THE IMPLICATIONS OF CYANOBACTERIA BLOOMS ON THE BASE OF THE FOOD WEB IN LAKE WINNIPEG

by

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A Thesis submitted to the Faculty of Graduate Studies of the

University of Manitoba

in partial fulfillment of the requirements of the degree of

Master of Science

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ABSTRACT

Over the past two decades, Lake Winnipeg has been experiencing increasingly rapid eutrophication, and large cyanobacterial blooms now form in the North Basin in most years in late summer or fall. Cyanobacteria are considered a relatively poor food source compared with other phytoplankton, but the impacts of these blooms upon the primary consumers in the lake have not previously been researched. A microscopic analysis of whole water samples found cyanobacteria to be scarcely present in summer 2012, with nitrogen-fixing and non-fixing cyanobacteria comprising 11.2% and 8.4% of the basin-wide biovolume, respectively, and all but absent in fall. Gut content analysis of chironomids found that cyanobacteria made up an almost negligible part of their diet. Stable isotope analysis revealed that nitrogen-fixing cyanobacteria reduced phytoplankton $\delta^{15}N$ values, and that this same reduction could be traced through the zooplankton, but not down to the sediments or chironomids.

ACKNOWLEDGEMENTS

Many hands still could not make this work light, but I would not have been able to carry it without all of them, and I am extremely grateful. Many thanks:

- to Brenda, without whom, this work would neither have begun nor finished, and without whose guidance, I would probably still be thinking instead of doing;

- to Gordon and Mark, for shaking their heads at all the wrongs things at the right times, and for answering my naïve questions as well as my cynical ones;

- to Karen Scott, Marianne Geisler, Desiree Stratton, and the crew of the M.V. Namao, for facilitating my field work, and collecting for me when real life got in the way;

- to Katie and Daigo, for making the first half of this degree far easier to bear than the second;

- to Andrew, for putting up with my neuroses and patiently answering all the questions I probably should have been asking somebody else;

- to my family, near and far, for helping keep me warm and full;

- and to Rachel, for her support, patience, understanding, and, well, everything.

This work would not have been possible without generous funding support from the Natural Sciences and Engineering Research Council in the form of a Canada Graduate Scholarship, the Faculty of Sciences at the University of Manitoba, and the Lake Winnipeg Research Consortium in the form of their Honours and Graduate Student Scholarship.

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CHAPTER 1: INTRODUCTION

Cyanobacteria have been present on earth for at least 2.8 billion years (Schopf and Walter 1982). Despite their ancient origins, they are well adapted to modern conditions, as shown by their increased prominence in lakes that have been enriched either naturally or anthropogenically (Gibson and Smith 1982). Some species of cyanobacteria are able to fix atmospheric nitrogen rather than relying on dissolved sources; such species are particularly well suited to take advantage of increased concentrations of phosphorus in lakes (Schindler et al. 2008). There is also evidence that cyanobacteria have become more dominant in shallow lakes in regions that have warmed in recent years (Kosten et al. 2012). At high density, certain species of cyanobacteria can form surface scums or floating mats, usually in late summer or autumn (Gibson and Smith 1982). These mats supplant other phytoplankton by limiting the available light and their structure may render them an unsuitable food source for herbivorous zooplankton (Tillmanns et al. 2008). When temperatures drop, the cyanobacterial mats sink to the bottom, where they may become a suitable food source for benthic invertebrates (Frouz et al. 2004, Nascimento et al. 2008). The mucilage surrounding the colonies may enhance their overwintering survival on the lake bottom (Gibson and Smith 1982) and may deter or prevent grazing. However, if the cyanobacteria are available as a food source for benthic grazers, then an increase in cyanobacterial production at the expense of other phytoplankton species could create an alternate energy pathway, supplementing the benthic food web to the detriment of the pelagic one. This alternate pathway would also be seasonally shifted from the traditional one, as the zoobenthos would be feeding on cyanobacteria that had settled in the fall and winter, rather than the zooplankton feeding on other phytoplankton throughout the open-water season.

Over the past 20 years, Lake Winnipeg has been impacted by multiple human and environmentally induced perturbations, including increasing commercial and recreational fisheries activities, invasive species, climate change and cultural eutrophication. Lake Winnipeg is the sixth largest lake in Canada by surface area, and is divided into two distinct but connected basins: the relatively small South Basin (10% of the lake volume) and the larger North Basin (81% of the lake volume), connected by a small passage termed The Narrows (9% of the lake volume) (Lévesque and Page 2011). The lake is relatively shallow for its size; the mean depths of the North and South basins are 13.3 m and 9.7 m, respectively (Patalas and Salki 1992). Though Lake Winnipeg has historically been mildly eutrophic, major increases in phosphorus inputs into the lake have led to rapid eutrophication since the mid-1990s (Schindler et al. 2012). As a result, large cyanobacterial mats now form most years in the summer and autumn, primarily in the North Basin, but occasionally in the South Basin as well. The extent of the blooms varies annually, with some years, e.g., 2006, having massive blooms in summer and fall, while other years, e.g., 2007, either have small blooms exclusively in fall or are dominated by phytoplankton other than cyanobacteria (Figure 1, Page 2011).

As well as annual variation, seasonal variation is also seen in the dominant phytoplankton taxa in the lake. When mean water temperature is cooler, i.e., at times other than summer and autumn, diatoms usually dominate, sometimes forming large blooms in spring or under the ice in winter (Kling et al. 2011). Cyanobacteria dominance is generally correlated with surface water temperature (Page 2011), though it is not clear why cyanobacteria are able to outcompete diatoms at higher temperatures. Since silicon dissolution rates increase with water temperature (Kling 1998), it is possible that cyanobacterial dominance is less a function of their improved performance than a function of decreased diatom performance. It is also possible that allelopathic cyanotoxins play a significant role in cyanobacterial dominance (Christofferson 1996).

The dominant phytoplankton taxa in any given season should represent the available food source for both the zooplankton and the zoobenthos, though there is likely a lag effect for the benthos as the phytoplankton must sink to be available. It is possible that bacteria in the water column degrade sinking phytoplankton to the point that only resistant structures (e.g. silicious diatom frustules, cyanobacteria akinetes) sink to the lake bottom, though it is unlikely that this process is completely effective as there would be no food for the zoobenthos.

The analysis of the gut contents of aquatic insects has long been used to attempt to identify the components of their diet (Mecom and Cummins 1964), and can be used to determine whether they are able to feed upon cyanobacteria or other phytoplankton taxa, and to compare the composition of their diet with that of the phytoplankton community. Chironomids, non-biting midges of the insect order Chironomidae, make up a considerable proportion of the benthic invertebrate community in Lake Winnipeg (Hann 2011). The larvae inhabit the sediments, overwinter as pupae, then hatch into aerial adults in the spring. Different members of the order have different feeding modes; some chironomids are grazers, non-selectively feeding on detritus and surficial sediments (Ingvason et al. 2004), some are gatherers or scrapers, eating the periphyton growing on rocks (Mihuc and Toetz 1994), and others are predatory (Pinder 1986), though most taxa are thought to be generalists, using more than a single feeding mode (Pinder 1986). Chironomids were selected for the gut content analysis portion of this study because of their prevalence in Lake Winnipeg, the existence and availability of previously collected specimens, and their being large enough for dissection to be feasible. Traditional gut content analysis looks at the organic components of the diet and allows for the identification of short-term dietary composition, typically of material that has been ingested in the last 24 hours (Mihuc and Toetz 1994). Freshly captured or killed (Sierszen et al. 2003), or preserved (Mihuc and Toetz 1994) specimens are dissected, and

their gut contents are examined microscopically and identified. Depending on the purpose of the study, the gut contents may simply be scanned for the presence of a certain component, or the relative or total contribution of each constituent may be quantified.

Stable isotope analysis has become the tool of choice to study seasonal and longer-term consumption patterns. This method has been used to describe food web structure and energy flow in both terrestrial and aquatic ecosystems (Gu et al. 1996; Ponsard and Arditi 2000). The stable isotope compositions of various body tissues are determined and quantitatively compared with isotopic signatures from all potential food items. Organisms with varying diet sources in each season will acquire different signatures in their tissues, provided that each diet type has a distinct isotopic signature allowing the relative contribution of different foods to be quantified in the diet within a particular period. Thus, the contribution to the overall diet of benthic organisms from phytoplankton in the spring and early summer can be assessed relative to the contribution from cyanobacteria during late summer and fall when blooms are prevalent. Combining stable isotope analysis with gut content analysis allows the relative energetic importance of all dietary relationships to be elucidated (Vander Zanden and Rasmussen 1996).

The stable isotope ratios of nitrogen and carbon are used most frequently in food web studies (Deines et al. 2009) and to represent the trophic structure of aquatic systems (Peterson and Fry 1987; Cabana and Rasmussen 1996; Fry 2006). Stable nitrogen isotope ratios ($\delta^{15}N$) are useful for identifying the trophic position of consumers (Vander Zanden et al. 1997; Post et al. 2000). The $\delta^{15}N$ value of a consumer depends on both the lake $\delta^{15}N$ baseline value ($\delta^{15}N$ of the primary producers), which is influenced by the available dissolved nitrogen sources' $\delta^{15}N$ value, and the trophic level of the

consumer, with an enrichment in $\delta^{15}N$ equal to approximately 3.4‰ for each trophic level in aquatic food webs (Post 2002). Therefore, nitrogen isotope signatures of primary and secondary consumers scale to isotopic signatures of primary producers within a particular ecosystem. In contrast, stable carbon isotopes are not used to determine trophic level as $\delta^{13}C$ changes little from primary producer to consumer with mean trophic fractionation of ~0.39‰ in aquatic food webs (Post 2002). Instead, the $\delta^{13}C$ value of a consumer depends on baseline values of the primary producers, which depend on the origin of dissolved inorganic carbon. Benthic, littoral, and pelagic primary producers discriminate differently against ^{13}C so that, with the same dissolved atmospheric carbon source, the benthic producers show higher $\delta^{13}C$ values than the pelagic producers, e.g. –27‰, and –31‰ respectively (Sierszen et al. 2003). Therefore, $\delta^{13}C$ values reflect the location of the diet in the water column. Overall, benthic invertebrate nitrogen and carbon isotopic signatures will be lake-specific and changes in isotopic ratios will reflect changes in diet, e.g., trophic shift from a littoral or benthic to a pelagic food source or among trophic levels (Vadeboncœur et al. 2001; Vander Zanden and Vadeboncœur 2002).

Stable isotope work on Lake Winnipeg is in the early stages of study. Previous research has investigated the trophic level of predatory fish (Gewurtz et al. 2006) and examined the feeding location of migratory birds (Ofukany et al. 2012). Hobson et al. (2010) have also published a chemical baseline of $\delta^{13}C$ for dissolved inorganic carbon and $\delta^{15}N$ for nitrate along with their analysis of stable isotope ratios of fish from the lake. Though their data were robust at a basin-wide scale across two years of sampling, they found that the stable isotope ratios varied both spatially and at a seasonal scale (Hobson et al. 2010). There were also differences in the stable isotope values of primary producers between the two basins, which the authors hypothesize were caused by differences in algal growth rate due to differing turbidity and average temperatures of the basins

(Hobson et al. 2010). Such environmental conditions are likely to vary annually, highlighting the importance of resampling primary producers to acquire current, accurate baselines for comparing their stable isotope ratios to those of primary consumers.

It is important to understand how the increased abundance of cyanobacteria affects the base of the food web in Lake Winnipeg. If the cyanobacteria are unavailable as a food source to zooplankton, but are available to the zoobenthos upon sinking, then there may be a shift from a system based predominantly upon planktonic secondary producers, to a system based upon benthic ones. Because cyanobacteria dominate in Lake Winnipeg at only certain times of the year, the extent of this shift may vary seasonally. Overall, this project attempts to determine whether cyanobacteria are available as a food source for both zooplankton and chironomids. Chapter 2 describes the phytoplankton community present in Lake Winnipeg during summer and fall of 2012. Chapter 3 presents the results of stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ values from phytoplankton, zooplankton, chironomids, and sediments collected from Lake Winnipeg during the open-water season of 2012, as well as gut content analysis performed on chironomids from 2006 and 2007.

FIGURES

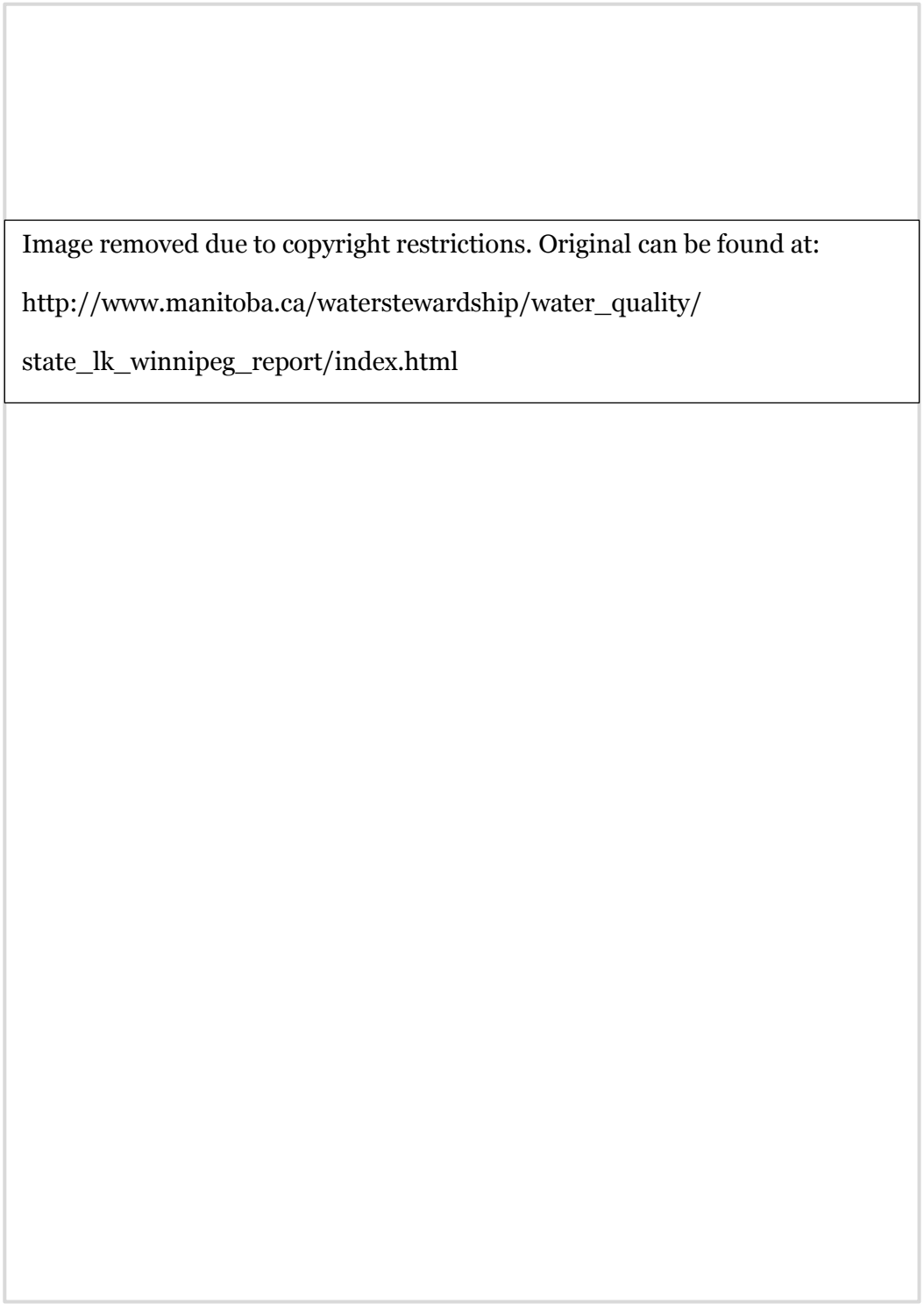

Figure 1.1. Seasonal and spatial variation in chlorophyll-a concentrations in Lake Winnipeg in 2006 and 2007. Black dots identify monitoring stations. Adapted from Figure 8.3 in Page (2011).

CHAPTER 2: SEASONAL VARIATION IN THE PROPORTION OF CYANOBACTERIA IN LAKE WINNIPEG IN 2012

INTRODUCTION

Cyanobacteria have constituted a larger proportion of lentic phytoplankton communities in shallow water ecosystems across the globe in recent years, largely because of global increases in surface water temperatures and phosphorus concentrations (Kosten et al. 2012). However, cyanobacterial dominance is not inevitable; rather, it depends upon a set of ideal conditions including high nutrient loading, a low N:P ratio, and appropriate hydrological and light conditions (Elser 1999). Though cyanobacteria are commonly regarded as a poor to inedible food source (Tillmanns et al. 2008), there has been evidence that some *Daphnia* and small-bodied cladocerans are able to graze on filaments (Fey et al. 2010). Thus, inhibition of *Daphnia* and other potential grazers is important for cyanobacterial dominance to occur (Elser 1999).

Since the mid-1990s, increases in phosphorus loading into Lake Winnipeg have led to rapid eutrophication (Schindler et al. 2012). The Red River is the main contributor of phosphorus to the lake, and its inputs have nearly doubled during this time, primarily due to increased runoff and flood frequency (McCullough et al. 2012). This has resulted in large blooms of filamentous, nitrogen-fixing cyanobacteria (predominantly *Anabaena* and *Aphanizomenon*) often appearing in the North Basin (Kling et al. 2011). However, the extent of these blooms varies seasonally, annually, and spatially (Page 2011). Historical reports suggest that Lake Winnipeg has been mesotrophic to eutrophic (Kling 1996), with occasional blooms of cyanobacteria since observations have been recorded (Kling et al. 2011). Microfossil remains from cores taken from the lake confirm that

though it has traditionally been oligotrophic to mesotrophic, it has generally been dominated by cyanobacteria in hot, dry years, and diatoms in cool, wet ones, but that since the last half of the previous century, primary productivity has increased and species composition has shifted towards cyanobacterial dominance (Kling 1998, Kling et al. 2011).

Despite the general trend towards eutrophication, it is important that continued monitoring of the phytoplankton of Lake Winnipeg occurs, owing to the natural variation in the location, timing, and extent of cyanobacterial blooms. Such resampling is particularly important for food web studies concerned with the potential impacts of cyanobacteria, and should be conducted with at least the same frequency and spatial resolution as other food web components. This paper presents the proportion of the phytoplankton community comprising of cyanobacteria as determined by a microscopic examination of samples collected from the North Basin of Lake Winnipeg in the summer and fall of 2012.

METHODS

Phytoplankton samples were collected during the summer (July 20-28) and fall (September 16-26) 2012 research cruises at each of 25 sites in the north basin of Lake Winnipeg. Samples consisted of 125 mL taken from a larger water sample collected 1 m below the surface by Niskin bottle from the rosette sampler on board the M.V. Namao. Samples were preserved immediately with 5 mL of 10% formalin.

In the laboratory, samples were serially concentrated three times, with volumes reduced to approximately 25 mL, 7 mL, and 1 mL, respectively. Samples were homogenized by gentle shaking then allowed to settle for at least 100 hours before the top liquid was pipetted off. To match the spatial resolution of the stable isotope analysis

(Chapter 3), the samples were combined into eight clusters (Figure 2.1). A slide was made from each cluster and 1/3 of each was examined under 400x magnification on a compound microscope. Such a magnification allows for identification of net phytoplankton, but may miss picoplankton and smaller cells. Cells appearing in the subsampled area were identified to genus and counted. In order to calculate biovolumes, the diameter or length and width of ten organisms of each genus were measured (Grace Analytical Laboratory 1994) using a digitizing pad and a *camera lucida* attached to the compound microscope. Biovolumes were calculated following the shapes and formulae given for each genus by Hillebrand et al. (1999) (Table 2.1) and the proportion of the total biovolume in each cluster in each season was analysed graphically. Genera were then categorized as nitrogen-fixing cyanobacteria (determined by the presence of heterocysts), non-fixing cyanobacteria, or other phytoplankton taxa, and the proportion of the total biovolume each of these taxa comprised in each cluster in each season was calculated and analysed graphically.

RESULTS

Measured dimensions and calculated biovolumes of the phytoplankton genera observed are presented in Table 2.2. The phytoplankton community composition in the North Basin in Lake Winnipeg varied spatially in both summer and fall (Figure 2.2). In summer, cyanobacteria were found north of Long Point, though they did not dominate the phytoplankton in any of the clusters (Figure 2.2a, 2.3). The clusters were generally dominated by diatoms in the summer, though *Cryptomonus* dominated the southernmost clusters. The diatoms were primarily dominated by *Stephanodiscus*, though *Fragilaria* contributed largely to the biomass in the northernmost and north-westernmost parts of the lake in summer. In fall, the phytoplankton community was completely dominated by diatoms (Figure 2.2b). *Stephanodiscus* dominated north of

Long Point, though *Fragilaria* again made large contributions in clusters 1 and 2. *Aulacoseira* made up a large part of the biomass in clusters 3 and 5, and dominated the phytoplankton biomass south of Long Point.

When the genera were examined in their aggregated taxa, taxa other than cyanobacteria dominated in both seasons, though both nitrogen-fixing and non-fixing species of cyanobacteria were present north of Long Point in the summer. The highest observed proportion of cyanobacteria was 0.348 in cluster 2, near Grand Rapids, in the summer. South of Long Point in the summer and in the entire North Basin in the fall, cyanobacteria made a negligible contribution to the total phytoplankton biovolume. The overall proportions of N-fixing and non-fixing cyanobacteria were 0.112 and 0.084 in the summer, 6.37×10^{-4} and 2.60×10^{-3} in the fall, and 0.013 and 0.012 overall.

DISCUSSION

In recent years, Lake Winnipeg has repeatedly been presented in the media as sick, toxic, and home to massive blooms of cyanobacteria. While there is scientific literature to support the idea that cyanobacterial blooms have increased in frequency and intensity since the 1990s (McCullough et al. 2012, Schindler et al. 2012), the findings of this current study suggest that care must be taken against making large-scale generalizations, and that there is spatial heterogeneity even within the North Basin. In particular, there appears to be a distinction between the areas north and south of Long Point. Hydrodynamic modelling suggests the presence of cyclonic gyres north and south of Long Point, while the predominant currents along the eastern shore flow north to the Nelson River (Yerubandi and Zhao 2011). These gyres could explain why cyanobacteria were only seen north of Long Point, as there may not be any mixing from the north to the south. Both the seemingly inverse relationship between cyanobacteria and cryptophytes

in the summer, and the diatom spatial gradient in the fall suggest underlying physical or chemical gradients. The spatial shift between *Fragilaria* sub-dominance in the northwestern regions of the lake and *Aulacoseira* sub-dominance and dominance to the south of the north basin in the fall in particular suggests that the inputs from the Saskatchewan River vary from those flowing up from the South Basin enough to support different phytoplankton taxa. Indeed, the Saskatchewan River has been modified by the creation of a number of reservoirs in recent decades, such that the water flowing into Lake Winnipeg is less turbid and less nutrient-rich than it previously was (Schindler et al. 2012). It has been suggested that these changes have contributed to the increasing cyanobacterial blooms, as they are favourable for the taxa that form the blooms in the North Basin (Schindler et al. 2012), so it is likely that they have had effects on other phytoplankton taxa as well. The South Basin of Lake Winnipeg is turbid and nutrient-rich (McCullough et al. 2012), so it is intuitive that there would be different environmental conditions in regions of the North Basin fed by the Narrows and by the Saskatchewan River, and that there would be a gradient between the two. This current study is limited by the aggregation of research stations into clusters; further research at a finer spatial scale, could reveal interesting differences in phytoplankton composition, particularly north and south of Long Point.

Most studies on the phytoplankton community in Lake Winnipeg thus far have presented the mean phytoplankton biomass averaged spatially over either the entire lake or the two basins, and temporally, ranging from seasonal to multi-annual averages (e.g. Kling et al. 2011, Page 2011). Seasonal averaging makes sense, as it reflects the frequency of samples. However, in a lake that produces spatially and temporally limited cyanobacterial blooms, the other averages can be misleading. This study found that in 2012, cyanobacteria were only present in substantial quantities north of Long Point in the summer. However, these quantities were not sufficient to increase the basin-wide

proportion of cyanobacteria to previously seen levels, even in summer when they were most prevalent. It is worth noting that the current study focused only on summer and fall samples, while previous studies include spring data in their averages. Because cyanobacteria are generally not observed in spring (Kling et al. 2011), annual averages including spring data would show a lower contribution from cyanobacteria than the current study. Perhaps the high overall proportion observed in past years was driven by extremely high densities at individual sites. Indeed, when such a spatial breakdown is provided (e.g. Figures 8.5 and 8.6 in Page 2011), it is obvious that overall proportions are being skewed by the presence of blooms at single sites, which appear to have densities an order of magnitude higher than outside the blooms. It has been suggested that cyanotoxins can play an allelopathic role, supressing the growth of non-cyanobacteria (Christofferson 1996), thus reducing competition and allowing high densities of cyanobacteria within blooms. Alternatively, high bloom densities could be caused by physical patterns, with buoyant cyanobacterial filaments being pushed together by wind and currents.

Variation in sampling methods may also explain some of the discrepancy seen between the results of this study and those of previous work on Lake Winnipeg. Previous samples of phytoplankton in Lake Winnipeg have been conducted as vertical tows of a phytoplankton net or as tube samples of the entire euphotic zone (Page 2011). One component of cyanobacterial dominance is their ability to regulate their buoyancy such that many taxa are able to float higher than other phytoplankton taxa, allowing them to block their competitors from light (Bucka and Wilk-Woźniak 2005). Consequently, one would expect cyanobacterial blooms to be concentrated near the surface. Samples collected by Niskin bottle from 1 m below the surface could be sampling a different phytoplankton community than samples integrating the entire euphotic zone. Thus, the different sampling methods are useful in addressing different research topics: net

sampling or euphotic zone sampling may provide a more accurate picture of the entire phytoplankton community, i.e., they can answer which organisms are present to utilize the available nutrients; whereas, Niskin bottle sampling can provide a picture of the portion of the phytoplankton community that is available for grazers, as they are normally not located directly at the surface (Kamada 2012) and would also be unavailable to feed upon the top surface of a thick mat of cyanobacteria, but would only have access to the bottom-most layer.

Even if differences in sampling methods can explain some of the incongruities in cyanobacterial contributions to the phytoplankton community observed in this study and past ones, ship-board observations suggest that cyanobacterial blooms were not present when the Namao was in the North Basin in summer (M. E. Geisler, Lake Winnipeg Research Consortium, Winnipeg, Manitoba, personal communication) or fall. Satellite photographs taken with Moderate Imaging Spectoradiometers (MODIS) from 2012 provide corroborating evidence that this was a poor year for cyanobacterial blooms in the North Basin relative to previous years (Images of Lake Winnipeg available from http://lakewinnipegresearch.org/

blog/). With a lack of published hydrological, environmental, and chemical data from the lake and its watershed from the 2012 field season, it is difficult to determine which step on Elser's (1999) framework of cyanobacterial bloom development was unfulfilled. It seems unlikely that either the hydrological conditions in the watershed or the chemical inputs to the lake differed strongly enough from those of the last 20 years that have contributed to the lake's eutrophication (McCullough et al. 2012, Schindler et al. 2012); thus it seems likely that both the first (high nutrient inputs) and second (low N:P ratio) steps in the framework remained in place, and that cyanobacterial dominance was prevented by more immediate environmental conditions.

The 2012 field season, particularly the fall sampling season, was exceptionally windy (Personal observation). High winds could mix the water column to the extent that the buoyancy regulation that aids in cyanobacterial suppression of other taxa was impossible. The satellite photos also show higher than normal turbidity in the North Basin in the summer and fall, most likely caused by the high winds. The filamentous forms of cyanobacteria that have previously dominated the North Basin require relatively clear water (O'Neil et al. 2012). It has been suggested that the onset of large blooms in the North Basin coincided with the development of reservoirs along the Saskatchewan River that lowered the inputs of particulate matter, resulting in the basin being less turbid (Kling et al. 2011). Higher turbidity could have reduced their ability to dominate this year. Higher temperature is often cited as a cause of increased cyanobacterial dominance (Kosten et al. 2012), with the implication that lower than average temperatures could suppress cyanobacteria. Though the lake-wide surface temperature was higher in the spring of 2012 than at any time in the past five years, summer and fall average surface temperatures were not abnormal (Figure 2.3), suggesting that temperature was not a factor in the low proportion of cyanobacteria observed this year.

TABLES

Table 2.1. Shapes and formulae used in calculating biovolumes of phytoplankton genera identified in samples. Shapes and formulae were those presented by Hillebrand et al. (1999). *Height was immeasurable, so biovolume was calculated for *Fragilaria* cells as if they were cylinders.

Genus	Shape	Formula
Aphanizomenon	Cylinder	$\frac{\pi}{4} \times d^2 \times h$
Anabaena	Sphere	$\frac{\pi}{6} \times d^3$
Microcystis	Sphere	$\frac{\pi}{6} \times d^3$
Planktothrix	Cylinder	$\frac{\pi}{4} \times d^2 \times h$
Aulacoseira	Cylinder	$\frac{\pi}{4} \times d^2 \times h$
Stephanodiscus	Cylinder	$\frac{\pi}{4} \times d^2 \times h$
Fragilaria	Elliptic prism*	$\frac{\pi}{4} \times a \times b \times c$
Scenedesmus	Prolate spheroid	$\frac{\pi}{6} \times d^2 \times h$
Cryptomonus	Prolate spheroid	$\frac{\pi}{6} \times d^2 \times h$

Table 2.2. Average dimensions of observed phytoplankton. Diameter and height were measured using a *camera lucida* and a digitizing pad and represent an average of ten measurements, while biovolume was calculated using the shapes and formulae from Table 2.1. *Height was immeasurable and was estimated to be the same as the diameter of *Aulacoseira*.

Genus	Diameter (μm)	Height (μm)	Biovolume (μm^3)
Aphanizomenon	2.442	4.076	19.090
Anabaena	5.91		108.083
Microcystis	4.449		46.109
Planktothrix	4.008	3.547	44.742
Aulacoseira	6.12	16.411	482.756
Stephanodiscus	49.82	6.12*	11930
Fragilaria	4.04	64.088	821.541
Scenedesmus	3.253	13.396	74.224
Cryptomonus	5.553	8.657	139.772

FIGURES

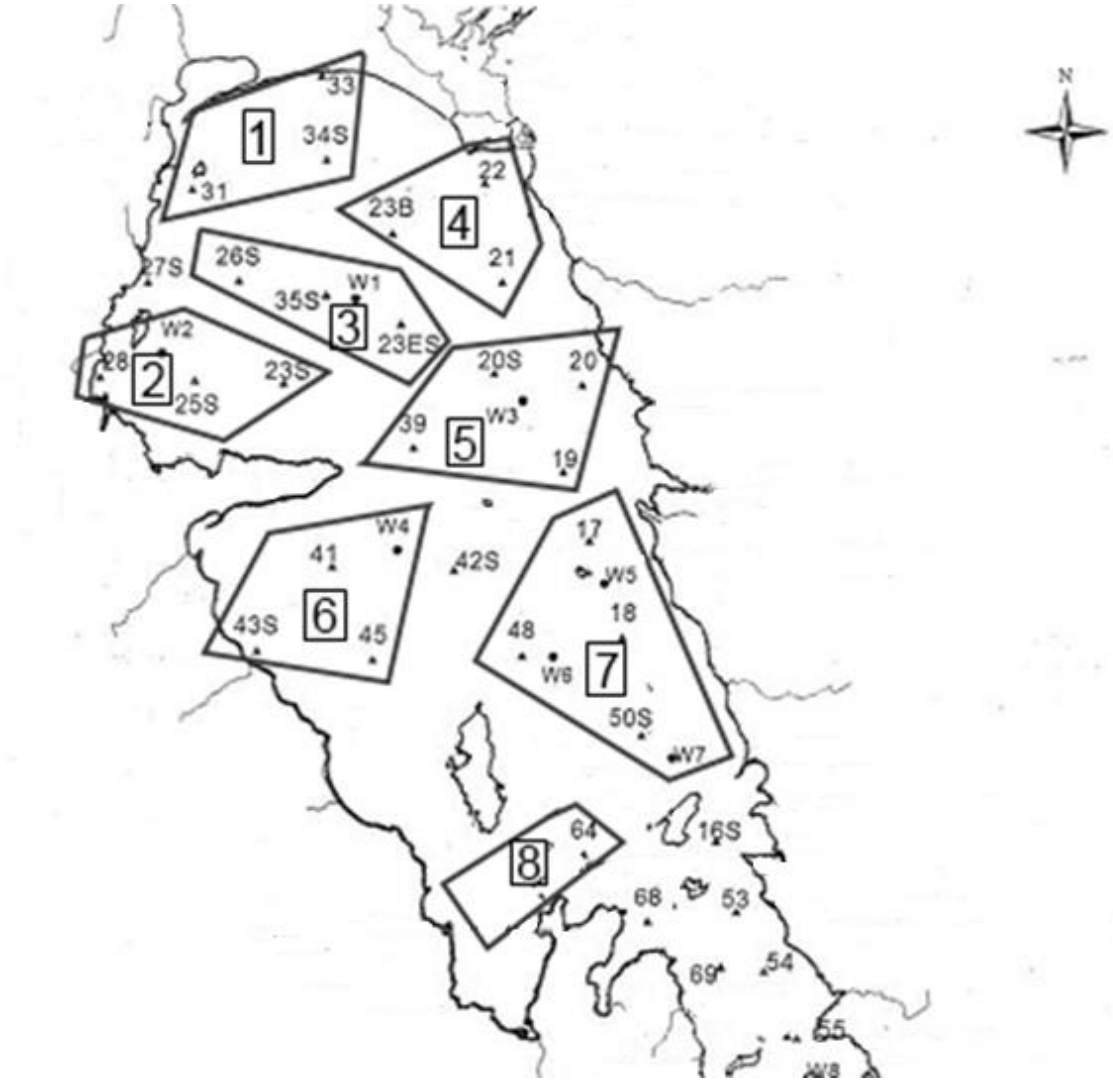

Figure 2.1. The North Basin of Lake Winnipeg, showing the locations of the eight clusters used and the research stations contributing to them. Boxed numbers indicate the cluster designation. Adapted from Lake Winnipeg Research Consortium Research Station Map, available at http://www.lakewinnipegresearch.org/pdf%20files/LK%20Wpg%20Station%20Map.pdf

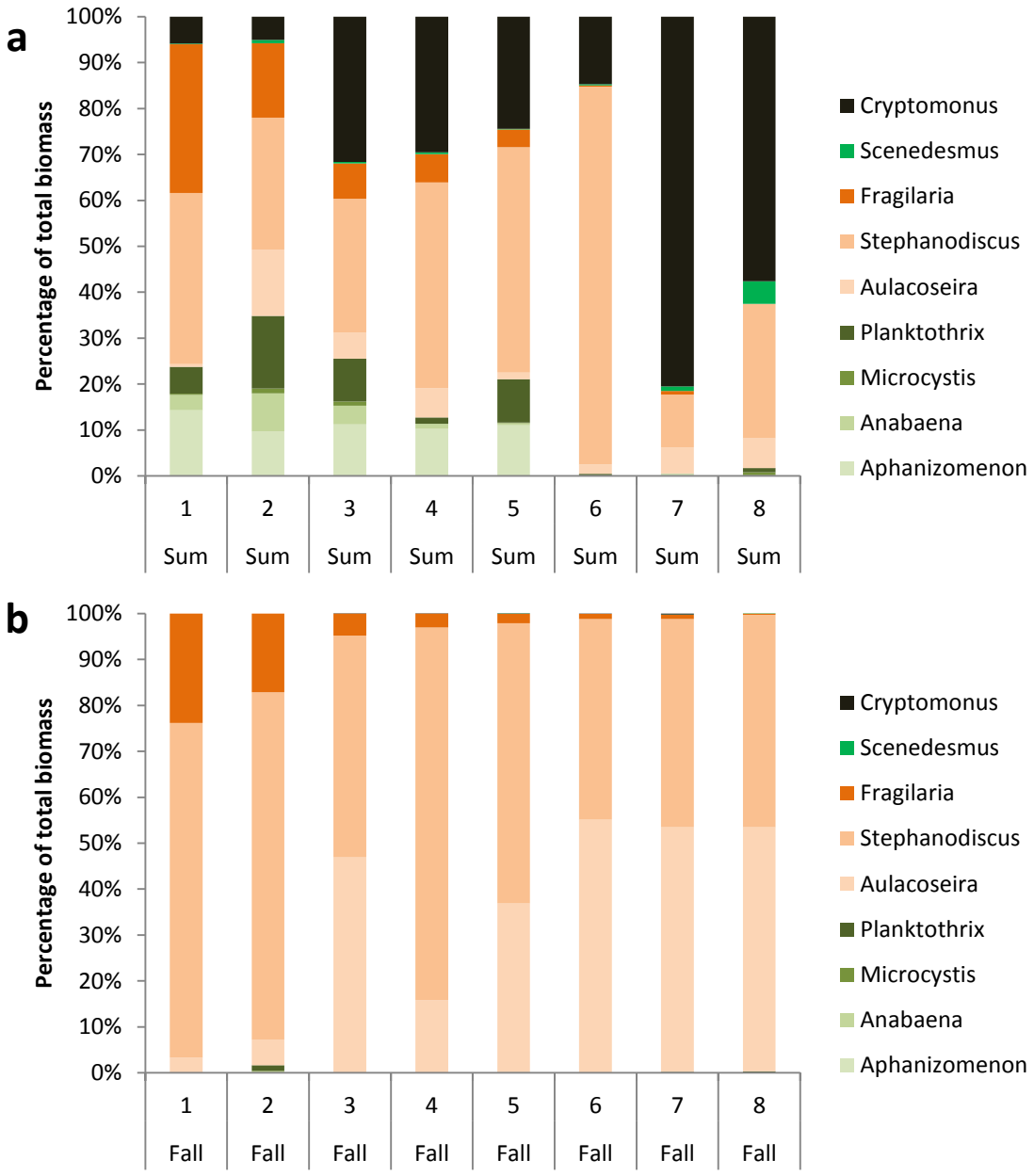

Figure 2.2. Proportion of the total phytoplankton biomass composed of each genus observed in each cluster in summer (a) and fall (b).

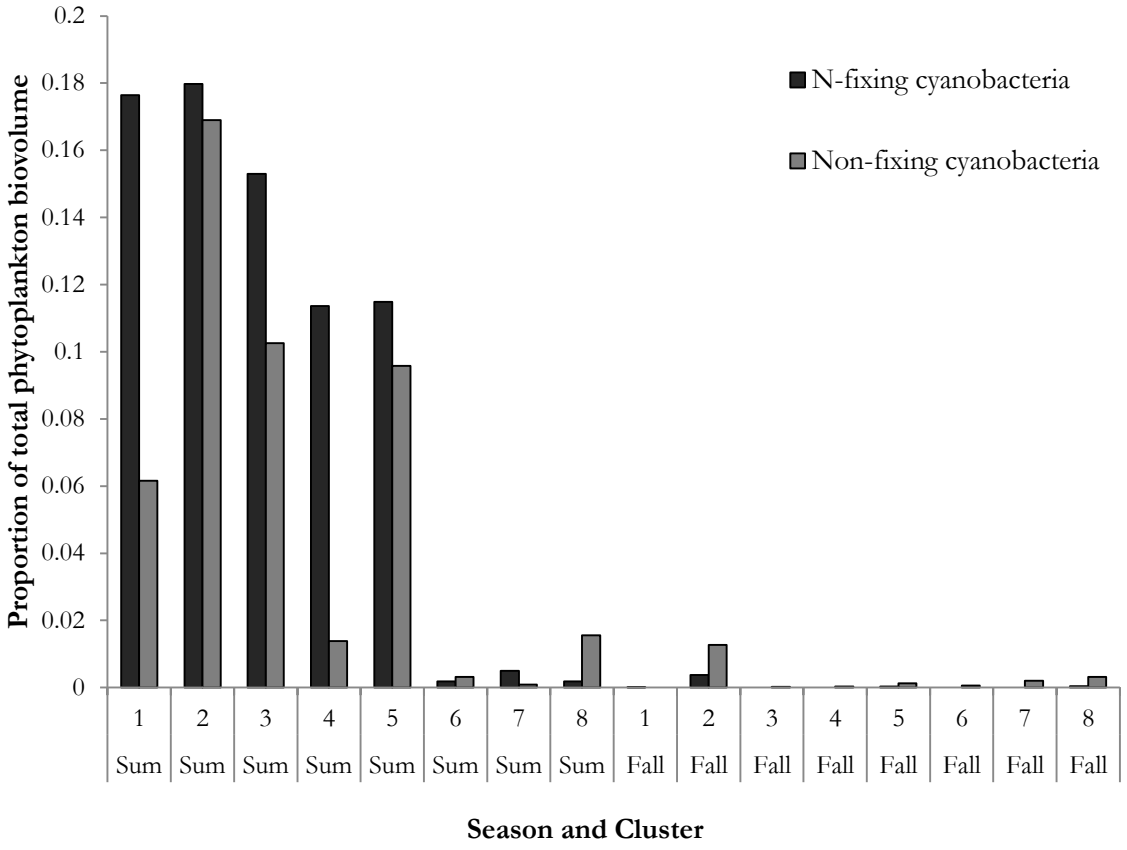

Figure 2.3. Cluster breakdown of the proportion of the total measured phytoplankton biovolume comprised by nitrogen-fixing and non-nitrogen-fixing cyanobacteria. (Sum = summer)

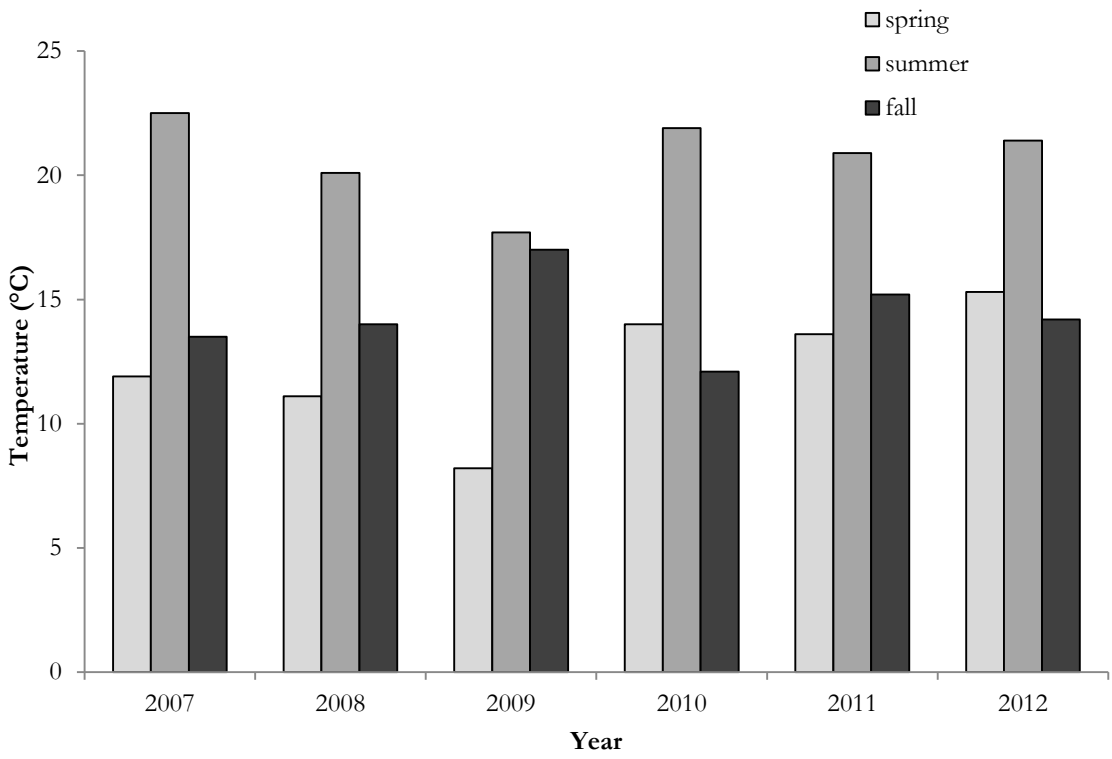

Figure 2.4. Lake-wide average surface temperatures observed in each seasonal research cruise onboard the M.V. Namao. Data aggregated from ship's log-books, publicly available at http://www.lakewinnipegresearch.org/documents%20internal.html. (spring: early June; summer: mid July-early August; fall: mid September-early October).

CHAPTER 3: BENTHIC AND PLANKTONIC INTERACTIONS AT THE BASE OF THE LAKE WINNIPEG FOOD WEB

INTRODUCTION

Though the phytoplankton (Kling et al. 2011), zooplankton (Patalas and Salki 1992; Olynyk 2013), and benthos (Hann 2011) of Lake Winnipeg have been extensively studied, research into the interactions between them has been lacking. Such research into food web reconstruction has been started at higher trophic levels (Sheppard et al. 2011, Olynyk 2013, Sheppard 2013). The acceleration of eutrophication of the lake over the last decade (McCullough et al. 2012; Schindler 2012) makes it vital to understand the base of the food web, in order to predict how the accompanying cyanobacterial blooms will impact both the zooplankton and the benthos. Cyanobacteria are generally regarded as an unsuitable food source for zooplankton (Tillmanns et al. 2008), but become available to the benthos upon settling (Nascimento et al. 2008). A shift in the phytoplankton community to species that are inedible to zooplankton could mean a shunt of energy from the planktonic-pelagic system to the benthos, with possible implications for the entire Lake Winnipeg food web. A seasonal diet reconstruction, using a combination of both traditional gut content analysis and stable isotope analysis, would allow for the identification of the impacts of cyanobacteria on the primary consumers.

Gut content analysis provides a direct assessment of the most recent components of an organism's diet (Mecom and Cummins 1964). Preserved specimens can be dissected, and the contents of their alimentary tracts can be identified (Mihuc and Toetz 1994). This allows for analysis of the short-term dietary composition. For this study, larval chironomids have been selected, due to their relatively high abundance in the

sediments of Lake Winnipeg, and because of the availability of organisms collected during a known bloom. Much of the recent literature on larval chironomid gut content analysis focuses on sediment toxicity or heavy metal analyses, wherein the chironomid tissues and organic gut contents are simply digested away using hydrogen peroxide or nitric acid (Brooke et al. 1996, Chapman 1985). However, with care, the entire alimentary tract of the organism can be removed and the contents can be examined and identified (Mihuc and Toetz 1994). Caution must be taken in identification, as digestion alters gut contents, and may alter different items at different rates (Ingvason et al. 2004). Another factor that may influence the results of gut content analysis is the problem of purging. Depending upon preservation techniques, chironomids may regurgitate their gut contents (Davies and McCauley 1970). However, Ingvason et al. (2004) found that anaesthetizing chironomids with ethanol prevented regurgitation. The chironomids used in this study were preserved using a low dose of formalin, which should have the same effect, though this assumption was not tested.

In contrast to gut content analysis, stable isotope analysis allows for long-term diet reconstruction. Organisms integrate the stable carbon isotope ratios ($\delta^{13}C$) of their food sources, with very little fractionation occurring between trophic levels. This fractionation is usually on the order of 0.39‰ in aquatic food webs (Post 2002). The $\delta^{13}C$ of an organism depends upon the baseline values of the dissolved inorganic carbon, and the location of the primary producers within the water column, as benthic, littoral, and pelagic primary producers fractionate carbon at different rates (Sierszen et al. 2003) Therefore, examining the $\delta^{13}C$ of an organism and its food sources can allow for quantification of the contributions of those food sources to the diet of the organism. Stable nitrogen isotope ratios ($\delta^{15}N$) can be used to identify the trophic level of an organism, as there is an enrichment of approximately 3.4‰ for each trophic level (Post 2002). However, identification of an organism's trophic position requires calibration

against a system's baseline $\delta^{15}N$ level. In an aquatic system, this is the $\delta^{15}N$ value of the primary producers. Changes in the $\delta^{15}N$ baseline can reflect changing sources of nutrients. This is particularly useful when considering nitrogen-fixing taxa, e.g. certain species of cyanobacteria. Because these species use atmospheric nitrogen, their $\delta^{15}N$ values are closer to the atmospheric value of 0‰ than the $\delta^{15}N$ values of species that cannot fix nitrogen (Mayer and Wassenaar 2012). This new baseline is reflected in species at higher trophic levels that feed upon the nitrogen-fixing cyanobacteria. Therefore, by comparing the isotopic signatures of primary producers and their consumers in the presence and absence of nitrogen-fixing cyanobacteria, it is possible to determine whether said cyanobacteria are a viable food source. The following study examines spatial and seasonal trends in the stable carbon and nitrogen isotopes of phytoplankton, zooplankton, chironomids, and sediments from the North Basin of Lake Winnipeg, and compares them with seasonal variation in the gut contents of previously collected chironomids from the same region.

METHODS

To answer the question of whether or not chironomids ingest cyanobacteria, individuals were examined that had been sampled from summer 2006-spring 2007 from 10 stations in the North Basin, along an East-West transect across the lake from a region that has previously produced large surface mats of cyanobacteria, to one where mats have been scarce or absent (Figure 3.1; Transect includes stations 20, 20S, 21, 23S, 23ES, 25S, 26S, 27S, 28, 35S). The three largest individuals were subsampled from each station at each season, and the length of each was recorded prior to dissection. Individuals were not identified to below the Family level prior to dissection.

For the preparation of the gut contents, a modification of the methods of Mihuc and Toetz (1994, modified from Mecom and Cummins 1964) was used. The entire chironomid alimentary tract was removed and bisected longitudinally. The stomach contents were diluted with deionized water, shaken for 1 minute, then filtered through a 0.45 μm Millipore filter (25 mm diameter). The filter was cleared for 6-24 h with immersion oil then mounted on a slide with glycerin and examined microscopically. The approximate composition of the gut contents was determined by estimating the percent covered by different components in 25 fields of view of each filter at 400x magnification. The components were identified as one of three diatom genera (*Aulacoseira*, *Stephanodiscus*, or *Fragilaria*), as cyanobacteria, or as detritus and other unidentifiable material.

For stable isotope analysis, chironomids, phytoplankton, zooplankton, and sediment samples were collected during the spring (June 12-17), summer (July 21-28), and autumn (September 16-26) research cruises of 2012 from all stations in the North Basin of Lake Winnipeg. Chironomids were live-picked from Ekman dredges. Zooplankton were collected from the water column with a vertical tow of a 73 μm Wisconsin net from 1 m off the bottom to the surface. Both zooplankton and chironomids were kept alive for 6-24 hours to allow the purging of gut contents (Brooke et al. 1996), and then frozen without water in Whirlpaks™. Phytoplankton samples were whole-water subsamples taken from 1 m below the surface by Niskin bottle, filtered onto quartz fibre filters and frozen on board. The surficial layer of detritus (1-2 cm) was collected from the top of the Ekman dredge, and was frozen on board in in a Whirlpak™. Both freezing and chemical preservation affect stable isotope ratios of carbon and nitrogen, so preservation is generally to be avoided (Syvaranta et al. 2011). However, it is particularly inadvisable to compare samples that have been preserved using different methods (Barrow et al. 2008). Since all samples collected needed to be preserved to

avoid degradation before they could be transported to the laboratory for processing, they were all frozen. This likely invalidates comparisons with previously collected samples, but was the most practical option for comparing the different types of samples in this study. All samples were freeze-dried until they reached a constant mass as determined experimentally in the laboratory. Samples from each station were combined into the clusters used in Chapter 2 (Figure 2.1) before being ground with a mortar and pestle, and then packed into tin capsules. The capsules were sent to the GLIER Stable Isotope laboratory at the University of Windsor for analysis. There was not enough chironomid material available for analysis from cluster 5 in the summer, or clusters 5 and 8 in the fall. Results of the stable isotope analysis are given in δ notation in parts per thousand (‰) relative to Vienna Pee Dee Belemnite and atmospheric standards for carbon and nitrogen, respectively.

RESULTS

Chironomid gut contents covered a higher proportion of the filters in spring than in summer or fall (Figure 3.2). Cyanobacteria were observed in small quantities in the gut contents in all seasons, though they were most prevalent in the fall (Figure 3.2). There were no spatial trends to cyanobacteria presence, as they were found in gut contents from across the entire transect. Chironomid lengths were bimodally distributed in all seasons, though there were more large individuals in the spring (Figure 3.3). Larger insects had the capacity for more material in their alimentary tract, though this capacity was not always met (Figure 3.3).

On average, phytoplankton and sediments were less enriched for $\delta^{15}N$ than zooplankton and chironomids, though there was extensive spatial variation in $\delta^{15}N$ for all samples (Figure 3.4). Phytoplankton $\delta^{13}C$ values decreased in the summer then increased

in the fall in all clusters (Figure 3.5a). Phytoplankton $\delta^{15}N$ values decreased in the summer only in clusters north of Long Point, but increased in fall in all clusters except cluster 2 (Figure 3.5b). Zooplankton $\delta^{13}C$ changes matched those of the phytoplankton, consistently decreasing in summer then increasing in fall (Figure 3.6a). Zooplankton $\delta^{15}N$ values decreased in summer in all clusters except cluster 7, though the clusters with the largest change were those north of Long Point (Figure 3.6b). In fall, $\delta^{15}N$ values increased in clusters 2 and 3, decreased in clusters 5, 6, and 8, and were relatively consistent in clusters 1, 4, and 7 (Figure 3.6b). Clusters 1 and 2 had sediment $\delta^{13}C$ values that were consistently much higher than the rest of the clusters (Figure 3.7a). There were no spatial trends to the seasonal changes in $\delta^{13}C$ values. The sediment $\delta^{15}N$ value for cluster 1 was the lowest observed, while the value for cluster 2 was the highest observed. Sediment $\delta^{15}N$ values were consistent seasonally, except for an increase from summer to fall in clusters 1 and 2 (Figure 3.7b). Chironomid $\delta^{13}C$ values generally decreased in summer and increased in fall (Figure 3.8a), while chironomid $\delta^{15}N$ values followed the opposite trend, increasing in summer and decreasing in fall, with the exception of clusters 3 and 6 (Figure 3.8b).

There was no clear, linear relationship between $\delta^{13}C$ and $\delta^{15}N$ in the individual samples or between trophic levels. In spring, zooplankton were enriched for $\delta^{15}N$ over phytoplankton by an average of 3.01±0.53‰ (mean±standard error), but less enriched for $\delta^{13}C$ than phytoplankton (Figure 3.9a). In summer, zooplankton were enriched for $\delta^{15}N$ by 1.94±0.38‰, but there was no trend for $\delta^{13}C$ (Figure 3.9b). In fall, zooplankton were not consistently enriched for $\delta^{13}C$, but were enriched by 1.06±0.54‰ for $\delta^{15}N$ compared to phytoplankton, though clusters 1 and 6 had zooplankton that were less enriched for $\delta^{15}N$ than their phytoplankton food source (Figure 3.9c). Chironomids usually had $\delta^{13}C$ values intermediate between those of sediments and phytoplankton in

all seasons, and were always enriched in $\delta^{15}N$ relative to both potential food sources, though the extent of this enrichment varied both spatially and seasonally (Figure 3.10).

DISCUSSION

Little is known about the energy pathways at the base of the Lake Winnipeg food web. With the recent increases in cyanobacterial dominance in the lake, it is important to know whether or not cyanobacteria are a viable food source for the primary consumers. This study presents the first exploration of the availability of cyanobacteria as a food source for chironomids in Lake Winnipeg using gut content analysis, and for both chironomids and zooplankton using stable isotope analysis.

The analysis of the chironomid gut contents suggests that cyanobacteria make up a negligible portion of chironomid diet, regardless of the time of year. This result is unexpected, particularly with the large blooms present in the summer and fall of 2006 (Page 2011). Traditional gut content analysis provides a valuable comparison with stable isotopic studies of diet, but it is not without limitations. The main concern with gut content analysis is that it provides a very short-term view of what an animal has been eating (Sierszen et al. 2003). Chironomids generally have a gut residence time of less than twelve hours (Brooke et al. 1996). Therefore, it would be necessary to catch a chironomid shortly after it had been feeding upon intact cyanobacteria in order to identify the food source accurately. There is the additional concern when sampling chironomids, as common preservation methods may lead to regurgitation of the gut contents (Davies and McCauley 1970). Cyanobacteria lack the rigid frustules found in diatoms, and are likely to be easily digested by chironomids. The diatom frustules found in the chironomid gut contents appeared to be empty, so it is unclear whether the chironomids were ingesting living diatoms or just the frustule remains mixed in with the

sediments. Thus, the large portion of the gut contents that was unidentifiable could have been a mixture of partially digested diatoms, cyanobacteria, or sediments.

Phytoplankton showed a depression in $\delta^{15}N$ values in the summer, moving closer to atmospheric values, suggesting the presence of nitrogen-fixing cyanobacteria, particularly north of Long Point. The concurrent $\delta^{13}C$ depression may reflect changes in growth rate rather than community composition (Gu and Shelske 1996), though there is also evidence that cyanobacteria fractionate carbon more strongly than other phytoplankton (Wainright and Fry 1994). The depression in zooplankton $\delta^{15}N$ values in summer may reflect them feeding upon nitrogen-fixing cyanobacteria, particularly because the largest depressions happened in clusters north of Long Point, in the same clusters that had the largest reductions in phytoplankton $\delta^{15}N$ values. However, the community composition of zooplankton changes throughout the year, with predatory copepods present in higher abundance in the spring, and cladoceran grazers present in higher abundance in the summer (Olynyk 2013), so the reduced $\delta^{15}N$ values may reflect the zooplankton feeding at a lower average trophic level. The spike in zooplankton $\delta^{13}C$ values in the fall may again reflect a change in growth rate, or it may be the result of them feeding upon phytoplankton with higher $\delta^{13}C$ values. The extremely enriched $\delta^{13}C$ values from the sediment samples from clusters 1 and 2 may have been caused by the presence of a high proportion of inorganic carbon, which has a more positive value than organic carbon (Komada et al. 2008). There is a large amount of wind-driven erosion from the north shore into the lake (Greg McCullough, University of Manitoba, Winnipeg, Manitoba, personal communication), which could increase the inorganic carbon content in cluster 1. Cluster 2 is fed by the relatively clear water from Cedar Lake and the Saskatchewan River (Schindler et al. 2012), which could alter its sediment composition relative to the other studied clusters. However, it seems more likely that there is not an addition of inorganic material in clusters 1 and 2 relative to the others in the lake, but

rather a lack of organic material, due to the dominant flow and wind patterns that mix the North Basin. Data on the organic and inorganic carbon content of the sediments of Lake Winnipeg were not publicly available at the time of publication. The seasonal consistency of isotopic values in the sediments requires that the seasonally variable planktonic inputs are being averaged out, likely by bacteria either during or after settling. The presence of identifiable phytoplankton remains in cores (Kling 1998) suggests that at least some of this isotopic levelling occurs within the sediments rather than during settling. Due to sampling limitations, chironomid length was not measured for the stable isotope analysis portion of this study. However, in the gut contents portion, it was observed that the chironomids from the North Basin of Lake Winnipeg had a bimodal distribution of lengths (Figure 3.3). This suggests that either at least two distinct instars or at least two different taxa were present. Chironomids have been found to occupy different trophic levels even when sampled from within the same microhabitat (Reuss et al. 2013). Thus, a bulk sample of chironomids, such as the one used for the stable isotope analysis, might be incorporating biomass from multiple taxa occupying more than a single trophic level. Such a bulk sample would give an average $\delta^{15}N$ value for the chironomids, rather than reflecting the trophic position of each taxon. A true recreation of the benthic food web in Lake Winnipeg would require identification of chironomids to the genus level before the stable isotope analysis (Reuss et al. 2013).

The standard trophic discrimination factor when moving up the food web is 3.4‰ for $\delta^{15}N$, and 0.39‰ for $\delta^{13}C$ (Post 2002). It was expected that the planktonic food web would follow these values. The closest approximation was in spring, though even this fell short of the normal trophic discrimination factor for $\delta^{15}N$, and the fractionation for $\delta^{13}C$ was negative instead of positive. Zooplankton have been found to fractionate at atypical rates, occasionally appearing to undergo negative fractionation, possibly caused by lipid accumulation or changes in $\delta^{13}C$ values with depth (del Giorgio and France

1996). There is also potentially an issue in using size to differentiate between phytoplankton and zooplankton. One rotifer and several zooplankton fragments were observed in the whole water samples during the phytoplankton identification, and there were likely large-bodied and filamentous phytoplankton contaminants in the zooplankton samples. Such cross-contamination would bring the observed stable isotope values closer together, but would not explain the negative fractionation of $\delta^{13}C$ observed in zooplankton. Perhaps the filters were absorbing or adsorbing dissolved inorganic carbon, which has been found to be in the -9.0 to -4.0‰ $\delta^{13}C$ range (Hobson et al. 2010), artificially raising their $\delta^{13}C$ values. The $\delta^{13}C$ values for phytoplankton are expected to be lower than those of the DIC, as phytoplankton selectively use the lighter isotope, increasing the concentration of the heavier one in the DIC, and thus raising its $\delta^{13}C$ value (Trojanowska et al. 2008).

The chironomids also did not follow the expected fractionation for $\delta^{13}C$, unless they were ingesting only phytoplankton. However, their lack of seasonal variation in either $\delta^{13}C$ or $\delta^{15}N$ precludes this possibility. It is likely that the chironomids were feeding almost entirely on sediments rather than intact phytoplankton, but that all sediment samples contained inorganic carbon, skewing their values less negative (Komada et al. 2008). If chironomids were using sediments as their major food source, then their discrimination factor for $\delta^{15}N$ was also less than the expected 3.4‰. Though the chironomids were held to allow them to purge their gut contents, this purging may not have been fully effective. It is also possible that the chironomids were covered in the same microbe community that inhabited the sediments. Both of these factors would reduce the observed difference between chironomid and sediment signatures.

Previous research into stable isotopes in Lake Winnipeg has focused on nutrient pools (Hobson et al. 2010), fish (Gewurtz et al. 2006), and migratory birds (Ofukany et

al. 2012). This study thus fills an important knowledge gap, and provides a valuable baseline of stable isotopes of carbon and nitrogen for the base of the Lake Winnipeg food web. It is apparent that nitrogen-fixing cyanobacteria can be identified by their distinct lowering of the bulk phytoplankton $\delta^{15}N$ signature, and that this signature can be traced through the zooplankton, suggesting that they are available as a viable food source. However, no clear signal can be traced from the phytoplankton or the zooplankton down to the sediments, suggesting that there is an active microbial alteration of their isotopic signatures within the water column or in the sediments, and that different research techniques are required if the impacts of cyanobacteria on chironomids are to be determined.

FIGURES

Figure 3.1. Location of monitoring stations on Lake Winnipeg and the transect of Lake Winnipeg Sampling Stations from which chironomid gut contents were examined. W Stations (hollow circles) were not used unless they were combined with other regular stations. Modified from Figure 2.1 in the State of Lake Winnipeg Report (Environment Canada and Manitoba Water Stewardship 2011).

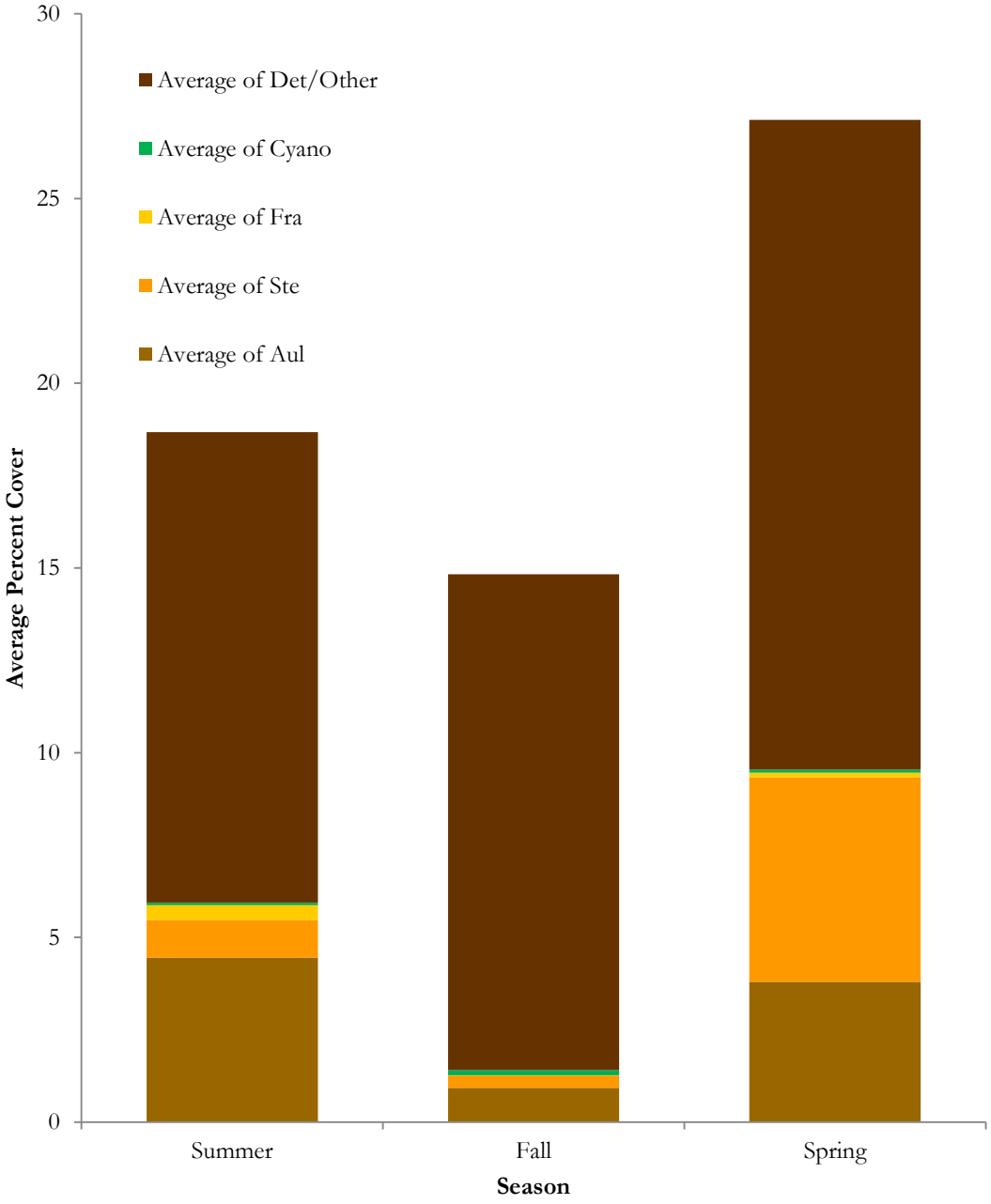

Figure 3.2. Overall seasonal averages of the percentage covered by different taxa on the filters of chironomid gut contents from the transect across the North Basin 2006-2007 (Aul: *Aulacoseira*, Ste: *Stephanodiscus*, Fra: *Fragilaria*, Cyano: Cyanobacteria, Det/Other: detritus and unidentifiable material).

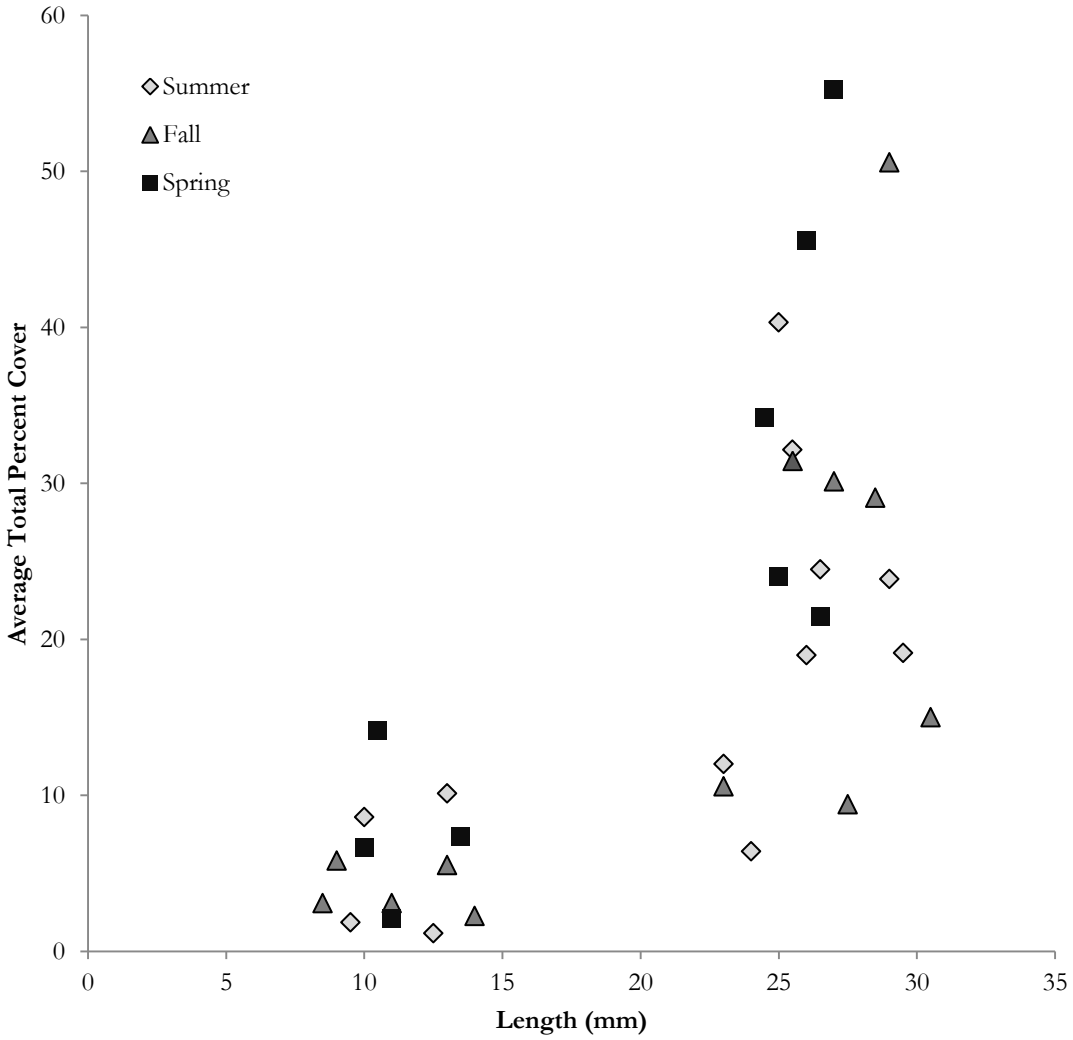

Figure 3.3. The percentage of each filter covered by chironomid gut contents in each season as a function of their length. Longer chironomids had larger guts, and thus had more capacity for more food, though this capacity was not always reached.

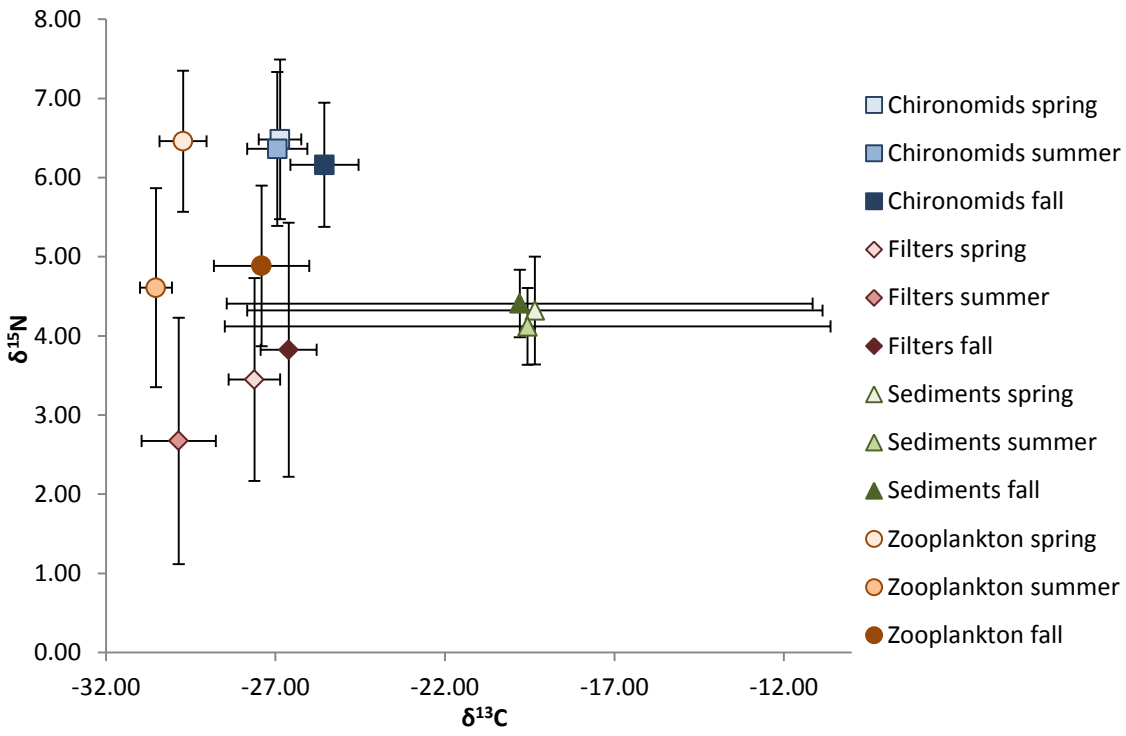

Figure 3.4. Seasonal average $\delta^{13}C$ and $\delta^{15}N$ values for all samples**.** Error bars show ± 1 standard deviation.

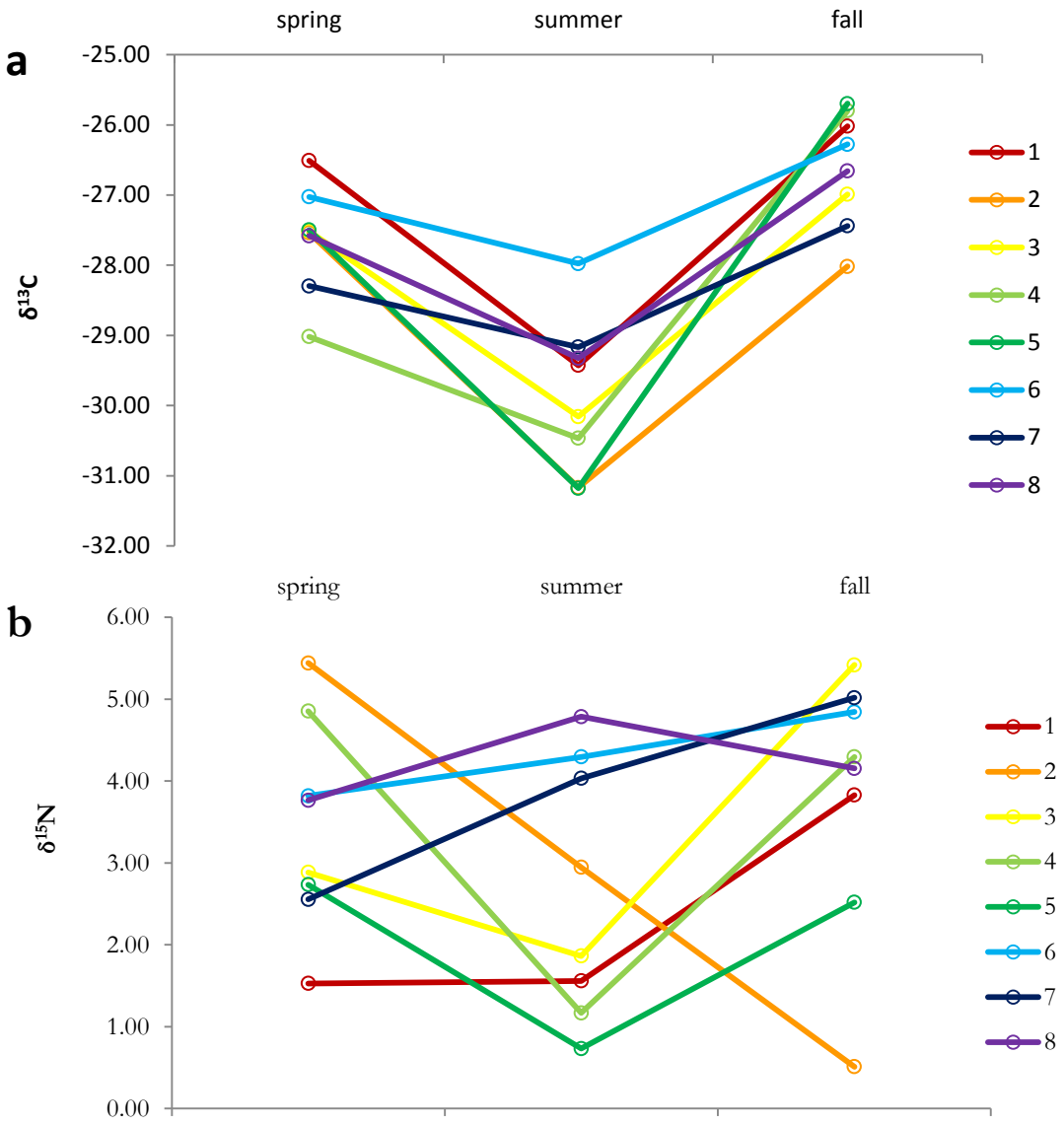

Figure 3.5. Seasonal trends in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) for phytoplankton in the eight clusters in the North Basin.

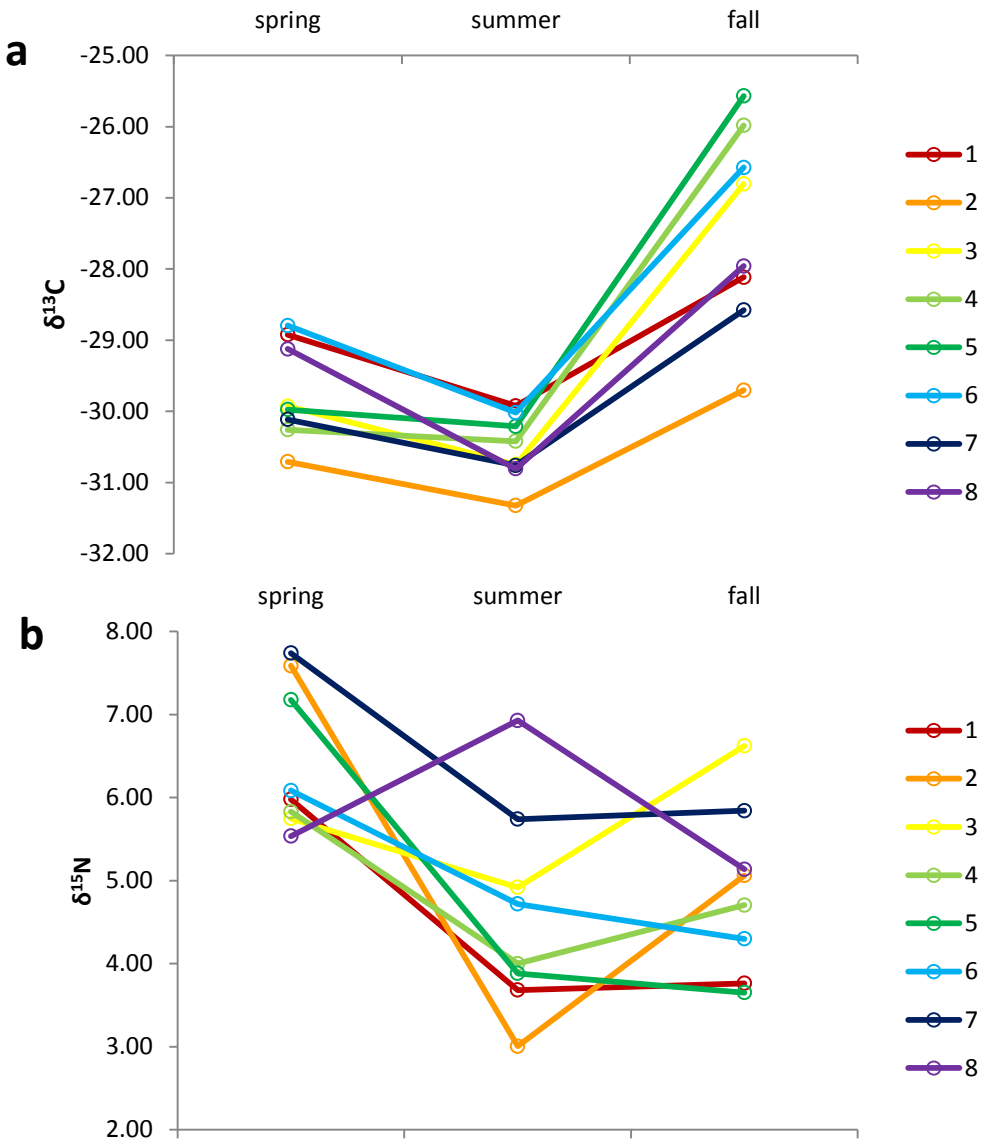

Figure 3.6. Seasonal trends in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) for zooplankton in the eight clusters in the North Basin.

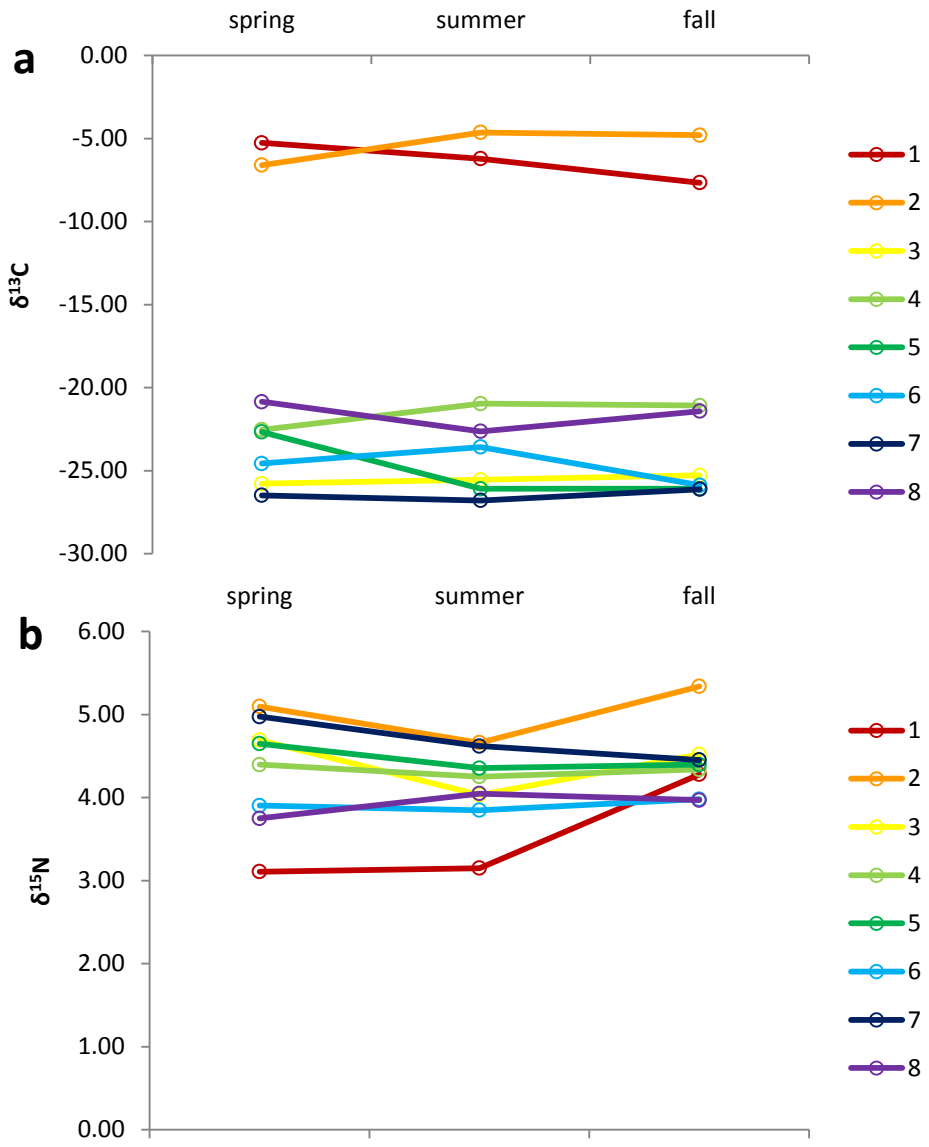

Figure 3.7. Seasonal trends in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) for sediments in the eight clusters in the North Basin.

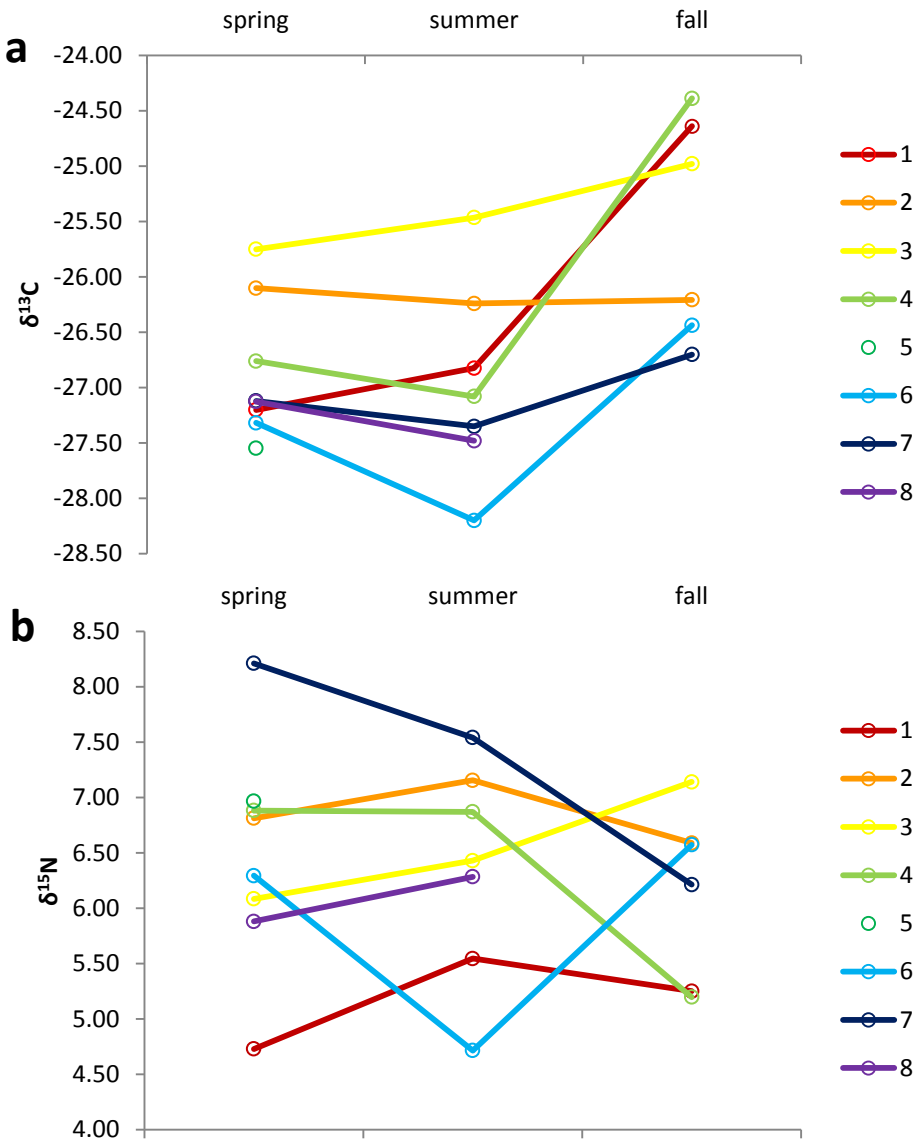

Figure 3.8. Seasonal trends in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) for chironomids in the eight clusters in the North Basin.

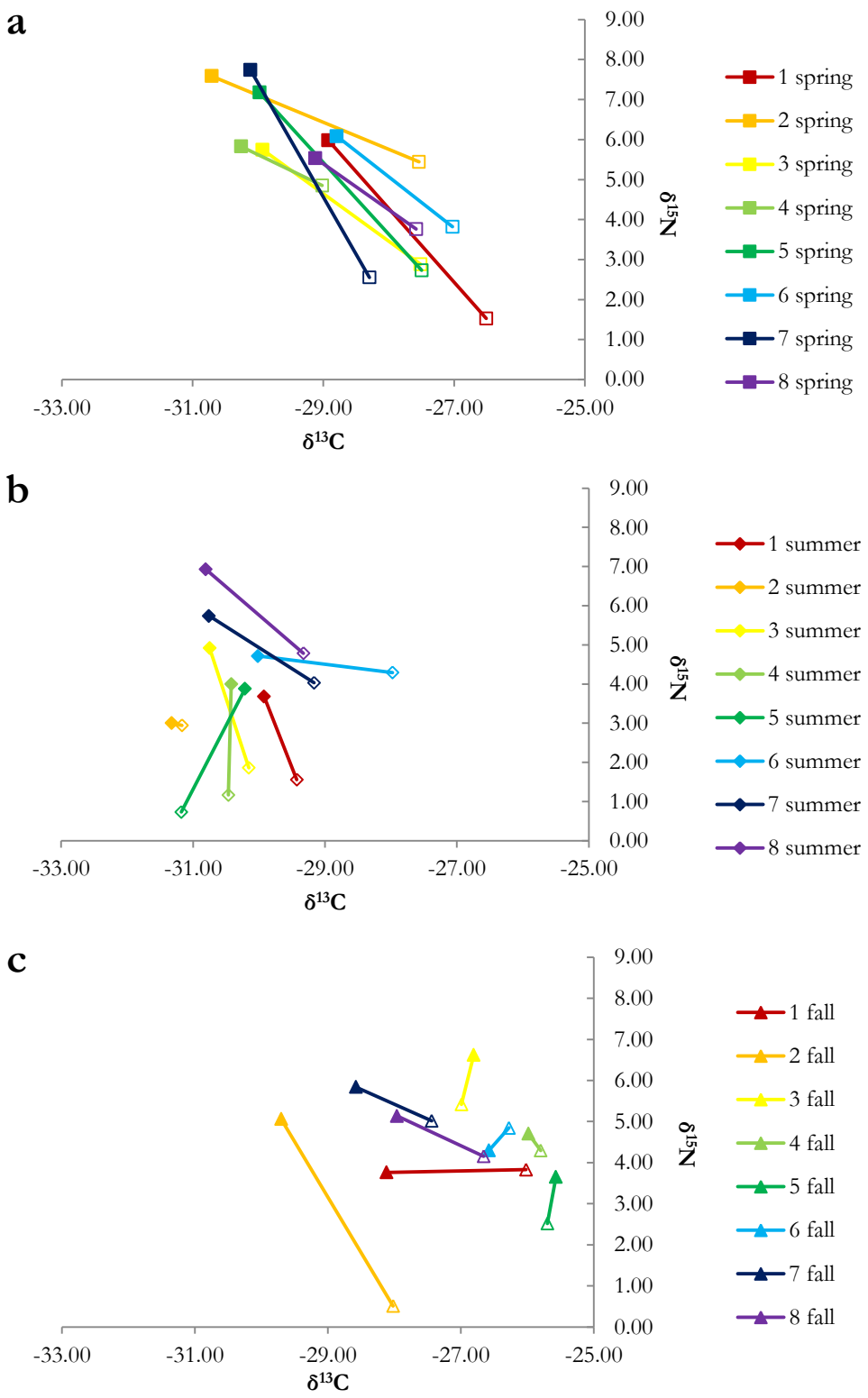

Figure 3.9. Relationship between $\delta^{13}C$ and $\delta^{15}N$ of phytoplankton (open symbols) and zooplankton (filled symbols) within each cluster in the North Basin in spring (a), summer (b), and fall (c).

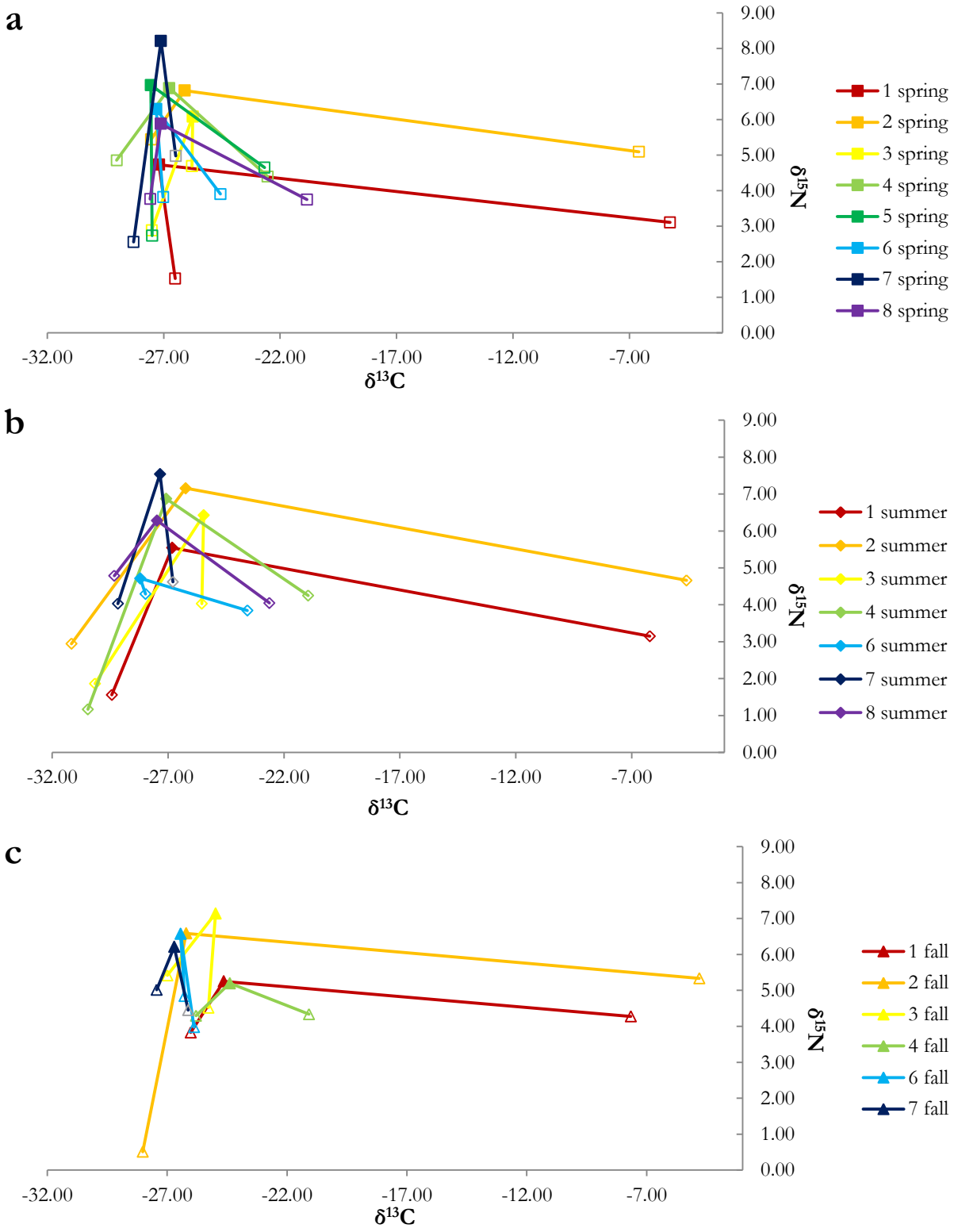

Figure 3.10. Relationship between $\delta^{13}C$ and $\delta^{15}N$ of chironomids (filled symbols), and phytoplankton and sediments (both open symbols, phytoplankton is always less enriched, i.e. more negative, than sediments) within each cluster in the North Basin in spring (a), summer (b), and fall (c).

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

Cyanobacterial blooms, although not to the extent reported in previous years, formed in the summer of 2012 north of Long Point in the North Basin of Lake Winnipeg. These blooms consisted in part of genera known to fix atmospheric nitrogen (i.e. *Aphanizomenon* and *Anabaena*). Bulk, filtered phytoplankton in this region at this time also displayed a reduction in $\delta^{15}N$ values, rendering them closer to atmospheric values. There was a negative relationship between the observed proportion of the phytoplankton that was nitrogen-fixing cyanobacteria in each cluster and the $\delta^{15}N$ values of the filtered phytoplankton in the cluster, but no obvious relationship with any other phytoplankton taxa (Figure 4.1). There was a deviation from this trend with cluster 2 in the fall, which had the lowest observed $\delta^{15}N$ value while not having any identifiable nitrogen-fixing cyanobacteria (Figure 4.1b). Though no intact nitrogen-fixing cyanobacterial cells were observed at this location in the fall, they were observed in the summer. It is possible that the cyanobacteria present in the summer lowered the $\delta^{15}N$ values of the nutrient pool available to other taxa in the fall (Rolff 2000). The $\delta^{13}C$ values for phytoplankton in the summer were positively correlated with the $\delta^{15}N$ values, so they also appeared to be negatively related to the proportion of nitrogen-fixing cyanobacteria. There is evidence that cyanobacteria fractionate carbon more efficiently than other taxa, leading to them having lower $\delta^{13}C$ values than other phytoplankton (Wainright and Fry 1994, Rolff 2000).

Previous research has found minimal fractionation of carbon between trophic levels, which has allowed $\delta^{13}C$ values to be used as a tracer in food web studies (Post 2002). As discussed in Chapter 3, this conservation of values was not found to be the case in this study. The planktonic food web reconstruction may have been impacted by

the mismatch in vertical distribution of the phytoplankton and zooplankton sampled, as phytoplankton were collected only at the surface, while zooplankton integrated the entire water column. Isotopic values of zooplankton and phytoplankton have been found to change with depth (del Giorgio and France 1996), even over a small scale in shallow lakes (Gu and Shelske 1996). Further studies could take advantage of the rosette sampler on board the M.V. Namao by collecting and filtering water samples from discrete depths throughout the water column. This could provide a vertical profile of changes in $\delta^{13}C$ values of the phytoplankton with depth. In order to provide a direct comparison, it would be valuable to conduct similarly stratified sampling of the zooplankton. Such sampling could also provide insight into why the stable isotope values for the sediments were seasonally consistent when their inputs (i.e. phytoplankton and zooplankton) were seasonally variable. The seasonal stability of the sediments may have been due to bacterial degradation of all material prior to sedimentation, though the presence of identifiable phytoplankton material in sediment core samples (Kling 1998) suggests that such degradation cannot be complete. Alternatively, the stability might be driven by the presence of a high proportion of invariable inorganic material (Komada et al. 2008). Regardless of the cause, it is clear that further work is required to identify how isotopically variable phytoplankton and zooplankton inputs generate stable sediments, which host variable chironomids.

The initial goal of this research was to determine whether cyanobacteria were a viable food source for chironomids. The $\delta^{15}N$ signatures provided some evidence that cyanobacteria were actually being ingested by zooplankton, but it was not possible to trace either the $\delta^{15}N$ or the $\delta^{13}C$ signatures from the phytoplankton to the chironomids. There has been some research into the use of $\delta^{34}S$ values as a more reliable discriminant between planktonic and sedimentary food sources in benthic diet, avoiding the fractionation problems sometimes associated with $\delta^{13}C$ values (Croisetière et al. 2009).

There has also been work into using $\delta^{2}H$ in reconstructing benthic food webs, though this is primarily of value in discriminating between organisms eating methanotrophic bacteria and those utilising other food sources (Deines et al. 2009). Both $\delta^{2}H$ and $\delta^{18}O$ have limited use in food web reconstruction for chironomids, as they are heavily influenced by the isotopic signatures of the overlaying water as well as diet (Wang et al. 2009). Further research could use additional isotopes to better track cyanobacteria through the food web.

Alternatively, there could be value in conducting culturing experiments, feeding chironomids nitrogen-fixing cyanobacteria for varying proportions of their lifecycle and observing how their $\delta^{15}N$ signatures changed. Laboratory-raised chironomids have been found to fractionate carbon and nitrogen at 0.7‰ and 3.4‰ respectively, when fed a constant food source (Wang et al. 2009). Both $\delta^{13}C$ and $\delta^{15}N$ values have been found to change seasonally and between instars in chironomids (Grey et al. 2004), so a rigorous dataset examining the effects that varying diet had upon tissue signatures would be a valuable tool in explaining naturally occurring variation. Benthic macroinvertebrates in laboratory cultures have been shown to suffer high mortality rates after ingesting toxic species of cyanobacteria (Frouz et al. 2004), so care would need to be taken to avoid using toxin-producing species.

The cyanobacteria in the North Basin of Lake Winnipeg have been found to produce cyanotoxins, albeit intermittently (Kling et al. 2011). Initial research into cyanotoxins suggested that they were a potential cause of zooplankton avoiding ingesting cyanobacteria (Christoffersen 1996). These cyanotoxins can exert acute and chronic lethal and sub-lethal effects on a range of organisms, including aquatic and terrestrial wildlife, domestic animals, and humans who ingest or come into contact with them (White et al. 2005). Cyanotoxins may persist in the environment for as long as four days

before being broken down by bacteria (Hyenstrand et al. 2003), and may also be absorbed or adsorbed by other phytoplankton, creating another possible mechanism for uptake by consumers (Hyenstrand et al. 2003).

The $\delta^{15}N$ signatures of the zooplankton north of Long Point in this study appeared to reveal that the zooplankton were feeding upon nitrogen-fixing cyanobacteria. Cyanotoxin concentrations were not measured for this study. Therefore, it is possible that the cyanobacteria were not producing them, as cyanotoxin production is temporally variable (Sarnelle et al. 2010). It is also possible that the effects of cyanotoxins are less severe than was initially hypothesized. The literature is divided on the importance and extent of the impacts that cyanotoxins have upon invertebrates. A meta-analysis by Tillmanns et al. (2008) found no difference in the impacts of toxic and nontoxic cyanobacteria. Feeding upon toxic species of cyanobacteria was found to negatively affect *Daphnia*, but only in their first two weeks of life (Sarnelle et al. 2010). Contrastingly, grazing by zooplankton has been found to be important in controlling the size of cyanobacterial blooms in tropical lakes (Kâ et al. 2012). *Daphnia galatea* in Lake Constance, Europe, were found to have evolved additional phenotypic plasticity as a response to the presence of toxic cyanobacteria in less than two decades (Hairston et al. 2001). It is possible that the invertebrates of Lake Winnipeg have developed similar resistances to the toxic cyanobacteria species present.

Cyanobacterial blooms are only one of the major changes Lake Winnipeg is currently facing. The climate, hydrology, and nutrient inputs within the watershed continue to be altered by natural and anthropogenic causes (Schindler et al. 2012). The non-native Rainbow Smelt have already become established and appear to have altered the food web in Lake Winnipeg (Sheppard et al. 2011). *Bythotrephes*, an invasive, predatory zooplankter, has been found in the lake (Kim et al. 2012). Zebra mussels occur

in the watershed and are slowly approaching the lake itself (Wassenaar and Rao 2012). It is difficult to predict what impacts these new invasive species will have, but studies like this one, offering insight into the existing food web structure, will provide a valuable baseline for future research.

FIGURES

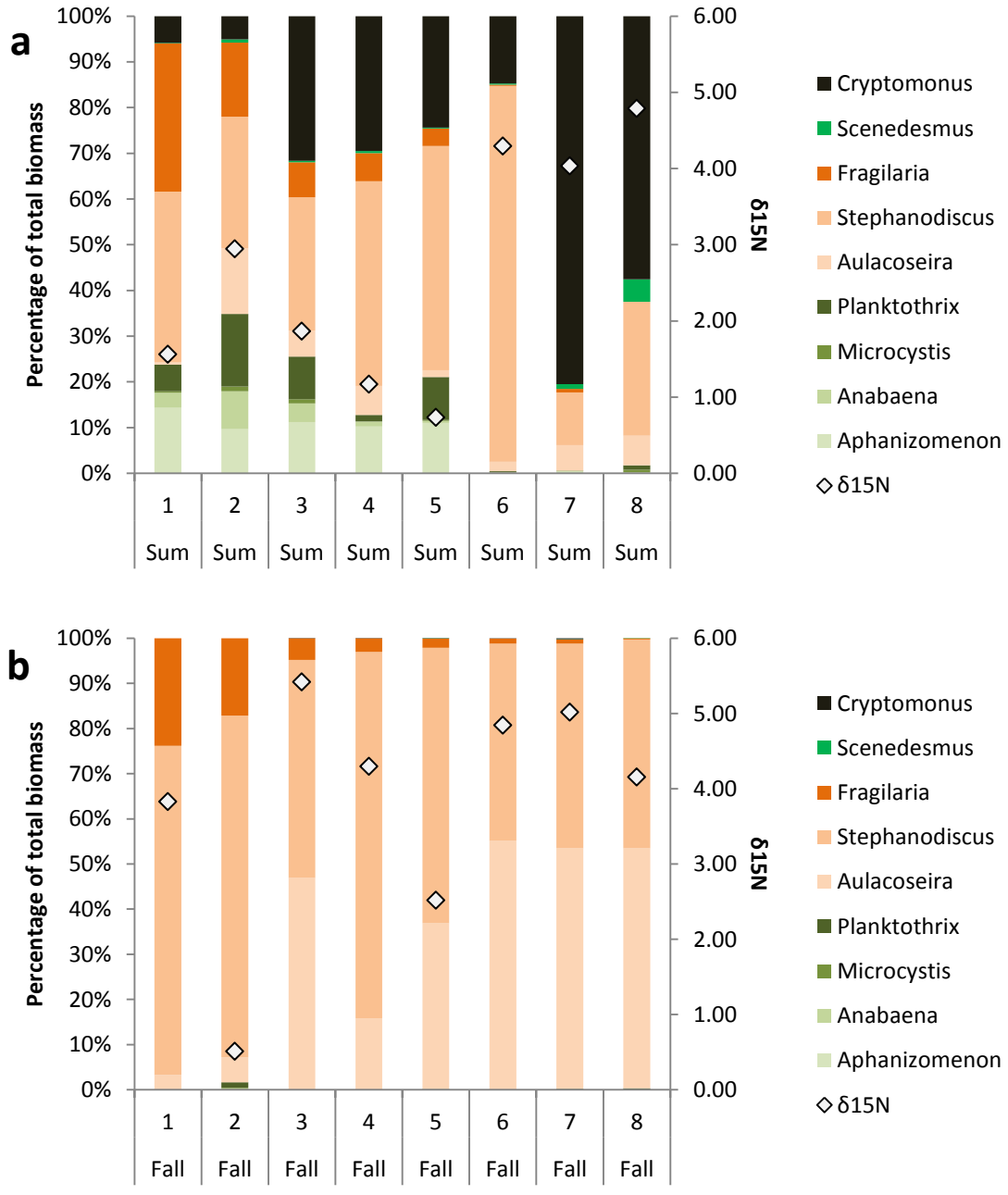

Figure 4.1. Comparison of phytoplankton composition and $\delta^{15}N$values at each of the eight clusters in the North Basin of Lake Winnipeg in summer (a) and fall (b). Phytoplankton use the left axis and $\delta^{15}N$ values use the right axis.

CHAPTER 5: LITERATURE CITED

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