Testing the Robustness of Pelagic Zooplankton as Indicators of Land Use Impacts on Small Lakes

by

Kevin Rieberger
B.Sc., University of Victoria, 1994

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Abstract

I investigated the utility of nitrogen stable isotope signatures in zooplankton as water quality indicators in small lakes. The $\delta^{15}N$ of organisms such as fish, mussels, insects, and aquatic plants have been linked to land use in several studies, however, I believe pelagic zooplankton in lakes provide comparable information to these with the benefit of being commonly available and easier to collect. To determine the potential use of this tool in water quality assessments, I collected samples from a total of 61 lakes throughout British Columbia.

To investigate the seasonal patterns and inter-annual consistency of calanoid copepods and *Daphnia* $\delta^{15}N$, I analyzed data collected from eight coastal lakes over several years. Seasonal variability in zooplankton $\delta^{15}N$ was observed for several small, temperate lakes with peak values generally occurring in the winter and spring. Sampling schedules should therefore include this critical period of maximum $\delta^{15}N$. Calanoid $\delta^{15}N$ was consistently higher and less variable than *Daphnia* $\delta^{15}N$, and therefore selected as the preferred taxonomic group. A strong relationship between mean $\delta^{15}N$ and density of septic systems was demonstrated and spring calanoid $\delta^{15}N$ was consistent over time in the absence of changes in land use. Consistent seasonal patterns in zooplankton $\delta^{15}N$ on a year-to-year basis support the application of this parameter in water quality trend analysis.

To determine the physical factors that influence spring calanoid copepod $\delta^{15}N$, the role of several watershed and limnological characteristics in 22 British Columbia lakes was investigated. The density of residential lots, as a proxy for septic density, within the riparian zones of lakes and their tributary streams was the only consistent significant predictor of $\delta^{15}N$. While this suggests residential land use and septic density influenced calanoid $\delta^{15}N$ in these lakes, there are likely additional factors that contribute to the final signature measured. These factors include: the contribution of different N sources and subsequent isotopic mixing, the physical characteristics of individual lakes and differences in lake-specific chemical or biological processing of N.

Some weaknesses in the study were identified. Most of the lakes studied had residential development as the dominant land use and a better representation of other land use types, such as agriculture, would have been beneficial. The lakes studied were exposed to relatively low or high levels of septic densities and more lakes exposed to moderate levels of development may have explained more variation. Finally, the majority of lakes studied were located in coastal areas and greater regional representation of lakes may have illustrated regional differences in seasonal trends.

In my research, I have demonstrated an application for calanoid $\delta^{15}N$ in water quality assessments and resource management. When used in conjunction with other information, such as land use and water chemistry, $\delta^{15}N$ provides insight to nutrient sources for a particular lake, tracks changes in water quality over time, and can help guide management decisions.

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Chapter 1: General Introduction

Introduction

Anthropogenic activities have greatly altered the global nitrogen cycle, especially in the last 50 years, with non-point source inputs of inorganic N (e.g., septic seepage, agricultural and urban runoff) contributing to the degradation of freshwater, estuarine and marine ecosystems (Carpenter et al 1998; Carmago and Alonso 2006). Septic failures have been linked with many water quality and human health impacts (Moore et al. 2003) and for lakeshore residents, inputs from septic systems are often a concern. There are about 250,000 on-site sewage (septic) systems in British Columbia, which are generally effective at treating household wastes if designed and installed properly in appropriate soil and maintained regularly. An ongoing concern for water management and regulatory agencies is that failing or improperly installed septic systems cause contamination of ground and surface waters. Septic tank effluent can typically contain several contaminants of concern including pathogens, chemical and biochemical oxygen demanding compounds, nitrogen compounds and phosphorus compounds (US EPA 1980, 2002). Septic systems can be a source of both N and P in groundwater and surface water via groundwater. Removal rates for nutrients in the absorption of conventional septic systems range from 0% to 40% for N and 50% to 85% for P (Potts et al. 2004). Estimated loadings from septic systems to Canadian surface and ground waters for N and P are 154,000 tonnes/year and 1,900 tonnes/year, respectively (Environment Canada 2001).

The average lifespan of most septic systems ranges from 10 to 30+ years, depending on the design and installation, quality and quantity of wastewater and level of maintenance employed. Installation practices and homeowner maintenance of septic systems play critical roles in system longevity (Day 2004) and will influence the impact of these systems on nearby waterbodies. Although P is usually the nutrient limiting productivity in lakes (Schindler 1977), N also plays a limiting role (Elser et al. 1990; Vander Zanden et al. 2005) and so it is essential that nutrient inputs be sufficiently managed to protect water quality. Phosphorus tends to be effectively retained by most soils, moving slowly downward through the soil matrix. Under certain conditions, such as high rainfall rates, small but significant amounts of P can move through preferential flow paths, bypassing the soil matrix (Day 2004). Nitrogen, especially in the form of nitrates, tends to be more mobile, due to its high solubility and weak retention by soil.

There has been an increasing emphasis on lake stewardship across North America in recent years. Those who live on lakeshores and within developing watersheds are being challenged to be more accountable for their actions and take responsibility for the potential impacts on their lakes and streams. In British Columbia, volunteer stewardship groups are being encouraged to participate in activities such as water quality monitoring, habitat restoration and education and outreach to their neighbours. Development of new water quality monitoring and assessment techniques and approaches is critically important to support these efforts.

One issue of particular concern has been the evaluation of impacts of domestic on-site sewage disposal, including bacteriological contamination and increased nutrient loadings, on surface waters. Moore et al. (2003) concluded that of the 30 lakes they surveyed, those with homes on septic systems had higher P and chlorophyll *a* concentrations than did undeveloped lakes or lakes with homes serviced by sewers. Eutrophication often leads to lakes that are less economically beneficial and aesthetically desirable to humans (Moore 2003), in part because of the dominance of cyanobacteria, which has been correlated with increasing nutrient concentrations in lakes (Downing et al. 2001). There is also human health risks associated with eutrophication. For example, Giani et al. (2005) found that the amount of toxin-producing cyanobacteria increased with total P and total N concentrations in lakes across a range of trophic conditions.

Residents and other lake users need to know whether septic systems are having an impact on the water quality and biota of their lakes and municipal and regional governments need to know if new land-use zoning or better waste management practices are required. Given the limited resources available to volunteer groups and government agencies alike, establishing an impact or determining a level of contamination is difficult because of the associated analytical costs. With respect to microbiological sampling, a fairly rigorous monitoring program is required to establish bacteria levels (BC water quality guidelines require a minimum of five weekly samples collected within 30 days for statistical purposes). Even then, results can be greatly influenced by factors beyond the control of the sampler (e.g., localized microplumes of fecal indicators from specific sources such as wildlife) and determining the source of contamination can be difficult and costly. Although volunteers have collected many samples in British Columbia, the results rarely meet the statistical requirements of the established water quality guidelines, because of budgetary constraints, and are of little value as water quality indicators.

Stable isotopes of nitrogen may provide a simple indicator to help assess lake condition and determine whether there are impacts associated with human land-use. Nitrogen has two stable isotopes: ^{14}N and ^{15}N, with the average abundance of $^{15}N/^{14}N$ in air being constant at 1/272. Nitrogen isotope ratios are generally reported in per mil (‰) relative to N_2 in air, using the symbol δ (delta) to indicate a difference between a sample and a standard (e.g., atmospheric N) (Kendall 1998). Nitrogen derived from residential sources has a distinct range of isotopic values (McKinney et al. 2002) and it has been established that the ratio of ^{15}N to ^{14}N is higher in sewage-derived N compared to other sources (Cabana and Rasmussen 1996). The high $\delta^{15}N$ signature is attributed to the high trophic position of humans and the fractionation that occurs during nitrogen cycling leading to greater proportionate losses of the lighter ^{14}N isotope. Several researchers have shown that higher $\delta^{15}N$ values associated with human land-use are transferred to the biota of the receiving waters (Cabana and Rasmussen 1996; Yelenik et al. 1996; McClelland et al. 1997; McClelland and Valiela 1998; McKinney et al. 2001, 2002; Cole et al. 2004, 2005; Steffy and Kilhan, 2004; Vander Zanden et al. 2005; Kaushal et al. 2006). While there can be high fluctuations in the $\delta^{15}N$ signatures of primary producers (Cabana and Rasmussen 1996), primary consumers incorporate these fluctuations into an integrated value near the base of the food chain for the ecosystem in question (McKinney et al. 2002).

Despite the obvious interest in using $\delta^{15}N$ in water quality assessments, very little work has been done on the use of freshwater zooplankton $\delta^{15}N$ signatures specifically to assess water quality. Zooplankton provides a ubiquitous and easily obtained group of organisms in which to measure impacts of human land-use. They are also relatively short-lived organisms, and therefore provide a more accurate reflection of the water chemistry at the time of sampling. In this thesis, I focus on the use of zooplankton $\delta^{15}N$ signatures as indicators of water quality in small lakes. I investigate the $\delta^{15}N$ signatures in calanoid copepods and *Daphnia* (Cladocera) over time, the watershed characteristics that may influence zooplankton $\delta^{15}N$ signatures, and the application of zooplankton $\delta^{15}N$ signatures in water quality assessments.

A significant amount of the work done on $\delta^{15}N$ signatures in lentic biotas has focused on food web structure and trophic relationships between organisms (Fry 1991; Vander Zanden et al. 1999; Post et al. 2000; Matthews and Mazumder 2003; Perga and Gerdeaux 2006). In order to determine the trophic position of an organism within its food web using its $\delta^{15}N$ signature, the base level $\delta^{15}N$ of the system must be known. This has led many researchers to investigate the influence of land use on $\delta^{15}N$ signatures and the application of this information in resource management.

McKinney et al. (1999, 2002) acknowledged the variability of primary producers related to the source of N and the fractionation of N that occurs during uptake and metabolism and followed up on Cabana and Rasmussen's (1996) suggestion that unionid mussels be used to determine base level $\delta^{15}N$. They assumed that freshwater mussels, like marine mussels, integrate the $\delta^{15}N$ of primary producers over time and could provide information on the sources of N in aquatic ecosystems. Trends in changing land use should therefore be reflected in the $\delta^{15}N$ signatures of the biota. McKinney et al. (2002) found that mussel isotope values were correlated with long-term average nitrate concentrations and that land use within a 200 m buffer (particularly residential and natural vegetation) explained most of the variation in the data. In this paper, the authors recognized the shortfalls of the produced model (e.g., inaccurate land use data) and raised the question of how isotopic signatures are influenced by site-specific characteristics such as soils, natural vegetation and atmospheric patterns.

Overall, there is general agreement that unionid mussels are a good integrator of $\delta^{15}N$ signatures near the base level of aquatic food webs. Using these organisms avoids the temporal (seasonal) and spatial (e.g., within a lake) variability seen in the $\delta^{15}N$ signature of primary producers. There are also practical advantages to using mussels. Lake et al. (2001) found no relationship between mussel size and $\delta^{15}N$ signature, which would simplify the collection of individuals to assess a waterbody or group of waterbodies. McKinney et al. (1999) concluded that the $\delta^{15}N$ signature of a mussel from a single site within a pond could be used to estimate the baseline $\delta^{15}N$ of the whole pond. This same study, however, also showed significantly different signatures within a single pond, and the authors recognized that mussels sampled from one location in a non-homogeneous system may not be representative of the whole system. This could be problematic if only one site is being used to assess the condition of the lake and that site is subject to disturbance or change over time. It appears however, that with sufficient background information and careful site selection, one can determine the baseline $\delta^{15}N$ signature of a lake based on the analysis of a single mussel.

But can this baseline signature be used in assessing the water quality of a given lake? When assessing lake water quality, we consider various physical, chemical and biological parameters to draw conclusions on the condition of the lake at the time of sampling, and over time we use this information to identify trends in water quality and develop appropriate management strategies. It is well known that nutrient concentrations can fluctuate considerably in lakes, both temporally (i.e. seasonally) and spatially (within the water column). In stratified lakes, there is often an accumulation of nutrients in the hypolimnion, resulting from internal loading, while epilimnetic concentrations are reduced through biological assimilation. In British Columbia, provincial water quality monitoring programs often use water chemistry results measured during the spring overturn period to determine the overall condition of the lake and track changes over time. If mussel $\delta^{15}N$ signatures were to be used as part of this assessment, it would be difficult to relate this information to water chemistry data because we would be unsure of the time frame that mussel $\delta^{15}N$ signatures represent, while water chemistry results specifically relate to conditions at the time of sample collection.

A number of researchers have used the $\delta^{15}N$ signatures of aquatic organisms as indicators of nutrient inputs to waterbodies. Cabana and Rasmussen (1996) were among the first to suggest the $\delta^{15}N$ signatures of aquatic organisms could be used as an indicator of impacts on aquatic ecosystems from human land use. They presented data from many different studies, using many different organisms, to show an increasing trend in organism $\delta^{15}N$ with human population and suggested that $\delta^{15}N$ signatures at the base of the food chain provide a useful indicator of nutrient inputs such as human sewage. While human density accounted for most of the among-lake variation (68%) in this study, the authors also recognized the influence of other sources of variability such as denitrification, nitrogen fixation and the input of low $\delta^{15}N$ from synthetic fertilizers. The relationship is also flawed because the signatures of many different organisms from many different water bodies are used and since that work, within-lake and between-lake variations in the $\delta^{15}N$ of aquatic organisms have been demonstrated (Lake et al. 2001; Matthews and Mazumder 2003; Cole et al. 2005).

McClelland et al. (1997) used the $\delta^{15}N$ signatures of estuarine plants and animals to link sources of N from watersheds to the N in food webs of adjoining estuaries. They found that $\delta^{15}N$ in the biota increased with urbanization, and although atmospheric and fertilizer sources contribute, increases in wastewater loading appear to drive the change in $\delta^{15}N$ in estuaries. They concluded that this tool could be used to assess N inputs over time and suggested it can be used to provide early detection of nutrient inputs and prevent profound ecological impacts. The authors note the influence of mixing different N sources on the final $\delta^{15}N$ measured in biota but fail to incorporate this into their conclusions in which they suggest the potential for $\delta^{15}N$ as an early indicator of eutrophication. For example, they compare the $\delta^{15}N$ signatures of two primary producers, *Zostera marina* and *Gracilaria tikvahiae*, from five different estuaries of varying size and development. From this they show a good relationship between wastewater load (as a percentage of the total load) and $\delta^{15}N$. Two of the five estuaries (Eel Pond and Childs River) had very similar N loading contributions from atmospheric sources, fertilizers and wastewater (approximately 25%, 12% and 65%, respectively), hence similar $\delta^{15}N$ values (*Z. marina* ~ 6‰, *G. tikvahiae* ~ 8‰), but very different total N loading rates (88 kg N ha^{-1} yr^{-1} and 467 kg N ha^{-1} yr^{-1}). A third estuary (Quashnet River) had a relatively high total N loading rate (390 kg N ha^{-1} yr^{-1}) with a higher contribution from atmospheric sources (45%) and fertilizers (26%), but a lower contribution from wastewater (28%), resulting in lower $\delta^{15}N$ values (*Z. marina* ~ 0‰, *G. tikvahiae* ~ 6‰). Linear regression of these data showed a significant ($p = 0.0001$) relationship between the contribution of wastewater to the total N load and $\delta^{15}N$. The authors concluded that comparisons of $\delta^{15}N$ values between pristine and impacted estuaries, or monitoring $\delta^{15}N$ signatures over time at the same estuary would identify relatively low wastewater inputs. Based on $\delta^{15}N$ signatures alone, one could conclude that the third estuary is less affected than the other two and, in fact, might appear to be improving if fertilizer inputs increased without a similar increase in wastewater inputs. On the other hand, because denitrification rates tend to increase as a function of nitrogen concentration, high loadings of inorganic nitrogen fertilizer could still result in elevated $\delta^{15}N$ in biota (Vander Zanden et al. 2005). This illustrates the need to exercise caution when interpreting isotope data, as well as the need for a complete understanding of land use and nutrient budgets within the watershed. Furthermore, $\delta^{15}N$ data may be more applicable on a site-specific basis than as a general indicator of impact across sites.

Lake et al. (2001) measured $\delta^{15}N$ signatures in fish, mussel and sediment samples from 17 small freshwater sites to examine food chain length and trophic position across sites affected by differing levels of anthropogenic activity. Their results showed a logarithmic relationship between dissolved inorganic N (DIN) concentrations and $\delta^{15}N$ and concluded that $\delta^{15}N$ is a sensitive indicator of eutrophication at sites with low DIN. There are some difficulties with this interpretation, however, in that the authors compared a relatively dynamic characteristic (i.e., DIN) with a more stable one (mussel and fish $\delta^{15}N$) which integrates the $\delta^{15}N$ of all nitrogen sources over an undetermined period of time. Additionally, the fact that the observed relationship between $\delta^{15}N$ and DIN was logarithmic, with the greatest increases in $\delta^{15}N$ seen at low DIN levels, suggests that at higher loadings, the mixing of several N sources may complicate interpretation of $\delta^{15}N$ signatures. This study also found the fraction of residential land (but not other land use types) in buffer zones surrounding sites to be correlated with fish $\delta^{15}N$, and concluded that urban development, and presumably human wastewater, resulted in elevated $\delta^{15}N$ values in these small freshwater systems. From this conclusion they are assuming that a higher N loading translates to a higher $\delta^{15}N$ in the biota; this may not be accurate, as was evident in McClelland et al. (1997).

Cole et al.'s (2005) investigation of macrophyte $\delta^{15}N$ signatures concluded these organisms could be excellent indicators of anthropogenic N in aquatic systems. In this work, the authors determined the isotopic signatures of macrophytes collected from freshwater and estuarine waterbodies and compared mean signatures for rooted and non-rooted species from both environments to wastewater loadings. Of the species tested, only two were common to all three freshwater ponds sampled. Furthermore, there was considerable variation between the individual rooted species measured at each pond: Ashumet Pond ranged from 6.4‰ to 12.3‰ (n = 5, wastewater = 80%); Coonamessett Pond ranged from 0.5‰ to 6.8‰ (n = 6, wastewater = 17%); and Miacomet Pond ranged from 2.9‰ to 7.3‰ (n = 8, wastewater = 27%). Depending on the mix of plants analysed, it would be possible to obtain mean values that are very close to one another from these systems which are subject to a considerable range of wastewater inputs. Variation in macrophyte species-specific signatures among systems has been demonstrated (Inglett and Reddy 2006; Jones et al. 2004) and suggests a limitation to the use of a bulk macrophyte signature in assessing anthropogenic impacts on aquatic ecosystems. However, in estuarine and marine environments, macrophytes may provide the most suitable organism for this purpose (e.g., Savage 2005).

Vander Zanden et al. (2005) assessed the utility of macroinvertebrate primary consumer $\delta^{15}N$ signatures as an indicator of lake trophic status, nutrient loading and watershed land use. They measured the $\delta^{15}N$ signatures of primary consumers ($PC_{\delta 15N}$) belonging to several taxa (Bivalvia, Gastropoda, Amphipoda, Isopoda, Ephemeroptera, Trichoptera, Chironomidae, Corixidae, Tipulidae and Oligochaeta), calculated a mean value for each lake and developed models to predict $PC_{\delta 15N}$ based on a number of physical, limnological, nutrient loading and nutrient source parameters. They found that $PC_{\delta 15N}$ reflected land use closer to the lake or its tributaries and that the among-lake variation was likely due to multiple processes including N loading, nutrient source and perhaps broader scale catchment processes. Vander Zanden et al. (2005) concluded that primary consumer $\delta^{15}N$ provides a useful monitoring tool because it integrates the underlying variability of DIN and reflects anthropogenic nutrient loading over a wide range of conditions. There is some question, however, as to the application of this particular approach because of method used to determine $PC_{\delta 15N}$; several organisms were used to determine $PC_{\delta 15N}$ for each lake and the effect of this on the assumed isotopic signature of primary consumers is not discussed. Other studies have shown taxon-specific variation in $\delta^{15}N$ of zooplankton (Matthews and Mazumder 2003), macrophytes (Cole et al. 2005) and fish (Lake et al. 2001) and there is no reason not to expect the same with aquatic macroinvertebrates. Secondly, the signatures represent only one season from a single year and so any temporal variation has not been quantified. The authors addressed this to some degree in that they measured nutrient concentrations and loadings for a period prior to collecting the macroinvertebrates, however detecting trends in water quality requires a longer period of time. It is possible that the $\delta^{15}N$ signatures of shorter lived organisms and corresponding water quality conditions may provide a more sensitive water quality indicator.

There are other problems with using macro-invertebrates and fish, mostly dealing with ease of sample collection. Mussels are not abundant in some all lakes (see McKinney et al. 2002). Some researchers have collected samples by diving, however safety has to be a consideration, especially if volunteers are called on to collect information. Another factor is the overall stability of mussel populations; over half of all North American freshwater mussel species are considered at risk (Lee 2000) making the collection and use of these organisms as environmental indicators somewhat questionable. The collection of macro-invertebrates is also labor-intensive and it would be desirable to have consistent collections, in terms of species and life stages analyzed, in order to track changes over time. Fish $\delta^{15}N$ signatures can be difficult to interpret because of their generally higher trophic position and the fact that signatures may be influenced by other factors such as food chain length, naturally occurring variation in $\delta^{15}N$ values within populations, and the effects of environmental perturbation (Lake et al. 2001). There is also the added requirement of collection permits often required by regulatory agencies.

For $\delta^{15}N$ signatures of aquatic biota to be useful indicators of water quality certain considerations must be taken into account:

- the test organism must be near the base of the food web to simplify interpretation of the results;
- the $\delta^{15}N$ signature must be representative of conditions (e.g., water chemistry) at the time of sampling;
- land use within the watershed must be reasonably understood; and
- the test organism must be abundant and easy to collect so that monitoring can be replicated in the future.

Structure of this Thesis

My main goal in conducting this research was to develop a robust indicator of septic inputs to lake ecosystems and investigate the application of this indicator in lake water quality assessments. To do this, I proposed to model the variability in $\delta^{15}N$ signatures in zooplankton as a function of human sewage inputs to lakes to produce a simple model and associated understanding of co-varying factors that could be used by water managers and volunteer lake stewardship groups alike to monitor and manage the health of lake ecosystems. Specifically, I wanted to test the hypothesis that calanoid copepod and *Daphnia* $\delta^{15}N$ signatures are stable over time in the absence of changes in human land-use. Secondly, I wanted to measure the variability in the $\delta^{15}N$ of calanoid copepods and *Daphnia* to determine if one taxon provides a more stable signal over time. Finally, I wanted to investigate the factors (watershed, morphometric and water chemistry) that influence zooplankton $\delta^{15}N$.

Chapter 2: Water quality assessments of small lakes using stable isotope signatures of pelagic zooplankton

Seasonal changes in zooplankton $\delta^{15}N$ have been demonstrated (Matthews and Mazumder 2005), presenting possible complications with respect to the timing of sample collection. In this chapter, I explore the seasonal patterns and inter-annual consistency of calanoid copepods and *Daphnia* $\delta^{15}N$ and assessed the potential use of this tool in water quality assessments of lakes. My goals were to identify seasonal patterns in zooplankton $\delta^{15}N$ that would suggest preferential times for collecting zooplankton for isotopic analysis. I wanted to investigate the consistency of $\delta^{15}N$ from year to year, to assess its potential value in detecting long-term water quality trends. And finally, I wanted to determine the difference in variability of calanoid and *Daphnia* $\delta^{15}N$ to determine whether one group of species provides a more consistent signature.

Chapter 3: Linking residential development to calanoid nitrogen isotope signatures

The $\delta^{15}N$ of aquatic biota have been quantitatively linked to anthropogenic impacts in a number of studies (McClelland et al. 1997; Lake et al. 2001; Vander

Zanden et al. 2005) and must be demonstrated to effectively use zooplankton $\delta^{15}N$ in water quality assessments. In Chapter 2, I demonstrate that calanoid copepod $\delta^{15}N$ is less variable than *Daphnia* $\delta^{15}N$ over time and, therefore, provides a more consistent water quality indicator. In this chapter, I investigate the link between calanoid copepod $\delta^{15}N$ and a number of watershed and limnological characteristics in 22 British Columbia lakes. I propose that, because of the availability and ease of collection of calanoid copepods and the influence of residential development on $\delta^{15}N$, calanoid N isotope data provide a valuable supplement to the water quality assessments of small lakes.

Chapter 2: Water Quality Assessments of Small Lakes Using Stable Isotope Signatures of Pelagic Zooplankton

Abstract

I explored the seasonal patterns and inter-annual consistency of zooplankton (calanoid copepods and *Daphnia*) $\delta^{15}N$ and assessed the potential use of this measure in water quality assessments of lakes. Samples were collected from eight coastal lakes over three to five years. A general seasonal pattern was noted whereby $\delta^{15}N$ peaked in the winter and spring, followed by a decrease in summer and subsequent increase through fall to similar levels the next winter and spring. This pattern was also demonstrated in calanoid copepod data collected from 61 lakes throughout British Columbia. Calanoid copepod $\delta^{15}N$ was consistently higher and less variable than *Daphnia* $\delta^{15}N$. A strong relationship between mean $\delta^{15}N$ and the density of septic systems was demonstrated, and calanoid copepod $\delta^{15}N$ was generally consistent over time in the absence of changes in land use. Consistent interannual patterns in zooplankton $\delta^{15}N$ on suggest an application for this parameter in trend analysis. Overall, my results provide support for the use of calanoid copepod $\delta^{15}N$ in water quality assessments when used in conjunction with other data.

Introduction

Stable isotopes are increasingly being used, in combination with more conventional techniques, to address ecological questions in freshwater research (Adams and Sterner 2000; Grey 2006). A common application is the use of δ^{15}N and δ^{13}C signatures of aquatic organisms to determine trophic relationships and energy pathways in aquatic ecosystems (Fry 1991; Vander Zanden et al. 1999; Post et al. 2000; Matthews and Mazumder 2003, 2006; Perga and Gerdeaux 2006). Since nitrogen isotopic signatures can also be used to help identify sources of nitrogen, it is a potentially useful tool in water quality assessments. Cabana and Rasmussen (1996) were among the first to propose this application and, subsequently, several studies have linked the δ^{15}N of various organisms (e.g., mussels, aquatic plants, macroinvertebrates and fish) with anthropogenic impacts (McClelland et al. 1997; McClelland and Valiela 1998; Fry 1999; Costanzo et al. 2001; Lake et al. 2001; McKinney et al. 2002; Steffy and Kilham 2004; Cole et al. 2005; Savage 2005; Schlacher et al. 2005; Vander Zanden et al. 2005; Anderson and Cabana 2006; Kaushal et al. 2006).

Despite the promising results reported to date, there are some potential problems with the approaches described in the literature. For example, sample replication may be difficult when composite samples of several species (e.g., Cole et al. 2005; Vander Zanden et al. 2005) are used to measure δ^{15}N. For higher trophic level organisms such as fish (e.g., Lake et al. 2001), interpretation of results can be complicated by the turnover rate and subsequent expression of isotope signatures in animal tissue (Grey 2006) and variations in isotopic expression of different tissue types within an individual (e.g., Perga and Gerdeuax 2005). Finally, while several studies have used unionid bivalves as an indicator of baseline $\delta^{15}N$ (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; McKinney et al. 2002; Vuorio et al. 2007), they are not available in all freshwater systems and can be difficult to collect (Grey 2006).

For $\delta^{15}N$ signatures of aquatic biota to be useful indicators of water quality, certain considerations must be taken into account. First, the test organism should be near the base of the food chain so that variation in food chain length does not influence the interpretation of $\delta^{15}N$ patterns. Second, from a resource management perspective, $\delta^{15}N$ signatures should ideally be representative of conditions (e.g., water chemistry) at the time of sampling, or over a time period of interest to managers. Consumers are rarely in isotopic equilibrium with their food sources (Matthews and Mazumder 2005), however, so this may be difficult to accomplish. Aquatic organisms with a shorter turnover time may be more appropriate to use in this application of stable isotopes. Third, land use and nitrogen sources within the watershed, and the hydrology of the watershed must be reasonably well understood so that isotope results can be properly interpreted. Finally, the test organism must be abundant, widespread and easy to collect so that monitoring can be easily replicated in the future.

The $\delta^{15}N$ of the various components of the inorganic nitrogen pool are highly variable, which leads to high temporal variability in the $\delta^{15}N$ of particulate organic matter and primary producers such as phytoplankton (Lehman et al. 2004). In order to use $\delta^{15}N$ in water quality assessments, it is therefore important to measure the $\delta^{15}N$

of an organism that integrates the δ^{15}N of different sources, both spatially and temporally, to simplify data interpretation. In light of the above considerations, the δ^{15}N signatures of crustacean zooplankton have potential for the purpose of assessing land use impacts on lakes. There are species-specific differences in δ^{15}N signatures between zooplankton taxa (Matthews and Mazumder 2003) which justifies ensuring the same component of the community be measured each time, as opposed to measuring signatures of bulk samples with variable species composition. The life cycles of most zooplankton are relatively short (weeks), so that the signatures measured are more reflective of the water chemistry and other conditions at the time of sampling than are the δ^{15}N of longer lived organisms like mussels. Groups of zooplankton species, such as calanoid copedods and cladocerans, are usually abundant and easy to collect with no collection permits required. The samples consist of a composite of individual whole animals so processing is usually easier than taking a portion of an animal (e.g., fish tissue) or grinding up the whole animal in order to get a composite of the different tissue types (e.g., mussels).

The overall objective of this study was to determine whether the δ^{15}N signatures of crustacean zooplankton could be used to detect changes in human land use over time, potentially enhancing water quality assessments in lakes. Many nitrogen sources have distinct isotopic signatures (Heaton 1986) that can be used to help determine the sources of inputs to receiving waters (Vander Zanden et al. 2005). For example, the δ^{15}N of N from atmospheric sources or synthetic fertilizers is typically near 0‰, ranging between -3‰ and 8‰ (Vander Zanden et al. 2005), compared to values between 10‰ and 20‰ for wastewater (McCelland and Valiela

1998). While the seasonal variation of zooplankton $\delta^{15}N$ has been shown (Grey et al. 2001; Matthews and Mazumder 2005; Perga and Gerdeaux 2006) I am not aware of any studies, other than Syväranta et al. (2008), that present site-specific $\delta^{15}N$ signatures for more than 2 years. Understanding the factors that influence seasonality and inter-annual variation in $\delta^{15}N$ will be crucial if $\delta^{15}N$ is to be used in trend assessments. The goals of this study were to (i) determine if there is an optimal period for sample collection given the seasonal variation in $\delta^{15}N$ signatures; (ii) identify differences in the variability of calanoid copepod and *Daphnia* $\delta^{15}N$ signatures to help determine if one taxonomic group provides a more consistent indicator over time; and (iii), determine if calanoid copepod and *Daphnia* $\delta^{15}N$ signatures are consistent, in the absence of land use change or alterations to a lake, from year to year.

Methods

Study Sites

Zooplankton samples were collected from 61 lakes throughout British Columbia (Appendix 2.1). Samples were collected as part of several projects, therefore, the number of samples collected from each lake and the timing of collection was dictated by the primary goals of each project. More intense sampling was conducted on eight small coastal lakes located on southern Vancouver Island, British Columbia, specifically for this study, over a period of three to five years. Durrance, Fork, Prospect, Langford, Florence, and Glen lakes were sampled in each of 2003 to 2007; Kemp Lake was sampled from 2004 to 2007; and Cusheon Lake from 2005 to 2007. The Cusheon Lake data were supplemented with additional data collected in 2002 and 2003 (Matthews and Mazumder 2003). In addition, data from another two lakes unaffected by urban development, Sooke and Council (Perga et al. 2006), were included to serve as reference sites; these lakes are located within the protected watershed supplying drinking water to Victoria, British Columbia. The dominant calanoid copepod species were identified for these lakes. *Daphnia* were not identified to species.

A summary of morphological, limnological and physical characteristics for each lake is provided in Table 2.1. All lakes (except Florence) are monomictic, experiencing distinct thermal stratification from approximately May through October and a completely mixed water column from approximately November to March each year. Florence Lake does not exhibit strong thermal stratification because of its relatively shallow depth. Each lake receives significant fall and winter rain and rarely experiences complete surface ice cover. Langford and Glen lakes have aerators that operate from May to October each year. The aerators were installed in the mid-1980's in an attempt to minimize internal phosphorus loading which contributed to increasing eutrophication. Each lake (except Council and Sooke) is also exposed to some degree of urban development with on-site septic systems providing the main method of sewage disposal. Florence, Glen and Langford lakes have recently undergone extension of centralized sewer systems and lakeshore residents are now required by local government to connect to this service. Additionally, the land near the inflow to Langford Lake underwent development (from wetland and ex-agricultural land to future municipal park plus light industrial) over the period of study. There were no other major land use changes in close proximity to any of the other lakes.

Sample collection and analysis

Zooplankton samples were collected using an 80 μm mesh, 30 cm diameter Wisconsin net. Samples were collected as part of several studies which resulted in some data gaps. My goal, however, was to get quarterly representation for each lake on an annual basis. Initially, samples were collected at established deep stations using repeated 10 m vertical tows to ensure enough biomass was collected to allow stable isotope analysis. After some poor catches, however, horizontal tows behind the boat were used to increase the catch efficiency. Vertical tows were made occasionally when phytoplankton growth was high to provide a cleaner sample. On four occasions early in the study, both vertical and horizontal tows were done to compare calanoid copepod isotopic signatures between the two methods.

After collection, samples were kept on ice and frozen as soon as possible without preservation, usually within six hours of collection. Guts were not cleared prior to processing or analysis as previous studies suggest gut clearance has a negligible impact on overall isotopic values of organisms (Anderson and Cabana 2006). Samples remained frozen until being thawed for processing. Individual *Daphnia* and calanoid copepods were picked from each sample using a binocular microscope. Neither taxa were separated by species due to the difficulty and time involved in species identification while obtaining enough tissue to conduct the analyses. In addition, the general taxonomic groupings of "*Daphnia*" and "calanoid copepod" have been used in previous isotopic studies (e.g., Matthews and Mazumder 2003). Following Matthews and Mazumder (2003), the goal was to obtain approximately 1 mg of dried tissue per sample for isotopic analysis. All developmental stages were included in the samples and the number of individuals within a sample depended on the dominant life stage present at the time of sampling. For some samples, individual zooplankton were counted to allow the calculation of the average weight per individual in the sample. Samples were then freeze dried, packed in 6x4 mm tin cups and analyzed by the University of Victoria Department of Biology, Water and Aquatic Science Research Program's laboratory using a *DELTA^{plus}* Advantage Isotope Ratio Mass Spectrometer. Samples were analyzed for $\delta^{15}N$, $\delta^{13}C$, percent carbon and percent nitrogen. Lipid extraction was not performed, therefore, to account for lipid-related biases in $\delta^{13}C$, the mathematical normalization method recommended by Smyntek et al. (2007) was followed:

$$\delta^{13}C_{ex} = \delta^{13}C_{bulk} + 6.3((C{:}N_{bulk} - 4.2)/\ C{:}N_{bulk})$$

where $\delta^{13}C_{ex}$ is the lipid-extracted $\delta^{13}C$ value, $\delta^{13}C_{bulk}$ is the $\delta^{13}C$ value of the non-extracted sample and $C{:}N_{bulk}$ is the C:N ratio of the non-extracted sample.

As each sample consists of different individual zooplankton, I wanted to determine the variability in $\delta^{15}N$ between replicate samples collected on the same date. Triplicates were picked and analyzed when there were sufficient numbers of each taxonomic group available in the sample.

Water Chemistry

Water samples were collected near the top (0.5 m) and bottom (within 1 m) of the water column and occasionally at the mid-point of the water column. Surface grab samples were collected by hand and deep-water samples were collected using a Van Dorn bottle. All water samples were kept in coolers on ice and shipped within 24 hours to Maxxam Analytics in Burnaby, British Columbia for analyses of total nitrogen (TN), ammonia (NH_4), nitrate+nitrite (NO_3+NO_2), total phosphorus (TP), dissolved organic carbon (DOC) and total organic carbon (TOC). The following analytical techniques were used to determine parameter concentrations: TN by Alkaline Persulfate Digestion, UV radiation; NH_4 by the Automated Phenate method; NO_3+NO_2 by the Automated Cadmium Reduction method; TP by the Ascorbic Acid method; and TOC and DOC by the Persulfate Oxidation method. Values of one half the detection limit were used for concentrations that were reported as less than the detection limit. Average concentrations for the whole water column were calculated for each parameter on each sampling date and these values were used in subsequent data analyses. Dissolved oxygen (DO) and temperature profiles were performed using a YSI 550A DO meter and Secchi depths were measured with a standard Secchi disc.

Land Use Characterization

Information on individual septic systems was not readily available for the study lakes, so the density of residential lots was used as a proxy. At the time of sampling, on-site septic systems were the primary method of sewage disposal for the area around each developed lake, therefore it was assumed each lot represented a single home with its own septic system (this is a reasonable assumption for southern Vancouver Island). Lots were categorized by size (i.e. $\leq$0.25 ha, $\leq$0.5 ha, $\leq$2.5 ha). Densities (i.e. number of lots per km^2) were calculated for each size category in the 200 m buffer zone around each lake and the 200 m buffer zone around each lake and its tributaries, counting each lot contained at least partly in that buffer. Previous studies (Valiela et al. 1997; Lake et al. 2001; McKinney et al. 2002) suggest that residential land use within a buffer zone around a lake, versus the whole watershed, provides the strongest relationship with organism δ^{15}N. Cadastral data, which illustrate the boundaries and ownership of land parcels, were obtained from the Province of British Columbia's Land Resource Data Warehouse using ArcView 8.

Statistical Analysis

Pearson's product moment correlation was used to determine the relationships between water chemistry variables and zooplankton δ^{15}N. Water chemistry results were $\log_{10}$ transformed prior to performing the tests, to better approximate a normal distribution. Average concentrations for the whole water column were calculated on any given sampling date and used in these analyses. To characterize spring total N and total P concentrations for each lake, median values were determined from the data available for periods when the water column was mixed. Nutrient concentrations can vary considerably from year to year due to site-specific conditions at the time of sampling. Using the median values avoided the influence of unusually high or low nutrient concentrations in a given year and provided a more appropriate indication of typical conditions for each lake. Simple linear regression was used to determine the relationships between zooplankton weight and $\delta^{15}N$. JMP® 5.1 statistical software was used for these statistical tests.

To identify trends over time in zooplankton $\delta^{15}N$, the nonparametric seasonal Kendall trend test (Hirsch et al. 1982), which accounts for seasonal trends, non-normal distributions and missing data (Gilbert 1987), was used. The null hypothesis is that there is no monotonic trend while the alternative hypothesis is that there is a monotonic trend, either upward or downward. A strong advantage of this test is that one does not have to make assumptions, apart from monotonicity, about the functional form of any trend that may be present (e.g, linear, exponential); the test only considers whether within-season/between-year differences tend to be monotonic (Smith et al. 1996). These tests were performed using programs available from the US Geological Survey (Helsel et al. 2006) specifying four seasons (i.e. equal time periods) per year.

To test for seasonal differences in calanoid $\delta^{15}N$, data from all lakes were pooled and organized according to the time of year the sample was collected. The lakes sampled represent a wide range of geographical and climatic conditions. Seasonal periods vary between coastal regions and southern and northern interior regions and it would be difficult to determine seasonal classifications that would apply on a provincial basis. Therefore I arbitrarily assigned quarters based on the Gregorian calendar to represent seasons: quarter (Q) 1 = January, February and March; Q2 = April, May and June; Q3 = July, August and September; and Q4 = October, November and December. Mean $\delta^{15}N$ signatures were calculated for each month and quarter. Preliminary analysis of the data suggested that calanoid $\delta^{15}N$ was greatest in the spring, typically around March. The data did not meet the assumptions of conventional parametric tests (e.g., randomly sampled, normally distributed, equal variances), therefore, a nonparametric randomization test (Potvin and Roff 1993; Bulté and Onghena 2008) was used to determine if the mean Q1 $\delta^{15}N$ signature was statistically higher than the others. With randomization tests, the researcher can use any test statistic considered appropriate (Bulté and Onghena 2008) and the observed test statistic used here was the difference between the mean signature of the highest quarter and the mean signature of the remaining quarters. The data were randomized among quarters 100,000 times using a procedure written specifically for this application with R statistical software. The test statistic was calculated after each iteration of the randomization test. The one-sided *p*-value for the test was calculated as the proportion of iterations the calculated test statistic was greater than the observed test statistic. Thus, a proportion of less than 0.025 would allow rejection of the null hypothesis that all quarters have an identical probability distribution.

Results

The dominant species of calanoid copepod identified in each lake were as follows: *Hesperodiaptomus franciscanus* (Durrance, Fork, Glen, Langford, Prospect); *Leptodiaptomus tyrelli* (Sooke, Council); *Skistodiaptomus oregonensis* (Cusheon, Kemp); and *Onychodiaptomus hesperus* (Florence).

Calanoid copepod signatures were measured in samples collected on the same date from horizontal and vertical tows once in each of Council, Fork, Glen and

Prospect lakes. Differences ranged between 0.2‰ (Fork Lake) and 0.6‰ (Prospect Lake), with each method providing a higher signature twice; horizontal tows provided higher signatures in Council and Fork lakes, vertical tows provided higher signatures in Glen and Prospect lakes. From this, I concluded there was no appreciable difference in calanoid $\delta^{15}N$ signatures collected from vertical and horizontal tows, and used mostly horizontal tows throughout the study.

For replicate samples collected on the same date, standard deviations ranged from 0.02‰ to 0.84‰ for calanoid copepods and from 0.02‰ to 2.16‰ for *Daphnia*. 47% of the calanoid samples and 32% of the *Daphnia* samples had standard deviations of less than 0.10‰. For the calanoids, 76% of all sample means had standard deviations less than 0.30‰ and for the *Daphnia*, 72% had standard deviations less than 0.30‰. With this low level of variation among replicates from the same sample, I was confident that a single sample analyzed from a given sampling date would provide an accurate $\delta^{15}N$ signature for that date, thereby reducing the processing time.

Zooplankton Isotopic Signatures Among Lakes

Temporal trends in $\delta^{15}N$ signatures for each lake are illustrated in Figures 2.1a through 2.1j, and a summary of these results is provided in Table 2.2. Calanoid $\delta^{15}N$ signatures were consistently higher than *Daphnia*, which agrees with previously reported results (Leggett et al. 2000; Matthews and Mazumder 2003; Karlsson et al. 2004; Perga and Gerdeaux 2006; Syväranta et al. 2006) suggesting either that calanoid copepods occupy a higher trophic level than *Daphnia* or they have a different isotopic baseline in these lakes (Matthews and Mazumder 2003). Mean *Daphnia* $\delta^{15}N$ signatures showed a higher coefficient of variation (CV) for all lakes where both taxa were found except Florence Lake and Glen Lake, where the CVs were equal (Table 2.2). The variability in $\delta^{15}N$ signatures between taxa is evident in Figure 2.1, however the seasonal patterns of copepod and *Daphnia* $\delta^{15}N$ were similar; that is, when one increased in $\delta^{15}N$, so did the other. Lakes exposed to a higher level of development (e.g., Florence and Glen) showed less variability in $\delta^{15}N$ between taxa, while lakes exposed to lower levels of development (e.g., Kemp and Fork) showed a greater degree of variability.

Overall, zooplankton $\delta^{15}N$ increased with the level of residential development (Table 2.1, 2.2), which is consistent with other studies (Cabana and Rasmussen 1996; Lake et al. 2001; McKinney et al. 2002). The reference lakes (Council and Sooke) showed the lowest $\delta^{15}N$ signatures for calanoid copepods. The lowest *Daphnia* $\delta^{15}N$ signatures were measured in Sooke Lake. *Daphnia* were not found in sufficient numbers in Council Lake, although a mean value of 2.4‰ has been reported for this lake (Matthews and Mazumder 2007).

Durrance Lake showed higher average signatures than expected, based on the low level of residential development around this lake (given the lack of development around this lake, I would have expected to see values closer to those of Council and Sooke lakes); this was due, in part, to elevated signatures of 17.4‰ and 15.1‰ for calanoids, and 10.7‰ and 12.3‰ for *Daphnia* in March 2004 and March 2006, respectively. Even without these higher measurements, Durrance Lake $\delta^{15}N$ zooplankton signatures were higher than expected; although there are no homes within the 200 m buffer zone of Durrance Lake, there are two homes in close proximity to the inflow of the lake with a history of septic system failures.

Linear regression was used to explore the relationship between zooplankton weight and isotopic signature (Table 2.3). Although some significant relationships were noted, the results varied by lake. Cusheon Lake, for example, showed a significant negative relationship between calanoid weight and $\delta^{15}N$, and a positive relationship between *Daphnia* weight and $\delta^{15}N$. Kemp Lake showed a significant relationship between *Daphnia* weight and $\delta^{15}N$, but not for calanoid $\delta^{15}N$. The overall results for both taxa were significant, yet weak, and were not consistent enough across all lakes to suggest that $\delta^{15}N$ increased with zooplankton weight.

A summary of $\delta^{13}C$ results for all lakes is provided in Table 2.4. While mean values between taxa for each lake were generally similar, there was some variation among lakes. Sooke Lake showed the most enriched mean values for both taxa, and Glen Lake showed the most depleted values. Generally, the more developed lakes showed more depleted mean $\delta^{13}C$ values, except Durrance Lake, which had the second most depleted signatures.

Seasonal Trends in Zooplankton Isotopic Signatures

All $\delta^{15}N$ results for each lake were pooled by month to identify seasonal patterns within each lake (Tables 2.5 and 2.6). Generally, each lake showed higher $\delta^{15}N$ signatures in winter and early spring, peaking between January and March, followed by a decrease through the summer and fall months, after which point $\delta^{15}N$ values increased again. The magnitude of seasonal variation varied between lakes with Durrance and Florence lakes showing the greatest range in calanoid $\delta^{15}N$ signatures (5.5‰ and 5.0‰, respectively) and Kemp and Fork lakes showing the least range (1.6‰ and 1.9‰, respectively). The exception to this was Glen Lake with the highest monthly mean measured in November and the lowest mean measured in December. The different pattern could be related to Glen Lake being aerated. Syvränta et al. (2008) noted that nitrification can result in ^{15}N-depleted NO_3^- and ^{15}N-enriched NH_4^+, even at very low oxygen concentrations. An aerated hypolimnion, such as that of Glen Lake, has the potential for high rates of nitrification, which could lead to distinct isotopic signatures between inorganic N pools, as described by Syvränta et al. (2008). ^{15}N-enriched NH_4^+ would be available for uptake throughout the water column of Glen Lake in the fall, after the aerator is shut down for winter and mixing of the water column occurs. This would result in peaks in zooplankton $\delta^{15}N$ before the other lakes. Langford Lake is also aerated, however the effectiveness of this system is questionable as a distinct thermal stratification and low hypolimnetic DO concentrations were measured in close proximity to the diffuser. The seasonal $\delta^{15}N$ pattern in Langford Lake is more similar to the other non-aerated lakes suggesting that the ^{15}N enrichment of NH_4^+ may not be occurring in this lake.

Daphnia $\delta^{15}N$ values also peaked by March, with the exception of Cusheon (July), Fork (October) and Glen (November) lakes. The range in values was greatest in Langford Lake, at 7.9‰, and lowest in Cusheon Lake, at 1.1‰.

To explore the seasonal $\delta^{15}N$ pattern further, calanoid $\delta^{15}N$ signatures were pooled across all lakes and monthly mean signatures were calculated for the entire data set (Table 2.7). Of the 61 lakes (Appendix 2.1) 14 were sampled in each of the four quarters, seven were sampled in three quarters, nine were sampled in two quarters and 31 lakes had samples from one quarter only, for a total of 256 isotope signatures. The highest mean signatures were seen in February, March and April (11.2‰, 12.1‰ and 11.0‰, respectively). This peak was followed by a gradual decrease through late spring and summer, followed by a gradual increase in the fall and winter months before peaking again in the spring.

Recognizing that variations in regional climatic conditions would limit monthly isotopic comparisons, seasonal (i.e. quarterly) mean calanoid $\delta^{15}N$ signatures were also calculated. Of the 61 lakes, 25 were sampled in Q1, 36 in Q2, 44 in Q3 and 21 in Q4. Mean signatures for each of the quarters are listed in Table 2.8. Q1 had the highest mean signature at 11.6‰, while mean signatures for the other quarters were all lower.

The observed test statistic used in the randomization test was the difference between the mean $\delta^{15}N$ of Q1 (11.6‰) and the mean of means for Q2, Q3 and Q4 (9.4‰), which is 2.2‰. The results showed that the probability distribution was not the same for all quarters ($p < 0.001$) and it was concluded that the mean calanoid $\delta^{15}N$ was higher in Q1 than at other times of the year. To determine whether a specific month was influencing this result this procedure was repeated for data from January, February and March (Table 2.7). The observed test statistic used was the difference between the highest monthly mean $\delta^{15}N$ (March at 12.1‰) and the mean of means for the other months (10.6‰). The results showed the probability distribution was not significantly different between Q1 months ($p = 0.377$), and therefore the mean March $\delta^{15}N$ was not significantly greater than the mean signatures for January or February.

Water Chemistry and Zooplankton $\delta^{15}N$

Zooplankton δ^{15}N was compared to water chemistry results using correlation analysis (Table 2.9). Overall, there was little evidence of any relationship between water chemistry parameters and zooplankton δ^{15}N. Weak, but significant, positive relationships were found between calanoid copepod δ^{15}N and log TN ($r = 0.497$, $p < 0.0001$) and log NO_3+NO_2 ($r = 0.467$, $p = 0.0005$). *Daphnia* δ^{15}N was also correlated with log TN ($r = 0.332$, $p = 0.0049$) and log NO_3+NO_2 ($r = 0.354$, $p = 0.0172$). In every lake, TN, at times, consisted of a considerable proportion of NO_3+NO_2, so the fact that both parameters were correlated with zooplankton δ^{15}N is not surprising. A significant positive relationship was found between lot density (<0.25 ha within the 200 m lake buffer zone) and log spring TN ($r = 0.77$, $p = 0.0267$), but not and spring log NO_3+NO_2 ($p > 0.0500$) or log spring NH_4 ($p > 0.0500$).

Residential Development and Zooplankton $\delta^{15}N$

The results of simple linear regression analyses between lot density and mean zooplankton δ^{15}N for each lake are listed in Table 2.10. Significant positive relationships were found between both calanoid copepod δ^{15}N and *Daphnia* δ^{15}N and all lot density classifications within the 200 m lake buffer. No significant relationships were found between zooplankton δ^{15}N and lot densities within the 200 m buffer of the lakeshore and tributaries.

To test the relationship between mean zooplankton δ^{15}N and small lot density, data for an additional 11 Vancouver Island lakes were added (Table 2.11). All of the additional lakes are on septic systems except Long Lake, which is sewered. It could not be confirmed that all homes are connected to the centralized sewer service and it was assumed that 10% of the homes in this area are using on-site septic systems. The results are illustrated in Figure 2.2. Although less variation in zooplankton δ^{15}N is explained with the additional data included in the model, the relationships remain significant between lot density and both calanoid copepod δ^{15}N ($R^2 = 0.65$, $F_{20, 0.05} = 35.5$, $p < 0.0001$) and *Daphnia* δ^{15}N ($R^2 = 0.79$, $F_{20, 0.05} = 68.3$, $p < 0.0001$). Of the 21 lakes represented in Figure 2.3, nine have a lot (<0.25 ha) density of 0 lots/km^2, and six of these (Butchart, Council, Goldstream, Jump, Old Wolf and Sooke) have no residential development at all within the 200 m buffer or the watershed. For these six lakes, the average calanoid δ^{15}N ranges from 4.8‰ (Council) to 6.9‰ (Jump) and the average *Daphnia* δ^{15}N ranges from 2.8‰ (Butchart) to 7.1‰ (Old Wolf). The other three lakes with no small lots (Mitchell, Durrance and Pease) all have some residential development in close proximity and higher calanoid copepod δ^{15}N, ranging from 9.2‰ (Mitchell Lake) to 10.6‰ (Durrance), but comparable *Daphnia* δ^{15}N, ranging from 2.4‰ (Mitchell) to 6.0‰ (Durrance) suggesting that low levels of development may be expressed in calanoid copepod δ^{15}N.

Inter-Annual Trends in Zooplankton $\delta^{15}N$

The results of seasonal Kendall trend test analyses for calanoid δ^{15}N (using data presented in Figure 2.1) are provided in Table 2.12. No significant trends were seen in *Daphnia* δ^{15}N at any of the lakes ($p > 0.10$), possibly reflecting the higher variance seen in the isotopic signatures for this taxon. Significant downward trends in calanoid copepod δ^{15}N were seen in Florence ($p = 0.0875$) and Langford ($p =$

0.0367) lakes; however these results should be viewed with caution. For Florence Lake, the results represent only nine of 20 "seasons" defined in the seasonal Kendall trend analysis (four seasons per year for five years), which may be insufficient to determine if a trend exists. The Langford Lake data represent 16 of 20 seasons and March calanoid $\delta^{15}N$ signatures decreased steadily from 15.9‰ in March 2003 to 14.3‰ in March 2007; however in April 2007 the calanoid $\delta^{15}N$ was 15.4‰. When the number of seasons per year was increased to six (i.e., groupings of two months) neither lake showed a significant trend (p>0.1). There have been some recent land use changes near the inflow to Langford Lake, including stormwater management measures such as detention ponds, and this could be reducing inputs of nitrogen with an elevated $\delta^{15}N$ signature. However, no significant decrease in TN concentrations was noted (p = 0.6069).

None of these lakes, except Glen, underwent any land use changes during the period of study that would be consistent with decreasing anthropogenic inputs of N. The Glen Lake data showed an insignificant downward trend (p = 0.5690) despite a continuous decrease in calanoid $\delta^{15}N$ from 25.9‰ in March 2003 to 18.9‰ in March 2007 (Figure 2.1j). Like Florence, these data represent only nine of 20 seasons, which may be insufficient. The City of Langford has recently extended centralized sewer service to the areas of Florence, Langford and Glen lakes and requires lakeshore residents to connect to this service by 2009. In addition, all new homes built must also connect to this service. Glen Lake was the first to be offered this service beginning in 2004. By the end of 2006 (prior to the final sampling effort in March 2007), 22% of homes within the 200 m buffer zone around Glen Lake had converted from septic to centralized sewer service. Similarly, 21% of shoreline residences had converted to centralized sewer. TN did not decrease significantly in Glen Lake over this period ($p = 0.5201$).

Discussion

Seasonal Variation in Zooplankton $\delta^{15}N$

Zooplankton δ^{15}N varies both within lakes (temporally and between taxa) and between lakes and is influenced by many factors including: the N sources entering the lake (Jones et al. 2004; Gu et al. 2006), N cycling within the lake (Karlsson et al. 2004; Lehmann et al. 2004), differences in site-specific isotopic baselines (Matthews and Mazumder 2003), fractionation of N isotopes during biologically-mediated transformations of N within the lake (Adams and Sterner 2000; Jones et al. 2004; Karlsson et al. 2004; Lehmann et al. 2004), isotopic differences in the forms of N preferred by pelagic autotrophs (Leggett et al. 2000; Syränata et al. 2008), differences in zooplankton diet and feeding behavior (Grey et al. 2001; Karlsson et al. 2003; Matthews and Mazumder 2007), and, lake-specific physical and chemical characteristics (Perga and Gerdeaux 2006). This range of factors underscores the need to consider potential within-lake spatial and temporal variability of isotope ratios in studies of individual lakes or multiple lake comparisons that utilize stable isotope analyses, as recommended by Syväranta et al. (2006).

The variable nature of zooplankton δ^{15}N, both within and between lakes, was demonstrated in this study; however, some conclusions can be drawn from the results. The data showed between-lake variation in seasonal patterns, with zooplankton δ^{15}N generally peaking in the winter and spring (November through April), followed by a decrease through the summer months and a variable increase through the fall, returning to peak again the following winter and spring. While this general trend may not have been demonstrated in all lakes covered in this study, it has been described elsewhere (Leggett et al. 2000; Grey et al. 2001). My results illustrate the seasonal variability of zooplankton $\delta^{15}N$ and the need for adequate sampling in order to capture this variability, which should include the period from January to March when maximum $\delta^{15}N$ signatures in a lake are likely to occur. There is considerable variation between the lakes sampled and factors such as local climate (e.g., precipitation, ice cover, air temperature), land use and hydrology undoubtedly play some role in determining the $\delta^{15}N$ of biota and contribute to inter-lake variability. This is not to suggest, however, that sampling should be limited to once per year. Sampling on a more frequent basis would be desirable in order to identify lake-specific seasonal trends. While monthly measurements would be desirable for the purpose of general water quality assessments, it is not always feasible and so quarterly sampling should be considered as a minimum to identify any seasonal differences in zooplankton $\delta^{15}N$ values. With adequate sampling over time for a given lake, the preferred sampling periods may become more evident.

Three hypotheses are proposed to explain the seasonal peak in $\delta^{15}N$. First, allochthonous inputs of nutrients and organic materials to these lakes are greatest in the fall and winter months with high levels of precipitation, therefore the contribution of organic material with elevated ^{15}N due to runoff, such as sewage and animal wastes, would be greatest. Lehmann et al. (2004) found high $\delta^{15}N$ values in winter for particulate organic nitrogen and noted this had also been observed in other lakes

(Hodell and Schelske 1998; Teranes and Bernasconi 2000). Grey et al. (2001) observed a seasonal shift in zooplankton isotopic signatures, reflecting a dietary switch from a reliance on allochthonous C derived from particulate organic matter (POM) during winter and early spring, to a heavy dependence on algal production during summer. Zooplankton $\delta^{15}N$ will therefore fluctuate seasonally with the $\delta^{15}N$ of the preferred forms of inorganic N available for uptake by phytoplankton.

The second contributing factor is the mixing of the water column. The $\delta^{15}N$ of bottom water NO_3^- can increase substantially during summer months. Lehmann et al. (2004) observed an increase from 8‰ to 27‰, which they associated with the development of anaerobic conditions coupled to decreasing $[NO_3^-]$, indicating active denitrification in the hypolimnion. They speculated that mixing of the water column, including ^{15}N-enriched bottom water NO_3^- (due to microbial NO_3^- reduction), may have contributed to the increase in surface water $\delta^{15}N$ values of NO_3^- observed after November. Conversely, Leggett et al. (2000) observed a reduction in NO_3^- $\delta^{15}N$ and concluded that fall mixing should "reset" the $\delta^{15}N$ of NO_3^- to reflect the lower, less-enriched values of the hypolimnetic NO_3^- pool on a seasonal basis. Syvränta et al. (2008) observed distinct differences in NO_3^- $\delta^{15}N$ (depleted) and NH_4^+ $\delta^{15}N$ (enriched), which they attributed to increased rates of hypolimnetic nitrification resulting from lake aeration. The resetting of isotopic values, following destratification and mixing of the water column, appears to be a consistent phenomenon and is clearly influenced by site-specific conditions at each lake. Furthermore, it demonstrates the potential use of zooplankton $\delta^{15}N$ signatures in long-term water quality trend assessments.

A third possible reason for the seasonal peak could be related to food availability for zooplankton. Over-wintering female calanoid copepods rely heavily on stored lipids for reproduction during the spring isothermal period, and can exist for long periods at extremely low food levels (Bundy et al. 2005). It is believed that nutritional stress results in the preferential excretion of ^{14}N from internal sources, leading to an increase in organism $\delta^{15}N$ (Adams and Sterner 2000; Karlsson et al. 2004). Either lack of food or deterioration of food quality (i.e. reduced N content) during late fall and winter could contribute to increasing zooplankton $\delta^{15}N$. However, given the productive nature of these lakes it seems unlikely that this would contribute significantly to seasonal shifts in zooplankton $\delta^{15}N$ on southern Vancouver Island.

The following decrease observed in zooplankton $\delta^{15}N$ was likely related to the $\delta^{15}N$ signatures of NO_3^- available to phytoplankton. During spring, zooplankton biomass grows rapidly and excretion of $\delta^{15}N$-impoverished inorganic N is high. Inorganic N is rapidly assimilated by primary producers, with preferential uptake of $^{14}NH_4^+$ (Perga and Gerdeaux 2006) and $^{14}NO_3^-$ (Syväranta et al. 2006) that leads to more negative values for organic matter. Lehmann et al. (2004) noted decreases in particulate organic N $\delta^{15}N$ of more than 10‰, which they associated with increasing primary production. This decrease is eventually reflected in the zooplankton $\delta^{15}N$ (Perga and Gerdeaux 2006). As the NO_3^- concentration in the photic zone progressively decreases during the productive period, residual NO_3^- becomes progressively enriched in ^{15}N and, in turn, newly produced phytoplankton display a steadily increasing $\delta^{15}N$ (Lehmann et al. 2004), which, again, should be reflected in higher trophic level organisms utilizing this material, as shown in Syväranta et al. (2006).

There are other factors that can also contribute to the ongoing seasonal variation of zooplankton $\delta^{15}N$. The occurrence of N_2-fixing cyanobacteria will decrease the $\delta^{15}N$ of POM because of the reduced fractionation associated with N_2 fixation. Lakes with low dissolved inorganic nitrogen (DIN) are more reliant on N_2 fixation and are associated with a low $\delta^{15}N$ of POM; conversely, lakes with high DIN concentrations show little N_2 fixation and high $\delta^{15}N$ of POM (Gu et al. 2006). This is consistent with the positive correlation I observed between [NO_3+NO_2] and zooplankton $\delta^{15}N$ (Table 2.7). Gu et al. (2006) observed low $\delta^{15}N$ of POM in warm months and high $\delta^{15}N$ in cold months and attributed this to the dominance of N_2-fixing cyanobacteria during the growing season. Inter-specific variation in zooplankton $\delta^{15}N$ within a single lake over time or between a group of lakes may also result from differences in feeding behavior as the availability of resources changes (Perga and Gerdeaux 2006). Variation resulting from diet and feeding habit in zooplankton is discussed further in the next section, however it is very likely that this also contributed to the seasonal variation seen in $\delta^{15}N$ between zooplankton taxa in the lakes sampled.

While there are a number of factors that contribute to seasonal shifts in zooplankton $\delta^{15}N$, it appears that mixing of the water column integrates the signatures of different sources, at least for a brief period. Lehmann et al. (2004) reported a constant $\delta^{15}N$ value for nitrate throughout the water column following winter holomixis, which prevailed in the mid waters throughout the year, but varied substantially in the photic zone and near-bottom waters. They found the $\delta^{15}N$ value for the water column matched the weighted average $\delta^{15}N$ for the inflows suggesting that external sources control the isotopic composition of the whole-basin nitrate pool. Inter-annual consistency in the seasonality of zooplankton $\delta^{15}N$ has been demonstrated (Syränta et al. 2008); the seasonality of zooplankton $\delta^{15}N$ shown in some of the lakes covered by this study culminated in a peak around March that was fairly consistent for some lakes on a year-to-year basis. Similarly, Grey et al. (2001) also noted strong consistency of zooplankton $\delta^{15}N$ between February values for successive years.

Between-Taxon Variation in $\delta^{15}N$

Substantial inter-taxon variability in zooplantkton $\delta^{15}N$ (Matthews and Mazumder 2003; Perga and Gerdeaux 2006;) would complicate the use of bulk isotopic zooplankton $\delta^{15}N$ signatures in water quality trend assessments, as the overall signature would be most influenced by the dominant taxon in the sample (Syvränta et al. 2006). It would therefore be important to determine which, if either, taxon provided a more consistent $\delta^{15}N$ signature over time and therefore a more consistent water quality indicator. My results showed greater variance in *Daphnia* $\delta^{15}N$ than calanoid copepod $\delta^{15}N$ for most of the lakes we sampled (Figure 2.1, Table 2.2), although the isotopic signatures of both taxa followed the same general trends over time. Vander Zanden and Rasmussen (2001) found greater fractionation of ^{15}N in carnivores than herbivores, and that herbivore $\delta^{15}N$ was more variable than carnivore $\delta^{15}N$. Most copepods are omnivores (Schulze and Folt 1990) and, although herbivorous at least during some life stages, represent one of the main groups of invertebrate predators in both limnetic and littoral regions in inland waters (Brandl 2005). Therefore, it is reasonable to expect the $\delta^{15}N$ of omnivorous calanoid copepods to be higher and less variable than herbivorous *Daphnia*.

The difference in $\delta^{15}N$ between *Daphnia* and calanoid copepods is likely related to differences in feeding habits and seasonal shifts in diet; compared to the more herbivorous cladocerans, calanoid copepods feed more readily on larger prey from the microbial food chain which could lead to a higher trophic position of copepods compared to cladocerans (Matthews and Mazumder 2007). Phytoplankton isotopic signatures are influenced by growth rate, cell size and geometry, and physical and environmental characteristics such as pH, temperature, light intensity and day length (Vuorio et al. 2006). In lakes where *Daphnia* are heavily reliant on phytoplankton, this variation in primary producer $\delta^{15}N$ will be reflected in the signature of the primary consumers and several studies have documented large seasonal variation in the $\delta^{15}N$ of individual zooplankton taxa. Matthews and Mazumder (2005) noted an increase of more than 10‰ in *Daphnia* $\delta^{15}N$ over the course of a summer. Matthews and Mazumder (2007) reported the rapid increase in $\delta^{15}N$ of herbivorous zooplankton (*D. pulex* and *Holopedium gibberum*) that surpassed the $\delta^{15}N$ of the more predacious zooplankton (*Epischura nevadensis* and *Chaoborus trivittatus*) with little variation in the $\delta^{15}N$ of the presumed basal food sources. The data presented here were consistent with this and showed *Daphnia* $\delta^{15}N$ at times approached that of calanoid copepods (e.g., Figures 2.1d and 2.1g), and in one instance at Fork Lake, actually exceeded calanoid $\delta^{15}N$ (Figure 2.1f).

Copepods, on the other hand, usually prefer nanoplankton and microplankton, including microzooplankton, but are inefficient feeders on picoplankton (Karlsson et al. 2004; Bundy et al. 2005). While both calanoid copepods and *Daphnia* can be omnivorous, to what extent likely depends on lake conditions (Matthews and Mazumder 2003). So within-lake variation in $\delta^{15}N$ between *Daphnia* and calanoid copepods will depend on the dietary contribution of primary producers, bacteria and nonphotosynthetic prey (ciliates, heterotrophic nanoflagellates, rotifers and other microzooplankton) (Matthews and Mazumder 2003) to each and between-taxa variation should be expected. This was not the case for all lakes sampled, the most obvious of which is Glen Lake (Figure 2.1j). Both calanoid copepod and *Daphnia* $\delta^{15}N$ had CVs of 0.1 (Table 2.2) with a difference of approximately 4‰ between the two for all samples. The between-taxa synchronicity in $\delta^{15}N$ seen in some of the other lakes (Figures 2.1c, 2.1g and 2.1i) could be an indication of simpler food chains (Matthews and Mazumder 2007) and similar seasonal patterns for zooplankton $\delta^{15}N$ could be an indication of change at the base of a food chain (Syvränta et al. 2006) shared by both taxa. Additional information, such as phytoplankton and zooplankton community composition at the time of sampling, could provide further insight to this aspect in single-lake and between-lake investigations.

Another potential cause of the variation seen in *Daphnia* $\delta^{15}N$ could be related to body size. Matthews and Mazumder (2007) found *D. pulex* $\delta^{15}N$ to be positively related to body size, therefore, an inconsistent mix of life stages in the samples analyzed could contribute to seasonal variation. My results, however, did not show any strong evidence of a consistent relationship between zooplankton $\delta^{15}N$ and estimated body weight (Table 2.3).

Finally, one other point to consider is the distribution and seasonal occurrence of each group. *Daphnia* were not present, at least in the surface waters, of all lakes on each sampling date (e.g., Figures 2.1a, 2.1f and 2.1i) whereas there were always enough calanoid copepods to allow isotopic analyses. Availability of the desired test organism for isotopic analyses is an obvious consideration.

The exact mechanisms for the isotopic variance between zooplankton taxa are surely complex and beyond the scope of this study, however, the overall higher variation of *Daphnia* $\delta^{15}N$ suggests that calanoid copepods may provide a more appropriate group of organisms for the application of $\delta^{15}N$ in water quality assessments, although in some situations (such as Glen Lake) a case could be made for either taxon. Matthews and Mazumder (2003) noted that the variable degree of omnivory among calanoids may complicate the interpretation of $\delta^{15}N$ among lakes or the temporal interpretation of $\delta^{15}N$ within a lake. While this may be a hindrance for the purpose of establishing the isotopic baseline of a lake, the apparent lower variation in $\delta^{15}N$ signatures provided by omnivory, coupled with a consistent presence in the zooplankton community, may make calanoid copepods the more practical taxonomic group for the application of stable isotope analysis in trend assessments. Although *Daphnia* $\delta^{15}N$ provides a good baseline for determining the trophic position of primary consumers (Matthews and Mazumder 2003), it changes too quickly to be useful in water quality monitoring on a monthly schedule. There may be instances however, such as monitoring inputs from first-flush events or localized sampling within a specific area of a lake, where a more intensive sampling effort in a shorter time frame is warranted and the quicker response of *Daphnia* may provide a more appropriate indicator organism.

Using Zooplankton $\delta^{15}N$ to Detect Change

The relationship between increasing land use and organism δ^{15}N has been demonstrated in several studies (Lake et al. 2001; McKinney et al. 2002; Steffy and Kilham 2004; Anderson and Cabana 2006) and the findings presented here are consistent with these results. A significant relationship between zooplankton δ^{15}N and lot density within a 200 m buffer zone was demonstrated, although this must be viewed with caution, as lake-specific circumstances, such as mixing of N sources with different isotopic signatures, may have been a factor. For example, Langford and Prospect lakes have had significant inputs of fertilizers over the years and Cusheon Lake experiences frequent cyanobacteria blooms. Gu et al. (2006) speculated that regeneration of N derived partly from previous N_2 fixation contributed to the low δ^{15}N of the DIN pool in a small, eutrophic lake. A similar isotopic mixing of N sources may be occurring in these lakes, lowering the δ^{15}N below what might be expected based soley on waste disposal practices around the lake. This might help to explain the decrease seen in spring calanoid δ^{15}N in Langford Lake, from 15.9‰ to 14.3‰, over the period of study. Langford Lake typically experiences cyanobacteria blooms in the fall which may be reflected in spring zooplankton δ^{15}N. Prospect Lake (Figure 2.1g) showed noticeable variation in δ^{15}N from year to year which may have been influenced by the contribution of low δ^{15}N fertilizers from a golf course located adjacent to the lake and its main inflow, both from direct runoff and regenerated from sediments. Without the input of low $\delta^{15}N$ sources, the average zooplankton $\delta^{15}N$ may have been higher in these lakes, decreasing the strength of the relationship illustrated in Figure 2.2.

It therefore seems reasonable to ask if these results accurately reflect the impact from varying degrees of on-site septic disposal, or whether the relationship is a result of chance, recognizing that the isotopic mixing of various N sources is contributing to the final signature measured in the biota. It may be a combination of both; while the relationship is significant, it is clear there are site-specific factors influencing zooplankton $\delta^{15}N$. Given the level of residential development around Long Lake (Table 2.11) we would expect a higher zooplankton $\delta^{15}N$ than what was measured. However, although some of the homes in this area are serviced by septic systems, the majority are on centralized sewer so this result is not surprising. Conversely, Elk Lake had a higher isotopic signature than expected, based on residential development, which could be related to livestock (predominantly horses) within the watershed. This illustrates the need for more comprehensive assessments in terms of land use and nutrient budgets for single-lake investigations, to avoid the potential for misinterpretation of results over time. A decrease in septic inputs to a lake can result in a seemingly large reduction in $\delta^{15}N$, which is also influenced by the greater proportional contribution from regenerated N with a lower $\delta^{15}N$. Conversely, a decrease in N loadings from sources with a low $\delta^{15}N$ (e.g., cyanobacteria, synthetic fertilizers) can result in an increase in $\delta^{15}N$, which may actually represent an overall improvement to the lake. Unfortunately, determining nutrient budgets for each of these lakes was beyond the scope of this study, but should be an important consideration for single-lake assessments.

It has previously been shown that unionid mussels provide an integrated near-base level $\delta^{15}N$ that can be used to recognize trends in $\delta^{15}N$ values with changing land use characteristics (McKinney et al. 2002). The results for Durrance Lake (Figure 2.1c) suggest that zooplankton $\delta^{15}N$ may be more sensitive and therefore even more valuable in detecting change before serious environmental degradation occurs. The overall zooplankton $\delta^{15}N$ measured in this lake was higher (even if the spikes measured in 2004 and 2006 are ignored) than would be expected, given the low level of residential development, when compared to reference lakes. The magnitude of the spikes measured in 2004 and 2006 are an interesting anomaly that would not be evident in the $\delta^{15}N$ signature of long-term integrator such as mussels. Determining the cause of these spikes would be necessary to confirm whether the higher zooplankton signatures result from anthropogenic activities or lake-specific temporal variation. While they could be the result of septic failures occurring at properties near the inflow of the lake during the previous winter (which have been verified by dye tests in the past), it seems unlikely that the contribution of N from one or two failing systems could cause such substantial spikes in zooplankton $\delta^{15}N$. The spikes could be the result of starvation or nutritional stress, however this seems unlikely as both calanoid copepod and *Daphnia* $\delta^{15}N$ increased similarly and low food production is more likely to have a larger effect on cladoceran $\delta^{15}N$ than on copepod $\delta^{15}N$ (Karlsson et al. 2004). Zooplankton size may have contributed to the elevated $\delta^{15}N$ signatures, as a significant relationship with zooplankton $\delta^{15}N$ was seen in

Durrance Lake (Table 2.3), although the lack of a consistent relationship between weight and isotopic signatures in all lakes suggests this may not be a major factor.

The other possible explanation could be that these spikes are a reflection of increased $\delta^{15}N$ of nitrate in the hypolimnion during periods of anoxia. Discrimination against ^{15}N in lakes may occur during microbial transformation of organic N to inorganic N, during uptake of inorganic N by bacterioplankton and phytoplankton, and by nitrification and denitrification. All of these processes raise the $\delta^{15}N$ of the residual N pool, and in the case of denitrification, the whole lake pool (Karlsson et al. 2004). It is possible that hypolimnetic DIN $\delta^{15}N$ increased (Lehmann et al. 2004; Syvränta et al. 2008) in Durrance Lake during stratification and was incorporated into the biota after holomixis and reflected in the zooplankton $\delta^{15}N$. However, changes in zooplankton $\delta^{15}N$ caused by changes in N source can be extremely rapid (Syvränta et al. 2008) and because Durrance Lake is typically isothermal by November, we might expect to see an increase in zooplankton $\delta^{15}N$ caused by high $\delta^{15}N$ of hypolimnetic DIN closer to turnover than what was measured (calanoid $\delta^{15}N$ was 8.5‰ on January 29, 2004 and 17.4‰ on March 16, 2004). Clearly, more frequent and complete (e.g., determination of DIN isotopic ratios) sampling would be required to determine the cause of such spikes.

Although one should be cautious about accepting the hypothesis that septic failures could manifest into such substantial spikes in zooplankton $\delta^{15}N$ in Durrance Lake, it is equally hard to accept any of the other possibilities as the sole cause of isotopic enrichment because spikes of similar magnitude were not observed in any of the other lakes, even in the more developed lakes. Elevated zooplankton $\delta^{15}N$ was found in other lakes exposed to low development (e.g., Mitchell and Pease lakes, see Table 2.11), supporting the notion that small inputs can be detected. If these spikes do represent septic failures, it supports the use of zooplankton $\delta^{15}N$ for detecting changes in water quality before they develop into more obvious problems.

Conclusions

In summary, this study documented seasonal variability in zooplankton $\delta^{15}N$ for several small, temperate lakes with peak values generally occurring in the winter and spring. The most important factors influencing seasonal changes are likely shifts in the $\delta^{15}N$ of N sources preferred by primary producers and land use within the 200 m buffer around the lake in question. Seasonality of zooplankton $\delta^{15}N$ dictates that multiple sampling should occur on an annual basis; although frequent sampling (i.e. monthly) would be best to identify seasonal trends, this is not often feasible. For the purpose of trend assessment, quarterly sampling should be sufficient, but more frequent sampling would be desirable when possible. Statistical methods such as the seasonal Kendall trend test can be used to determine whether a change is occurring over time.

Calanoid copepods appear to be the preferred taxonomic group for this purpose over a moderate time period (e.g., months), whereas *Daphnia* may be more appropriate for short time periods (e.g., weeks) and long-term $\delta^{15}N$ integrators, such as mussels, may be more appropriate for investigations covering a period of years. Calanoid copepod signatures are less variable than *Daphnia* because of their assumed higher trophic position and omnivorous diet. *Daphnia* were not present in all lakes, which may limit their use in trend assessments. The wide distribution of calanoid copepods, ease of collection, storage and processing, and relative stability of $\delta^{15}N$ in the absence of land use changes support their use in this application of stable isotopes.

This study presents evidence that, as has been shown with other aquatic biota, zooplankton $\delta^{15}N$ is influenced by land use. The strongest relationships seen were in the 200 m buffer zone around the lake indicating that efforts to deal with water quality should be focused on this area, as previously suggested by Vander Zanden et al. (2005). My results also suggest that zooplankton $\delta^{15}N$ may be quite sensitive to small sewage inputs, but this requires further investigation to confirm. The application of zooplankton $\delta^{15}N$, with respect to in-depth single lake assessments, would be greatly enhanced with an accurate N budget for the lake in question. Overall, zooplankton $\delta^{15}N$ signatures appear to provide a useful tool which can enhance lake water quality assessments and help demonstrate the benefits of responsible land use practices around lakes.

Table 2.1: Summary of morphological, limnological and physical characteristics of the the study lakes. Shoreline development is defined as the density of small lots (<0.25 ha) within a 200 m buffer zone around each lake. Total N and P concentrations are median concentrations for each lake, for data collected when the water column was completely mixed. The number of samples used to determine median concentrations is provided in parentheses.

Lake	Mean Depth (m)	Maximum Depth (m)	Perimeter (km)	Surface Area (ha)	Volume (dam^3*)	Watershed Area (km^2)	Elevation (m)	Shoreline development ($lots/km^2$)	Spring Total N (μg/L)	Spring Total P (μg/L)	Secchi (m)
Council	5	17	3	13	826	2.7	402	0	200 (1)	4 (1)	7.0
Sooke	20	67	21	428	92,700	86.1	173	0	$85^{\dagger}$	$3^{\dagger}$	$8.0^{\dagger}$
Durrance	6	16	2	8	514	1.8	134	0	300 (6)	9 (6)	6.0
Kemp	5	11	3	25	1,229	5.2	33	36	590 (3)	14 (3)	2.8
Cusheon	4	10	4	28	1,160	7.5	99	37	710 (2)	16 (2)	2.5
Fork	2	10	1	2	92	1.5	216	63	260 (3)	7 (3)	2.7
Prospect	7	14	5	68	4,040	8.6	48	123	420 (5)	16 (5)	3.3
Langford	9	17	5	61	3,980	2.2	67	235	560 (7)	22 (7)	4.0
Florence	4	6	2	7	400	1.7	76	241	550 (4)	24 (4)	3.0
Glen	6	14	2	17	1,229	5.8	67	558	790 (4)	16 (4)	3.0

†: Values reported in Davies et al. 2004.

* dam^3 = cubic decametre

Table 2.2: Summary of zooplankton δ15N signatures by lake (SD = standard deviation, CV = coefficient of variation).

	Calanoid $\delta^{15}N$ (‰)						*Daphnia* $\delta^{15}N$ (‰)					
Lake	Mean	SD	CV	Min	Max	n	Mean	SD	CV	Min	Max	n
Council	4.8	1.5	0.3	1.7	7.3	19	—	—	—	—	—	—
Sooke	6.0	0.7	0.1	4.8	7.5	32	3.1	0.8	0.3	1.4	4.9	32
Durrance	10.6	2.8	0.3	7.5	17.4	13	6.0	3.1	0.5	2.3	12.3	13
Kemp	8.3	0.7	0.1	7.5	9.4	9	4.2	2.1	0.5	1.7	8.5	9
Cusheon	8.2	1.2	0.1	6.3	10.0	12	4.1	0.8	0.2	2.6	5.2	12
Fork	7.2	1.1	0.2	5.0	8.5	10	3.5	2.3	0.7	0.3	7.8	8
Prospect	9.5	1.8	0.2	7.0	13.4	12	5.1	3.0	0.6	-0.3	9.5	12
Langford	13.9	0.9	0.1	12.3	15.9	24	10.0	1.9	0.2	5.2	13.0	24
Florence	11.5	2.6	0.2	6.8	14.7	9	9.6	2.0	0.2	6.2	11.7	6
Glen	21.5	2.3	0.1	18.9	25.9	9	17.5	2.3	0.1	14.4	21.9	9

Table 2.3: Results of linear regression between zooplankton weight and $\delta^{15}N$. Zooplankton weight is measured as the average weight (µg) per individual in the sample analyzed.

		Calanoid					*Daphnia*					
Lake	Size range (µg)	R^2	F	Slope	*p*	n	Size range (µg)	R^2	F	Slope	*p*	n
Cusheon	3.2 - 8.1	0.77	16.5	-0.45	0.0098	7	20.5 - 37.7	0.76	19.3	0.07	0.0046	8
Durrance	2.8 - 22.0	0.59	26.2	0.45	<0.0001	20	3.1 - 68.5	0.52	18.5	0.11	0.0005	19
Florence	5.9 - 10.8	0.11	1.2	-0.31	0.3015	12	2.6 - 22.8	0.19	1.9	0.15	0.2078	10
Fork	5.0 - 9.0	0.00	0.0	0.01	0.9144	16	6.0 - 11.0	0.02	0.1	-0.18	0.7520	8
Glen	4.7 - 14.7	0.04	0.4	0.11	0.5247	12	3.1 - 64.6	0.11	1.1	0.03	0.3246	11
Kemp	2.4 - 9.4	0.07	0.9	0.09	0.3526	13	15.5 - 83.3	0.42	10.3	0.07	0.0063	16
Langford	2.3 - 9.0	0.25	13.3	0.28	0.0007	43	3.0 - 58.2	0.24	21.3	0.08	<0.0001	70
Prospect	4.0 - 10.7	0.77	58.9	0.84	<0.0001	20	2.4 - 39.5	0.28	10.8	0.11	0.0028	30

Table 2.4: Summary of δ^{13}C results for all lakes. δ^{13}C values are the mean of all results for each lake (SD = standard deviation; CV = coefficient of variation).

	Calanoid δ^{13}C (‰)					*Daphnia* δ^{13}C (‰)				
Lake	Mean	SD	CV	Range	n	Mean	SD	CV	Range	n
Council	-34.1	1.4	-0.04	5.6	19	—	—	—	—	—
Sooke	-31.8	0.6	-0.02	2.5	32	-31.1	1.2	-0.04	4.8	32
Durrance	-38.9	4.0	-0.10	11.3	13	-38.7	4.3	-0.11	12.0	13
Kemp	-34.3	2.9	-0.08	8.3	9	-33.4	3.3	-0.10	8.1	9
Cusheon	-36.2	2.2	-0.06	7.4	12	-34.2	2.8	-0.08	8.3	12
Fork	-35.5	1.6	-0.05	5.1	10	-34.6	1.9	-0.05	5.4	8
Prospect	-34.1	2.9	-0.09	8.2	12	-32.9	3.1	-0.09	9.0	12
Langford	-33.9	3.6	-0.11	13.0	24	-32.2	3.5	-0.11	12.5	24
Florence	-37.9	2.3	-0.06	7.8	9	-37.0	2.1	-0.06	5.9	6
Glen	-39.9	1.8	-0.05	5.2	9	-38.6	2.0	-0.05	6.4	9

Table 2.5: Monthly calanoid copepod δ15N signatures (‰) for all lakes. Average signatures were calculated where data were available for multiple years. Standard deviations are provided in parentheses.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Council	6.1	5.5 (0.9)	5.8 (0.6)	5.5	4.2 (0.9)	–	5.0 (2.1)	3.0	2.2	3.1 (2.0)	5.3 (0.9)	5.3
Sooke	7.3	7.4 (0.1)	6.9	6.1	5.6 (0.1)	5.2 (0.3)	5.6 (0.1)	5.7 (0.1)	6.0 (0.1)	6.4 (0.1)	6.4 (0.1)	6.8
Durrance	8.5	–	13.0 (3.1)	–	11.2	–	9.3	7.5	8.9	–	9.0 (1.2)	–
Kemp	–	7.6	9.2 (0.3)	–	8.8	–	–	8.7	–	8.1	7.6 (0.2)	8.3
Cusheon	–	9.2 (1.1)	8.7	–	6.8 (0.6)	7.4	7.7	8.8	–	9.0 (1.5)	–	9.1
Fork	7.4	–	8.2 (0.4)	–		7.7	–	6.3 (1.8)	–	6.6 (1.2)	6.2	–
Florence	–	–	13.8 (0.6)	–	10.5	–	–	6.8	–	–	9.8 (1.5)	11.1
Prospect	8.9	–	11.2 (1.7)	–	10.4	–	9.9	9.1	7.7	–	8.0 (0.9)	–
Langford	14.2 (0.2)	13.4	15.0 (0.7)	14.7 (0.7)	12.7	14.2	14.6 (0.3)	13.2 (0.8)	13.0 (0.1)	13.2	13.3 (0.1)	13.3
Glen	–	–	21.8 (3.2)	–	20.5	–	–	21.8	–	–	22.7 (0.6)	18.8

Table 2.6: Monthly *Daphnia* δ15N signatures (‰) for all lakes. Average signatures were calculated where data were available for multiple years. Standard deviations are provided in parentheses.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Council	–	–	–	–	–	–	–	–	–	–	–	–
Sooke	2.8	3.9 (1.3)	4.9	2.9	3.2 (1.2)	3.2 (0.9)	3.5 (0.5)	3.1 (0.9)	2.0 (0.4)	2.8 (0.3)	3.4 (0.2)	3.4
Durrance	4.7	–	9.0 (0.4)	–	6.9	–	4.8	4.5	2.4	–	3.1 (0.9)	–
Kemp	–	3.5	6.6 (2.7)	–	5.5	–	–	3.9	–	5.5	2.5 (0.1)	1.7
Cusheon	–	4.4	4.7	–	4.0 (0.4)	3.9	4.7	2.6	4.0	4.0 (1.6)	–	4.5
Fork	–	–	4.1 (0.7)	–	–	3.0	–	3.7	–	4.4 (4.9)	0.3	–
Florence	–	–	10.8 (0.7)	–	8.2	–	–	–	–	–	–	6.2
Prospect	3.2	–	8.3 (0.8)	–	6.8	–	5.5	2.4	3.6	–	2.0 (2.0)	–
Langford	9.7 (1.5)	9.1	12.5 (0.4)	11.5 (0.6)	10.5	11.0	10.4 (0.3)	9.8 (0.3)	9.1 (0.6)	9.8	7.7 (1.3)	5.2
Glen	–	–	18.3 (2.2)	–	15.4	–	–	17.6	–	–	18.7 (0.5)	14.4

Table 2.7: Mean monthly calanoid $\delta^{15}N$ signatures (‰) by month for 61 BC lakes (SD = standard deviation)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
mean	10.1	11.0	12.1	11.2	9.3	9.5	9.8	9.3	7.9	7.8	10.5	10.5
SD	2.9	2.8	4.4	3.2	3.4	3.0	2.7	3.6	3.2	3.1	4.3	4.0
upper 95%	12.7	12.7	13.4	13.4	10.7	10.9	10.9	10.6	9.3	9.2	12.4	13.8
lower 95%	7.4	9.4	10.8	9.1	7.8	8.2	8.8	7.9	6.5	6.2	8.7	7.1
n	7	14	46	11	24	22	30	30	22	18	24	8

Table 2.8: Mean calanoid $\delta^{15}N$ signatures (‰) by quarter for 61 British Columbia lakes (SD = standard deviation).

	Q1	Q2	Q3	Q4
mean	11.6	9.7	9.1	9.5
SD	4.0	3.2	3.2	4.0
upper 95%	12.6	10.6	9.8	10.7
lower 95%	10.7	8.9	8.4	8.4
n	67	57	82	50

Table 2.9: Results of Pearson's product moment correlation analyses between zooplankton $\delta^{15}N$ signatures and water chemistry characteristics for 10 Vancouver Island lakes (listed in Table 2.1). Water chemistry values were the average concentrations for the whole water column measured on the day of sampling.

	Calanoid			*Daphnia*		
	r	n	*p*	*r*	n	*p*
$\log_{10}$ TN	0.497	88	<0.0001	0.332	80	0.0049
$\log_{10}$ NH_4	0.249	55	0.0671	-0.086	48	0.5591
$\log_{10}$ NO_3+NO_2	0.467	52	0.0005	0.354	45	0.0172
$\log_{10}$ TP	0.128	87	0.2381	-0.083	79	0.4677
$\log_{10}$ TOC	-0.192	66	0.1223	-0.122	59	0.3585

Table 2.10: Results of linear regression analyses between lot density and mean zooplankton $\delta^{15}N$ from 10 Vancouver Island lakes (Table 2.1). The mean of all $\delta^{15}N$ results for each lake is used.

Taxon	Lot size (ha)	Buffer	Regression Equation	df	R^2	F	*p*
Calanoid	<0.25	within 200 m of lakeshore	$y = 6.8 + 0.03x$	9	0.88	58.9	<0.0001
Calanoid	<0.5	within 200 m of lakeshore	$y = 6.7 + 0.02x$	9	0.87	53.8	<0.0001
Calanoid	<2.5	within 200 m of lakeshore	$y = 6.4 + 0.02x$	9	0.84	42.9	0.0002
Calanoid	<0.25	witihin 200 m of lakeshore and tributaries	$y = 8.3 + 0.04x$	9	0.33	4.0	0.0799
Calanoid	<0.5	witihin 200 m of lakeshore and tributaries	$y = 8.2 + 0.03x$	9	0.34	4.1	0.0784
Calanoid	<2.5	witihin 200 m of lakeshore and tributaries	$y = 8.0 + 0.03x$	9	0.33	3.9	0.0826
Daphnia	<0.25	within 200 m of lakeshore	$y = 3.4 + 0.02x$	8	0.93	98.6	<0.0001
Daphnia	<0.5	within 200 m of lakeshore	$y = 3.2 + 0.02x$	8	0.92	80.1	<0.0001
Daphnia	<2.5	within 200 m of lakeshore	$y = 3.0 + 0.02x$	8	0.87	46.2	0.0003
Daphnia	<0.25	witihin 200 m of lakeshore and tributaries	$y = 5.0 + 0.03x$	8	0.35	3.8	0.0911
Daphnia	<0.5	witihin 200 m of lakeshore and tributaries	$y = 4.9 + 0.03x$	8	0.35	3.8	0.0910
Daphnia	<2.5	witihin 200 m of lakeshore and tributaries	$y = 4.8 + 0.03x$	8	0.33	3.5	0.1030

Table 2.11: Additional isotope data used to test the relationship between zooplankton $\delta^{15}N$ and lot (< 0.25 ha) density. Mean $\delta^{15}N$ values were calculated from all available data (SD = standard deviation).

Lake	Lot Density (lots/km^2)	Calanoid $\delta^{15}N$ (‰)			*Daphnia* $\delta^{15}N$ (‰)		
		Mean	SD	n	Mean	SD	n
Butchart	0	6.1	0.5	9	2.8	0.2	5
Old Wolf	0	6.1	1.2	2	7.4	—	1
Goldstream	0	6.3	0.8	13	3.2	0.8	10
Jump	0	6.9	0.7	8	4.1	0.6	7
Mitchell	0	9.2	1.1	6	2.4	3.1	2
Pease	0	9.5	—	1	5.7	—	1
Teanook	11	8.6	1.4	6	5.8	1.3	4
Elk	21	12.2	2.2	14	8.1	3.3	16
Weston	22	8.7	2.5	6	5.1	4	4
Quamichan	41	14.6	—	1	5.6	8.1	2
Long	44	11.6	—	1	5.3	—	1

Table 2.12: Results of seasonal Kendall trend tests for calanoid $\delta^{15}N$ data based on 4 seasons per year. S represents the Kendall test statistic and Z is the standard normal deviate determined from S and the variance of S.

Lake	Kendall's tau	S	Z	*p*
Cusheon	0.250	2	0.327	0.7434
Durrance	-0.143	-2	-0.217	0.8286
Florence	-0.778	-7	-1.708	0.0875
Fork	-0.429	-3	-0.693	0.4884
Glen	-0.333	-3	-0.539	0.5690
Kemp	-0.667	-4	-1.108	0.2679
Langford	-0.520	-13	-2.089	0.0367
Prospect	0.000	0	0.000	1.0000

Figure 2.1: Calanoid copepod and *Daphnia* δ^{15}N signatures over time. Solid circles represent calanoid copepods and open circles represent *Daphnia*.

Figure 2.1a: Council Lake.

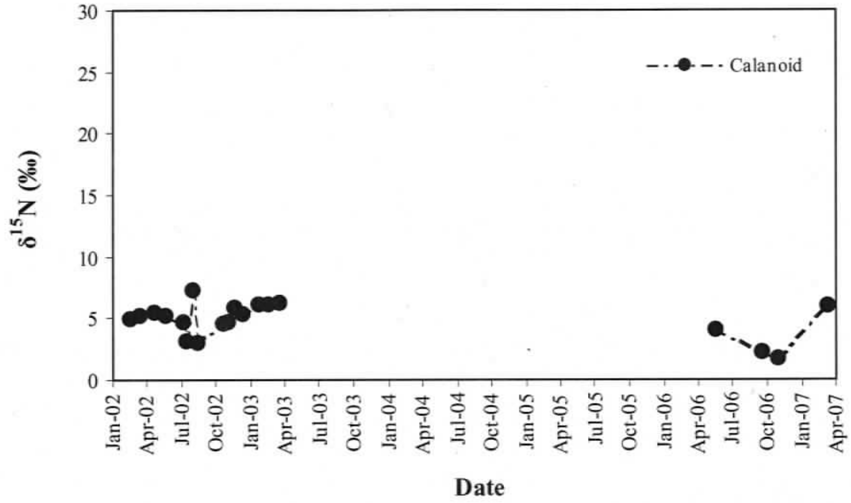


Figure 2.1b: Sooke Lake.

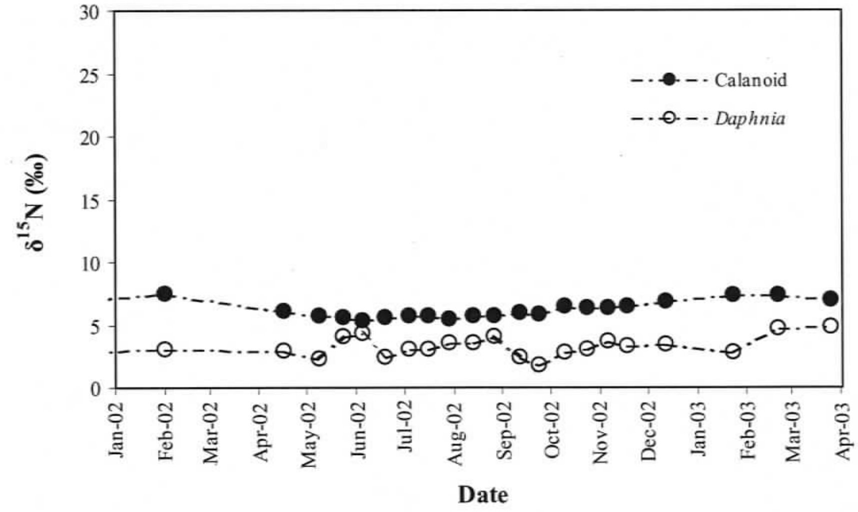


Figure 2.1c: Durrance Lake.

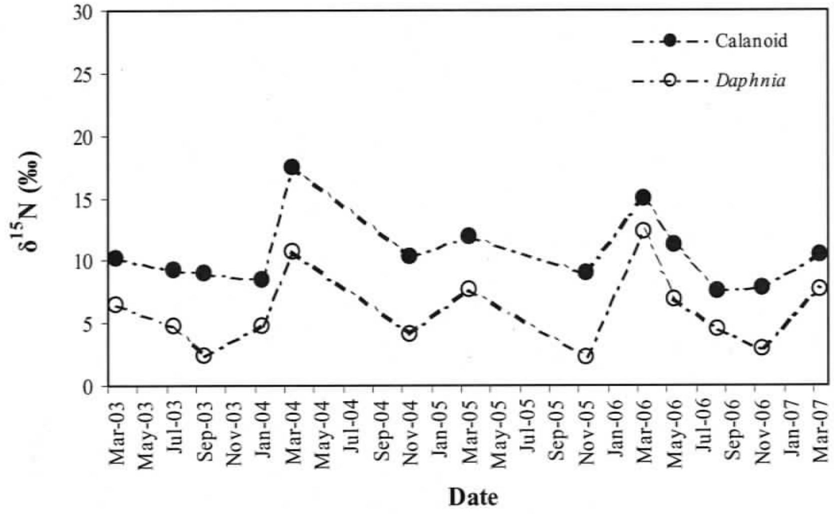


Figure 2.1d: Kemp Lake.

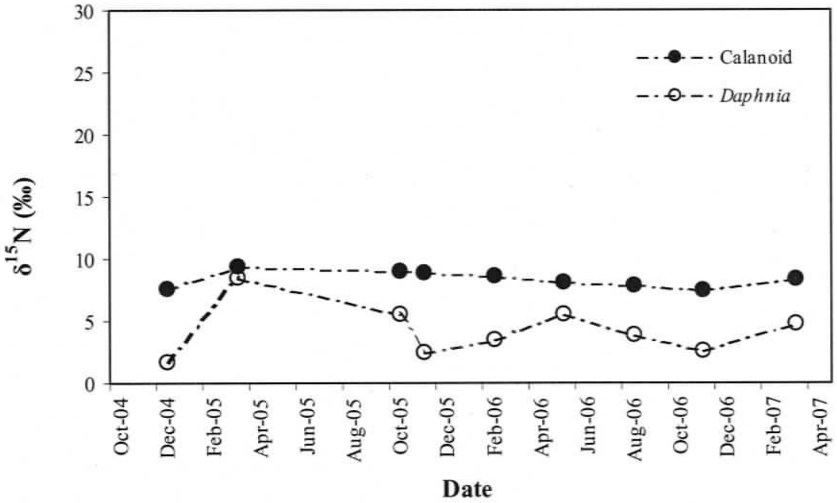


Figure 2.1e: Cusheon Lake.

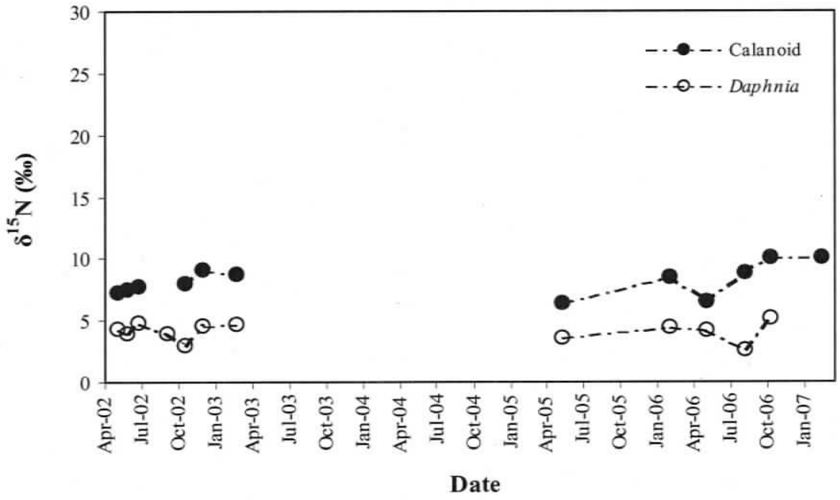


Figure 2.1f: Fork Lake.

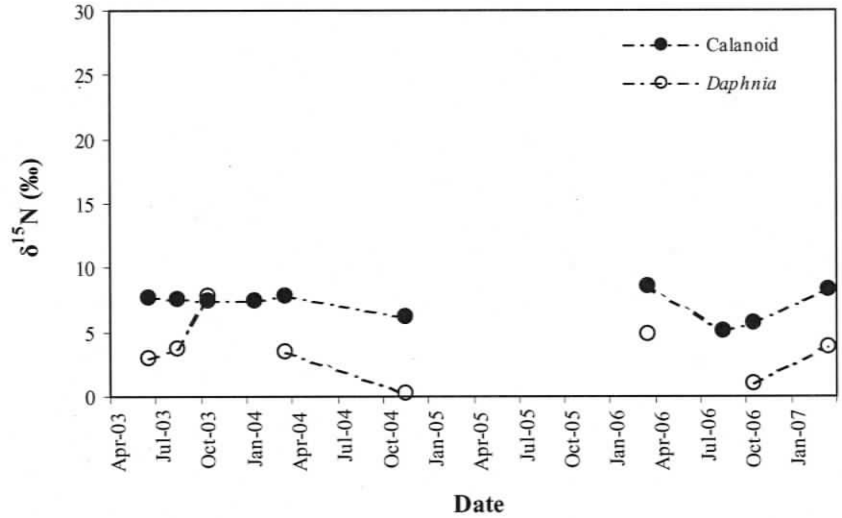


Figure 2.1g: Florence Lake.

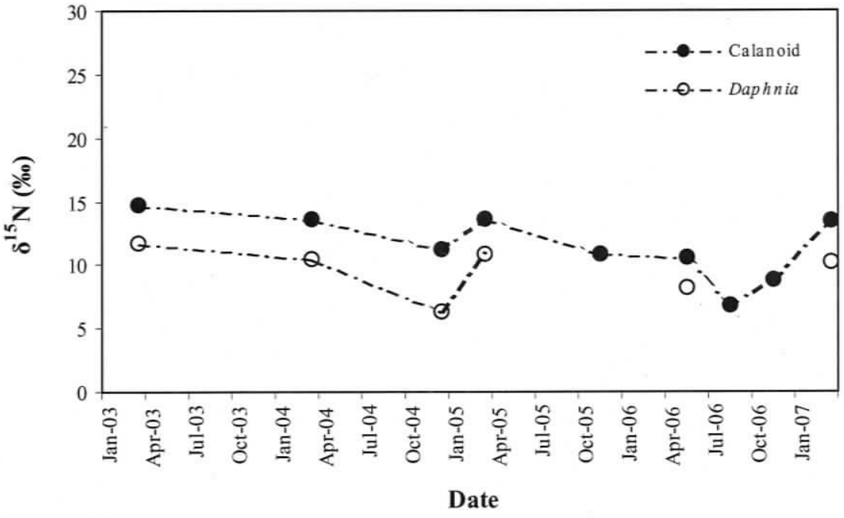


Figure 2.1h: Prospect Lake.

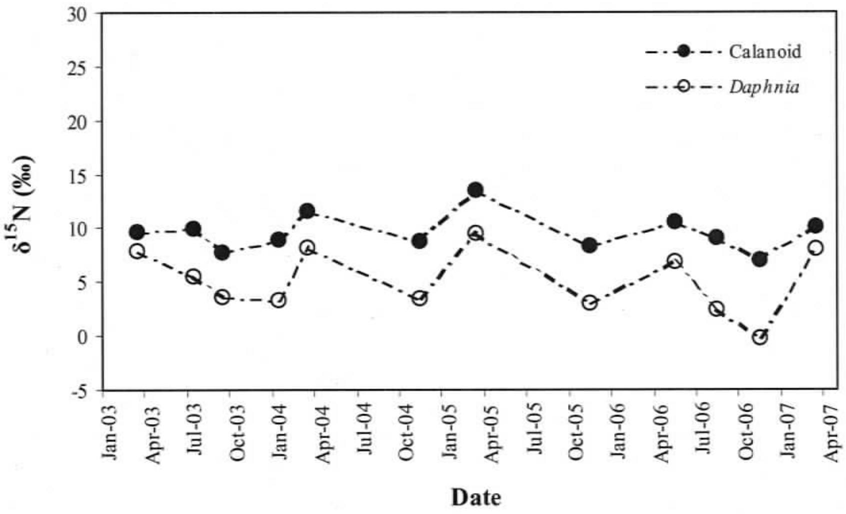


Figure 2.1i: Langford Lake.

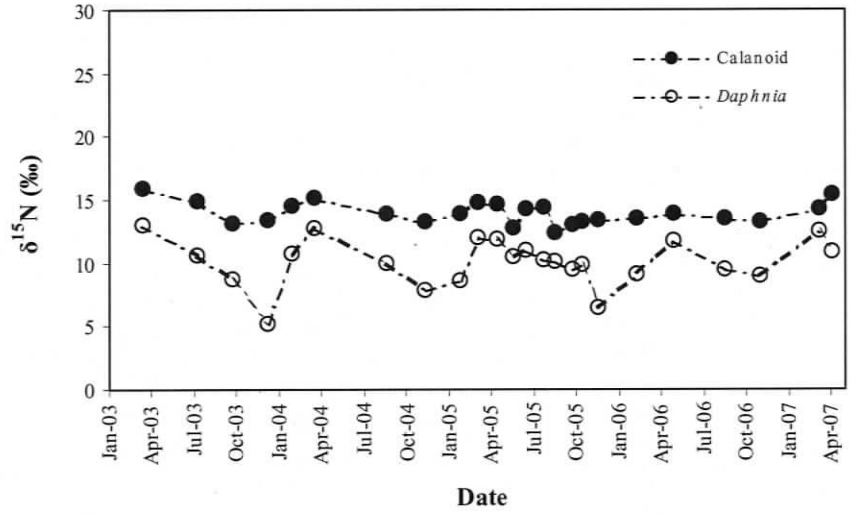


Figure 2.1b: Glen Lake.

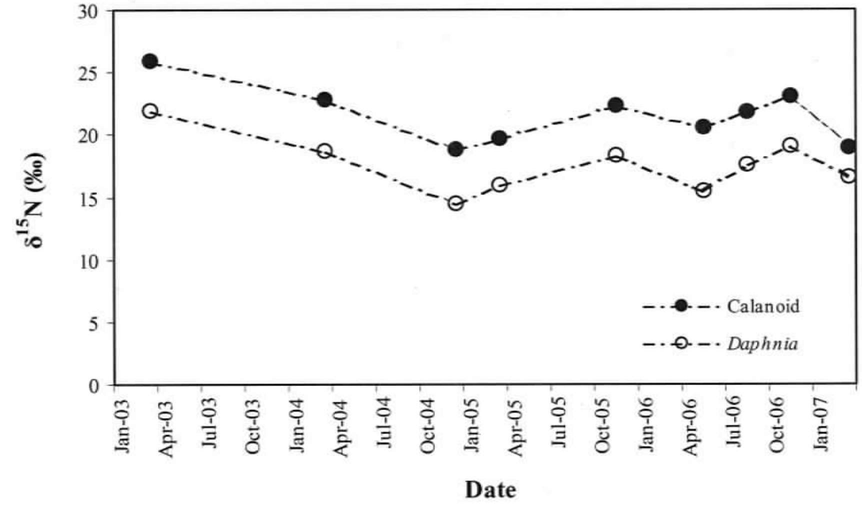
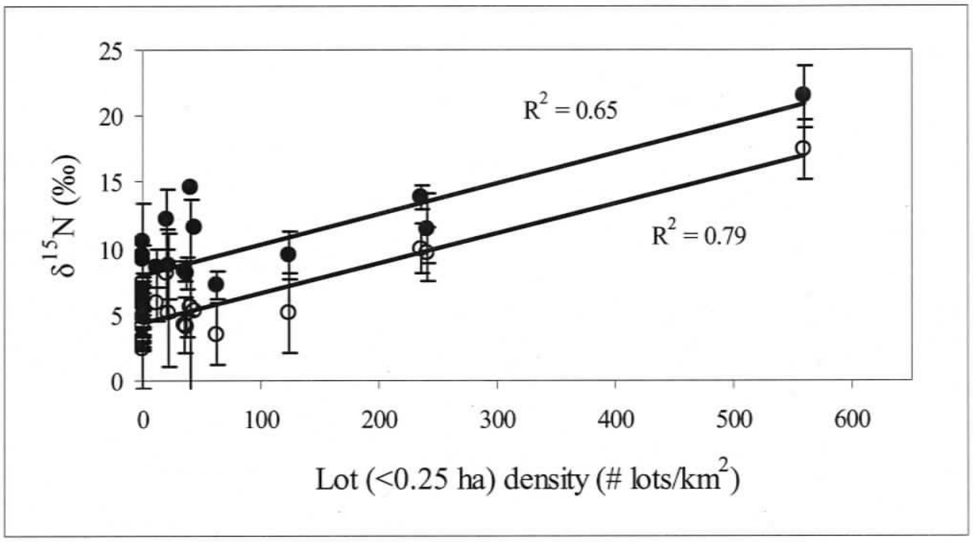


Figure 2.2: Zooplankton $\delta^{15}N$ versus residential lot density within a 200 m buffer zone for 21 coastal lakes. Error bars represent ± 1 standard deviation. Closed circles represent calanoid copepod $\delta^{15}N$ ($R^2 = 0.65$, $F_{20, 0.05} = 35.5$, $p < 0.0001$) and open circles represent *Daphnia* $\delta^{15}N$ ($R^2 = 0.79$, $F_{20, 0.05} = 68.3$, $p < 0.0001$).



Chapter 3: Linking Residential Development to Calanoid Copepod Nitrogen Isotope Signatures

Abstract

The nitrogen isotope signatures ($\delta^{15}N$) of aquatic biota have been quantitatively linked to anthropogenic impacts in a number of studies. While several different groups of organisms have been used in this application, practical considerations, such as collection and availability of certain organisms, may make replication of studies difficult over time. Calanoid copepods are common in many lakes and easy to collect and may thus provide an alternative to less abundant organisms such as unionid mussels. In this study, the links between calanoid copepod $\delta^{15}N$ and various watershed and limnological characteristics in 22 British Columbia lakes are investigated. The density of residential lots, as an index of septic inputs, within the riparian zones of lakes and their tributary streams was the only consistent significant predictor of $\delta^{15}N$. While this suggests that residential land use influenced calanoid $\delta^{15}N$ in these lakes, there are likely several other factors that contribute to the final signature measured. The availability and ease of collection of calanoid copepods and their relationship to residential development support the use of this tool in water quality trend assessment of small lakes.

Introduction

Phosphorus is usually the nutrient that limits primary productivity in lakes (Schindler 1977), although nitrogen can also play an important role (Elser et al. 1990; McKinney et al. 2002; Vander Zanden et al. 2005; Camargo and Alonso 2006; Schindler 2006). Diffuse nutrient sources represent a significant contribution to overall nutrient inputs to aquatic system (Leavitt et al. 2006; Schindler 2006; Bunting et al. 2007) and managing these effectively has proven challenging in light of increasing development, changing land use practices, and, potentially, climate warming (Schindler 2001). One area of concern is on-site waste disposal practices. Water managers are increasingly concerned that failing or improperly installed septic systems contaminate ground and surface waters with pathogens, nutrients and biologically active compounds (Potts et al. 2004), and it has been shown that lakes serviced with septic systems can have higher concentrations of P, chlorophyll *a* and inedible algae (Moore et al. 2003). In freshwater systems, N can also play an important role in net primary production (Camargo and Alonso 2006), so it is essential that both N and P inputs be managed sufficiently to protect water quality. Land development can contribute to eutrophication, which often impacts habitat and water quality (Schindler 2006), resulting in lakes that are less economically beneficial and aesthetically desirable to humans (Moore et al. 2003). Therefore, it is important for resource managers and lake residents to understand how land use decisions affect lake water quality and what can be done to minimize impacts. Linking anthropogenic sources in watersheds to N in lakes and ponds could provide important information for managing and regulating nutrient enrichment (McKinney et al. 2002).

A growing body of evidence supports the use of stable N isotope signatures of biota as indicators of land use impacts on both freshwater and marine aquatic ecosystems (Cabana and Rasmussen 1996; McClelland et al. 1997; Costanzo et al. 2001; Lake et al. 2001; McKinney et al. 2002; Steffy and Kilham 2004; Anderson and Cabana 2005; Cole et al. 2005; Vander Zanden et al. 2005; Anderson and Cabana 2006; Kaushal et al. 2006). A primary advantage is that $\delta^{15}N$ signatures may provide a sensitive indicator that allows the detection of slight changes in N sources prior to ecosystem level impacts (McClelland and Valiela 1998; Vander Zanden et al. 2005). Differences in $\delta^{15}N$ in inorganic N sources are reflected in the biota that consumes the N, which can be easily measured (Vander Zanden et al. 2005). A variety of organisms have been used in this application, including unionid mussels (McKinney et al. 2002), benthic invertebrates (Vander Zanden et al. 2005) and fish (Lake et al. 2001), recognizing that these organisms act as integrators of the seasonal variation in $\delta^{15}N$ seen in primary producers and consumers (Vander Zanden et al. 2005). There are, however, potential problems with this approach. First, the $\delta^{15}N$ of organisms is rarely in equilibrium with local N sources (Matthews and Mazumder 2005), therefore it is difficult to relate organism $\delta^{15}N$ to conditions, such as water chemistry, at the time of sampling because longer-lived organisms reflect the range of isotopic signatures over a longer period of time. Second, mean $\delta^{15}N$ signatures of taxonomic or functional groups (e.g., Vander Zanden et al. 2005) may vary in multi-year studies because of the species-specific signatures of the individual organisms used in the sample. Inter-annual comparisons would require consistent composition of bulk samples from year to year, and this may be difficult to replicate. Finally, the limited distribution of certain organisms (e.g., mussels) makes inter-lake comparisons difficult over a broad geographical range.

In certain regions, calanoid copepods may provide a practical option for several reasons. Short life spans means they are more reflective of the water chemistry at the time of sampling. For example, *Eudiaptomus gracilis* has a mean adult longevity of approximately five weeks (Berger and Maier 2001), suggesting that the $\delta^{15}N$ measured in a sample would reflect the $\delta^{15}N$ of inorganic N available within the past few months. Calanoid copepods are usually abundant in lakes and easy to collect, with no collection permits required. Finally, the samples analyzed consist of a composite of individual whole animals, so processing is usually easier than taking a portion of an animal (e.g., fish tissue) or grinding up the whole animal in order to get a composite of the different tissue types present (e.g., mussels). While there is the potential for several species of calanoid copepods to be included in a sample, similar feeding habits among species (Schulze and Folt 1990; Brandl 2005) support the use of general taxonomic groupings, such as calanoid copepods, which have been used in previous stable isotope studies (Matthews and Mazumder 2003). However, seasonal variability in zooplankton $\delta^{15}N$ has been demonstrated (Matthews and Mazumder 2005) so temporal consistency in sample collection would be desirable for multi-year comparisons. In Chapter 2, seasonal differences in zooplankton $\delta^{15}N$ were observed with the highest signatures measured in the first quarter of the year, and lower signatures measured in the summer and fall months. This trend has also been described in other studies (Leggett et al. 2000; Grey et al. 2001). Therefore, if calanoid copepod $\delta^{15}N$ is to be used in water quality assessments of lakes, sample collection should at least include samples from the period when the $\delta^{15}N$ reaches a maximum each year. For British Columbia lakes, this appears to be between January and March.

The goal of this study was to determine which watershed and limnological characteristics influence spring calanoid copepod $\delta^{15}N$ signatures. While a general case can be made for calanoid copepods as an isotopic integrator for water quality assessments based on availability and ease of collection, it is necessary to demonstrate a link between calanoid $\delta^{15}N$ and anthropogenic impacts to validate the use of this tool.

Methods

Sample collection and analysis

Zooplankton samples were collected from 22 lakes (Table 3.1) using an 80 μm mesh, 30 cm diameter Wisconsin net. Samples were collected between January and March, from 2002 to 2007 as part of several projects; therefore, timing of collection was not consistent between lakes. Of the 22 lakes, nine were sampled only once, while the other 13 lakes were sampled from two to five times. After collection, samples were kept on ice and frozen as soon as possible without preservation (usually within six hours of collection). Guts were not cleared prior to processing or analysis as previous studies suggest gut clearance has a negligible impact on overall isotopic values of organisms (Anderson and Cabana 2006). Samples remained frozen until thawed for processing. Individual calanoid copepods were picked from each sample using a binocular microscope. Samples were not separated by species due to the difficulty and time involved in species identification while obtaining enough tissue to conduct the analyses. Following Matthews and Mazumder (2003), the goal was to obtain approximately 1 mg of dried tissue per sample for isotopic analysis. All developmental stages were included in the samples and the number of individuals within a sample depended on the dominant life stage present at the time of sampling. Although there are physiological differences associated with body size that could lead to positive relationships between zooplankton size and $\delta^{15}N$, no studies have attempted to resolve this (Matthews and Mazumder 2007). Samples were then freeze dried, packed in 6x4 mm tin cups and analyzed for $\delta^{15}N$ by the University of Victoria Department of Biology, Water and Aquatic Science Research Program's laboratory using a *DELTA^{plus}* Advantage Isotope Ratio Mass Spectrometer.

Water Chemistry

Water samples were collected near the top (0.5 m) and bottom (within 1 m) of the water column. Surface grab samples were collected by hand and deep water samples were collected using a Van Dorn bottle. All water samples were kept in coolers on ice and shipped to Maxxam Analytics in Burnaby, British Columbia for analyses of total nitrogen (TN) and total phosphorus (TP). TN was determined by Alkaline Persulfate Digestion, UV radiation and TP by the Ascorbic Acid method. Values of one half the method detection limits were used for concentrations that were reported as less than the method detection limit. Average concentrations for the whole water column were calculated for each parameter on each sampling date and these values were used in subsequent data analyses. Dissolved oxygen (DO) and temperature profiles were obtained using a YSI 550A DO meter and Secchi depths were measured with a standard Secchi disc. For lakes and sampling dates where water chemistry data were incomplete, historical data from the British Columbia Ministry of Environment's Environmental Monitoring System database were used to complete the dataset.

Land Use Characterization

Information on individual septic systems was not readily available for the lakes I examined, so the density of residential lots was used as a proxy, assuming each lot represented a single home with its own septic system. Lots were categorized based on size (i.e. $\leq$0.25 ha, $\leq$0.5 ha, $\leq$2.5 ha). Densities (lots per km^2) were calculated for each size category within the 200 m buffer zone around each lake only, and the 200 m buffer zone around each lake and its tributaries, counting each lot contained at least partly in that buffer. With the exception of Long Lake (which is sewered), all lakes have on-site septic systems as the primary means of waste disposal. Discussions with local government staff indicated that while most homes are connected to the centralized sewer service in this area there are likely some that are not, so it was assumed that 10% of the homes around Long Lake are using on-site septic systems. Previous studies (Valiela et al. 1997; Lake et al. 2001; McKinney et al. 2002) suggest that residential land use within a buffer zone around a lake (versus the whole watershed) provides the strongest relationship with organism δ^{15}N. In Chapter 2, the strongest relationship was between zooplankton δ^{15}N and the smallest lot size (<0.25 ha), which would result in the greatest densities of homes. Lot density in this study, therefore, refers to the density of residential lots less than 0.25 ha for both the 200 m buffer zone around each lake and the 200 m buffer zone around each lake and its tributaries. Agricultural land use for each lake was estimated by the amount of Agricultural Land Reserve (ALR) land in the buffer for each lake and its tributaries (ha ALR/km^2). The ALR is a provincial land use classification in which agriculture is recognized as the priority use. Specific agricultural land use data (e.g., animal densities, crop type) were not readily available and so analyses were limited to the general measurement of ALR density.

Land use data were obtained from the Province of British Columbia's Land Resource Data Warehouse using ArcView 8. Cadastral data layers, which provide information on lot size and ownership, were not available for some areas of the province. For the southern interior of BC, cadastral information available online and Google Earth was also used to estimate the number of small lots (<0.25 ha) within the 200 m buffer of the lake. Lot densities for the buffer areas around tributary streams were not calculated for these lakes.

Lake Residence Time

Lake residence time was not available for all lakes. The time to replace the volume of water in each lake was estimated by calculating the amount of precipitation to fall on the whole watershed using monthly precipitation and temperature normals (Environment Canada 2005) for the station nearest each lake. Potential evaporation and evapotranspiration was accounted for by applying the Thornthwaite method, which uses air temperature as an index of the energy available for evapotranspiration, assuming that air temperature is correlated with the integrated effects of net radiation and other controls of evapotranspiration, and that the available energy is shared in fixed proportion between heating the atmosphere and evapotransipiration (Dunne and Leopold 1978). In several instances, the estimated residence time was very close to the reported residence time, indicating that this method provided a reasonable estimate for this application.

Statistical Analysis

JMP® 5.1 statistical software was used to run stepwise multiple linear regression with calanoid $\delta^{15}N$ as the dependent variable and several predicator variables (e.g., land use, water chemistry, lake morphology) to determine which factors may be influencing calanoid copepod $\delta^{15}N$. For each lake on each sampling day, average concentrations for the water column were calculated for TN, TP, N:P, water temperature and DO. Average concentrations for the whole water column were calculated on any given sampling date because most of these lakes have completely mixed water columns during the first quarter of the year. Median values for each parameter in each lake were determined and used in subsequent multiple regression analyses. Median concentrations provide values that are more indicative of conditions typical for a given lake, whereas the average concentrations of all sampling dates could be influenced by atypically high or low values resulting from conditions at the time of sampling in any given year. Similarly, median values were also determined for calanoid $\delta^{15}N$ and Secchi depth. Predictor variables (except temperature and DO, which did not require transformation) were log-transformed to better approximate a normal distribution. Prior to running the stepwise regression analysis, a multivariate correlation matrix (using Pearson's product moment correlation) was constructed to identify multi-collinearity between predictor variables. Using an r value of 0.7 as a cutoff (McGarigal et al. 2000), predictor variables showing the highest level of collinearity were systematically removed until only those with r values less than 0.7 remained. These were then used in a stepwise multiple linear regression analysis to develop models to predict calanoid $\delta^{15}N$ ($p < 0.1$ to include, $p < 0.05$ to leave). Normal distribution of the residuals was checked using a Shapiro-Wilk test. Akaike information criterion (AIC) values, provided by the statistical software, were used to rank the resulting models. AIC values provide a measure of the goodness of fit of an estimated statistical model and a tool for model selection for models from a given data set, with lower values indicating stronger models.

Results

Spring calanoid $\delta^{15}N$ signatures ranged from 6.0‰ (Council Lake) to 21.2‰ (Glen Lake) (Table 3.2). Of the 22 lakes, two are not exposed to residential development (Council and Old Wolf) and showed similar $\delta^{15}N$ values (6.0‰ and 6.1‰, respectively). Another three lakes, Durrance, Mitchell and Pease have no residential development on small lots (<0.25 ha), but there is development on larger lots and, for Mitchell and Pease lakes, some livestock present. These three lakes showed higher calanoid $\delta^{15}N$ (11.1‰, 8.7‰ and 9.5‰, respectively) than the lakes with no development.

Lot density within the 200 m lake buffer ranged from 0 to 558 lots/km^2; within the 200 m buffer of the lake and tributaries, lot density ranged from 0 to 235 lots/km^2. ALR land density ranged from 0 to 56 ha/km^2 within the lakeshore buffer, and from 0 to 60 ha/km^2 within the lake and tributaries buffer. TN ranged from 138 (Old Wolf Lake) to 786 μg/L (Glen Lake) and TP from 1 (Mabel and Mara lakes) to 41 μg/L (Elk Lake).

The first stepwise regression used data from all 22 lakes within a 200 m buffer zone. The correlation matrix for multi-collinearity is provided in Appendix 3.1 and resulted in the selection of the following predictor variables: log elevation, log watershed area:lake surface area (WSA:SA), log residence time, log lot density, log ALR, log total N, log N:P, log Secchi depth, temperature, and DO. The results of this regression produced a model with one significant predictor variable, log lot density ($t = 3.6$, $p = 0.002$), with an R^2 value of 0.39 ($F_{21,\ 0.05} = 12.6$, $p = 0.002$) (Figure 3.1a) and an AIC value of 46.7.

Of the 22 lakes, 17 are located on the west coast of Vancouver Island, while the remaining five are located in the southern interior of BC (Kalamalka, Mabel, Mara, Skaha and Wood). To determine whether removing regional differences would improve the model, a second stepwise regression using only the 17 coastal lakes was performed. The procedure to remove multi-collinearity was repeated and resulted in the following predictor variables in the stepwise regression: log elevation, log mean depth, log maximum depth, log WSA, log WSA:SA, log lot density, log ALR, log TP, log N:P, log Secchi depth, temperature, and DO (Appendix 3.2). Again, the model produced had log lot density as the only significant predictor variable ($t = 3.3$,

$p = 0.0054$) and was slightly stronger than the previous model with an R^2 of 0.41 ($F_{16, 0.05} = 10.6, p = 0.0054$) (Figure 3.1b) and an AIC value of 38.3.

Finally, to determine whether land use within the buffer zone around the lake and its tributary stream could explain more of the variation in $\delta^{15}N$, a third stepwise regression was run. Cadastral data were not available for the Kalamalka, Mabel, Mara, Skaha and Wood lake watersheds, so only the 17 coastal lakes were included. The test for multi-collinearity was repeated, with lot density and ALR data for each lake plus their tributaries. The predictor variables selected were the same as the previous stepwise regression with the exception of TP (Appendix 3.3). The model produced by this stepwise multiple regression also had log lot density as the only significant predictor variable ($t = 3.9, p = 0.0014$) with a greater R^2 value of 0.50 ($F_{16, 0.05} = 15.2, p = 0.0014$) and an AIC value of 35.5. The residuals, however, were not normally distributed ($W = 0.8759, p = 0.0274$) so the dependent variable ($\delta^{15}N$) was log-transformed and the stepwise multiple regression was repeated with the same predictor variables. Again, log lot density was the only significant predictor ($t = 4.3$, $p = 0.0006$) and the resulting model had an R^2 of 0.56 ($F_{16, 0.05} = 18.8, p = 0.0006$) (Figure 3.2), normally distributed residuals ($W = 0.9431, p = 0.358$) and an AIC value of -81.2.

Discussion

Many factors are involved in establishing the $\delta^{15}N$ value at a site, but studies have related increases in $\delta^{15}N$ in aquatic systems to the extent of anthropogenic activity in watersheds and the input of wastewater (Lake et al. 2001). In this study, lot density was the only consistent significant predictor of calanoid $\delta^{15}N$, while physical variables showed little explanatory power, similar to previous reports (Vander Zanden et al. 2005). The amount of variability explained, and the strength of the model produced, increased when the density of residential land use within the 200 m buffer for the lake and its tributaries was considered (AIC = -81.2) as opposed to only the lake buffer (AIC = 38.3). In comparing fish $\delta^{15}N$ to various land use categories (forest, agricultural, wetlands and residential), Lake et al. (2001) found significant relationships only with residential land use; elevations in $\delta^{15}N$ were consistent with increased dissolved inorganic N concentrations, which probably resulted from wastewater inputs. Lake et al. (2001) concluded that N stable isotopes are useful indicators of anthropogenic impacts on freshwater systems. Similarly, McKinney et al. (2002) found residential land use to be the only significant predictor of mussel $\delta^{15}N$ when also compared to other land uses (livestock, row-crop agriculture and natural vegetation). The results presented here also demonstrate a connection between residential land use and the $\delta^{15}N$ of aquatic biota, providing a complimentary measure to traditional water quality monitoring parameters. For example, Baker et al. (2008) suggested that shoreline development was not necessarily a good predictor of impacts to lakes, such as P loads and water clarity. Morphological factors (WSA:SA, water residence time) and improved P management policies may counteract increased shoreline development. When coupled with water chemistry and land use data, $\delta^{15}N$ values provide additional information on the impacts of human land use to lakes that would be beneficial to resource managers in terms of identifying nutrient sources and trends over time.

Agricultural land use was not the dominant land use in any of the watersheds included and not a strong predictor of calanoid $\delta^{15}N$. It is possible that the measure I used, ALR density, was too broad a classification for this purpose or that there simply was not enough agricultural land use in the watersheds we studied to influence calanoid $\delta^{15}N$. Causes of eutrophication vary across large spatial scales in response to different types of land use, land cover and geologies; where agriculture is a major component of the landscape, studies have identified runoff from agriculture as a principle cause of eutrophication (Moore et al. 2003). Anderson and Cabana (2006) showed livestock manure (measured as the percent contribution to the total inorganic N load) to be the best predictor of organism $\delta^{15}N$, but noted that the predominance of livestock manure as an N source in their watersheds may have masked the effects of other sources of N. Even if more precise data were available to quantify the amount and type of agricultural land use in the watersheds studied, it may not have been possible to explain any more of the variation seen in calanoid $\delta^{15}N$ because residential land use was the predominant land use type. Therefore, the dominant land use type in watersheds may limit the modelling of $\delta^{15}N$ on a regional basis.

Several factors may contribute to the unexplained variation in the model presented here. Different N sources with different $\delta^{15}N$ signatures are likely combining to provide a signature that is reflective of the mixing of several sources. Runoff from developed watersheds can include a number of N sources such as septic sewage, synthetic fertilizers, pet and wildlife wastes, and atmospheric sources. The relative contribution of each can alter the $\delta^{15}N$ measured in the biota (e.g., McClelland et al. 1997). Although specific N loading data were not available for the watersheds in this study, some qualitative conclusions can be drawn. Synthetic fertilizers are initially low in $\delta^{15}N$, however site-specific N cycling processes, such as denitrification and volatilization, can result in elevated $\delta^{15}N$ values (Vander Zanden et al. 2005; Anderson and Cabana 2006). McLelland et al. (1997) showed higher $\delta^{15}N$ values for estuarine plants in sites whose N loading was dominated by wastewater (~65% wastewater, 12% fertilizer) than in sites with an equal contribution from both sources (28% wastewater, 26% fertilizer), demonstrating the effect of mixing inorganic N sources with different $\delta^{15}N$ values.

Nitrogen derived from synthetic fertilizers may be reflected by lower $\delta^{15}N$ values in organisms (McKinney et al. 2002) depending on the rate of fertilizer application at a given site. For example, Prospect Lake (Table 3.2) has a golf course within the 200 m buffer of the lake. Synthetic fertilizers are presumably a source of N inputs to this lake, which may act to lower the overall $\delta^{15}N$ below what might be expected based on the density of septic systems around the lake. Different rates of fertilizer application on residential properties would also have a varying effect on organism $\delta^{15}N$. Similarly, low $\delta^{15}N$ from atmospheric sources via N_2-fixing cyanobacteria may also contribute to dampening overall isotopic signatures, as has been suggested in other studies (e.g., Gu et al. 2006). This was illustrated in the results for Cusheon Lake (Table 3.2), which experiences frequent cyanobacteria blooms.

Another source of variation may be that calanoid $\delta^{15}N$ appears to be sensitive to low levels of anthropogenic inputs. This study included lakes with very low lot densities but relatively high calanoid $\delta^{15}N$. Of the lakes considered here, Council and Old Wolf were the only lakes without residential development and showed the lowest $\delta^{15}N$ values (approximately 6‰, see Table 3.2). Durrance, Mitchell and Pease lakes have no development on small residential lots (<0.25 ha), but all have some level of development on larger residential lots serviced by on-site septic systems. In addition, Mitchell and Pease lakes have some livestock present in low densities. When these three lakes were removed from the regression analysis, the strength of the relationship between log lot density (lakes and tributaries) and log calanoid $\delta^{15}N$ increased substantially ($R^2 = 0.74$, $F_{13,\,0.05} = 34.5$, $p < 0.0001$, AIC = -71.4). Thus, processes influencing calanoid $\delta^{15}N$ at low levels of development appear to introduce considerable variation into the final model.

Determining the exact mechanisms of these influences is an area requiring further study. While traditional measures of water quality, such as TN, TP, and water clarity, suggest that these lakes are not impacted, calanoid $\delta^{15}N$ (8.7‰ – 11.1‰) indicates that human land use is contributing to the nutrient loading of the lakes, when compared to results from undeveloped lakes, and that calanoid $\delta^{15}N$ is sensitive to low levels of development. This is consistent with previous observations that the $\delta^{15}N$ associated with a particular N source outweighs the effects of gross N loadings to lakes (Vander Zanden et al. 2005).

Finally, the potential differences in $\delta^{15}N$ between species of calanoid copepod and life stage were not taken into account. Of the 22 lakes included in this study, the dominant calanoid copepod species was identified in 17 with *Hesperodiaptomus franciscanus* being the most common in coastal lakes and *Leptodiaptomus ashlandi* the dominant species in Okanagan lakes (Table 3.2). There may be considerable interspecific and intraspecific variation in copepod ecology, both between lakes and within lakes with differences in behaviour between individuals at different stages of development as well as between sexes of adults (Fryer 1998); this may have contributed to some variation in $\delta^{15}N$. However, the general consistency of inter-annual signatures discussed in Chapter 2 and the difficulty involved in separating calanoid copepod by species for each lake supports the approach outlined here. Additional sources of variation identified in other studies include differences in the range of residential land use around the test lakes, differences in the scale and characterization of land use data, physical characteristics of the lakes and ponds, and chemical or biological processing of N (e.g., rates of denitrification and N fixation) (McKinney et al 2002).

In using biota $\delta^{15}N$ to assess lake water quality, the availability and ease of collection of the test organism should be a consideration. Although mussels appear to provide representative organisms for such applications, they are not available in all freshwater systems and can be difficult to collect (Grey 2006). McKinney et al. (2002) recognized that the selection of lakes and ponds used to test their model was limited by the accessibility and the availability of mussels, demonstrating this point. Studies using a range of ages and/or sizes of organisms, or a composition of organisms (e.g., mean primary consumer $\delta^{15}N$) may be difficult to replicate over time. This is an important consideration in light of recent studies demonstrating changes to biotic communities in lakes with increasing shoreline development (Scheuerell and Schindler 2004; Brauns et al. 2007). In my experience, calanoid copepods were readily available across a range of trophic conditions in lakes and could be collected with relative ease using a standard Wisconsin net. Although samples must be picked in order to perform the isotopic analysis, there are generally no other special considerations, such as specific tissue type to be analyzed or functional feeding group classification. While the time frame in question may help in the choice of organism used (e.g., *Daphnia* for short-term studies, mussels for long-term studies), calanoid copepods offer a reasonable option for the consistent measurement of $\delta^{15}N$ over multiple years to track impacts of land use on aquatic ecosystems.

Conclusions

The restoration of lakes can take a long time after external nutrient loading is reduced. Although eutrophication can sometimes be reversed, it is an expensive process and success rates are extremely variable; thus, it is much easier to pre-emptively avoid eutrophication than to attempt restoration of eutrophic lakes (Schindler 2006). The fact that lakes with centralized sewer systems have been shown to be less eutrophic than lakes on septic suggests that lakes will recover with improvements in waste management (Moore et al. 2003). While $\delta^{15}N$ appears to be reflective of anthropogenic nutrient loading to aquatic systems under a wide range of conditions, suggesting utility as a monitoring tool for anthropogenic nutrient loading (Vander Zanden et al. 2005), its use as a tracer of individual sources may be limited (Anderson and Cabana 2006). Despite this, $\delta^{15}N$ can provide useful insight into problems resulting from anthropogenic stress within specific ecosystems and could be of value to managers and regulators in developing general policies or strategies regarding monitoring and assessment of eutrophication in lakes (McKinney et al.

2002). The results presented here show that calanoid δ^{15}N signatures are influenced by residential land use, specifically the density of on-site septic systems. Therefore, calanoid δ^{15}N may provide an early warning sign to changes in lake water quality and a measure of improvement over time that can help guide resource managers and encourage local stewardship of lakes.

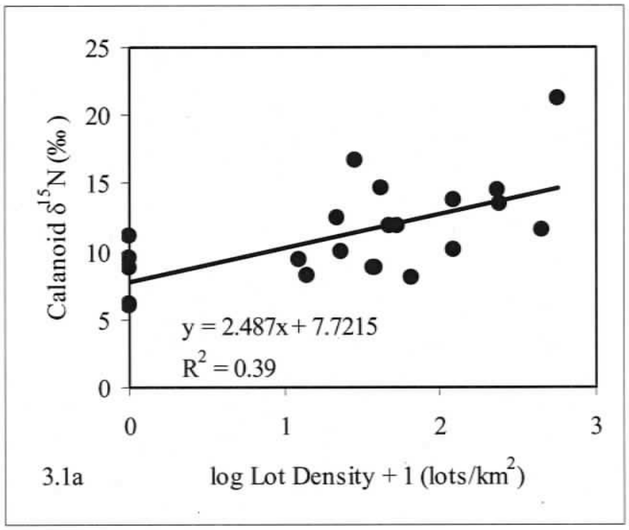
Table 3.1: List of lakes sampled and the physical characteristics for each.

Lake	Longitude	Latitude	Elevation (m)	Mean Depth (m)	Maximum Depth (m)	Residence Time (years)	Surface Area (ha)	Watershed Area (ha)	WSA:SA
Council	123° 40' 15"	48° 31' 44"	402	5	17	0.55	13	265	20
Cusheon	123° 28' 8"	48° 48' 58"	99	4	10	0.21	28	754	27
Durrance	123° 28' 37"	48° 32' 51"	134	6	16	0.50	8	181	22
Elk	123° 23' 52"	48° 31' 41"	65	8	19	4.55	247	734	3
Florence	123° 30' 45"	48° 27' 31"	381	4	6	0.41	7	173	25
Fork	123° 29' 4"	48° 31' 9"	216	2	10	0.11	2	151	76
Glen	123° 31' 20"	48° 26' 15"	67	6	14	0.38	17	579	34
Kalamalka	119° 19' 27"	50° 10' 36"	391	59	142	10.52	2,569	54,415	21
Kemp	123° 46' 50"	48° 22' 46"	33	5	11	0.07	25	520	21
Langford	123° 31' 51"	48° 26' 58"	67	9	17	3.24	61	217	4
Long	124° 1' 2"	49° 12' 39"	111	6	14	1.79	39	131	3
Mabel	118° 43' 53"	50° 33' 0"	396	120	200	6.57	5,790	396,602	68
Mara	119° 2' 0"	50° 45' 23"	347	18	46	0.31	1,850	538,683	291
Mitchell	123° 30' 17"	48° 30' 43"	160	2	8	0.02	2	612	255
Old Wolf	123° 40' 14"	48° 30' 4"	340	4	13	1.41	25	132	5
Pease	123° 29' 50"	48° 32' 43"	139	3	6	0.10	4	184	45
Prospect	123° 26' 26"	48° 30' 51"	48	7	14	0.83	68	856	13
Quamichan	123° 39' 45"	48° 48' 0"	26	4	8	1.58	289	1,211	4
Skaha	119° 35' 16"	49° 25' 0"	339	27	57	8.41	1,817	62,225	34
Teanook	123° 30' 28"	48° 29' 18"	108	2	8	0.66	3	21	7
Weston	123° 25' 31"	48° 47' 4"	69	6	12	0.32	18	316	17
Wood	119° 23' 23"	50° 4' 47"	394	16	34	2.15	883	23,050	26

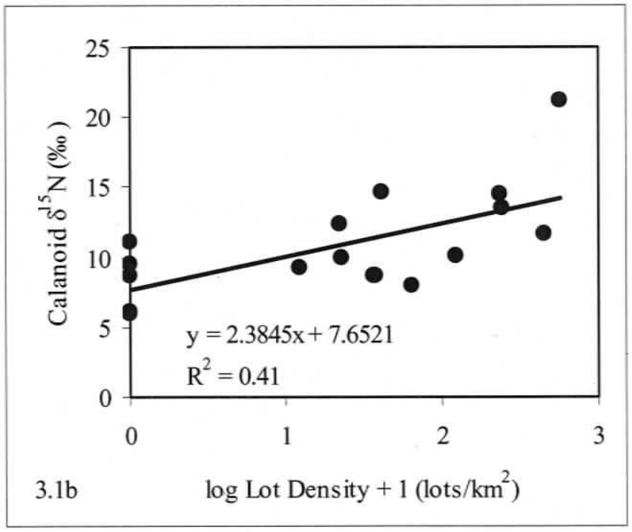
Table 3.2: Results of N isotope analyses, land use characterization and water chemistry sampling. These data were used in subsequent stepwise multiple regression analyses. TN, TP, N:P, Secchi depths, water temperature and DO represent median values for all daily averages of results collected in Q1 (January, February, March).

Lake	Calanoid $\delta^{15}N$ (‰)	Dominant species	Lot Density (lake only) (lots/km^2)	Lot Density (lake + tribs) (lots/km^2)	ALR (lake only) (ha/km^2)	ALR (lake + tribs) (ha/km^2)	Total N (µg/L)	Total P (µg/L)	N:P	Secchi Depth (m)	Temperature (°C)	Dissolved Oxygen (mg/L)
Council	6.0	*Leptodiaptomus tyrelli*	0	0	0	0	197	4	54	7	6	12
Cusheon	8.7	*Siskodiaptomus oregonensis*	37	11	20	33	705	16	46	3	5	10
Durrance	11.1	*Hesperodiaptomus franciscanus*	0	1	0	0	290	8	40	6	5	11
Elk	12.4	*Hesperodiaptomus franciscanus*	21	18	39	43	675	41	17	3	5	13
Florence	13.5	*Onychodiaptomus hesperus*	241	127	0	0	543	24	27	3	7	10
Fork	8.0	*Hesperodiaptomus franciscanus*	63	24	0	4	266	7	40	3	7	9
Glen	21.2	*Hesperodiaptomus franciscanus*	558	88	0	4	786	16	52	3	8	9
Kalamalka	11.8	*Leptodiaptomus ashlandi*	53	—	15	17	387	5	73	12	4	10
Kemp	8.7	*Siskodiaptomus oregonensis*	36	11	19	14	590	12	53	3	6	11
Langford	14.5	*Hesperodiaptomus franciscanus*	235	235	0	0	555	19	27	3	7	11
Long	11.6	not identified	444	44	0	0	437	7	60	5	4	8
Mabel	8.2	*Leptodiaptomus ashlandi*	13	—	0	10	150	1	150	16	4	12
Mara	11.8	*Leptodiaptomus ashlandi*	46	—	8	15	170	1	170	7	3	11
Mitchell	8.7	*Hesperodiaptomus franciscanus*	0	0	6	1	235	5	48	5	6	11
Old Wolf	6.1	*Hesperodiaptomus franciscanus*	0	0	0	0	138	4	31	5	4	13
Pease	9.5	not identified	0	0	0	0	190	5	41	4	5	12
Prospect	10.1	*Hesperodiaptomus franciscanus*	123	21	4	1	419	16	26	4	5	14
Quamichan	14.6	not identified	41	55	56	60	477	36	13	4	5	10
Skaha	13.8	*Leptodiaptomus ashlandi*	120	—	10	13	213	7	30	6	3	8
Teanook	9.3	*Hesperodiaptomus franciscanus*	11	11	0	0	310	8	41	3	8	8
Weston	10.0	not identified	22	9	32	27	785	15	51	2	5	10
Wood	16.6	not identified	27	—	26	18	370	34	11	5	4	12

Figure 3.1: Results of stepwise multiple regression analyses to determine factors influencing calanoid $\delta^{15}N$ in 22 British Columbia lakes. Figure 3.1a includes land use data within the 200 m buffer zone of the lakeshore for all 22 lakes and Figure 3.1b includes data for only the 17 Vancouver Island lakes.

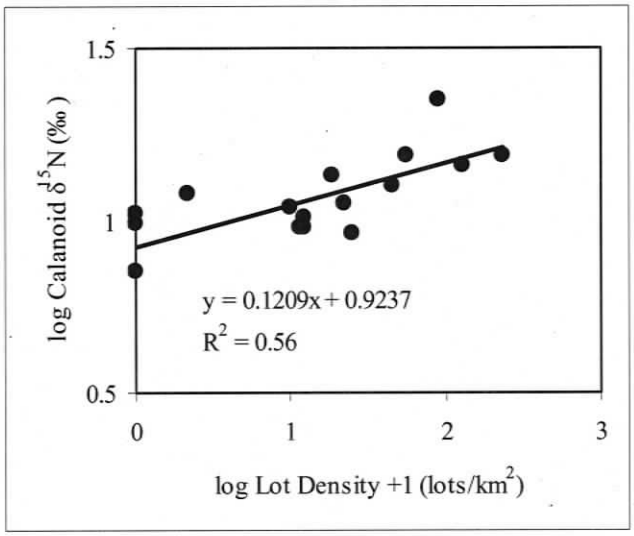


3.1a



3.1b

Figure 3.2: Results of stepwise multiple regression analyses to determine factors influencing calanoid $\delta^{15}N$ in 17 Vancouver Island lakes. The x-axis represents land use (lots/km^2) data for the 200 m buffer around the lakeshore and the tributaries for each lake. $\delta^{15}N$ data (y-axis) were log-transformed to achieve normal distribution of the residuals.



Chapter 4: General Conclusions

My goal in this research was to determine the utility of zooplankton $\delta^{15}N$ for water quality assessments of small lakes, specifically to help identify sources of nutrient inputs and identify changes over time. Several researchers have demonstrated the relationship between biota $\delta^{15}N$ and anthropogenic inputs from land use (e.g., Cabana and Rasmussen 1996; McKinney et al. 2002; Cole et al. 2005; Vander Zanden et al. 2005). The main problem that I perceived with the approaches reported in the literature concerned the test organisms used. While organism $\delta^{15}N$ is undoubtedly influenced by human land use, the collection of test organisms and the replication of sample collection can be difficult. Unionid mussels are one of the preferred organisms for this application of stable isotope analysis (Cabana and Rasmussen 1996; McKinney et al. 2002), however, in my experience, mussels are not universally abundant or easily collected in BC lakes. Other studies have used bulk signatures of organisms, such as macroinvertebrates (Vander Zanden et al. 2005) and macrophytes (Cole et al. 2005). The species-specific variability of $\delta^{15}N$ dictates that the species composition of bulk samples should be consistent each time in order to allow temporal comparisons; however, this may be difficult to achieve. I believe that pelagic zooplankton in lakes provide information comparable to other organisms and have the benefit of being commonly available and easy to collect.

The first aspect I needed to address regarding the use of zooplankton in stable isotope studies was the seasonality and inter-annual consistency of zooplankton $\delta^{15}N$. Seasonal changes have been documented in several studies (Leggett et al. 2000; Grey et al. 2001; Perga and Gerdeaux 2006) and it is crucial to understand these patterns for proper timing of sample collection. Syväranta et al. (2008) concluded that restricted sampling of organisms at lower trophic levels represents only a "snapshot" of values within the biota, with high seasonal variability, which can potentially lead to misinterpretation of results. In my work, I observed a seasonal pattern in zooplankton $\delta^{15}N$ from BC lakes, whereby values peak in the winter and spring, decline through summer, increase through the fall, and peak again the following winter or spring. This pattern was observed for several lakes over a number of years and, in the absence of any significant change in land use, spring zooplankton $\delta^{15}N$ was consistent from year to year. Syväranta et al.'s (2008) examination of zooplankton $\delta^{15}N$ in Finnish lakes showed the annual peak to occur in October, presumably because of the more northerly location of Finland compared to Vancouver Island, but the inter-annual consistency is clear. In my work, $\delta^{15}N$ values for calanoid copepods were shown to be significantly higher in the first quarter of the year than in other quarters. Therefore, January through March would be the critical period for sample collection assuming that the $\delta^{15}N$ peak is due, in part, to watershed runoff in winter carrying human-derived N to a lake. This is not to suggest, however, that only one sample should be collected. A minimum of quarterly sampling, and preferably monthly sampling, would be required to identify seasonal $\delta^{15}N$ patterns for a specific lake and avoid misinterpretation of results.

Next, I wanted to determine whether one taxon, calanoid copepods or *Daphnia*, would provide a more consistent $\delta^{15}N$ signature, thereby supporting the use of that taxonomic grouping for this purpose. Substantial between-taxon variation in zooplankton $\delta^{15}N$ has been demonstrated (Matthews and Mazumder 2003: Perga and Gerdeaux 2006; Syväranta et al. 2008), therefore the $\delta^{15}N$ of bulk zooplankton samples would be influenced by the dominant taxon in the sample (Syväranta et al. 2006). During my initial analyses of zooplankton samples, I measured the $\delta^{15}N$ for calanoid copepods, *Daphnia* and bulk samples consisting of a mix of the different taxa present. The $\delta^{15}N$ of bulk samples always fell somewhere between the higher calanoid $\delta^{15}N$ and lower *Daphnia* $\delta^{15}N$, with values closer to one taxon suggesting either a greater contribution of calanoids or *Daphnia* to the overall result, or the influence of another species present (e.g., *Chaoborus*, which were generally slightly lower in $\delta^{15}N$ than calanoid copepods) . The variability seen in these bulk samples dictate that a taxon-specific $\delta^{15}N$ be used. On a lake-specific basis, my results showed similar seasonal patterns between *Daphnia* and calanoid copepod $\delta^{15}N$, although *Daphnia* were more variable. In addition, *Daphnia* were not available in all lakes at all times, adding support for the use of calanoid copepod $\delta^{15}N$ in water quality assessments. From this I concluded that calanoid copepods were generally a better indicator organism than *Daphnia* for N isotope analysis, however, the goal of a particular study should guide which organism is used. For example, *Daphnia* may be a more appropriate choice for studies focussing on a short period (e.g., weeks), such as first-flush events; calanoids may be more appropriate for studies focussing on a moderate time period (e.g., months); and, if available, longer-lived organisms like unionid mussels may be more appropriate for longer-term multi-year studies.

Finally, in order to demonstrate the value of calanoid $\delta^{15}N$ in lake water quality assessment work, the isotopic signatures had to be quantitatively linked to human land use. Residential development was the predominant anthropogenic land use around most of the lakes included in this study. My results consistently showed a significant relationship between calanoid $\delta^{15}N$ and residential development in the form of small lot density, which I used as a proxy for septic tank density. This was the only significant predictor variable identified in the study, explaining about 56% of the variation seen in the calanoid copepod $\delta^{15}N$ measured between January and March. Clearly there are other lake-specific factors influencing zooplankton $\delta^{15}N$ including: varying degrees of individual septic system maintenance around a lake, other lands use types (e.g., agricultural), contribution from low $\delta^{15}N$ sources such as fertilizers and N-fixing cyanobacteria, pets and wildlife wastes, and the seasonal recycling of historic N sources liberated from bottom sediments.

When one considers all of these confounding factors, developing a universal model to predict $\delta^{15}N$ appears to be a very difficult task. For example, of the lakes examined in Chapter 3, Prospect and Cusheon had $\delta^{15}N$ signatures that were likely influenced by the mixing of low $\delta^{15}N$ sources in those systems. Prospect Lake has a golf course situated directly beside the lake and Cusheon Lake experiences frequent cyanobacteria blooms. Without the contribution of these sources, the zooplankton $\delta^{15}N$ measured may have been quite different. Dominant land use, either residential or agricultural (Anderson and Cabana 2006, for example), appears to be the main driver of organism $\delta^{15}N$; therefore the use of $\delta^{15}N$ in water quality assessments may be best applied at a local or lake-specific level.

Overall, I did not find a strong relationship between water chemistry variables and zooplankton $\delta^{15}N$. This is not surprising when one considers the dynamic nature of many of the water chemistry measures used in lake assessments, and the length of time required to see these changes reflected in the biota. In Chapter 2, mean $\delta^{15}N$ was significantly correlated with total nitrogen (TN) and nitrate+nitrite, but this analysis did not take the seasonality of isotopic signatures into account. In Chapter 3, I found no significant relationship between spring calanoid $\delta^{15}N$ and TN or total phosporus (TP). Even though N and P concentrations explained very little of the variation seen in my data, this information is necessary for the proper interpretation of $\delta^{15}N$ results in this context. For example, my data set included calanoid $\delta^{15}N$ results for Bouchie Lake (located near Williams Lake in central BC) ranging from 3.3‰ (October 2005) to 9.6‰ (April 2007). Bouchie Lake has a residential lot (<0.25 ha) density of 2 lots/km^2 (serviced by septics) and an ALR density of 33 ha/km^2. Based on the $\delta^{15}N$ results, Bouchie Lake does not appear to be heavily affected by land development. However, TN was 1,585 µg/L and 1,400 µg/L and TP was 242 µg/L and 130 µg/L, respectively, for the two sampling dates, illustrating impacts to water quality in this lake. Bouchie Lake also experiences frequent cyanobacteria blooms, which introduce atmospheric N with a low $\delta^{15}N$ to the N pool. The relatively low calanoid $\delta^{15}N$ measured in Bouchie Lake illustrates the point made by Vander Zanden et al. (2005) that the $\delta^{15}N$ associated with a particular N source has a stronger influence than gross N loadings to a waterbody in determining organism $\delta^{15}N$. In this case, it is likely that the low $\delta^{15}N$ of atmospheric N and the recycling of low $\delta^{15}N$ sources in the sediments (e.g., Gu et al. 2006) have resulted in a lower isotopic signature in the biota. An improvement in water quality demonstrated by a reduction in N-fixing cyanobacteria might actually translate to an increase in $\delta^{15}N$, but this would have to be assessed in conjunction with other information such as nutrient concentrations, phytoplankton taxonomy, Secchi depths and dissolved oxygen profiles.

It is recognized that there are several aspects that could have strengthened this work. First, most of the lakes sampled had residential development as the dominant land use type. It would have been beneficial to include more lakes with agriculture as the dominant land use. Additionally, information on the different types of agricultural land use (e.g., crop, livestock, range) around lakes may have helped to better explain some of the variation in lakes where these activities occur. With respect to septic density, a better representation of lakes with varying degrees of development may have strengthened my final model. Of the 22 lakes sampled in Chapter 3, five had lots densities of 0 lots/km^2 and six had lot densities greater than 100 lots/km^2. The other 11 lakes had densities between 11 lots/km^2 and 63 lots/km^2 (Table 3.2), and it would have been useful to have included more lakes at the medium to high densities of development. Finally, most of the detailed analysis in this research focussed on results from coastal lakes and more specifically on southern Vancouver Island lakes. A better representation of lakes from the interior of the province with quarterly sampling would have been useful to identify regional differences. It is possible that I would have seen differences in seasonal patterns given that interior lakes behave much differently than coastal lakes in terms of hydrology, stratification patterns and ice cover. For example, coastal lakes are exposed to extensive periods of precipitation in the fall and winter, are generally monomictic, and experience little, if any, periods of ice cover. Conversely, interior lakes experience a spring freshet mainly driven by the melting snow pack, are often dimictic, and can experience extensive periods of ice cover. These factors contribute significantly to differences in chemical, physical and biological processes in lakes which could alter isotopic signatures in zooplankton.

In my research, I have demonstrated an application for calanoid copepod $\delta^{15}N$ signatures in water quality assessments and resource management. When used in conjunction with other information, such as land use and water chemistry, $\delta^{15}N$ signatures provide insight into nutrient sources for a particular lake, tracks changes in water quality over time and can help to guide management decisions. To compliment this work, I am fortunate to have a unique opportunity to test the effectiveness of calanoid $\delta^{15}N$ signatures as water quality indicators with the extension of centralized sewer service to the areas of Glen, Langford and Florence lakes and the local requirement for connection of shoreline homes to these systems by the end of 2009. In the period covered by this study, 22% of residences within the 200 m buffer of Glen Lake decommissioned their septic systems and connected to centralized sewer. Over this time, I measured decreases in calanoid copepod and *Daphnia* $\delta^{15}N$ of 7.0‰ and 5.3‰, respectively. It seems unlikely that the relatively recent decommissioning of septic systems could result in decreases of this magnitude in such a short time frame, however, the resulting proportional increase of inputs from low $\delta^{15}N$ sources (e.g., fertilizers, N-fixing cyanobacteria) could help explain these results. My data collection began before the sewer service was extended and the continuation of regular sampling should help determine how zooplankton $\delta^{15}N$ responds to changing sewage disposal practices. The development of accurate nutrient budgets for these lakes could provide additional clarification for the results seen to date.

The Glen Lake results are encouraging in terms of being able to demonstrate the benefits of more appropriate waste disposal methods, such as centralized sewer service, close to lakes. Data collection should continue here, and in Florence and Langford lakes as well, to confirm the effectiveness of this tool for lake water quality assessments. If it can be demonstrated to lakeshore residents and resource managers that land use practices, such as wastewater disposal methods, are having a positive impact on lake water quality, we are more likely to encourage responsible behaviour and practices by homeowners and changes in management strategies, including bylaws.

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Appendix 2.1: Summary of physical and limnological characteristics of lakes sampled for zooplankton $\delta^{15}N$. Time of year sampled is identified in columns Q1 (January, February, March), Q2 (April, May, June), Q3 (July, August, September) and Q4 (October, November, December). Values in these columns refer to the total number of samples collected per quarter.

Lake	Longitude	Latitude	Mean Depth (m)	Surface Area (ha)	Volume (dam^3)	Watershed Area (km^2)	Elevation (m)	Total N (g/L)	Total P (g/L)	Q1	Q2	Q3	Q4
Badger	120° 8' 9"	51° 2' 4"	6	86	2,147	5.4	1,091	—	—		1		
Bleeker	120° 9' 50"	50° 30' 7"	7	38	1,870	22.7	1,048	1,500	42			1	
Bouchie	122° 37' 34"	53° 2' 1"	4	136	4,984	68.3	762	1,800	210	1	3	4	2
Burns	125° 43' 59"	54° 12' 27"	9	1,179	106,551	711.2	701	597	29			1	
Butchart Reservoir	123° 38' 11"	48° 32' 24"	12	69	—	2.7	547	—	—	1	2	3	3
Council	123° 40' 15"	48° 31' 44"	5	13	826	2.7	402	60	4	1	2	2	1
Cusheon	123° 28' 8"	48° 48' 58"	4	28	1,160	7.5	99	855	19	3	4	2	3
Decker	125° 50' 25"	54° 17' 22"	9	1,180	105,950	427.9	699	603	34			1	
Dragon	122° 25' 18"	52° 57' 1"	7	541	14,447	34.9	579	805	28		1		
Durrance	123° 28' 37"	48° 32' 51"	6	8	514	1.8	134	270	10	6	1	3	3
Elk	123° 23' 52"	48° 31' 41"	8	247	18,900	7.3	65	677	36	7	1	2	4
Florence	123° 30' 45"	48° 27' 31"	4	7	7,800	1.7	381	601	29	4	1	1	3
Fork	123° 29' 4"	48° 31' 9"	2	2	92	1.5	216	260	7	4	1	2	3
Fuller	123° 43' 15"	48° 54' 33"	9	24	1,081	0.8	455	500				1	
Glen	123° 31' 20"	48° 26' 15"	6	17	1,229	5.8	67	750	12	4	1	1	3
Glimpse	120° 16' 21"	50° 14' 51"	—	90	—	22.3	1,219	635	52			1	
Goldstream Reservoir	123° 37' 44"	48° 30' 31"	7	72	7,128	11.2	57	—	—	1	2	3	3
Hallamore	120° 7' 42"	51° 30' 12"	13	29	2,111	18.7	660	—	—		1		
Horn	124° 42' 16"	51° 47' 59"	19	195	31,472	46.2	1,015	332	7		2		
Horne	124° 41' 59"	51° 35' 20"	20	847	—	104.1	123	—	—			1	
Horse	121° 7' 5"	49° 20' 32"	15	1,229	176,628	777.7	914	414	37		3	2	1
Jump Lake Reservoir	124° 14' 14"	49° 0' 39"	—	184	—	48.4	—	—	—	2	1	2	3
Kalamalka	119° 19' 27"	50° 10' 36"	59	2,569	1,500,686	544.2	391	270	3	1		1	
Kathlyn	127° 12' 20"	54° 49' 30"	5	132	7,749	16.9	510	330	15			1	
Kemp	123° 46' 50"	48° 22' 46"	5	25	1,229	5.2	33	610	14	3	1	1	4
Lac des Roches	120° 35' 10"	518° 29' 23"	22	663	95,479	43.7	1,128	290	14		1		
Langford	123° 31' 51"	48° 26' 58"	9	61	3,980	2.2	67	520	33	7	5	7	5
Long	124° 1' 2"	49° 12' 39"	6	39	1,427	1.3	111	440	8	1			
Loon	121° 14' 9"	51° 6' 43"	27	609	173,706	307.9	825	605	82		1		
Mabel	118° 43' 53"	50° 33' 0"	120	5,790	7,177,900	3,966.0	396	110	10	1		1	
Mara	119° 2' 0"	50° 45' 23"	18	1,850	357,700	5,386.8	347	160	2	1		1	
Middle Quinsam	125° 28' 55"	49° 55' 21"	4	75	2,817	10.5	280	120	3			2	
Mitchell	123° 30' 17"	48° 30' 43"	2	2	53	6.1	160	242	5	4	1	1	
Nimpo	125° 11' 17"	52° 20' 14"	12	924	104,432	642.7	1,097	453	40		1		
Nukko	123° 0' 30"	54° 4' 14"	7	608	28,882	70.5	757	600	24		1		1
Old Wolf	123° 40' 14"	48° 30' 4"	4	25	1,050	1.3	340	185	5	2			
Paul	120° 6' 53"	50° 44' 25"	29	268	74,393	145.3	769	420	15			1	
Pease	123° 29' 50"	48° 32' 43"	3	4	99	1.8	139	425	53	1			
Pinantan	120° 1' 20"	50° 43' 27"	11	63	637	59.4	863	550	35		1		
Prospect	123° 26' 26"	48° 30' 51"	7	68	4,040	8.6	48	485	39	5	1	3	3
Quamichan	123° 39' 45"	48° 48' 0"	4	289	13,730	12.1	26	1,015	58	1			
Quennell	123° 49' 39"	49° 4' 47"	4	113	4,159	6.5	32	212	6		1		
Roche	120° 8' 57"	50° 28' 32"	26	162	6,797	33.1	1,145	960	4			1	
Ross	127° 31' 9"	55° 15' 35"	2	34	1,419	1.0	404	490	19		1	2	
Round	126° 55' 33"	54° 39' 20"	10	180	17,208	27.2	585	1,120	70		2	8	1
Seeley	127° 40' 57"	55° 11' 51"	4	22	365	5.4	306	300	21		1	2	
Seymour	127° 9' 35"	54° 44' 42"	5	87	855	17.2	533	520	71			2	
Shawnigan	123° 38' 23"	48° 38' 28"	12	541	64,057	72.2	118	224	6		1	1	1
Shumway	120° 14' 58"	50° 31' 23"	7	90	4,272	279.6	695	1,500	860			1	
Skaha	119° 35' 16"	49° 25' 0"	27	1,817	522,000	622.3	339	215	7	1	1		
Spider	124° 37' 37"	49° 20' 56"	4	55	1,795	2.2	140	230	8			1	
Stump	120° 22' 13"	50° 21' 50"	12	715	59,423	191.6	743	1,380	15		1	1	
Summit	122° 39' 30"	54° 16' 54"	2	1,339	89,000	143.1	707	340	18			1	
Swan	121° 18' 29"	53° 10' 44"	3	578	18,500	574.0	724	1,100	65			1	
Tabor	122° 32' 32"	53° 55' 1"	5	381	11,280	49.5	705	548	11		1		1
Teanook	123° 30' 28"	48° 29' 18"	2	3	78	0.2	108	380	6	3	1	1	
Tyhee	127° 2' 1"	52° 42' 46"	11	367	35,178	30.8	522	713	36			2	– –
Upper Quinsam	125° 33' 18"	49° 52' 30"	4	566	66,488	69.0	369	360	8			1	
Weston	123° 25' 31"	48° 47' 4"	6	18	719	3.2	69	815	15	2	2	1	1
Williams	122° 3' 50"	52° 7' 1"	12	700	87,978	2,421.8	565	717	37		4	3	1
Wood	119° 23' 23"	50° 4' 47"	16	883	129,700	230.5	394	370	34	1			

Appendix 3.1: Correlation matrix for predictor variables used in stepwise multiple regression analysis of calanoid $\delta^{15}N$ values. All lakes had data collected in the first quarter of the year (January, February, March) and land use data within the 200 m lakeshore buffer (n = 22). Variables in bold are those selected for the stepwise multiple linear regresion analyses. Italicized values indicate *r* values greater than 0.7, suggesting collinearity.

	Long.	Lat.	**log Elev.**	log Mean Depth	log max. Depth	log Perim.	log SA	log Vol.	log WSA	**log WSA:SA**	**log Res. Time**	**log Lot Density**	**log ALR**	**log TN**	log TP	**log N:P**	**log Secchi**	**Temp.**	**DO**
Longitude	1.000	*-0.854*	-0.576	*-0.787*	*-0.730*	*-0.826*	*-0.816*	*-0.762*	*-0.843*	-0.283	-0.633	-0.189	-0.198	0.348	0.299	-0.197	-0.648	*0.730*	0.115
Latitude	*-0.854*	1.000	0.537	*0.832*	*0.751*	*0.876*	*0.836*	*0.786*	*0.903*	0.379	0.500	0.146	0.178	-0.358	-0.442	0.453	*0.734*	*-0.738*	0.003
log Elevation	-0.576	0.537	1.000	0.425	0.622	0.382	0.271	0.347	0.431	**0.447**	**0.268**	**-0.242**	**-0.373**	**-0.650**	**-0.558**	**0.313**	**0.630**	**-0.411**	**0.003**
log Mean Depth	*-0.787*	*0.832*	0.425	1.000	*0.895*	*0.926*	*0.901*	*0.909*	*0.865*	0.148	*0.782*	0.217	0.114	-0.220	-0.324	0.376	*0.771*	-0.682	0.135
log Max Depth	*-0.730*	*0.751*	0.622	*0.895*	*1.000*	*0.833*	*0.759*	*0.868*	*0.773*	0.227	0.694	0.250	-0.027	-0.225	-0.304	0.356	*0.725*	-0.545	0.079
log Perimiter	*-0.826*	*0.876*	0.382	*0.926*	*0.833*	1.000	*0.982*	*0.969*	*0.936*	0.156	*0.755*	0.254	0.328	-0.222	-0.235	0.260	*0.712*	*-0.742*	0.157
log Surface Area	*-0.816*	*0.836*	0.271	*0.901*	*0.759*	*0.982*	1.000	*0.960*	*0.900*	0.035	*0.773*	0.282	0.405	-0.153	-0.126	0.146	0.639	*-0.753*	0.170
log Volume	*-0.762*	*0.786*	0.347	*0.909*	*0.868*	*0.969*	*0.960*	1.000	*0.867*	0.038	*0.791*	0.317	0.335	-0.102	-0.083	0.142	0.630	-0.678	0.194
log WSA	*-0.843*	*0.903*	0.431	*0.865*	*0.773*	*0.936*	*0.900*	*0.867*	1.000	0.467	0.542	0.162	0.303	-0.306	-0.356	0.371	0.697	*-0.740*	0.150
log WSA:SA	-0.283	0.379	**0.447**	0.148	0.227	0.156	0.035	0.038	0.467	1.000	**-0.306**	**-0.204**	**-0.129**	**-0.417**	-0.566	**0.540**	**0.311**	**-0.160**	**-0.015**
log Residence Time	-0.633	0.500	**0.268**	*0.782*	0.694	*0.755*	*0.773*	*0.791*	0.542	**-0.306**	1.000	**0.240**	**0.172**	**-0.098**	-0.002	**-0.062**	**0.580**	**-0.513**	**0.044**
log Lot Density	-0.189	0.146	**-0.242**	0.217	0.250	0.254	0.282	0.317	0.162	**-0.204**	**0.240**	1.000	**0.111**	**0.582**	0.384	**-0.050**	**-0.245**	**0.090**	**-0.411**
log ALR	-0.198	0.178	**-0.373**	0.114	-0.027	0.328	0.405	0.335	0.303	**-0.129**	**0.172**	***0.111***	1.000	**0.435**	0.508	**-0.358**	**-0.176**	**-0.327**	**0.143**
log TN avg	0.348	-0.358	**-0.650**	-0.220	-0.225	-0.222	-0.153	-0.102	-0.306	**-0.417**	**-0.098**	**0.582**	**0.435**	1.000	*0.794*	**-0.347**	**-0.650**	**0.354**	**-0.177**
log TP	0.299	-0.442	*-0.558*	-0.324	-0.304	-0.235	-0.126	-0.083	-0.356	-0.566	-0.002	0.384	0.508	*0.794*	1.000	*-0.837*	-0.653	0.308	-0.004
log N:P	-0.197	0.453	**0.313**	0.376	0.356	0.260	0.146	0.142	0.371	**0.540**	**-0.062**	**-0.050**	**-0.358**	**-0.347**	*-0.837*	1.000	**0.475**	**-0.206**	**-0.110**
log Secchi	-0.648	*0.734*	**0.630**	*0.771*	*0.725*	*0.712*	0.639	0.630	0.697	**0.311**	**0.580**	**-0.245**	**-0.176**	**-0.650**	-0.653	**0.475**	1.000	**-0.616**	**0.199**
Temp.	*0.730*	*-0.738*	**-0.411**	-0.682	-0.545	*-0.742*	*-0.753*	-0.678	*-0.740*	**-0.160**	**-0.513**	**0.090**	**-0.327**	**0.354**	0.308	**-0.206**	**-0.616**	1.000	**-0.187**
DO	0.115	0.003	**0.003**	0.135	0.079	0.157	0.170	0.194	0.150	**-0.015**	**0.044**	**-0.411**	**0.143**	**-0.177**	-0.004	**-0.110**	**0.199**	**-0.187**	1.000

Appendix 3.2: Correlation matrix for predictor variables used in stepwise multiple regression analysis of calanoid $\delta^{15}N$ values. All lakes are located on Vancouver Island, had data collected in the first quarter of the year (January, February, March) and land use data within the 200 m lakeshore buffer (n = 17). Variables in bold are those selected for the stepwise multiple linear regresion analyses. Italicized values indicate *r* values greater than 0.7, suggesting collinearity.

	Long.	**Lat.**	**log Elev.**	**log Mean Depth**	**log Max. Depth**	log Perim.	log SA	log Vol.	**log WSA**	**log WSA:SA**	log Res. Time	**log Lot Density**	**log ALR**	log TN	**log TP**	**log N:P**	**log Secchi**	**Temp.**	**DO**
Longitude	1.000	**-0.484**	**0.003**	**-0.078**	**0.028**	-0.120	-0.155	0.005	**0.142**	**0.309**	-0.126	**-0.151**	**0.164**	0.146	**0.271**	**-0.274**	**-0.307**	**0.216**	**0.335**
Latitude	**-0.484**	1.000	**-0.133**	**0.140**	**-0.159**	0.275	0.286	0.083	**0.045**	**-0.304**	0.172	**0.229**	**0.207**	0.181	**0.053**	**0.123**	**0.058**	**-0.576**	**-0.440**
log Elevation	**0.003**	**-0.133**	1.000	**-0.385**	**0.347**	-0.473	-0.610	-0.358	**-0.509**	**0.317**	-0.234	**-0.412**	**-0.645**	-0.621	**-0.611**	**0.300**	**0.445**	**0.035**	**-0.018**
log Mean Depth	**-0.078**	**0.140**	**-0.385**	1.000	**0.419**	0.671	*0.721*	*0.708*	**0.330**	**-0.633**	0.626	**0.395**	**0.151**	0.520	**0.498**	**-0.210**	**-0.009**	**-0.327**	**0.278**
log Max Depth	**0.028**	**-0.159**	**0.347**	**0.419**	1.000	0.267	0.130	0.545	**-0.018**	**-0.197**	0.269	**0.354**	**-0.217**	0.280	**0.356**	**-0.188**	**-0.013**	**0.074**	**0.097**
log Perimiter	-0.120	0.275	-0.473	0.671	0.267	1.000	*0.962*	*0.916*	0.619	-0.645	*0.736*	0.364	0.571	0.472	*0.728*	-0.652	-0.029	-0.373	0.341
log Surface Area	-0.155	0.286	-0.610	*0.721*	0.130	*0.962*	1.000	*0.868*	0.576	*-0.732*	*0.747*	0.349	0.572	0.466	*0.709*	-0.640	-0.068	-0.400	0.327
log Volume	0.005	0.083	-0.358	*0.708*	0.545	*0.916*	*0.868*	1.000	0.491	-0.647	*0.732*	0.417	0.444	0.537	*0.815*	-0.689	-0.131	-0.244	0.344
log WSA	**0.142**	**0.045**	**-0.509**	**0.330**	**-0.018**	0.619	0.576	0.491	1.000	**0.132**	0.048	**0.154**	**0.695**	0.430	**0.506**	**-0.321**	**-0.083**	**-0.325**	**0.398**
log WSA:SA	**0.309**	**-0.304**	**0.317**	**-0.633**	**-0.197**	-0.645	*-0.732*	-0.647	**0.132**	1.000	*-0.839*	**-0.299**	**-0.110**	-0.230	**-0.437**	**0.477**	**0.028**	**0.234**	**-0.075**
log Residence Time	-0.126	0.172	-0.234	0.626	0.269	*0.736*	*0.747*	*0.732*	0.048	*-0.839*	1.000	0.250	0.108	0.188	0.482	-0.615	0.144	-0.162	0.219
log Lot Density	**-0.151**	**0.229**	**-0.412**	**0.395**	**0.354**	0.364	0.349	0.417	**0.154**	**-0.299**	0.250	1.000	**0.056**	*0.747*	**0.612**	**-0.110**	**-0.573**	**0.311**	**-0.388**
log ALR	**0.164**	**0.207**	**-0.645**	**0.151**	**-0.217**	0.571	0.572	0.444	**0.695**	**-0.110**	0.108	**0.056**	1.000	0.516	**0.594**	**-0.363**	**-0.410**	**-0.328**	**0.252**
log TN avg	0.146	0.181	-0.621	0.520	0.280	0.472	0.466	0.537	0.430	-0.230	0.188	*0.747*	0.516	1.000	*0.808*	-0.109	*-0.722*	0.098	-0.200
log TP	**0.271**	**0.053**	**-0.611**	**0.498**	**0.356**	*0.728*	*0.709*	*0.815*	**0.506**	**-0.437**	0.482	**0.612**	**0.594**	*0.808*	1.000	**-0.670**	**-0.558**	**0.047**	**0.058**
log N:P	**-0.274**	**0.123**	**0.300**	**-0.210**	**-0.188**	-0.652	-0.640	-0.689	**-0.321**	**0.477**	-0.615	**-0.110**	**-0.363**	-0.109	**-0.670**	1.000	**0.041**	**0.045**	**-0.362**
log Secchi	**-0.307**	**0.058**	**0.445**	**-0.009**	**-0.013**	-0.029	-0.068	-0.131	**-0.083**	**0.028**	0.144	**-0.573**	**-0.410**	*-0.722*	**-0.558**	**0.041**	1.000	**-0.341**	**0.235**
Temp.	**0.216**	**-0.576**	**0.035**	**-0.327**	**0.074**	-0.373	-0.400	-0.244	**-0.325**	**0.234**	-0.162	**0.311**	**-0.328**	0.098	**0.047**	**0.045**	**-0.341**	1.000	**-0.358**
DO	**0.335**	**-0.440**	**-0.018**	**0.278**	**0.097**	0.341	0.327	0.344	**0.398**	**-0.075**	0.219	**-0.388**	**0.252**	-0.200	**0.058**	**-0.362**	**0.235**	**-0.358**	1.000

Appendix 3.3: Correlation matrix for predictor variables used in stepwise multiple regression analysis of calanoid δ^{15}N values. All lakes are located on Vancouver Island, had data collected in the first quarter of the year (January, February, March) and land use data within the 200 m buffer around the lakeshore and the lake's tributaries (n = 17). Variables in bold are those selected for the stepwise multiple linear regresion analyses. Italicized values indicate *r* values greater than 0.7, suggesting collinearity.

	Long.	**Lat.**	**log Elev.**	**log Mean Depth**	**log Max. Depth**	log Perim.	log SA	log Vol.	**log WSA**	**log WSA:SA**	log Res. Time	**log Lot Density**	**log ALR**	log TN	log TP	**log N:P**	**log Secchi**	**Temp.**	**DO**
Longitude	1.000	**-0.484**	**0.003**	**-0.078**	**0.028**	-0.120	-0.155	0.005	**0.142**	**0.309**	-0.126	**-0.040**	**0.195**	0.146	0.271	**-0.274**	**-0.307**	**0.216**	**0.335**
Latitude	**-0.484**	1.000	**-0.133**	**0.140**	**-0.159**	0.275	0.286	0.083	**0.045**	**-0.304**	0.172	**0.121**	**0.203**	0.181	0.053	**0.123**	**0.058**	**-0.576**	**-0.440**
log Elevation	**0.003**	**-0.133**	1.000	**-0.385**	**0.347**	-0.473	-0.610	-0.358	**-0.509**	**0.317**	-0.234	**-0.423**	**-0.626**	-0.621	-0.611	**0.300**	**0.445**	**0.035**	**-0.018**
log Mean Depth	**-0.078**	**0.140**	**-0.385**	1.000	0.419	0.671	*0.721*	*0.708*	**0.330**	**-0.633**	0.626	**0.401**	**0.192**	0.520	0.498	**-0.210**	**-0.009**	**-0.327**	**0.278**
log Max Depth	**0.028**	**-0.159**	**0.347**	**0.419**	**1.000**	0.267	0.130	0.545	**-0.018**	**-0.197**	0.269	**0.408**	**-0.200**	0.280	0.356	**-0.188**	**-0.013**	**0.074**	**0.097**
log Perimiter	-0.120	0.275	-0.473	0.671	0.267	1.000	*0.962*	*0.916*	0.619	-0.645	*0.736*	**0.416**	**0.547**	0.472	*0.728*	**-0.652**	**-0.029**	**-0.373**	**0.341**
log Surface Area	-0.155	0.286	-0.610	*0.721*	0.130	*0.962*	1.000	*0.868*	0.576	*-0.732*	*0.747*	0.400	0.549	0.466	*0.709*	-0.640	-0.068	-0.400	0.327
log Volume	0.005	0.083	-0.358	*0.708*	0.545	*0.916*	*0.868*	1.000	0.491	-0.647	*0.732*	0.500	0.456	0.537	*0.815*	-0.689	-0.131	-0.244	0.344
log WSA	**0.142**	**0.045**	**-0.509**	**0.330**	**-0.018**	0.619	0.576	0.491	1.000	**0.132**	0.048	**0.113**	**0.649**	0.430	0.506	**-0.321**	**-0.083**	**-0.325**	**0.398**
log WSA:SA	**0.309**	**-0.304**	**0.317**	**-0.633**	**-0.197**	-0.645	*-0.732*	-0.647	**0.132**	1.000	*-0.839*	**-0.387**	**-0.122**	-0.230	-0.437	**0.477**	**0.028**	**0.234**	**-0.075**
log Residence Time	-0.126	0.172	-0.234	0.626	0.269	*0.736*	*0.747*	*0.732*	0.048	*-0.839*	1.000	**0.378**	**0.093**	0.188	0.482	**-0.615**	**0.144**	**-0.162**	**0.219**
log Lot Density	**-0.040**	**0.121**	**-0.423**	**0.401**	**0.408**	0.416	0.400	0.500	**0.113**	**-0.387**	0.378	**1.000**	**0.240**	*0.733*	*0.728*	**-0.322**	**-0.559**	**0.406**	**-0.378**
log ALR	**0.195**	**0.203**	**-0.626**	**0.192**	**-0.200**	0.547	0.549	0.456	**0.649**	**-0.122**	0.093	**0.240**	1.000	0.626	0.655	**-0.326**	**-0.565**	**-0.189**	**0.057**
log TN avg	0.146	0.181	-0.621	0.520	0.280	0.472	0.466	0.537	0.430	-0.230	0.188	*0.733*	0.626	1.000	*0.808*	-0.109	*-0.722*	0.098	-0.200
log TP	0.271	0.053	-0.611	0.498	0.356	*0.728*	*0.709*	*0.815*	0.506	-0.437	0.482	*0.728*	0.655	*0.808*	1.000	-0.670	-0.558	0.047	0.058
log N:P	**-0.274**	**0.123**	**0.300**	**-0.210**	**-0.188**	-0.652	-0.640	-0.689	**-0.321**	**0.477**	-0.615	**-0.322**	**-0.326**	-0.109	-0.670	1.000	**0.041**	**0.045**	**-0.362**
log Secchi	**-0.307**	**0.058**	**0.445**	**-0.009**	**-0.013**	-0.029	-0.068	-0.131	**-0.083**	**0.028**	0.144	**-0.559**	**-0.565**	-0.722	-0.558	**0.041**	1.000	**-0.341**	**0.235**
Temp.	**0.216**	**-0.576**	**0.035**	**-0.327**	**0.074**	-0.373	-0.400	-0.244	**-0.325**	**0.234**	-0.162	**0.406**	**-0.189**	0.098	0.047	**0.045**	**-0.341**	1.000	**-0.358**
DO	**0.335**	**-0.440**	**-0.018**	**0.278**	**0.097**	0.341	0.327	0.344	**0.398**	**-0.075**	0.219	**-0.378**	**0.057**	-0.200	0.058	**-0.362**	**0.235**	**-0.358**	1.000