86$ %	$Z_A = 1$
$G_A = 3\%$ by volume	$Z_F = 1$
From Bada (1989) $k^{14}N / k^{15}N = 1.010 - 1.014$	
$k_P = 1.011$ for ^{14}N, 1 for ^{15}N	$H = 10^{-11}$
Fish are 18 % protein by volume	$Z_O = 1$
16 % of protein is nitrogen	$K_{OW} = 10^{-11}$
Let $V_F = 10cm^3 (1\ m^3/10^6 cm^3) P_a$	10^{11} and 10^{-11} are arbitrarily chosen as exceedingly large or small values = in lieu of measurements or calculated values.
$10cm^3 (1\ m^3/10^6 cm^3)(.18)(.16)$	

$$D_A f_A = D_V f_f + D_E + f_f + D_G f_f$$

$$f_f / f_A = D_A / D_V + D_E + D_G$$

Let $D_G = 0$ therefore $k_G = 0$

$$f_f / f_A = D_A / D_V + D_E$$

$$D_V = .9(G_A Z_A E_O k_P + V_F k_G Z_F) k_M$$

$$D_A = G_A Z_A E_O k_P$$

$$D_E = (1 - E_O) G_A Z_A (k_P) + 0.1 D_M$$

$$f_f / f_A = G_A Z_A E_O k_P / 0.9\ G_A Z_A E_O k_P\ k_M + (1 - E_O) G_A Z_A (1/k_P) + 0.1\ G_A Z_A E_O k_P k_M$$

$$f_f / f_A = E_O k_P / E_O k_P k_M + (1 - E_O)(1/k_P) \qquad \text{(A3.10)}$$

This equation reflects the fact that the isotope discrimination is unaffected by the rate of uptake.

Fugacities may be substituted into the 'Rayleigh equation' (A3.1)

$\alpha_{p/s} = R_P/R_S$

where R_P is the ratio of $^{15}N/^{14}N$ in the product (fish) and R_S is the ratio of $^{15}N/^{14}N$ in the reacting substrate (food).

Since f = CZ and Z for food and for fish are 1, C is nitrogen and therefore;

$^{15}f_f/\ ^{14}f_f = {}^{15}N/{}^{14}N$

and

$(^{15}f_f/\ ^{14}f_f) / (^{15}f_f/\ ^{14}f_A) = \alpha_{p/s}$, where the product is nitrogen waste, $1/\alpha_{p/s}$, which may be used to obtain the level of enrichment in fish relative to its food.

Where m is the unreacted substrate, and $R_{S,o}$ is the initial substrate in a reaction with first order kinetics,

$(\alpha_{p/s} - 1)\ln m = \ln R_S/R_{S,o}$

$R_S/R_{S,o} = m^{(\alpha p/s\ -1)}$

The following equations describe a first order reaction:

$^{14}N_s = {}^{14}N_{s,o} \times e^{-kt}$

$^{15}N_s = {}^{15}N_{s,o} \times e^{-k't}$

Substitution into the Rayleigh equation gives:

$m = e^{-kt}$, $\alpha_{p/s} = k'/k$

Therefore the ratio of fugacities of the heavy to light isotope in fish and food are equal to the overall rate constants of the heavy to light isotopes in fish and food respectively.

From the fish model we can also substitute;

$$^{14}f_f = {}^{14}f_{f,o} \times e^{-Dv t/VfZf}$$

and

$$^{15}f_f = {}^{15}f_{f,o} \times e^{-Dv' t/VfZf}$$

to get;

$m = e^{-Dv t/VfZf}$ and;

$\frac{^{15}f_f / {}^{15}f_A}{^{14}f_f / {}^{14}f_A} = {}^{15}D_v / {}^{14}D_v$ when considering only the fractionation related to transamination.

The Rayleigh equation can be used to derive:

$$1/\alpha_{p/s} = \ln(1-j)/\ln(1-j(R_p/R_S)) \qquad \text{(A3.11)}$$

where j is the fraction of the initial reactant that has been converted to product which is approximated by; E_o (0.9), since approximately 90% of the protein nitrogen taken up undergoes transamination in some form.

This equation can be rearranged into δ notation;

$$\delta_p = \{1-(1-j)^{\alpha p/s}/j\}(1000+\delta_{so}) - 1000 \qquad \text{(A3.12)}$$

Solve equation 1 for ^{15}N and for ^{14}N

$$^{15}f_f / {}^{15}f_A = E_O {}^{15}k_P / (E_O {}^{15}k_P {}^{15}k_M + (1 - E_O)(1/{}^{15}k_P))$$

$$^{14}f_f / {}^{14}f_A = E_O {}^{14}k_P / (E_O {}^{14}k_P {}^{14}k_M + (1 - E_O)(1/{}^{14}k_P))$$

$$\frac{{}^{15}f_f / {}^{15}f_A}{{}^{14}f_f / {}^{14}f_A} = \frac{\{E_O\,{}^{15}k_P / (E_o\,{}^{15}k_P\,{}^{15}k_M + (1 - E_O)(1/{}^{15}k_P))\}}{\{E_O\,{}^{14}k_P / (E_O\,{}^{14}k_P\,{}^{14}k_M + (1 - E_O)(1/{}^{14}k_P))\}}$$

$${}^{15}k_P = 1$$

$$\frac{{}^{15}f_f / {}^{15}f_A}{{}^{14}f_f / {}^{14}f_A} = \frac{\{E_O / EO\,{}^{15}k_M + (1 - E_O)\}}{\{E_O\,{}^{14}k_P / (E_O\,{}^{14}k_P\,{}^{14}k_M + (1 - E_O)(1/{}^{14}k_P))\}}$$

$$\frac{{}^{15}f_f / {}^{14}f_f}{{}^{15}f_A / {}^{14}f_A} = \frac{\{E_O / EO\,{}^{15}k_M + (1 - E_O)\}}{\{E_O\,{}^{14}k_P / (E_O\,{}^{14}k_P\,{}^{14}k_M + (1 - E_O)(1/{}^{14}k_P))\}}$$

From literature, $k^{14}N/k^{15}N$ for transamination of Glutamic acid to Aspartic acid is 1.0083

let $1.0085({}^{15}k_M) = {}^{14}k_M$ and ${}^{15}k_M = 1$, substitute $1.011 = {}^{14}k_P$ and $E_o = .86$

$$\frac{{}^{15}f_f / {}^{14}f_f}{{}^{15}f_A / {}^{14}f_A} = \frac{\{E_O / EO\,{}^{15}k_M + (1 - E_O)\}}{\{E_O\,{}^{14}k_P / (E_O\,{}^{14}k_P\,{}^{14}k_M + (1 - E_O)(1/{}^{14}k_P))\}}$$

$$\frac{{}^{15}f_f / {}^{14}f_f}{{}^{15}f_A / {}^{14}f_A} = \frac{\{.86/(.86 + (.14))\}}{\{.86(1.010)/(.86(1.010)(1.0083) + (.14)(0.9901))\}}$$

$$\frac{{}^{15}f_f / {}^{14}f_f}{{}^{15}f_A / {}^{14}f_A} = \frac{.86(.87581 + .1386)}{0.8686} = \frac{.87238}{.8686}$$

$$\frac{{}^{15}f_f / {}^{14}f_f}{{}^{15}f_A / {}^{14}f_A} = 1.0044 = R_P/R_{SO}, \text{ corrected for gut fractionation}$$

Substitute into;

$$1/\alpha_{p/s} = \ln(1-j)/\ln(1-j(R_p/R_S))$$

$$1/\alpha_{p/s} = \ln(1-j)/\ln(1-j(1.0044))$$

Where j is equal to .86(.9) = .774, the amount of nitrogen undergoing transamination

$$1/\alpha_{p/s} = \ln(1-0.774)/\ln(1-0.774(1.0044))$$

$1/\alpha_{p/s} = -1.4872/ \ln(.22259)$

$1/\alpha_{p/s} = -1.4872/ -1.5024 = .98988$

Substituting this value into equation A3.12 to obtain an enrichment factor in parts per mil, we let $\delta_{so} = 0$, and invert the equation to reflect that we are interested in the enrichment of the fish relative to the food.

$\delta_p = 1000 - \{1 - (1 - j)^{1/\alpha p/s} / j\} (1000 + \delta_{so})$

$\delta_p = \{1 - (1 - 0.774)^{0.98988} / 0.774\} (1000) - 1000$

$\delta_p = 1000 - \{0.771 / 0.774\} (1000)$

$\delta_p = 1000 - \{0.99612\} (1000)$

$\delta_p = 3.88$

The level of enrichment increases if the ratio of rates $k^{14}N/k^{15}N$ for transamination increases, and the enrichment observed in aquatic systems is on average 3.2 parts per mil. This suggests that on average the rate of fractionation is less than that observed for the conversion of glutamic acid to aspartic acid.

By substituting different values into the model, it can be observed that the following remains consistent:

if the ratio of the fugacities in the food decreases, $^{14}f_A$ increases relative to $^{15}f_A$ and the level of fractionation will increase and vice versa, i.e., enrichment occurs in $\delta^{15}N$ of feces if fractionation in the gut is somehow enhanced. The opposite is also true: elevated

efficiency in protein uptake would result in lower levels of fractionation between food and feces.

If the ratio of fugacities in the fish increases, $^{15}f_f$ increases and/or $^{14}f_f$ decreases, and subsequently the level of fractionation will decrease. A decreased level of metabolic fractionation, potentially due to more efficient protein utilization, produces lower signatures.

As more studies of protein specific fractionation in metabolism become available, the information obtained may be incorporated into Nitrofish to make it a more detailed and better a predictor of nitrogen fractionation factors. It may eventually be used in assessing feeding efficiencies by assessing proportional levels of amino acids assimilated.

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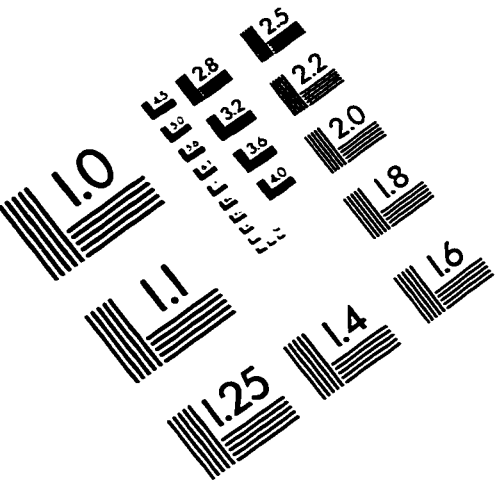
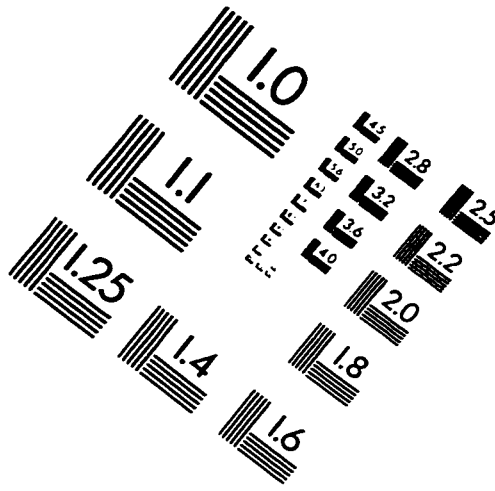
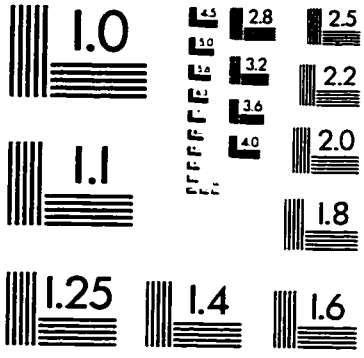
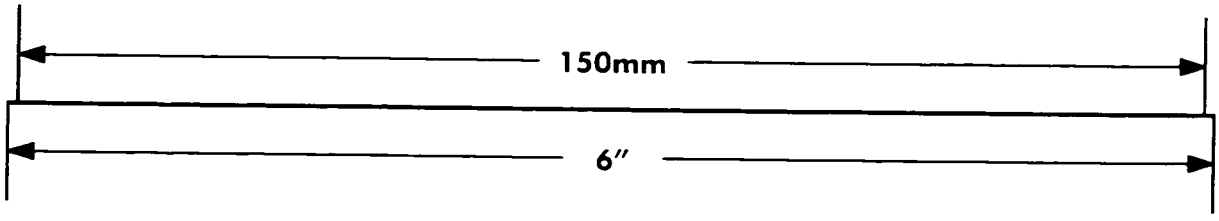
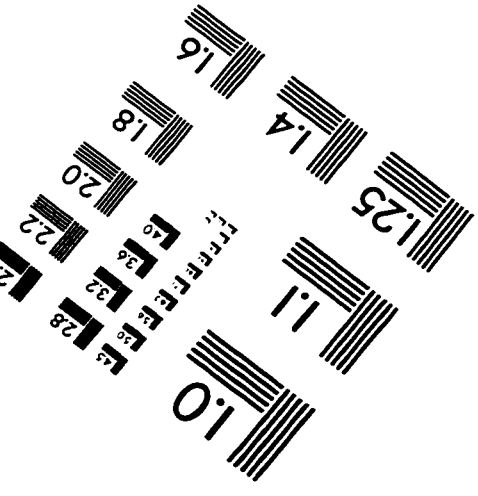
IMAGE EVALUATION TEST TARGET (QA-3)

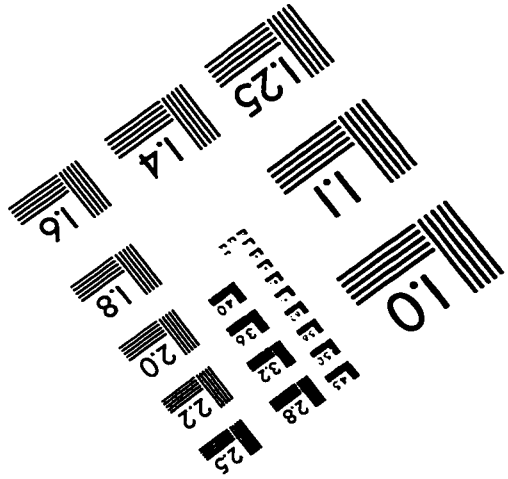












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